ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is spreading worldwide and becomes a major cause of mortality. In addition to major pathology in lungs such as pneumonia or respiratory failure, multiorgan failure and frequently haematological disorders such as thromboembolic manifestations are leading causes of mortality. This study reviews the interaction of the immune system with target cells, the role of cytokines and other components such as complements encountered in the pathophysiology of major disease processes and possible post-recovery complications. Although there are some clinical effects induced by strong immune reactions, long term immunity against the virus is found in the majority.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection imposes a major health problem in recent years and many people are affected socioeconomically. Coronaviruses belong to the family Coronaviridae which are RNA viruses. The virus is transmitted to humans mainly through the inhalation of droplets or aerosols and contact with contaminated surfaces. Viruses primarily affect the respiratory system ranging from mild infection to acute respiratory distress syndrome (ARDS) and multiple organs failure. Haematological disorders such as thrombosis contribute significantly to unfavourable prognosis in severely ill patients. These pathophysiologic reactions are
mediated by effector immune cells, cytokines including chemokines, complement system and sometimes these immune reactions can trigger serious pathologic complications in the body of the patients.

**Cytokine Storm and Thrombosis**

The occurrence of widespread macro and micro thrombosis is strongly associated with a grave outcome. The SARS-CoV-2 virus enters target cells by binding to angiotensin-converting enzyme-2 (ACE-2) receptors in the respiratory epithelium followed by endocytosis and replication. Subsequently, immune cells mainly macrophages are activated to produce cytokines including chemokines. A genome-wide association study (GWAS) revealed an association between the disease severity and ABO genes, SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6 and XCR1 in chromosome 3p21.31 in patients with severe COVID-19 (Ellinghaus et al., 2020).

Interleukin (IL)-6, IL-1B, IL-12 and tumour necrosis factor-α (TNF–α) are important cytokines involved in the pathogenesis of COVID-19. Excessive production of these cytokines during exposure to viral infection may create a cytokine storm causing severe pneumonia as well as systemic hyper-inflammation and multiple organ involvement. Various immune cells, lymphocytes and macrophages, are involved in this cytokine storm syndrome and it is also called macrophage activation syndrome (MAI) (McGonagle et al., 2020). TNF–α and IL-1 initiate the tissue factor production from macrophages which can activate the coagulation cascade and IL-1 and IL-6 activate the plasminogen activator inhibitor. A high level of IL-6 is a poor prognostic marker in predicting the clinical outcome of infection (Witkowski et al., 2016). It can be explained by the fact that IL-6 suppresses NK cell cytolytic function and subsequently prolong the interaction between innate and adaptive immune cells that further promotes cytokine storm, hemophagocytosis, and multi-organ dysfunction. Injured and dying cells produce damage-associated molecular patterns (DAMP), mainly high mobility group box-1 (HMGB-1) that initiates and perpetuates a powerful immune response producing proinflammatory cytokines (Cicco et al., 2020).

There is a possible role of lupus anticoagulants which is linked to proinflammatory state and hypercoagulability in COVID-19 although their significance is unclear. Antiphospholipid antibodies bind to endothelium and monocytes and these factors stimulate to express adhesion molecules which facilitate platelet adhesion, platelet activation and increased phospholipid of glycoprotein IIB-IIa (Tung et al., 2021). Viruses can cause damage to the endothelium contributing to the release of tissue factors from subendothelial cells for the initiation of the coagulation cascade (Tung et al., 2021). Tissue factor also activates the extrinsic pathway of coagulation and increase the risk of venous thromboembolism (VTE) (Terpos et al., 2020).

Human *in vitro* models of COVID-19 showed infected monocytes with a hyperexpression of procoagulant genes including fibrinogen, serine protease inhibitors (SERPINS), tissue factor, and factors II and X which induce coagulopathy (Giannis et al., 2020). Complement activation induced by infection results in the release of proinflammatory cytokines and microvascular damage (Diurno et al., 2020).

**Immuno-Thrombosis and Venous Thromboembolism**

Endothelial dysfunction is the basis of COVID-19 associated coagulopathy (CAC) dysfunction (Huertas et al., 2020). In an observational study of the clinical outcome of COVID-19 patients, a poor outcome is seen in patients with hypertension and diabetes which are associated with endothelial...
dysfunction compared to those without these comorbidities (Huertas et al., 2020). Endothelial disruption enhances platelet activation and aggregation with the formation of a plug which acts as a template for adhesion of coagulation factors.

