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Immunopathology of Covid 19 Infection

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Abstract

Covid 19 or SARS -COV-2 infection originated in Wuhan city of China towards the last week of 2019 which was declared as pandemic by WHO on February 11 2021. Corona virus belongs to Nido virus which is a large family of viruses causing a wide variety of infections ranging from common cold to major organ dysfunction such as acute respiratory syndrome and multi organ failure. As there was no immunity against the novel Covid 19 virus, this virus has affected the whole world through its mutant variants resulting in rapid spread. Clinical course is characterized by an initial viremic phase where most people remain asymptomatic, around 80% will have mild to moderate symptoms, 15% progress to severe covid and 5% become critically ill. Second stage which lasts for 4-10 days after symptom onset where viral replication persist and moderate disease develops resulting in viral pneumonitis, some times other organ also can be affected. Stage 3 occurs 6-15 days from the initial symptoms which is characterized by severe respiratory illness, multiorgan dysfunction, immune cell exhaustion and hyperinflammation resulting in cytokine storm in some patients. Hence it is desirable to look into the immuno pathology of Covid 19 infection and to assess the effectiveness of anti viral therapy, immunomodulation therapy and immune suppressant therapy apart from the respiratory and other supportive measures to these patients.

Keywords: Covid 19, immunopathology

Introduction

COVID-19 pandemic has affected 233,503,524 people globally, as of 5:45 pm CEST, 1st October 2021 and there have been 4,777,503 deaths reported by WHO so far [1]. It has originated in China towards the last week of December 2019 and was declared as a pandemic by WHO on March 11, 2020. It was reported to have jumped from bats to human without any identifiable intermediate host. SARS-CoV was first reported in China in 2003 and infected >8,000 individuals and it had resulted 774 deaths worldwide [2]. In 2012 MERS was reported in Saudi Arabia and infected more than 2,494 individuals and caused 858 deaths [3]. COVID-19 can present as mild upper respiratory infection, severe acute respiratory distress syndrome, multi organ failure and death especially in people more than 60 years of age and with co-morbid conditions like DM, CLD, CKD, COPD and Obesity. At present no age is impervious to infection with COVID-19; as the disease is still spreading fast through mutant COVID-19 virus. Still, we are in the process of learning the underlying mechanisms involved in the corona virus mediated diseases.

A vascular disease process is a contributory factor in COVID-19 pathogenesis. There are reports of increased respiratory dead space which suggests that pulmonary vascular thrombosis maybe occurring from thrombotic micro angiopathy or pulmonary embolism. Pulmonary embolism was noted in 40% of

hospitalized patients. Reported findings indicate that immunosuppression, endothelial activation and direct viral mediated endothelial damage rather than hyper inflammatory injury mediates COVID-19 induced organ injury [4].

Immunopathology of thrombosis

Most patients with COVID-19 presented with mild to moderate symptoms; approximately 15% progresses to severe pneumonia and about 5% develop acute respiratory distress syndrome, septic shock or multi organ failure [5].

SARS-CoV-2 can activate innate and adaptive immune responses. Uncontrolled inflammatory innate response and impaired adaptive immune response maybe responsible for harmful tissue damage both locally and systemically. Corona virus can also activate macrophage and dendritic cells. In patient with severe COVID 19, lymphopenia is associated with increase in neutrophil count and increased neutrophil to lymphocyte ratio, this usually indicate high disease severity and poor outcomes. Along with reduced number of CD4 and CD8 T cells, B cells, natural killer cells, there is elevated serum levels of pro-inflammatory cytokines including IL-6,IL-Iβ,IL-8, IL-17,G-CSF, IP10, MCPI, MIP1α, and TNFα Which will manifest as cytokine storm [6].

High level of pro-inflammatory cytokines can lead to shock and tissue damage in the heart, liver, kidney as well as respiratory failure and multi organ failure. Humoral response responsible for the production of specific antibodies IgM and IgG occurs between 14 - 21days after the onset of the disease and this level declines around 2-5 weeks after infection. Though the recovered individuals have a short period of immunoprotection lasting for 2 to 5 weeks, they are susceptible to reinfection by the same virus. SARS-CoV-2 has escape mechanism to prevent its destruction by the immune system by blocking the interferon signaling cascade responsible for the synthesis of type 1 interferon, which is needed for activating the antiviral status of an infected cell [7].

