Diagnostic, therapeutic, and immunological responses of SARS- COV- 2: A Review

Ruchi Tiwari, Ancha Kishore Babu[2], Shivam Verma[1], Juhi Mishra[1] and Gaurav Tiwari[1]*

[1]PSIT-Pranveer Singh Institute of Technology (Pharmacy), Kalpi Road, Bhauti, Kanpur-208020, India
[2]KPJ Healthcare University, Persiaran seriemas, Nilai, Negeri Sembilan, Malaysia-71800

***Corresponding Author:**
Dr. Gaurav Tiwari,
Professor & Research Co-ordinator,
Pranveer Singh Institute of Technology, Kalpi Road, Bhauti, Kanpur-208020, Uttar Pradesh, India.
Contact: tiwaridrruchi@gmail.com
+91-8299179267
ORCID: 0000-0003-2200-737X

Abstract

More than 150 million people worldwide are impacted by the coronavirus disease (COVID-19) coronavirus disease's initial wave, and 3 million die as a result. The amount of research on the cure for this virus has grown. Many repurposed medications that were shown successful in limited clinical trials were found to be useless in larger studies. This paper aims to provide an overview of the knowledge that is currently available on the treatment and effectiveness of immunomodulatory agents in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. In order to control inflammatory responses in their specific auto-immune condition, many people with the condition mentioned are receiving long-term treatment with agents like hydroxychloroquine, tumour necrosis factor (TNF-α) inhibitor drugs, other biological agents like monoclonal antibodies to IL-6, and Janus kinase inhibitors like baricitinib and tofacitinib. One randomized controlled trial (RCT) with anakinra was negative, one RCT with baricitinib+remdesivir was positive, and individual studies on various other drugs had intriguing, if early, findings. Hydroxychloroquine was not helpful at any illness stage. The SARS-Cov-2 infection's pathogenesis showed that multiorgan damage and immunological dysregulation predominated over hyperinflammation. Even yet, there is mounting evidence that immunomodulatory treatments may effectively treat COVID-19. RCTs contrasting glucocorticoids alone against glucocorticoids + anticytokine/immunomodulatory therapy are necessary since glucocorticoids seem to increase survival in select subgroups of patients.

Keywords: *COVID-19, Immunomodulation, immunology, immune system, cytokines, hyper inflammation, Janus kinase inhibitors.*

1. Introduction

SARS-CoV-2, which initially surfaced in Wuhan, China, in December 2019, is to blame for this extraordinary epidemic. A significant fraction of infected individuals may incur respiratory failure, acute respiratory distress syndrome, and mortality, despite the fact that the majority of patients only exhibit mild to moderate symptoms. Although there is a diagnostic test for polymerase chain reaction **(**PCR**)** testing of the virus by nasopharyngeal swab, there are cases where clinical symptoms like coughing, shortness of breath, and fever are present but a swab test result may be negative. These symptoms are frequently accompanied by laboratory changes like lymphopenia, elevated serum C-reactive protein (CRP), ferritin levels, and pulmonary infiltrate on chest radiographs. However, it may result in devastating diseases in high-risk people, including severe acute respiratory failure, multiorgan dysfunction, and death. In order to reduce mortality and morbidity related to COVID-19, it is crucial to have safe and effective pharmaceutical treatments (e.g., Long COVID). Nevertheless, COVID-19 treatment is still difficult despite the discovery of the SARS-CoV-2 vaccine being successful. Numerous medications that had been repurposed and shown beneficial in smaller clinical trials turned out to be unsuccessful in larger research. A vicious cycle that includes the release of proinflammatory mediators into the lungs, abnormal immune cell activation, coagulopathy, and histological evidence of hemophagocytosis in patients with more severe (1). Some characteristics of COVID-19 shown by the macrophage activation syndrome (MAS), also known as secondary haemophagocytic lymphohistocytosis, were found (sHLH). We go through the justification for the prospective use of immunomodulatory treatments in the treatment of SARS-CoV-2 in this overview. We will specifically investigate whether patient subgroups, based on the severity of the illness and the body's reaction, could benefit from immunomodulators. We investigate the known outcomes of immunosuppressive drugs (such as corticosteroids, interleukin (IL)-1 inhibitors, IL-6 inhibitors, and kinase inhibitors) and immunomodulators (such as IFN alpha, IFN beta, non-SARS-CoV-2 specific immunoglobulin, and convalescent plasma).

It is now clear that SARS-CoV-2 infection has two distinct clinical phases of infection: the initial viral infection and replication phase, followed by the inflammatory phase, which frequently causes rapid deterioration and worsening respiratory symptoms and frequently necessitates hospitalization to prevent deterioration. Immunosuppression is probably advantageous in cases of hyper inflammation even if corticosteroids aren't always advised and may make COVID-19-related lung damage worse. The present status of available treatment options that may be utilized to either directly target the virus or lessen its impact on the host response. In this article, we provide a brief summary of the research on the structure and life cycle of the virus and provide updated information on how the virus affects the host's innate immunity and inflammatory response, which are the main contributors to severe acute respiratory disease brought on by SARS-CoV-2 infections (2).

2. Structure of SARS- CoV- 2

Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus are the four genera that make up the Coronaviridae subfamily of the Coronaviridae family, which includes coronaviruses. The single-stranded positive-sense RNA (+ssRNA) virus CoVs has a bigger

genome than any other ribonucleic acid (RNA) virus (27–32 kb). Outside the genome, the nucleocapsid protein (N) formed the capsid, and the envelope that surrounds the genome is connected to three structural proteins: membrane protein (M), spike protein (S), and envelope protein (E). SARS-CoV-2, a recent member of the coronavirus family, has a genomic size of around 29.9 kb (Figure 1). SARS-CoV-2 has sixteen non-structural proteins (Nsp1–16) and four structural proteins (S, E, M, and N). Nsp1 mediates RNA replication and processing. Nsp2 modifies the host cell's survival signaling system. The translated protein is thought to be separated by Nsp3. Nsp4 alters ER membranes and has transmembrane domain 2 (TM2). Nsp5 takes involved in the replication-related polyprotein process. A presumed transmembrane domain is Nsp6. The interaction between nsp12 and template-primer RNA was greatly enhanced by the presence of nsp7 and nsp8. As a ssRNA-binding protein, Nsp9 serves a purpose. Nsp10 is essential for viral mRNA cap methylation. The RNA-dependent RNA polymerase (RdRp), an essential component of coronavirus replication and transcription, is found in Nsp12. Nsp13 interacts with adenosine triphosphate **(**ATP), and its zinc-binding domain is involved in transcription and replication. A proofreading exoribonuclease domain is Nsp14. Nsp15 possesses endoribonuclease activity that is Mn^{2+} dependent. A 2'-O-ribose methyltransferase is Nsp16. According to one research, NSP-mediated effects on protein trafficking, translation, and splicing may suppress host defenses. NSP16 interacts with the U1 and U2 snRNAs' messenger RNA (mRNA) recognition domains during SARS-CoV-2 infection to prevent mRNA splicing. In order to prevent mRNA from being translated, NSP1 binds to 18S ribosomal RNA in the mRNA entrance channel of the ribosome. In order to prevent protein from being transported to the cell membrane, NSP8 and NSP9 bind to the 7SL RNA at the Signal Recognition Particle (3-4).

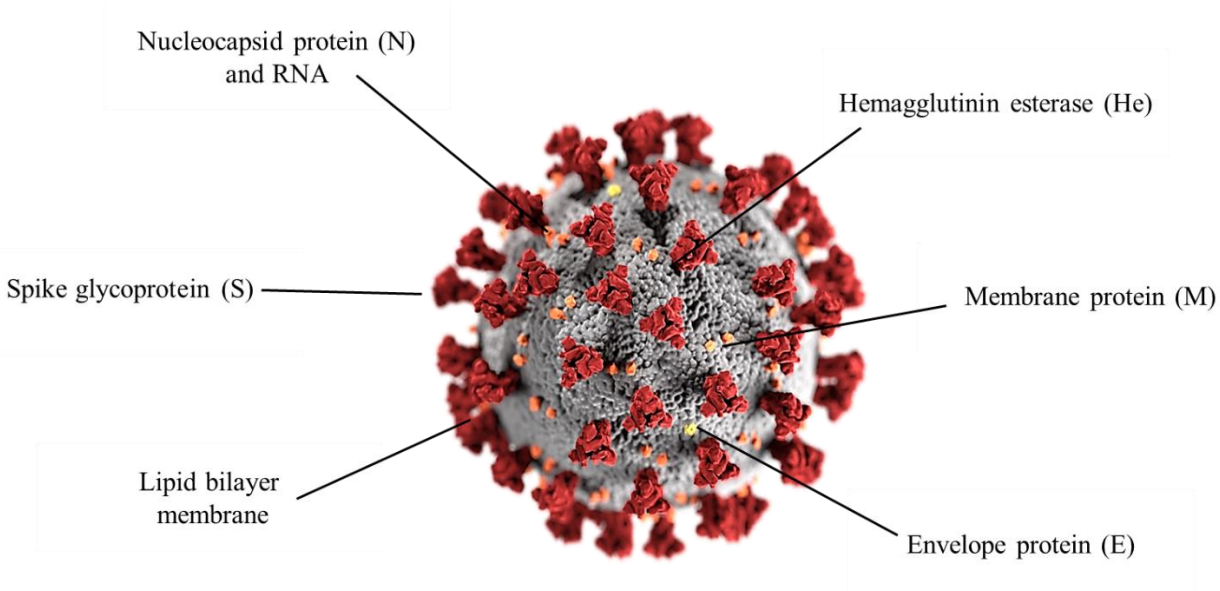

Figure 1: Structure of SARS-CoV-2.