Hypoxia which is a common manifestation of COVID-19 patients induces endothelial dysfunction and hypercoagulability. Hypoxia caused by severe COVID-19 pneumonia induces the expression of hypoxia-induced factors (HIFs) which are transcription factors expressed by vascular endothelium and immune cells (Palazon et al., 2014). HIFs together with cytokine TNF-α upregulate the tissue factor expression and promote the release of plasminogen activator inhibitor-1 (PAI-1) which inhibits tissue plasminogen activator (tPA) reducing the activity of plasmin consequently triggers thrombosis (Loo et al., 2021). Markers of sepsis-induced coagulopathy (SIC) such as prolonged prothrombin time (PT), increased fibrinogen and increased d-dimers are seen in 21.6% of patients (Price et al., 2020; Tang et al., 2020; Wang et al., 2021). The release of proinflammatory cytokines mainly IL-6 and cathepsin G, a serine protease, cause platelets activation and aggregation (Bautista et al., 2021).

Complement activation plays a major role in triggering thrombosis. The final complement complexes C5b-9 resulted from the activation and cleavage of the complement proteins act as a membrane attack complex. It can attack the target cells causing lysis of cells which increases the procoagulant activity (Wiedmer et al., 1986). C3b fragment of complement binds to CR1 receptor on platelet membranes triggering the release of short-chain polyphosphate (polyP) from platelets, which induces the expression of TF. Complement component C5a may also contribute to the recruitment of neutrophils (Loo et al., 2021).

Previous studies revealed that there was a high risk of VTE in 35-85% of COVID-19 inpatients compared to the overall risk of 2% in hospitalized patients in the general medical ward. A vascular filling defect on computed tomography pulmonary angiography (CTPA) may require a differential diagnosis of immune-thrombosis or local pulmonary thrombosis or classic pulmonary embolism (Klok et al., 2020). Incidence of formation of blood clots in leg veins, deep vein thrombosis (DVT) was slightly lower than pulmonary thrombosis and microthrombi were frequently seen in pulmonary capillaries (Ackerman et al., 2020; Klok et al., 2020; Longchamp et al., 2020).

**Neutrophil Extracellular Traps and Thrombosis**

Neutrophils play the first line of defence in acute inflammation. They act as antigen-presenting cells (APC) presenting the virus to immune effector cells such as T lymphocytes. Elevated neutrophils to lymphocyte ratio (NLR) and very low lymphocyte counts are common findings in severe COVID-19 patients. NLR is a poor prognostic marker in COVID-19 infected cases (Wang et al., 2021; Rangel et al., 2020). NETosis is a process in which neutrophil extracellular traps (NETs) are formed by neutrophil DNA materials that form networks with extracellular fibres to bind to pathogens and trap them. The role of NETosis in viral infection has been described (Jenne et al., 2013). When neutrophils are stimulated by viruses, toxins or proinflammatory cytokines such as TNF and IL-8, the nuclear membrane disintegrates and release chromatin material from the nucleus which is mixed with proteins of neutrophilic granules in the cytoplasm. After hypercitrullination of nuclear histones, DNA-protein complexes (NETs) are released into the extracellular spaces. Uncontrolled production of NETs is associated with diseases gravity and the extent of lung injury (Bendib et al., 2019). Histones, the major component of NETs, attract, activate and aggregate platelets which will lead to thrombosis. Tissue
factor is induced by activated platelets and trigger coagulation cascade augmenting the thrombus formation. Neutrophils also promote coagulation by releasing an enzyme, neutrophil elastase, which cleaves the tissue factor pathway inhibitor (TFPI) contributing to thrombosis (Loo et al., 2021).

Evidence of NETosis-related microthrombotic events have been seen in COVID-19 autopsied cases (Borges et al., 2020; Parra-Medina et al., 2021). Tracheal aspirate and plasma of COVID-19 patients show increased NET level, neutrophil-platelet aggregates and increased plasma MPO-DNA complexes show elevated NET that is associated with immunothrombosis and ARDS. The significant level of NETS induces heparin-induced thrombocytopenia and platelet/lymphocyte ratio is a poor prognostic factor (Zhou et al., 2020). Studies have shown that NETs entrap viruses by a DNA web. However, NET-induced hyper-inflammation and thrombosis are unwanted events leading to the poor clinical outcome (Funchal et al., 2015).