Type I and III interferonopathy

SARS-Cov-2 can suppress IFN signature significantly in severe Covid 19 compared to that in mild and moderate cases [8]. A multi-center observational study reported that the administration of IFN-beta 2b early in the course of Covid 19 infection improved in-hospital mortality [9]. Impaired viral clearance of SARS-Cov-2 due to the antagonism of IFN signaling may result in continuous Toll Like Receptor stimulation and pyroptosis of infected type 2 pneumocytes leading to hyper inflammatory state observed in severe Covid-19.

Neutrophils and neutrophil extracellular traps

Apart from cytokine storm, dysregulated macrophage recruitment, impaired natural killer cell and T cell response, there is a dysregulated myeloid response to SARS-Cov-2 in severe Covid. An elevated neutrophil/lymphocyte ratio have been reported to be prognostic of ARDS and death in Covid 19 [10].

Patients with severe Covid had increased levels of neutrophil extra cellular traps (NETS) [11,12]. NETS are webs of DNA material with antimicrobials and oxidant enzymes extruded by neutrophils to control infection. Serum samples collected from patients hospitalized with Covid 19 had higher levels of cell free DNA, myeloperoxidase (MPO)-DNA complexes and citrullinated histone H3 compared to those from patient with mild or moderate disease and healthy control. Elevated MPO - DNA complexes, have been associated with a decreased PaO_2/FiO_2 ratio resulting in increased demand for mechanical ventilation and further deterioration leading to death. NETs may contribute to the development of lung injury and microthrombi in Covid 19 possibly due to a NETs - platelet interaction.

Short telomeres and severe covid

The increased mortality due to Covid 19 among older adults has been attributed to a declining immunity with age; but the mortality is higher than what is seen in other viral or bacterial infections. Wang's study published on 29th July 2021 consists of 6,775 adults with Covid-19 and demonstrated that short hematopoietic cell Telomer length (TL) as expressed in leucocyte telomere length (LTL) preceded the onset of severe Covid 19. Because of the fact that the hematopoietic cell telomere length measurements had been already performed years before the SARS Cov-2 pandemic this study becomes significantly important [13].

Covid infection is associated with severe and prolonged lymphopenia which is due to fall in counts of T cells [14,15]. As hemopoietic cell telomere length shorten with age, T cells from older adult have diminished clonal expansion capacity compared to younger adults. A massive and rapid T cell clonal expansion may be necessary after contracting SARS-COV-2 infection.

A poor T cell response in patient with Covid 19 leaves the innate immune system relatively unchecked [15] resulting in cytokine storm and severe lung injury due to hyper inflammation. Short hemopoietic cell telomere length is associated with severe post covid pulmonary fibrosis [16] . Some adults in their thirties have short leucocyte telomere length similar to older adults and they may have heightened risk to severe Covid 19 [17].

Endothelial infection in Covid-19

SARS-COV-2 infects the host using the angiotensin converting enzyme 2 receptor which is expressed in several organs including the lung, heart, kidney, pancreas, intestine and endothelial cell [18].

Postmortem analysis of the transplanted kidney who had arterial hypertension and coronary artery disease and COVID-19 infection, revealed viral inclusion structures in the endothelial cells. Histological analysis revealed accumulation of inflammatory cells in endothelial cell are associated with apoptotic bodies in the heart, lungs and small bowel as well. Most small lung vessels appear congested. There was widespread thrombotic micro angiopathy with high incidence of deep vein thrombosis and pulmonary embolism reported from case series from China, Netherlands, France and Italy [19]. Postmortem histology also revealed lymphocytic endothelitis in lung, heart, kidney, intestine and liver as well as liver necrosis [20].

In the heart, there was histological evidence of myocardial infarction, but no sign of lymphocytic myocarditis. Histology of small intestine resection showed endothelitis of sub mucosal vessels and apoptotic bodies. ST segment elevation myocardial infarction can be the first presentation of COVID-19. Patient with underlying cardiovascular condition such as hypertension, diabetes mellitus and preexisting cardiovascular disease can develop high incidence of coronary thrombosis, heart failure and arrhythmia [21]. In the nervous system, there is a high incidence of ischemic stroke, metabolic encephalopathy due to hyponatremia, directly or indirectly causes infectious, para infectious and post infectious encephalitis and acute neuropathies like Guillain barre syndrome.

Arterial, venous and small vessels thrombosis occur in COVID-19 infection. This is a pro thrombotic state with high rates of thrombotic and micro vascular complication. In some patient APL antibodies also were detected along with elevated LDH, CRP, mild thrombocytopenia and D-dimer level. Procalcitonin level were normal in most of the inpatient except in one who had pneumonia. Hence in COVID-19 infection, there is coagulation abnormality, complement activation, cytokine storm, platelets hyperactivity and endothelial dysfunction contributing to the pathogenesis of thrombosis.