3. Detection methods

3.1. Viral shedding

The viral shedding in throat swabs and sputum peaks five to six days after the beginning of symptoms and ranges from 104 to 107 copies ml1. Higher viral levels in the respiratory tract are reflected by this. Nearly all nasal swabs from sick persons have viral RNA in them. Blood, saliva, and tears all tested positive at rates of 88, 78, and 16%, respectively. Large-scale population field testing using the chemiluminescence immunoassay, the enzyme-linked immunosorbent assay, and the lateral-flow immunochromatographic assay is made possible by the self-collection of naso- or oropharyngeal swabs. The lateral-flow immunochromatographic test offers a quick platform for point-of-contact serological detection using gold nanoparticles (AuNPs) and a colorimetric label. Here, a nanoparticle-conjugated SARS-CoV-2-specific antigen is used. SARS-CoV-2 Immunoglobulin G (IgG) and Immunoglobulin M (IgM) may bind to the SARS-CoV-2 antigen and antibody, which are identified colorimetrically, by loading blood or saliva specimens. The test is finished in 20 minutes and has a 90% accuracy rate. The shortest duration of viral shedding to date is 7 days following the beginning of symptoms, and viral infectivity is shown within 24 hours. Like the presence of serum neutralizing antibodies, SARS-CoV-2 detection falls to undetectable levels. Even in instances with concurrently high viral loads, 8 days after the beginning of symptoms, the live virus could not be reproduced in cell culture. These findings support the use of serological and quantitative viral RNA load measurements for determining when to stop using infection control measures.

3.2. RT–PCR

Although there are already protein, antibody, and nucleic acid-based diagnostic assays for the SARS-CoV-2 pandemic, viral nucleic acid detection by RT-PCR remains the gold standard. Over the currently available serological assays, nucleic acid testing offers increased sensitivity and specificity for virus identification. The use of reverse transcription–polymerase chain reaction (RT-PCR) as a sensitive, accurate, and specific viral detection method is required to distinguish SARS-CoV-2 from other common respiratory viruses. Despite the precision of the test, findings have not yet made it possible to control viral infection. The US Food and Drug Administration (FDA) allowed authorized labs to report internal SARS-CoV-2 diagnostic tests in February 2020. the isolation and conversion of viral RNA to complementary DNA at the start of the process (cDNA). After that, Taq DNA polymerase is used to ramp up the cDNA. The final overall procedure of the RT-PCR test, which measures viral load The whole turnaround time may go above two days and there is a chance of cross-contamination reducing specificity6. The examinations are often carried out in hospital labs (5).

3.3. Isothermal amplification using RT loop (RT–LAMP)

Nanotechnology is the foundation of Reverse Transcriptase loop-mediated isothermal amplification (RT-LAMP). The three by-products of the RT-LAMP system's three phases may be used as a model for the LAMP system's response. As LAMP reagents for creating the amplification mixes, step I uses solutions of deoxyribose adenosine triphosphate (dATP), polymerase (Bst 2.0), and avian myeloblastosis virus (AMV) transcriptase. The LAMP reagents' interaction with the open reading frame 1a/b (F1ab)-forward loop primer (LF) and

the nucleoprotein (np)-backward loop primer (LB) (np-LB*) is what initiates the isothermal amplification (RT-LAMP reaction in step (ii)). Products that may detect COVID-19 RT-LAMP are offered in step (iii). Step 1 displays the results of labeling F1ab-LF* and F1ab-LB* or np-LF* and np-LB* for digoxigenin and biotin, respectively. Step 2 displays fluorescein isothiocyanate **(**FITC)/biotin-labeled np-LAMP and FITC/biotin-labeled F1ab-LAMP amplicons (iii). The np-RT-LAMP is labelled with digoxigenin and biotin, whereas the F1ab-RT-LAMP product is labelled with FITC and biotin. In contrast, FITC is assigned to the F1ab primer set. Additionally, SARS-CoV-2 RNA is converted to cDNA using AMV-RT at 63 °C in 40 min, and the labelled F1ab-LF* and F1ab-LB* primers respond under the optimal circumstances. The FITC products and digoxigenin used in the RT-LAMP technology, which is used to detect F1ab and np primer, offer the raw materials for the following LAMP amplification. Easy COV RT-LAMP assays do not need centrifugation procedures, which are time-consuming, costly, and necessary for RNA extraction. The EasyCOV technique is an easy and uncomplicated test that doesn't need RNA extraction from the sample. EasyCOV's findings showed that it has a sensitivity of 72.7%. Individuals' infection profiles may be determined using LAMP methods on saliva. Due to its straightforward, quick, and painless patient process, EasyCOV is suitable for large-scale screenings of the general population and can identify SARS-CoV-2 in saliva.

3.4. Antigens for SARS-CoV-2

To find the presence of viral antigens produced by SARS-CoV-2 in samples taken from infected people's respiratory tracts, a quick diagnostic test was also created. In this test, antibodies attached to a paper strip within a plastic case bind to antigen contained in the sample. Within 30 minutes, this reaction produces a signal that may be seen. The tests may be used to detect acute or early infection since the identified antigen(s) are only produced if the virus is actively reproducing. Additionally, Abbott has launched a more widespread kind of fast diagnostic test for COVID-19 that looks for antibodies in the blood of infected people. On the ARCHITECT i1000SR and i2000SR laboratory apparatuses, which can perform between 100 and 200 tests per hour, Abbott's test can find the SARS-CoV-2 antibody. After one week of infection, SARS-CoV-2 antibodies start to develop. Any antibody response's potency is influenced by factors including age, nutritional state, illness severity, coexisting diseases, and medication.

3.5. Testing saliva

Saliva analysis has the potential to be used as a diagnostic tool since it can make it easier to find both the virus and the antibodies in COVID-19 patients. A significant possibility exists for screening for COVID-19 using human saliva samples. Using RT-PCR, it is possible to identify respiratory infections in saliva, including two seasonal human coronaviruses. In fact, saliva ($n = 37$) had mean SARS-CoV-2 titres (virus copies ml1) that were five times greater ($P < 0.05$) than nasopharyngeal swabs ($n = 46$). Additionally, none of the previously negative saliva samples became positive. On the other hand, in five cases, nasopharyngeal swabs first tested negative for SARS-CoV-2 and then became positive after a second test. However, with a considerable direction of present research activity, ever more trustworthy self-administered sample collection is still required (6-7).

4. Therapies based on cytokines

In cases of active rheumatic inflammation, targeted biologic treatments targeting certain cytokines have taken over as the preferred form of care. The management of conditions like rheumatoid arthritis, ankylosing spondylitis, and inflammatory bowel disease with cytokine-targeted biologic therapies has changed over the past few decades due to advances in the immunology of inflammatory diseases and technologies that allow for the mass production of biologic therapies. The production of cytokines such IL-1, IL-6, and tumor necrosis factor alpha **(**TNF-α) occurs quickly in persons hospitalized with SARS-CoV-2 infection, according to data from numerous organizations. Rapid start of the cytokine storm (CS) necessitates immediate treatment to avert multi-organ failure and mortality in the presence of additional concurrent risk factors such male gender, older age, immuno-compromise, and obesity. It has been reported that various risk variables, including advanced age, male gender, black or ethnic minority origin, obesity, diabetes mellitus, and cardiovascular disease, have been linked to the severity of SARS-CoV-2 and an increase in mortality. According to these observations, there may be genetic risk factors for cytokine release syndrome (CRS) or CS. For the treatment of active rheumatoid arthritis, juvenile idiopathic arthritis, and replasing or refractory giant cell arteritis, biologics targeting IL-6 are approved, such as tocilizumab, a humanized monoclonal antibody produced to the IL-6 receptor (GCA). Additionally, they have a license to treat cytokine release syndrome. The acute phase response, including CRP and ferritin, and fever are both mediated by IL-6. Immune system hyperactivation is a defining feature of COVID-19 severity. Numerous studies have shown that COVID-19 patients who need intensive care had higher levels of leukocytes, procalcionin, CRP, and other proinflammatory cytokines and chemokines (e.g., IL-1 and IL-6) and chemokines (e.g., CXCL10 and CCL2) (8). This kind of uncontrolled apoptosis, vascular leakage, thrombosis, multiorgan damage, and mortality are all possible outcomes of this hyperactive inflammatory response, which also starts the cytokine release syndrome.