Pulmonary embolism and deep venous thrombosis are the most frequent thromboembolic attacks in 20 – 30% of severe cases (Grimes et al., 2020; Connors et al., 2020). A thrombus may form in other organs such as cerebral circulation arterial and venous, coronary, mesenteric and peripheral arteries. Microvascular inflammation per se can cause acro-ischaemia (COVID-toes). Thromboinflammation plays an essential role in ARDS and determines the prognosis of severe cases. Platelets are also involved in the infectious process by triggering inflammation and thrombosis. When the platelet-toll-like receptors 2 (TLR-2) are activated there is cross-link formation between platelets and neutrophils. Activation of the tissue factor pathway of the coagulation cascade leads to the formation of thrombi in the microvasculature, subsequently resulting in total consumption of platelets and procoagulant factors, with the result of haemorrhage. Immuno-thrombotic events present with laboratory findings are distinct from DIC criteria. An elevated D-dimer at admission (≥ 20 mg/L) and constant rise in D-dimer are associated with increasing severity and in-hospital mortality (Yao et al., 2020; Zhou et al., 2020).

**Macrophage Activation Syndrome and Acute Respiratory Distress**

Features of macrophage activation syndrome (MAS) such as increased levels of cytokines namely IL-2, IL-7, TNF-α and serum ferritin have been observed in severe COVID-19 pneumonia (Huang et al., 2020). Hyperferritinemia, the marker of extensive macrophage activation, is a hallmark of COVID-19 pneumonia (Otsuka et al., 2020). MAS typically show signs of disseminated intravascular coagulation such as reduced fibrinogen, reduced platelets and increased d-dimers. High D-dimer was found in COVID-19 patients (McGonagle et al., 2020; Tang et al., 2020). MAS like hyper-inflammation plays an important role in the pathogenesis of severe pneumonia and ARDS. Approximately 20% to 40% of patients develop ARDS in the course of pneumonia (Huang et al., 2020, Wu et al., 2020).

Cell pyroptosis, a type of cell death, mediated by an activated cascade of NLRP-3 inflammasome was seen in COVID-19 associated lung inflammation (Hoffmann et al., 2020). High levels of cleaved gasdermine D (GSDMD), a pore-forming protein, which is a trigger of pyroptosis has been commonly observed in the lungs of severe COVID-19 patients compared to controls (Zhang et al., 2021). Alveolar macrophages are major cells that are highly positive for GSDMD staining on multiplex immunohistochemistry. The inflammation associated with cell death activates macrophages to produce pro-inflammatory cytokines and recruit T cells to the site. In addition, the entry of viral RNA into macrophages may directly activate them to produce cytokines (Malmgaard, 2004). Immune-mediated injury to parenchymal
and endothelial cells forms the tissue debris together with leaking from the newly formed capillaries (capillary leak syndrome) (Matthay et al., 2011), contributing to the formation of hyaline membranes in alveoli leading to insufficient oxygenation of capillary blood and ARDS (Bahloul et al., 2021). COVID-19 associated hypoxemia stimulates expression of hypoxia-inducible factors (HIFs) that activate macrophages to aggregate locally and express pro-inflammatory cytokines while HIF-1α enhances complement-mediated endothelial damage (Palazon et al., 2014).

### Renin-Angiotensin-Aldosterone System Dysregulation and Hypercoagulability

Angiotensin-converting enzyme 2 (ACE2) is highly expressed in kidneys, heart, pancreas, liver, small intestine, neurons and blood vessels. Autopsies of COVID-19 patients showed multisystem injuries, including acute lung injury, acute renal injury, cardiac injury, liver dysfunction and pneumothorax (Yang et al., 2020). The SARS-CoV-2 virus binds to ACE2 receptors which mediate the entry of the virus into the target cell. Upon binding, the transmembrane serine protease (TMPRSS2) cleaves ACE2 to promote viral uptake and metallopeptidase domain 17 (ADAM17) then cleaves ACE2 to cause ectodomain shedding (Heurich et al., 2014). The resultant ACE 2 downregulation disturbs the formation of angiotensin (Ang). Consequently, it will change the absolute level of Ang-II and increase the ratio of Ang II to Ang-(1-7). Increased Ang II level augments its effect on proinflammatory and prothrombotic processes. Ang II also activates the angiotensin type 1 receptor (AT1R) which enhances platelet activation and impairs fibrinolysis, resulting in hypercoagulability (Guo et al., 2001; Ni et al., 2020; Remkova et al., 2010).