Venous thrombosis in Covid infection is triggered by endothelial dysfunction characterized by increased level of Von Willebrand Factor, systemic inflammation, toll-like receptor activation, procoagulant state

by tissue factor activation. Lastly immune mediated damage by antiphospholipid antibodies may also contribute as speculated by Zang and colleagues [22].

Vasculitis in Covid-19

In Italy an epidemic of acute and self-limiting vasculitic lesions of hands and feet were reported in asymptomatic children and adolescents. They also developed various types of rashes and urticaria as well. Clinical manifestation affects the feet or hands as multifocal and often are asymmetric, appearing a few at a time in 2-3 days, then undergoing a different evolution from initial erythema to infiltration or ecchymosis and finally self-limiting in 12-20 days [23].

Covid-19 associated coagulopathy

In case series of 183 patients with Covid-19 with coagulopathy there was elevation of D-dimer levels, moderate to severe thrombocytopenia, prolongation of prothrombin time and decreased fibrinogen level in 71% of patients admitted to ICU and they did not survive; only 0.6% survivors met the ISTH criteria for diagnosis of DIC [24]. In critically ill Covid-19 patients, platelet count were normal or mildly elevated along with elevated fibrinogen levels and depletion of endogenous anticoagulants such as Antithrombin. Even though D-dimer levels were elevated, prolonged prothrombin time, platelet count less than 50,000/mm^2 (< 50 x109/l) and decreased fibrinogen were not reported in most patients with Covid-19 (25). Patients with Covid-19 have a hyper coagulation state with increased thrombin generation and hypo fibrinolysis [26].

Endothelial effects on coagulopathy in comparison with other disease, Covid-19 associated coagulopathy is associated with endotheliopathy and increased inflammatory response as evidenced by circulating markers of endothelial injury such as elevated von Willebrand factor (vWF), PAI1, soluble thrombomodulin, angiopoietin 2 and follistatin [27]. Most of these patients have increased mortality [28]. The endotheliopathy in Covid-19 infection is whether due to a result of direct invasion of endothelial cells or is it a consequence of virus induced inflammatory response is still uncertain. This endotheliopathy initiates 2 direct pathways. Initial mechanism is an inflammatory response marked by the release of interleukin 6. The second one is micro thrombotic pathway consequent to the release of large von willebrand factor which extend from the surfaces of endothelial cells and trap activated platelets. This micro thrombogenesis is supposed to be the pathophysiological mechanism responsible for acute respiratory distress syndrome (diffuse alveolar hemorrhage) and multi organ dysfunctional syndrome [29]. Not only micro thrombosis but macro thrombosis also occurs due to the merging micro thrombogenesis and coagulation pathway [30].

Covid-19 associated platelet dysfunction

Platelets are considered to be the first response to vascular injury. They are also involved in many critical cellular processes like autophagy, programmed cell death, and rapid de novo protein synthesis [31].

The incidence of thrombocytopenia in patients with severe C -19 has been reported to be on high as 35% (122/263) in a French study. But the incidence was lower than in patients with SARS or MERS. In severe cases of Covid-19 low fibrinogen levels and bleeding events are rare, but thrombotic complication are more common [32]. This is because platelets are hyper activated in patients with Covid-19.

In patients with cardiovascular comorbidities platelet apoptosis is likely to be an important mechanism of Covid-19 pathogenesis similarly tissue hypoxia, inflammation, endothelial activation immune system activation and apoptosis also contribute to increase thrombosis [33].

'Secondary capture' refers to the phenomenon of activated platelets binding to neutrophils and rolling of platelet bound neutrophils on the endothelium which has got an important role in immunothrombosis [34]. Platelet activation and apoptosis can contribute to the pathology of severe Covid-19, including

thrombosis and cytokine storm.

Co-morbid conditions like diabetes mellitus, COPD, ageing and obesity can lead to platelet hyperactivity and apoptosis through increased oxidative stress. Hence three major mechanisms of platelet clearance are platelet senescence, apoptosis and immune mediated platelet clearance by macrophage. Similarly SARS-COV2 induced production of antibodies against platelet surface antigen, antiphospholipid antibodies [35], hypoxia, direct infection of platelets through an endosomal toll like receptor mediated pathway may be contributing to SARS associated thrombocytopathy, apart from endothelial damage and thrombotic microangiopathy.