4.1. Inhibitors of the cytokine TNF-α

Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, juvenile arthritis, and inflammatory bowel disease are only a few of the inflammatory disorders for which biologic treatments that target the suppression of TNF-α were introduced in the 1990s. TNF-α inhibitors are being utilized in a broad range of formulations, including completely humanized biologics specific to TNF-α like adalimumab, etanercept, and infliximab. TNF-α efficacy as a therapy for a wide range of disorders has supported the finding that it is a crucial cytokine generated in a variety of conditions that cause inflammation, both in the acute and chronic phases. Blocking TNF-α causes a subsequent drop in IL-1 and IL-6, adhesion molecules, and angiogenic factors like vascular endothelial growth factor in diseases like rheumatoid arthritis (VEGF). The justification for using TNF-α inhibitors in hospitalized SARS-CoV-2 patients has been put forward[8]. Patients with inflammatory bowel disease and inflammatory arthritis are screened for cancer and tuberculosis (TB), and those who have a history of latent or active TB are put on TB eradication medication before initiating TNF-α inhibitors. Additionally, TNF-α inhibitors are often not prescribed to persons who have had cancer within the past five years. These

factors may not matter in the immediate aftermath of a Covid-19 infection, but they could have long-term effects and need to be taken into account while designing studies.

4.2. Inhibitors of janus kinase

Small compounds known as jakinibs, or janus kinase (JAK) inhibitors, stop the JAK-STAT (signal transducer and activator of transcription) signaling pathways implicated in the pathophysiology of several inflammatory and/or immune-mediated disorders. Janus kinase inhibitors have shown efficacy in treating a range of immune-mediated conditions that impact the skin, joints, and digestive system. Janus kinase inhibitors have also been used effectively in conditions such as polycythemia vera, essential thrombocythemia, and myelofibrosis that involve Janus kinase mutations (9). The Janus kinase inhibitors are frequently referred to as selective synthetic anti-rheumatic medications (tsDMARDs). JAK1, JAK2, JAK3, and tyrosine kinase 2 are the four receptor-associated tyrosine kinases that make up the Janus kinase family (TYK2). JAKs regulate gene transcription in the nucleus via phosphorylating intracytoplasmic STATs and cell membrane cytokine receptors when they are active. Each JAK and tyrosine detect a slightly different ATP-binding site, and ATP provides the phosphate. Autoimmune disorders and cancers may arise as a consequence of the overexpression of these pathways. JAK inhibitors (JAKi) fall into one of two categories: JAK1i (oclacitinib, upadacitinib, abrocitinib, itacitinib) and Pan-JAKi (delgocitinib, peficitinib). JAK1/2 and JAK3 inhibitors include baricitinib and ruxolitinib. JAK1/3 and JAK3i inhibitors include decernotinib and ritlecitinib. TYK2 inhibitors include deucravacitinib and brepocitinib.

Baricitinib treatment is now being tested in many clinical trials (NCT04320277 and NCT04321993) in comparison to anti-viral medications, but no results have been published as of yet. In recent research from the USA, 62% of participants were using a biologic medication or JAKi, but only 7% of those people were hospitalized. These subjects had both SARS-CoV-2 and an immune-mediated inflammatory disease. According to data from the US case series of persons who had SARS-CoV-2, using an immunomodulator did not seem to raise the probability of contracting SARS-CoV-2 characteristics that caused significant illness or death in this case Series (10-11).

ABL, numb-associated kinase, cyclin-dependent kinase, phosphoinositide 3 kinase/protein kinase B/mechanistic target of rapamycin (mTOR), extracellular signal-regulated kinase/mitogen- activated protein kinase, and Janus kinase (JAK) are a few kinases that are important for viral infection and are predicted to be (Figure 2). They have significant effects on viral replication, intracellular membrane trafficking, entrance, and the life cycle of the virus. They also have an immunomodulatory impact that may be helpful in preventing COVID-19-mediated hyperactive immune response.

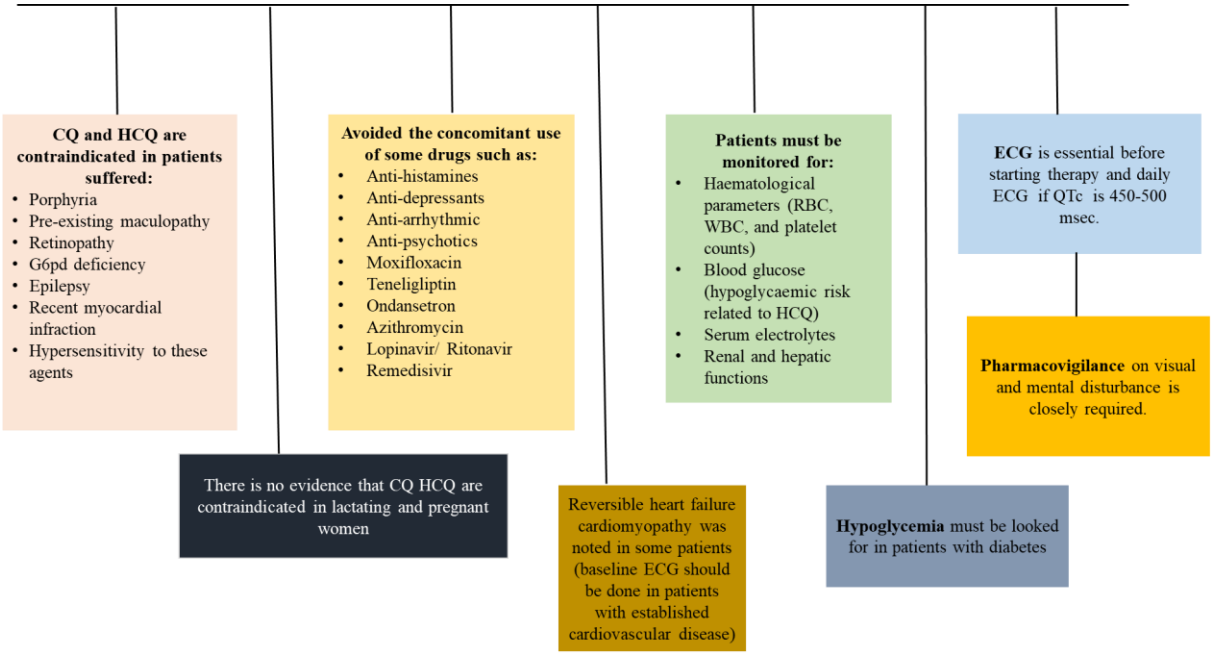

Figure 2: Function of Janus kinase inhibitors in the management of inflammatory and autoimmune illnesses

The following are a few examples of Janus kinase inhibitors that are offered in the US: Inrebic (fedratinib), Olumiant (baricitinib), Xeljanz (tofacitinib), Jakafi (ruxolitinib), Rinvoq (upadacitinib), Cibinqo (abrocitinib), and Opzelura are examples of drugs that are used to treat cancer (ruxolitinib)

The FDA granted Olumiant (baricitinib) approval in 2018. It includes an FDA black box warning for thrombosis, cancer, and cardiovascular problems. Olumiant comes as a 2 mg tablet, which should be taken once a day. The FDA rejected the 4mg dosage due to severe adverse effects. Upper respiratory infections and excessive cholesterol were uncommon, but were more common with baricitinib at increasing dosages, according to studies. Adults with moderately to severely active rheumatoid arthritis who did not previously respond adequately to methotrexate or tumour TNF-α inhibitor treatments are eligible to use olumiant. It has been approved in Europe for the treatment of COVID-19 based on research published in 2020 that demonstrated combining baricitinib with direct-acting antivirals might lower infectivity, viral replication, and inflammation related.

An article from Arthritis & Care Research from 2019 states that 4 mg of Olumiant monotherapy per day is useful for rheumatoid arthritis patients in controlling their condition. When methotrexate was added, the patient in the trial who didn't react well to baricitinib alone had better disease control.