The possibility of endothelial cell activation/damage due to the virus binding to ACE2 receptor may further increase VTE risk.

### Post-acute COVID-19 Complications

In the early recovery phase of COVID-19 illness, some patients experience complications. It is important to achieve early diagnosis and effective management to ensure a favourable outcome. Persistent hypoxemia due to restrictive diffusion as a result of pulmonary fibrosis with dyspnea is the most common sequelae. Resolution of acute renal injury during acute COVID-19 occurs in the majority of patients. However, a reduced estimated glomerular filtration rate (eGFR) has been reported at a 6-month follow-up (Nalbandian et al., 2021). Neuro-psychiatric manifestations such as anxiety, sleep disturbance and post-traumatic stress are common and early rehabilitation care and counselling are important for those cases. As the duration of the hyper-inflammatory state is unknown, there may be continuing risk of developing thromboembolic problems such as pulmonary embolism, intracardiac thrombus, thrombosed arteriovenous fistula and ischaemic stroke (Patell et al., 2020). Effects on the cardiovascular system such as myocardial fibrosis can result in cardiomyopathy or arrhythmia. Autonomic dysfunction occurs after a viral illness, resulting in postural orthostatic tachycardia syndrome and inappropriate sinus tachycardia (Yao et al., 2020). Studies showed that biomarkers of cerebral injury, such as elevated peripheral blood levels of neurofilament light chain have been found in patients with COVID-19 (Ameres et al., 2020). COVID-19 related secondary hemophagocytic lymphohistiocytosis (HLH) is a haematological disorder affecting hematopoietic tissues such as bone marrow, spleen, liver and lymph nodes. It is associated with fever, splenomegaly, cytopenia, hypofibrinogenemia, low or absence of natural killer (NK) cell activity and hyperferritinemia (Henter et al., 2007). The HLH syndrome can be seen as a result of dysregulated immune reactions after COVID-19 infection in patients during the recovery phase (Henter et al., 2007; Naous et al., 2021).
Treatment of COVID-19

Understanding the immunoopathophysiology of COVID-19 effects, several attempts have been in trial for treatment. Monoclonal antibodies were used to block the receptor of IL-6 to slow down the cytokine hyperproduction (Zhang et al., 2021). In a study, Eculicitumab acts to antagonize the complement C5 in which cases showed decreased inflammatory markers such as CRP and improved patient recovery (Diurno et al., 2020). An experimental study aimed to inhibit DAMP, monoclonal antibodies to HMGB, together with antineuramidase inhibitor revealed effective therapy (Hatayama et al., 2019). As SARS-CoV-2 uses ACE-2 receptor for entry into cells, human recombinant soluble ACE2 antibodies (hrsACE2) is found to be a promising treatment for severe infection (Zoufaly et al., 2020). Convalescent SARS serum samples can neutralize spike-driven virus entry showing that vaccine targeting spike proteins have promising benefits in prevention. As we have mentioned before, NETs are the main contributors to pathogenetic effects, thus, using drugs that can disrupt neutrophil activation and NETs formation are hopeful strategy (Hoffmann et al., 2020). Studies have shown that a better outcome was observed with anticoagulant and antiplatelet therapy for severe clinical cases with features of coagulopathy and those requiring mechanical ventilation (Godino et al., 2021).

CONCLUSION

The human immune system and immune cells fight against any challenging infection. The cytokines including chemokines produced by activated immune cells to recruit inflammatory immune cells at the site of injury aided by the complement system to enhance the protective effects. During the process of attempting to defend against COVID-19 infection, the powerful cytokine storm and hyperactive complement-mediated reaction result in some unwanted side reactions such as immunothrombosis and hyper-inflammatory reactions. Understanding these pathophysiological processes leading to hyperactive immune status will guide the clinicians to plan more effective management. Recent development in treatment remedies is in progress to modify these immune dysregulations so that better control of immune dysregulation can be achieved.

REFERENCES