There is an increase in the number of megakaryocytes in the lung, heart, kidney and bone marrow as evidenced by various autopsy studies [36]. Pulmonary megakaryocytes are increased in severely ill patients with COVID-19. This increase in megakaryocytes in bone marrow and lung may be a compensatory mechanism for rapid platelet consumption or loss.

Multisystem inflammatory syndrome in children and adolescents

In Covid 19 pandemic a hyper inflammatory vasculopathy was described in children known as multisystem inflammatory syndrome (MIS-C). All patients have presented with fever appearing 2-4 weeks after an infection with SARS-Cov-2. Most patients have serological evidence of infection. Most patients are older than those with classical Kawasaki disease and presented with hemodynamic instability, respiratory and gastrointestinal involvement and evidences of myocardial injury. Dysregulated immune response results in intense inflammatory response. The incidence of coronary aneurysm is lower than that seen in Kawasaki disease [37]. Some patients have presented with features of toxic shock syndrome, secondary hemophagocytic lymph histiocytosis or macrophage activation syndrome. Most patients so far have responded well to the same therapies used for classical Kawasaki disease. Immune response to SARS-Cov-2 may be responsible for this Kawasaki like disease in susceptible patient.

Multisystem inflammatory syndrome in adults

Hekimen et al describe 11 generally healthy patients aged 60 to 40 years (seven people aged >18 years) who presented with clinical and laboratory features of MIS with cardiac dysfunction requiring intensive care support that included vasopressor and inotropes. SARS-Cov-2 infection had occurred in a majority of cases several weeks prior followed by a post infection inflammatory syndrome. Six patients who underwent Cardiac MRI sowed evidence of edematous myocarditis. The presence of SARS-Cov-2 antibodies which develop within about 2 weeks of infection in most patients with Covid 19 infection associated with negative real time reverse transcription polymerase chain reaction (RT-PCR) in the setting of suggestive clinical history and presentation may be a clue to the diagnosis of MIS [38].

Management strategy

Potential immunotherapy in Covid-19

Asymptomatic Covid-19 carriers can transmit the disease to others and the virus has a wider range of incubation time than initially thought (0 -24 days) [39]. As immune modulation remain as an option for treatment, supportive and respiratory support remain the mainstay of therapy.

Repurposing of approved drugs is commonly employed to fight against newly emerging disease like Covid-19, as these drugs have known pharmacokinetics and safety profiles. Evidence of improvement has been observed with some antiviral therapies (Flavipiravir, Remdesivir) [40], antibodies (convalescent serum) [41], antiinflammatory agents (Dexamethasone) [42], immuno modulatory therapies (Tocilizumab) [43] and anticoagulants (Heparin) [44].

Despite these therapies, the case fatality rate of Covid-19 remains high in elderly patients [45].

Therapeutics targeting hyperinflammation

Corticosteroids have wide spread inhibitory effects on the immune system and are efficacious in managing acute inflammatory processes. The randomized controlled open label RECOVERY trial evaluated [42] the efficacy of dexamethasone in hospitalized Covid-19 patients. Dexamethasone 6 mg once daily was given for a period of 10 days. The study demonstrated a significantly lower 28 day mortality in patients randomized to receive dexamethasone versus usual care. In a prespecified subgroup analysis dexamethasone reduced 28 day mortality by 36% in patients on mechanical ventilation and by 18% in patients receiving oxygen without mechanical ventilation whereas dexamethasone had no benefit inpatients receiving no respiratory support. Dexamethasone was also more effective in patients randomized to receive treatment for more than 7 versus less than or equal to 7 days after the onset of COVID-19 symptoms ($P< 0.001$). Dexamethasone was the first therapy to improve survival in patients with Covid-19, suggesting that the patients were in a later disease stage dominated by immunopathology. It is also worth remembering that systemic steroids have been shown to impair viral clearance in patients with SARS- CoV-1 and MERS infection.

Biological immunomodulating drugs

IL-6 is a key inflammatory cytokine that has a critical part in inflammatory storm and elevated in Covid-19 [40-46]. Case reports have suggested that Tocilizumab is effective in the treatment of severe Covid-19, where patients had rapid decrease in inflammatory markers (i.e. C-reactive protein, Ferritin, D-Dimer) and improved oxygenation (i.e. PaO_2/FiO_2 ratio and lymphocyte count [44]. Somers et al reported that Tocilizumab use was associated with a 45% risk reduction for death versus supportive care in patients with Covid-19 on mechanical ventilation. Tocilizumab use was also associated with an increased risk of superinfection in patients who are mechanically ventilated.