Ruxolitinib, or Jakifi: 2011 saw the FDA's approval of jakafi. JAK1 and JAK2 are intended to be inhibited by it (12). Tablets of this medication come in strengths ranging from 5 mg to 25 mg. Since thrombocytopenia, anaemia (few red blood cells), and neutropenia are possible side effects of Jakafi, platelet counts must be checked both before and after commencing treatment. Clinical studies using roxolitinib are now being conducted to treat alopecia areata, pancreatic cancer, plaque psoriasis, and two different forms of lymphomas. The following myelofibrosis

conditions are among those that Jakifi is authorised to treat: - Primary myelofibrosis, post-polycythemia vera myelofibrosis, and post-essential thrombocythemia myelofibrosis
Adults with polycythemia vera who either did not react to or were intolerant to acute graft-versus-host disease in adults and children over the age of 12 who did not improve after receiving steroid therapy Graft-versus-host-disease. Ruxolitinib is being studied for a number of additional illnesses, including certain cancers, and may be used off-label for a number of other indications, including alopecia and plaque psoriasis. Upadacitinib, or Rinvoq: The FAD first authorized Rinvoq in 2019. The recommended dosage for this medication is one 15 mg tablet per day. Adults with moderately to severely active rheumatoid arthritis who did not react well to or were unable to take methotrexate may be treated with Rinvoq. Additionally, it has been licenced for the treatment of psoriatic arthritis, atopic dermatitis, and ulcerative colitis. Rinvoq is still being studied as a potential therapy for Crohn's illness
spondylitis with ankylosing, Psoriasis, Colitis of the bowels. Upadacitinib was efficacious and well-tolerated in persons with active ankylosing spondylitis who didn't tolerate or react well to non-steroidal anti-inflammatory medicines (NSAIDs). According to research released in late 2019. Cibinqo (abrocitinib) is a more recent medication in this class; it got FAD approval in 2022. Adults with moderate to severe atopic dermatitis that is unresponsive to other systemic medications, including biologics, may use Cibinqo (abrocitinib) to treat their condition. For oral use, this medication comes in 50 mg, 100 mg, and 200 mg tablets. Ruxolitinib Opzelura: Oral ruxolitinib received FAD approval in 2011, while ruxolitinib cream received approval in 2022. Opzelura has been given the all-clear to treat nonsegmental vitiligo in adults and children 12 years of age and older. The treatment of mild to severe atopic dermatitis is also permitted. Opzelura is offered as a 1.5% cream that must be administered twice daily. The maximum dosage is 100 g every two weeks or 60 g every week. Rheumatoid arthritis is one condition for which filgotinib is being evaluated as a therapy. arthritis psoriatic (Ulcerative colitis, Crohn's disease) Inflammatory bowel illness HIV infection. Being "very selective" indicates that it only targets a small number of JAK enzymes as opposed to many of them (13-14).

4.3. Hydroxychloroquine and chloroquine

Since its first synthesis in 1934, chloroquine has been widely used to treat autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus as well as to prevent and cure malaria.
Later, in 1955, hydroxychloroquine was released, and because of its enhanced safety profile, it immediately gained popularity. These medications' ability to combat plasmodium parasites is thought to be partially due to their interaction with DNA and their ability to prevent heme from polymerizing. A wide range of immunoregulation networks that have been thoroughly addressed in prior literature are connected to hydroxychloroquine's immunomodulatory effect. Antimalarial drugs like chloroquine and hydroxychloroquine are routinely used around the globe. The quinolone family includes the drugs chloroquine and hydroxylchloroquine. Although the therapeutic and toxic doses of these medications vary, they are related agents with a similar retinal toxicity profile. Rheumatic illnesses, such as systemic lupus erythematosus (SLE), are also treated with them. They are both weak bases: chloroquine and hydroxychloroquine. They are widely distributed and have a half-life of around 50 days.

It is still unclear exactly how chloroquine and hydroxychloroquine work to combat SARS-CoV-2. Chloroquine was first investigated in SARS-CoV, the virus that caused the SARS coronavirus outbreak in 2002–2003. Although SARS-CoV and SARS-CoV-2 have a genetic sequence that is 79% identical, SARS-CoV is expected to cause a more severe illness with a case fatality rate of 10% as opposed to 3%. According to research done originally on SARS-CoV, it is thought that SARS-CoV-2 penetrates cells through attaching to the angiotensin-converting enzyme-2 (ACE-2) receptor. Chloroquine may stop the virus from attaching to this receptor by blocking terminal glycosylation.

According to recent study, hydroxychloroquine may also block SARS-CoV-2 from binding to gangliosides, which may in turn impede interaction between the virion and the ACE-2 receptor. While some clinical studies are utilizing chloroquines at high doses, ranging from 500 to 1000 mg per day, for individuals with symptoms severe enough to warrant hospital admission, other reports indicate early usage to limit viral reproduction may be ideal. Figure 3 explains the warnings and contraindications for using chloroquine and hydroxychloroquine (15).

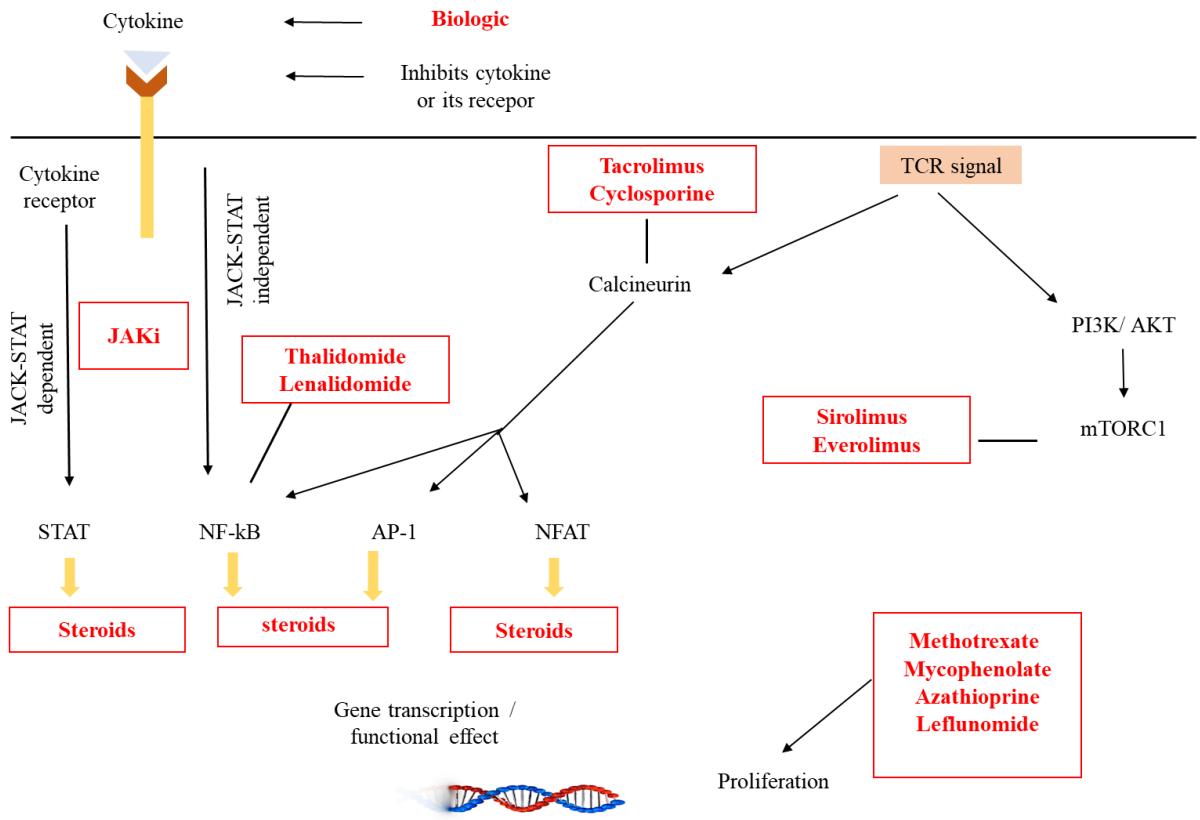

Figure 3: Caution and contraindication of chloroquine and hydroxychloroquine.

In the context of randomized controlled studies, the possible longer-term toxicity consequences of chloroquine in the setting of SARS-CoV-2, such as myocarditis arrhythmias, retinal damage, remain unknown. It has not been demonstrated that hydroxychloroquine is effective in reducing mortality or hospital stay duration, according to newly available data from the recovery trial, which included 1542 patients who were randomly assigned to receive hydroxychloroquine and 3132 patients who were assigned to receive usual care. Malaria and amebiasis have long been treated with chloroquine (N4-(7-Chloro-4-quinolinyl)-N1. N1-dimethyl-1, 4-pentanediamine).

However, widespread plasmodium falciparum resistance to it resulted in the development of other antimalarials, making it an option for the prevention of malaria. Acute poisoning and even death may result from a CQ overdose. CQ's manufacturing and market supply were significantly decreased in previous years owing to its limited use in clinical practice, at least in China. In order to create the derivative of CQ known as hydroxychloroquine (HCQ) sulphate, a hydroxyl group was first added to CQ in animals in 1946. More significantly, HCQ is still commonly used to treat autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus. It is simple to imagine that HCQ would be a strong option to cure infection by SARS-CoV-2 given that CQ and HCQ have comparable chemical structures and processes of functioning as a weak base and immunomodulator. Actually, as of February 23, 2020, seven clinical trial registries for employing HCQ to treat COVID-19 were discovered in the Chinese clinical trial registry. The experimental data to support the claim that HCQ is as effective as CQ in treating SARS-CoV-2 infection are currently lacking.

In order to achieve this, we compared the antiviral activity of HCQ to CQ against SARS-CoV-2 infection in African green monkey kidney VeroE6 cells (ATCC-1586). The results revealed that the CC50 values for CQ and HCQ were 273.20 and 249.50 m, respectively, and were not significantly different from one another. By measuring the viral RNA copy number in the cell supernatant at 48 hours after infection, the dose- response curves of CQ and HCQ against SARS-CoV-2 were calculated at four different multiplicities of infection (MOIs). This allowed for a more accurate comparison of the antiviral activity of the two drugs (16).

4.4. Corticosteroids

By lowering the activation of many inflammatory mediators generated by the body during infection and inflammation, corticosteroids have the capacity to control inflammation. In contrast, several investigations have shown that corticosteroid therapy may enhance the viral RNA content of SARS-CoV-2 when compared to placebo. Instead of treating ambulatory patients or those who merely need normal care for their infection, it may be more likely to utilize corticosteroids in an intensive care situation when individuals may be approaching a cytokine storm. In fact, preliminary analysis from the recovery trial, which involved 2104 patients randomly assigned to receive dexamethasone 6 mg once daily for 10 days (orally or intravenously), has shown that patients who require oxygen and/or are ventilated have a lower 28-day mortality rate when compared to patients who received usual care. Patients who did not need breathing assistance did not get any advantages. The publishing of this data for peer review is pending.

In comparison to those receiving standard treatment, patients who were randomised to receive dexamethasone 6 mg once a day for 10 days (orally or intravenously) showed a decrease in 28-day mortality among ventilated patients and oxygen-dependent patients. Patients who did not need breathing assistance did not get any advantages. The publishing of this data for peer review is pending. In hospitalized COVID-19 patients who need supplemental oxygen, multiple randomized trials show that systemic corticosteroid therapy improves clinical outcomes and lowers mortality. This is likely because it reduces the COVID-19-induced systemic inflammatory response, which can cause lung injury and multisystem organ dysfunction. In contrast, systemic corticosteroids have not been shown to be beneficial and may even be harmful in hospitalized COVID-19 patients who do not need additional oxygen.

Based on the findings from these clinical studies, the COVID-19 Therapy Guidelines Panel has recommended using corticosteroids in hospitalized COVID-19 patients. Systemic corticosteroids should not be used in COVID-19 patients who are not hospitalized, according to the available evidence (17).

5. The documents

The data from eight RCTs (7184 patients) comparing systemic corticosteroids to standard therapy in COVID-19 was examined by the panel on July 17, 2020 (Table 1). Recovery examined the effects of dexamethasone 6 mg given once daily (orally or intravenously) for up to 10 days in 6425 hospitalized patients in the United Kingdom (2104 were randomized, 16% were receiving invasive mechanical ventilation or extracorporeal membrane oxygenation: 60% were receiving oxygen only (with no non-invasive ventilation); and 24% were receiving extracorporeal membrane oxygenation). There are around 700 critically sick patients and 63 individuals who are not in critical condition in the data from seven additional smaller trials (definitions of critical illness varied across studies). For the latter, patients were recruited up to June 9th, 2020, with around 45% being mechanically ventilated invasively, half receiving corticosteroid medication, and the other half receiving no corticosteroid therapy. Corticosteroid regimens included: methylprednisolone 40 mg every 12 hours for three days, followed by 20 mg every 12 hours for three days (GLUCOVID); dexamethasone 20 mg daily for five days, then 10 mg daily for five days (two trials, DEXA-COVID, CoDEX); hydrocortisone 200 mg daily for four to seven days, followed by 50 mg daily for two to three days (one trial, CAPE-COVID); (one trial, steroids – SARI). While randomized, embedded, multifactorial, adaptive platform trial for community-acquired pneumonia (REMAP-CAP) was worldwide research, seven of the trials were carried out in separate nations (Brazil, China, Denmark, France, and Spain) (recruiting in 14 European countries, Australia, Canada, New Zealand, Saudi Arabia and the United Kingdom). Except for two studies that reported death at 21- and 30-days following randomization, all trials reported mortality after 28 days. The panel only looked at the data relating to the result of mechanical ventilation from one study (GLUCOVID, n=63) since the mortality statistics were not presented by subgroup (18).

Table 1: A list of significant RCTs reporting on the role of immunomodulation in the treatment of COVID-19

Investigators	**Sample size and characteristic**	**Results**
	Corticosteroids	
RECOVERY (dexamethasone) Hawiger et al (22)	2104 hospitalized COVID-19 patients receiving dexamethasone and 4321 patients with usual care	Dexamethasone lowered 28-day mortality in patients receiving either invasive mechanical ventilation or oxygen alone at randomized, but not among those without respiratory support.

Görlich et al (25)	299 COVID-19 patients with moderate/ severe ARDS were randomized (151 received dexamethasone with standard care and 148 with standard care only)	Dexamethasone plus standard care compared with standard care alone increased the number of ventilator free days over 28 days in COVID-19 patients with moderate/ severe ARDS.
Lin et al (26)	403 suspected or confirmed severe COVID-19 patients in the intensive care unit (ICU) (randomly assigned the fixed-dose (n=143), shock-dependent (n=152), and no (n=108) hydrocortisone groups	A 7-days fixed-dose hydrocortisone or shock-dependent hydrocortisone, compared with no hydrocortisone, were superior in improving organ support- free days within 21 days in severe COVID-19 patients.
Rodriguez-Garcia et al (27)	149 critically ill patients with SARS-CoV-2 infection and acute respiratory failure (76 with hydrocortisone and 73 with placebo)	Low-dose hydrocortisone, compared with placebo, did not significantly reduce death or persistent respiratory support at day 21 in critically ill patients with SARS-CoV-2 infection and acute respiratory failure.
	Kinase inhibitors	
De Luca (28)	1033 hospitalized COVID-19 patients underwent randomization (with 515 assigned to combination treatment and 518 to remdesivir alone)	Barcitinib/ remdesivir was superior than remdesivir alone in facilitating recovery and accelerating improvement in clinical status among COVID-19 patients, especially with high-flow oxygen or noninvasive ventilation. The combination was also associated with fewer major adverse events

An additional trial, which randomized hospitalized patients with suspected SARS-Cov-2 Infection published on 12 august 2020 (MetCOVID), was included as a supplement in the PMA publication, as it was registered after the searches of trial registries were performed. The supplement showed that inclusion would not change results other than reduce inconsistency.

6. SARS- CoV- 2 Reproduction and Transmission

The SARS-CoV-2 infection pathway is comparable to that of SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). By directly attaching to the spike protein, ACE2 serves as a crucial receptor via which SARS-CoV-2 enters the host cell. According to reports, ACE2 is expressed in the gastrointestinal system, nasal mucosa, oral mucosa, and lungs. According to research, SARS-RBD CoV-2's have a stronger affinity for ACE2 than SARS- CoV's. SARS-CoV-2 spike has a greater affinity for ACE2 due to the distinct biochemical components at the binding site between SARS-CoV-2 and SARS-CoV RBD regions, which are shown by a structural study. On the other hand, a silico molecular docking simulation indicates that the residue change brought on by single nucleotide polymorphisms (SNP) of ACE2 may improve ACE2's affinity to spike protein. One of the potential causes of SARS-greater CoV-2's spreadability and transmission capacity is its stronger affinity. According to recent research, SARS-CoV-2 can also infect T cells that lack ACE2. The promotion of viral entry into T cells and other ACE2-deficient cells by ectopic expression of CD147 points to a possible new pharmacological target for COVID-19 treatments.

The breakdown of the spike protein by the host proteases activates the entrance of SARS-CoV-2 after the spike protein has linked to ACE2. Transmembrane serine proteases of types II and IV (TMPRSS2/TMPRSS4) facilitate spike fusogenic activity and the cleavage of the spike protein, which helps the virus enter the cell. By causing structural changes in the S2 subunit and RBD opening, the spike protein aids in the fusion of the SARS-CoV-2 envelope with the cell membrane of alveolar type 2 cells via S1 binding to the receptor for glucose-regulated protein 78 (GRP78). Better spike protein binding to cell membrane receptors is made possible by these mechanisms. RNA from the virus is released into the cytoplasm of host cells where it is translated into the polyproteins pp1a and pp1b. These two proteins are split into 16 nonstructural protein (Nsps), which assemble the replication and transcription complex (RTC), attract membrane structures from the host cell, and then produce antisense negative-stranded subgenomic RNAs. There are two additional processes: 1) replication, which produces the sense strand RNA and repackages it in subsequent viruses; and 2) discontinuous transcription, which produces varying lengths of subgenomic mRNA by attaching to and starting transcription at various locations on the antisense template. In the ER-Golgi intermediate compartment, structural proteins (S, M, E, and N) and the viral DNA are put together to form virions after translation (ERGIC). This facilitates the new virion's fusion with the plasma membrane and subsequent release from the host cells, where it may then seek for more cells to infect. For instance, SARS-CoV-2 generally replicates in the respiratory tract, while it spreads via contact with infected people's saliva and other bodily fluids. SARS-CoV-2 infection and the dissemination of saliva droplets may both result via coughing, sneezing, and even breathing. Distance has an impact on transmission, and smaller droplets often go further since gravity has less of an impact on them. Once inside a vulnerable person's mouth, eyes, or nose, the virus might begin to replicate. Wearing a face mask, using an eye shield, and keeping your distance from other people may all reduce the chance of infection. Clarifying the processes behind immunological alterations in COVID-19 patients is crucial for directing treatment approaches. The following discussion examines the possible immunological alterations brought on by SARS-CoV-2 (19-20).