Tocilizumab is a first line drug for the treatment of cytokine release syndrome especially in patients with comorbidities. Animal studies have shown that interleukin-6 is required for the clearance of viruses and control of human pulmonary inflammation [45]. Hence blocking interleukin 6 (IL-6) could also interfere with viral clearance or exacerbation of lung inflammation.

Targeted synthetic immune suppressants

JAK I and JAK 2 inhibitors such as Baricitinib and Ruxolitinib could be potential treatments for the hyper inflammation seen in Covid-19 [46]. Baricitinib selectively inhibits the kinase activity of JAK I and JAK2. Baricitinib reduces the ability of SARS-CoV-2 virus to infect living cells. SARS-CoV-2 binds ACE 2 receptor on host cells and enters through a process of receptor mediated endocytosis. The immunosuppression function of Baricitinib may be beneficial to the hyperactive immune status in severe cases of Covid-19.

Monoclonal antibodies

Monoclonal antibodies specific to a viral protein are alternate treatment options for viral disease. During the last decade, severe targeted monoclonal antibodies were developed for the SARS-CoV spike protein to inhibit the viral fusion inside the host cell [47].

The SARS-Cov-2 genome encodes 4 major structural proteins spike (s), enevelole (E), membranes (M) and Nucleocapsid (N) as well as non structural and accessory proteins. The spike protein is further divided in to S1 and S2 that mediate the host cell attachment and invasion. Through its receptor binding domain (RBD), S1 attaches to ACE 2 receptor on the host cell and this initiates conformational change in S2 that results in virus - host cell membrane fusion and viral entry [48].

Anti SARS-Cov-2 monoclonal antibodies that target the spike protein have been shown to have a clinical benefit in treating Covid-19 infection [49].

Three anti-SARS-Cov-2 monoclonal antibody products have emergency use authorization from the Food and Drug administration for the treatment of mild to moderate Covid 19 non hospitalized patients with laboratory confirmed SARS-CoV-2 infection who are at high risk for progressing to severe disease and/or hospitalization.

These products are:

1. Bamlanivimab plus etesevimab

2. Casirivimab and indevimab

3. Sotrovimab 500 mg Intravenous (IV) infection

The Covid-19 treatment guidelines panel recommends using one of the following anti SARS Cov-2 monoclonal antibodies listed in the alphabetical order to treat non hospitalized patient with mild to moderate covid who are at high risk of clinical progression.

Covid 19 management strategy

Viremic phase	**Moderate disease (Immunological phase)**	**Stage of hyper inflammation**	**Stage of Immunosuppression**
1-7 days	4-10 days	6 - 15 days	15 -30 days
Antiviral	Steroid	Steroids	Treatment of bacterial + fungal sepsis as per guidelines
Monoclonal antibody cocktail	Anticoagulant therapy	Immunosuppressive therapy	Gradually reduce and stops immunosuppressive therapy
Symptomatic treatment	Immunomodulatory Therapy		

Supportive therapy

1. Oxygen with high flow nasal cannula or re-breathing mask
2. Ventilatory support with Bi PAP or with invasive ventilation with high PEEP
3. Prone ventilation

Conclusion

Covid 19 pandemic is still an international health emergency. Owing to the effects of vaccination, which still requires to be augmented , the stage is now fit for an escape from this covid pandemic. Still this can persist in some countries as endemic. This review presented the current evidence that a dysregulated host innate immune response is responsible for the hyper inflammatory syndrome in SARS -COV-2 infection .There is increased serum level of CRP ,Interleukin 6, reduced CD4,CD8 and T lymphocyte proportion and increase in other inflammatory cytokines and chemokines such as interleukin 2, interleukin 8 with an increased number of neutrophils and eosinophils capable of inducing immune abnormalities in Covid 19 patients . Hyper inflammatory stage shows overlapping features of hemophagocytic lymphohistiocytosis, macrophage activation , micro and macro immuno thrombosis without frank disseminated intravascular coagulation .Therapies modulating the immune response is necessary for reverting the immunopathology possible for preventing progression of the disease into multi organ failure. Due to the prolonged immune suppressant therapy with steroids and interleukin 6 blockers, there is an immuno suppressant state inviting the increased incidence of sepsis due to bacterial and fungal infections like mucor mycosis and aspergillosis. This has to be treated and prevented . Future research is warranted to find out further newer modality of immune therapy as well as vaccination strategy, and newer antiviral drugs, without targeting the importance of preserving the natural habitat of these microorganisms.

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