7. Immunological reactions to SARS-COV-2

7.1. Innate immune sensing of the SARS-CoV-2

The many mechanisms viruses use to inhibit innate immunity at various phases of infection serve as a reminder of the critical role that innate immunity plays in preventing viral infections in general and SARS-CoV-2 in particular. If effective, innate responses lead to prompt and efficient disease prevention or resolution, which happens in many cases of SARS-CoV-2 infection, including in adults with no or moderate symptoms, the young, and bats that carry the virus but aren't sick.

SARS-CoV-2, like other viruses, contains pathogen-associated molecular patterns (PAMPs) that are identified by the pattern recognition receptors of the innate immune system (PRRs). PAMPs for SARS-CoV-2 include double-stranded RNA intermediates that activate endosomal TLR3 and/or the cytoplasmic PRRs retinoic acid-inducible gene I (RIG) and MDA5, ssRNA that is recognised by endosomal TLRs 7 and 8, spike protein that directly activates TLR4, envelope protein that is sensed by TLR2, and spike protein that directly activates TLR4 (melanoma differentiation-associated protein 5). SARS-CoV-2 also directly activates innate immunity in epithelia via 2'-5'-oligoadenylate synthetase-like and RNA-activated protein kinase (PKR) (OASL). absent in melanoma 2/IFN-inducible protein 16 (AIM2/IFI16) and cGAS/STING (cyclic guanosine monophosphate-adenosine monophosphate synthase/stimulator of IFN genes) pathways are also indirectly triggered by self-DNA released into the cytoplasm during apoptosis, a common event in viral infections, including with SARS-CoV-2. In comparison to other viruses, SARS-CoV-2 includes less CpG-DNA patterns that are detected by TLR9 and/or the zinc-finger antiviral protein ZAP. Nuclear factor b (NF-β), activating protein 1 (AP-1), and IFN regulatory factors are among the transcription factors that are activated as a result of innate PRRs recognising viral PAMPs (IRFs). Proinflammatory cytokines including IL-6, TNF-α, and pro-IL-1 are canonically induced via the NF-β and AP-1 pathways. These cytokines need to be further processed by inflammasomes, immune cell regulators like GM-CSF, chemokines like IL-8, and other inflammatory mediators. Numerous effector genes, most notably the antiviral and immunomodulatory type I (subtypes) and type III (1/2/3) IFNs, are driven (or, in the case of IRF5, suppressed) by IRF1/3/5 and/or 7, with cell type-specific IRF expression controlling local IFN subtypes. By acting both upstream and downstream of type I IFNs, the PRR- and cytokine-inducible IL-32 supports antiviral defence.

7.2. Cytokines Storm

According to reports, people with severe COVID-19 symptoms exhibit the CS phenomenon, which is an unchecked release of pro-inflammatory cytokines. Infectious illnesses, rheumatic conditions, and tumour treatment may all cause CS, which often manifests as systemic inflammation and multiple organ failure. Acute respiratory distress syndrome (ARDS), which is a major cause of death in SARS-CoV/MERS-CoV patients, is one of the effects of CS on lung viral infections. Other effects include impaired T cell response, accumulation of alternatively activated macrophages, altered tissue homeostasis, and epithelial and endothelial cell apoptosis. In a research published by Huang, 41 COVID-19 patients' levels of inflammatory factors were assessed (13 ICU and 28 non-ICU patients) (21). In comparison to healthy adults, both ICU and non-ICU patients had higher levels of IL-1B, IL-1RA, IL-7, IL-

8, IL-9, IL-10, fibroblast growth factor (FGF), granulocyte-colony stimulating factor (G-CSF), interferon (IFN), interferon—inducible protein (IP10), monocyte chemoattractant protein (MCP1), macrophage inflammatory protein 1 al GCSF, IP10, MCP1, MIP1A, and TNF-α concentrations were greater in ICU patients than in non-ICU patients, indicating that the CS was linked to a more serious level of illness. Zhou's retrospective cohort research found that throughout the clinical course of COVID-19 disease, non-survivors had higher blood levels of IL-6 than survivors. IL-6 levels were shown to be higher in patients with severe COVID-19 according to other investigations. In a clinical study in Anhui, China, employing the IL-6 receptor-targeted monoclonal antibody (mAb) tocilizumab, 21 patients with severe COVID-19 reported improved respiratory function and quick fever control. Even two patients who were in severe condition all made a full recovery and were released from the hospital. This illustrates the requirement for anti-inflammatory therapy approaches for COVID-19 and the need of a balanced immune host response for the effective eradication of the SARS-CoV-2 infection (22).

7.3. NLRP3 suppression

Nucleotide-binding oligomerization domain-like receptors (NLRs; such as NLRP3) are a different class of PRRs that can activate inflammasomes. Lactate dehydrogenase (LDH), which is elevated in severe COVID-19, and the IL-1 family cytokines IL-1 and IL-18 are substrates that are released via gasdermin-D membrane pores by inflammasomes, which are huge protein complexes that, when activated, unleash proteases like caspase-1. The latter triggers IFN-, a type II IFN that is antiviral (Figure 4). SARS-CoV-2 stimulates NLRP3 inflammasomes like other highly virulent CoVs, and the presence of IL-18 and activated caspase-1 in patient sera is correlated with the severity of COVID-19 illness. Inflammasomes are activated by SARS-CoV-1 through the accessory proteins ORF8b and ORF3a. This is accomplished by ORF3a, which is functionally and genetically identical to SARS-CoV-1 and -2 and forms an ion channel that enhances K+ efflux. SARS-N CoV-2's protein has been demonstrated to suppress gasdermin-D-mediated cleavage in human monocytes while simultaneously activating NLRP3 in murine and human cells. One of the main processes that causes CRS is the excessive inflammasome activity that results from poorly managed innate immune responses, which is why it makes sense to look into the therapeutic use of inflammasome inhibitors in COVID-19 (23-24).

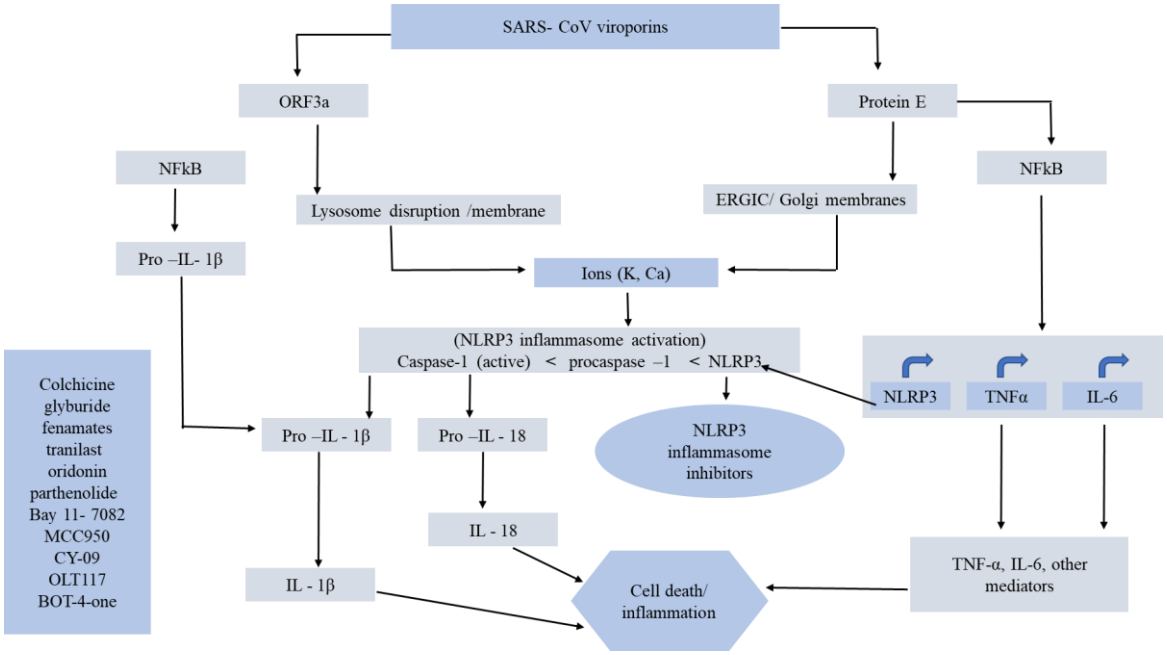

Figure 4: Coronavirus- induced NLRP3 inflammasome activation.

8. SARS-CoV-2–induced changes in innate immune cell composition and function

Neutrophia and monocytosis are observed to be associated with fewer and functionally worn-out natural killer (NK) and dendritic cells in the blood of adult COVID-19 patients after acute infection (DCs). Early on in the SARS-CoV-2 infection, neutrophilia is often seen, and neutrophils have the potential to exacerbate COVID-19 by generating NETs that cause thrombosis and organ damage NET by-products are elevated in COVID-19 patient sera. Additionally, early activation and the discharge of immature neutrophils are results of emergency myelopoiesis. A higher frequency of intermediate TNF-α and IL-6-producing CD14+CD16+ monocytes is seen in acute patients compared to convalescent patients, which may be caused by inflammation-induced compensatory myelopoiesis. Nonclassical CD14lowCD16high monocytes are reduced in acute patients compared to convalescent patients, possibly due to emigration into tissues. Increases in the cell cycle marker Ki-67, which is correlated with illness severity, provide credence to this notion. Blood DC frequency is low, and they perform poorly, producing less type I IFN among other things. Similar effects are seen in NK cells, which are crucial antiviral effectors. Their numbers are inversely linked with illness severity, and functional fatigue is suggested by elevations in the inhibitory receptors NKG2A (natural killer group 2A) and Tim-3 and reduced IFN- and granzyme B. When compared to convalescents, children with clinically mild illness had lower total monocyte, DC, and NK cell counts during the acute phase. SARS-CoV-2 causes fewer innate reactions in the lungs than other respiratory viruses, yet blood cells are consistently infiltrating the alveolar tissue. These cells generate large amounts of lytic proteases and reactive oxygen species, which impair gas exchange by causing tissue damage, capillary leak, and pulmonary edoema. In contrast to moderate COVID-19, which showed increases in alveolar macrophages and infiltration of plasmacytoid DC (pDC), severe COVID-19 was characterised by alveolar

macrophage depletion and infiltration of neutrophils, NK cells, proinflammatory FCN1+ macrophages, and SPP1+ profibrotic macrophages (26).

Type I and III IFN pathways and SARS-CoV-2 interactions Type I and III IFN pathways and SARS-CoV-2 interactions are complicated. On the one hand, studies have shown that type I and type III IFN responses in peripheral blood mononuclear cells (PBMCs) are sluggish and weakened, and that the degree of these effects correlates with the severity of the illness, the length of hospitalization, and mortality, correlating with earlier findings in SARS-CoV-1 infection. This decreased IFN response is linked to greater viral loads and inflammation, and it may be brought on by IFN induction and signalling that is antagonistic to SARS-CoV-2. Genetic susceptibility and pDC loss and dysfunction, the primary IFN-producing cells, may potentially play a role. For instance, loss-of-function mutations in IFN pathway genes were discovered in 3.5% of instances with severe COVID-19, and critical COVID-19 was also associated with high TYK2 and low IFNAR2 expression. Unexpectedly, neutralising autoantibodies against type I IFNs, namely IFN- and IFN-, were generated by 14% of very sick individuals. The fact that 90% of these patients were men may be a factor in the sex bias in severe infection. On the other hand, seemingly incompatible investigations have shown a robust type I IFN response in severe COVID-19. TNF-α /IL-1-driven inflammation was shown to be accompanied with increased type I IFN in classical monocytes, as determined by single-cell RNA sequencing. Interferon-regulated genes (IRGs) with proinflammatory qualities were more highly expressed in bronchoalveolar lavage (BAL) cells than were IRGs with other activities (27). In addition, COVID-19 autopsies showed two distinct phenotypes in lung tissue, including an IRG high signature linked to high viral loads and limited pulmonary damage but earlier mortality and an IRG low profile linked to reduced viral loads but severely damaged lungs and abundant CD8+ T cell and macrophage infiltration. According to one research, a greater IFN-to-type I IFN ratio was linked to shorter stays in intensive care units. IFN-, but not type I IFNs, were observed to correspond with quicker viral clearance in moderate to severe illness.

9. Immune evasion strategies of SARS-CoV-2

SARS-CoV-1 and MERS-CoV generate auxiliary proteins that enable the formation of double-membrane vesicles from the rough endoplasmic reticulum without Pathogen recognition receptors (PRRs), enabling viral replication and assembly without host detection in addition to inhibiting IFN activity. Additionally, MERS-CoV inhibits adaptive immune responses by altering epigenetic regulation and inhibiting both IFN-mediated antigen presentation and major histocompatibility complex (MHC) gene expression. Similar to how MHC-I proteins are tagged for lysosomal destruction by SARS-ORF8, CoV-2's which binds to them, makes it difficult for cytotoxic T cells to recognise and kill virus-infected cells. Type I and III IFNs exhibit strong antiviral effects, and viruses often evade these effects by decreasing their production and activity. SARS-CoV-2-encoded proteins' interactome analysis revealed numerous binding partners connected to the IFN pathway (e.g., the kinase TBK1, the ring finger protein RNF41, and the translocase TOMM70).

SARS-CoV-2 produces homologs of SARS-CoV-1 and MERS-CoV proteins that prevent the innate/IFN pathway from recognizing pathogens to producing effector proteins that are

encoded by the IRG. Using Nsp14 and Nsp16 to limit PRR identification, directly interfering with MDA5 recognition and IRF3 nuclear translocation, lowering STAT1 phosphorylation, and interfering with RIG-I signalling (through Nsp1/3) are a few examples. The tripartite motif-containing protein 25(TRIM25) and RIG-I were inhibited by the N protein of SARS-CoV-2, which prevented the activation of TBK1. Nsp1/3/6/12 to 15 and ORFs 6 and 9b are used by SARS-CoV-2 to suppress IFN production and signalling more effectively than MERS-CoV and SARS-CoV-1. Nsp6 and Nsp13 bind TBK1, preventing IRF3 nuclear translocation; ORF6 inhibits IRF3 and STAT1 nuclear trafficking; and ORF9b blocks RIG-I/MAVS (mitochondrial antiviral) (28-29).

10. Response that was generated by SARS Cov-2

SARS-CoV-2 has extensive impacts on adaptive immunity in addition to its effects on the innate immune system. Early lymphopenia of CD4+, CD8+, and regulatory T cells is often described in moderate-to-severe patient blood, although alterations to B cells seem less predictable. As a result, the ratio of neutrophils to lymphocytes or, alternatively, neutrophils to T cells (the latter being less exact but more easily quantifiable) were proposed as biomarkers indicative of severe illness. Increases in apoptosis and pyroptosis, an inflammatory type of programmed cell death, as well as inadequate replenishment may all contribute to T cell depletion. SARS-CoV-2 does not seem to productively infect T cells, in contrast to MERS-CoV, which infects T cells directly and causes pyroptosis in blood and lymphoid tissues. The growth of memory cells in response to SARS-CoV-2 infection was once believed to be compromised by dysregulated and worn-out T cell responses. Autopsies of COVID-19 patients revealed loss of germinal centres and lymphocyte depletion in the spleen and lymph nodes, indicating humoral immunity failure in the early stages of illness, which may help to explain why some people do not consistently produce antibodies. However, more recent research has shown that infections of rhesus macaques result in high germinal centre activation, which accumulates monocytes and promotes the proliferation of TFH cells with a TH1 profile that are selective for spike and nucleocapsid proteins. Furthermore, it was shown that IFN-+ cells remained functioning despite PD-1, a hallmark of fatigue, being expressed. In fact, recovered COVID-19 patients had memory T and B cells specific for SARS-CoV-2 that lingered for many months and could establish protective antiviral activities. While the number of memory B cells specific for the spike protein rose six months after infection, SARS-CoV-2-specific CD4+ and CD8+ T lymphocytes decreased after infection with a half-life of between three and five months. 83% of convalescents had memory T cells that grew in response to ex vivo stimulation with SARS-CoV-2 proteins, and asymptomatic patients' slightly lower proliferation was the only noticeable difference between them and symptomatic patients. Overall, the findings on T cell responses to SARS-CoV-2 infection are a little illogical, but quick CD4+ T cell responses seem crucial for efficient viral clearance (30).

11. Conclusion

The current knowledge of SARS-CoV-2, including its structure, entrance, and transmission pathways, is presented in this study. Then, we concentrated on the innate and inflammatory immune reactions brought on by the virus, particularly the cytokine storm. The Type I IFN

response is described, and SARS-tactics CoV-2's for dodging innate immune protection are highlighted. There has also been discussion of the viral proteins that inhibit type I IFN response. We recommend that more research be done to learn more about how SARS-CoV-2 uses a variety of tactics to modulate host innate immunity and inflammatory response because they are crucial for the virus' survival and transmission as well as the development of the illness and mortality of COVID-19 patients. We might solve the existing challenges of SARS-CoV-2 infection with the aid of this review. Additionally, we have studied the intriguing new medications that target innate immunological and inflammatory responses. Cocktail treatment is thought to increase the likelihood of a breakthrough. Tocilizumab and corticosteroids together have shown promising anti-inflammatory benefits with a better survival rate. IFN beta-1b, lopinavir-ritonavir, and ribavirin were used in early triple antiviral treatment. When compared to lopinavir-ritonavir treatment alone, the length of hospital stays and the amount of viral shedding were reduced following treatment with such a combination medication. The aforesaid combination treatment still has adverse effects, despite the promising results thus far. More factors, such as the patients' underlying health issues, phase, timing, whether mechanical breathing is used, medicine dosages, etc., should be taken into account in clinic settings. In order to determine the best COVID-19 treatment, studies should be conducted to confirm the safety and negative effects of medications.

AUTHORS' CONTRIBUTIONS

All authors contributed to review conception and design. Material preparation, data collection section was prepared by (Prof Ruchi Tiwari). The first draft was written by (Ms Juhi Mishra) (Mr Shivam Verma), reviewed by (Shivam Sharma) and edited by (Prof Gaurav Tiwari) and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

Authors would like to acknowledge Pranveer Singh Institute of Technology (Pharmacy), Kanpur for the support.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

FUNDING

None.

REFERENCES

1. Pan P, Shen M, Yu Z, Ge W, Chen K, Tian M, Xiao F, Wang Z, Wang J, Jia Y, Wang W. SARS-CoV-2 N protein promotes NLRP3 inflammasome activation to induce hyperinflammation. Nat Commun. 2021,12(1),1-17.
2. Schett G, Manger B, Simon D, Caporali R. COVID-19 revisiting inflammatory pathways of arthritis. Nature Reviews Rheumatology., 2020, 16(8), 465-470.
3. Yuan S, Jiang S.C., Zhang, Z.W., Fu, Y.F., Hu, J., Li, Z.L. Quantification of cytokine storms during virus infections. Front. Immunol., 2021, 1867.

4. Lin, T.Y., Hsia, S.H., Huang, Y.C., Wu, C.T., Chang, L.Y. Proinflammatory cytokine reactions in enterovirus 71 infections of the central nervous system. Clin. Infect. Dis., 2003, 36(3), 269-274.
5. Kennedy, J.R. Phosphatidylserine's role in Ebola's inflammatory cytokine storm and hemorrhagic consumptive coagulopathy and the therapeutic potential of annexin V. Med. Hypotheses., 2020, 135, 109462.
6. He, Y., Feng, Z., Wang, W., Chen, Y., Cheng, J., Meng, J., Yang, H., Wang, Y., Yao, X., Feng, Q., Chen, L. Global cytokine/chemokine profile identifies potential progression prediction indicators in hand-foot-and-mouth disease patients with Enterovirus A71 infections. Cytokine., 2019, 123, 154765.
7. Zhao, Z., Wei, Y., Tao, C. An enlightening role for cytokine storm in coronavirus infection. J. Clin. Immunol., 2021, 222, 108615.
8. Costela-Ruiz, V.J., Illescas-Montes, R., Puerta-Puerta, J.M., Ruiz, C., Melguizo-Rodríguez, L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev., 2020, 54, 62-75.
9. Elemam, N.M., Hammoudeh, S., Salameh, L., Mahboub, B., Alsafar, H., Talaat, I.M., Habib, P., Siddiqui, M., Hassan, K.O., Al-Assaf, O.Y., Taneera, J. Identifying Immunological and Clinical Predictors of COVID-19 Severity and Sequelae by Mathematical Modeling. Front. Immunol., 2022, 13.
10. Udugama, B., Kadhiresan, P., Kozlowski, H.N., Malekjahani, A., Osborne, M., Li, V.Y., Chen, H., Mubareka, S., Gubbay, J.B., Chan, W.C. Diagnosing COVID-19: the disease and tools for detection. ACS nano., 2020. 14(4), 3822-3835.
11. Wang, B., Li, R., Lu, Z., Huang, Y. Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. Aging (albany NY)., 2020, 12(7), 6049. Loeffelholz, M.J., Tang, Y.W. Laboratory diagnosis of emerging human coronavirus infections–the state of the art. Emerg. Microbes Infect., 2020. 9(1), 747-756.
12. Winichakoon, P., Chaiwarith, R., Liwsrisakun, C., Salee, P., Goonna, A., Limsukon, A., Kaewpoowat, Q. Negative nasopharyngeal and oropharyngeal swabs do not rule out COVID-19. J. Clin. Microbiol., 2020, 58(5), 00297-20.
13. Inauen, W., Suzuki, M., Granger, D.N. Mechanisms of cellular injury: potential sources of oxygen free radicals in ischemia/reperfusion. Microcirc Endothelium Lymphatics., 1989, 5(3-5), 143-155.
14. Fowler, A.A., Hyers, T.M., Fisher, B.J., Bechard, D.E., Centor, R.M., Webster, R.O. The adult respiratory distress syndrome: cell populations and soluble mediators in the air spaces of patients at high risk. Am. Rev. Respir. Dis., 1987, 136(5), 1225-1231.
15. Lu, Y.T., Hellewell, P.G., Evans, T.W. Ischemia-reperfusion lung injury: contribution of ischemia, neutrophils, and hydrostatic pressure. Am. J. Physiol. Lung Cell Mol. Physiol., 1997, 273(1), 46-54.
16. Matute-Bello, G., Liles, C.W., Frank Radella, I.I., Steinberg, K.P., Ruzinski, J.T., Hudson, L.D., Martin, T.R. Modulation of neutrophil apoptosis by granulocyte colony-stimulating factor and granulocyte/macrophage colony-stimulating factor during the course of acute respiratory distress syndrome. Crit. Care Med., 2000, 28(1), 1-7.

17. Martin, T.R., Galli, S.J., Katona, I.M., Drazen, J.M. Role of mast cells in anaphylaxis. Evidence for the importance of mast cells in the cardiopulmonary alterations and death induced by anti-IgE in mice. J Clin Invest., 1989, 83(4), 1375-1383.
18. Lamhamedi, S.A.L.M.A., Jouanguy, E.M.M.A.N.U.E.L.L.E., Altare, F.R.É.D.É.R.I.C., Roesler, J.O.A.C.H.I.M., Casanova, J.L. Interferon-gamma receptor deficiency: relationship between genotype, environment, and phenotype. Int J Mol Med., 1998, 1(2), 415-423.
19. Schottelius, A.J.G., Baldwin Jr, A.S. A role for transcription factor NF-kB in intestinal inflammation. Annu Rev Immunol., 1999, 14(1), 18-28.
20. Donald, R., Ballard, D.W., Hawiger, J. Proteolytic Processing of NF-κB/I κB in Human Monocytes: ATP-DEPENDENT INDUCTION BY PRO-INFLAMMATORY MEDIATORS. J Biol Chem., 1995, 270(1), 9-12.
21. O'Connell, M.A., Bennett, B.L., Mercurio, F., Manning, A.M., Mackman, N. Role of IKK1 and IKK2 in lipopolysaccharide signaling in human monocytic cells. J Biol Chem., 1998, 273(46), 30410-30414.
22. Hawiger, J., Veach, R.A., Liu, X.Y., Timmons, S., Ballard, D.W. IκB kinase complex is an intracellular target for endotoxic lipopolysaccharide in human monocytic cells. Blood,Am. J. Hematol., 1999, 94(5), 1711-1716.
23. Yang, R.B., Mark, M.R., Gray, A., Huang, A., Xie, M.H., Zhang, M., Goddard, A., Wood, W.I., Gurney, A.L., Godowski, P.J. Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. Nature., 1998, 395(6699), 284-288.
24. Kirschning, C.J., Wesche, H., Merrill Ayres, T., Rothe, M. Human toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide. J Exp Med., 1998, 188(11), 2091-2097. Tsytsykova, A.V., Goldfeld, A.E. Nuclear factor of activated T cells transcription factor NFATp controls superantigen-induced lethal shock. J Exp Med., 2000, 192(4), 581-586.
25. Görlich, D., Kutay, U. Transport between the cell nucleus and the cytoplasm. Annu Rev Cell Dev Biol., 1999, 15(1), 607-660.
26. Lin, Y.Z., Yao, S., Veach, R.A., Torgerson, T.R., Hawiger, J. Inhibition of Nuclear Translocation of Transcription Factor NF-κB by a Synthetic Peptide Containing a Cell Membrane-permeable Motif and Nuclear Localization Sequence. J Biol Chem., 1995, 270(24), 14255-14258.
27. Rodriguez-Garcia, J.L., Sanchez-Nievas, G., Arevalo-Serrano, J., Garcia-Gomez, C., Jimenez-Vizuete, J.M., Martinez-Alfaro, E. Baricitinib improves respiratory function in patients treated with corticosteroids for SARS-CoV-2 pneumonia: an observational cohort study. Rheumatology., 2021, 60(1), 399-407.
28. De Luca, G., Cavalli, G., Campochiaro, C., Della-Torre, E., Angelillo, P., Tomelleri, A., Boffini, N., Tentori, S., Mette, F., Farina, N., Rovere-Querini, P. GM-CSF blockade with mavrilimumab in severe COVID-19 pneumonia and systemic hyperinflammation: a single-centre, prospective cohort study. The Lancet Rheumatology., 2020, 2(8), 465-473.
29. Jackson, C.B., Farzan, M., Chen, B., Choe, H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol., 2022, 23(1), 3-20.
30. Meo, S.A., Meo, A.S., Al-Jassir, F.F., Klonoff, D.C. Omicron SARS-CoV-2 new variant: global prevalence and biological and clinical characteristics. Eur Rev Med Pharmacol Sci., 2021, 25(24), 8012-8018.