The interplay between hypovitaminosis D and the immune dysfunction in the arteriovenous thrombotic complications of the sever coronavirus disease 2019 (COVID-19) infection

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Thromboembolic complications including cerebrovascular accidents, pulmonary embolism, myocardial infarction, deep vein thrombosis and disseminating intravascular coagulopathy are serious encounters in sever coronavirus disease 2019 (COVID-19) infected patients. This worsens the prognosis and may lead to death or life long morbidities. The laboratory finding of the disturbed haemostasias and the hyperinflammatory response are almost invariably present in COVID-19 patients. Multiple treatment modalities are utilized by the healthcare professionals to overcome the cytokine storm, oxidative stress, endothelial dysfunction, and coagulopathy in these patients. The combined actions of vitamin D (VitD) as a steroid hormone with anti-inflammatory, immunomodulatory, and antithrombotic properties increase the potential of the possible involvement of hypovitaminosis D in the thromboembolic complications of COVID-19 infection, and stimulated researchers and physicians to administer VitD therapy to prevent the infection and/or overcome the disease complications. The current review highlighted the immunomodulatory, anti-inflammatory, antioxidative and hemostatic functions of VitD and its interrelation with the renin–angiotensin–aldosterone system (RAAS) pathway and the complement system. Additionally, the association of VitD deficiency with the incidence and progression of COVID-19 infection and the associated cytokine storm, oxidative stress, hypercoagulability, and endothelial dysfunction were emphasized. Normalizing VitD levels by daily low dose therapy in patients with hypovitaminosis D below (25 nmol/l) is essential for a balanced immune response and maintaining the health of the pulmonary epithelium. It protects against upper respiratory tract infections and decreases the complications of COVID-19 infections. Understanding the role of VitD and its associated molecules in the protection against the coagulopathy, vasculopathy, inflammation, oxidative stress and endothelial dysfunction in COVID-19 infection could lead to new therapeutic strategies to prevent, treat, and limit the complications of this deadly virus infection. ***Blood Coagul Fibrinolysis*** **34:000–000**

Blood Coagulation and Fibrinolysis 2023, **34**:00–00

Keywords: coronavirus disease 2019 (COVID-19), cytokine storm, infection, inflammation, thrombosis, vitamin D

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Received 25 January 2023 **Accepted** 11 February 2023

Background

Despite the extensive research related to the pathogenesis, clinical presentations, treatment, and prognosis of coronavirus disease 2019 (COVID-19) infections. There is insufficient knowledge about the exact mechanisms of the wide range of the homeostatic derangements and the sever complications associated with the disease. Several observational studies demonstrated a relationship between the low levels of vitamin D (VitD) and the unfavourable outcome of the COVID–19 patients. Simultaneously, the alarming high prevalence of VitD deficiency worldwide stimulated researchers to propose that VitD supplementation may help in delaying the acquisition of COVID-19, overcoming the pathological process of the infection, modifying the course of the disease, and decreasing its life-threatening complications; such as thrombosis and respiratory failure [1,2].

This review will highlight the physiological role of VitD in haemostasis and the potential role of hypovitaminosis D in the incidence and progression of COVID -19 infection and its associated vascular complications. The principle pathophysiological pathways of thrombosis in COVID-19 patients will be elaborated. The latter will include the inflammatory pathway, platelets abnormalities, the coagulation system aberration, the endothelial dysfunction (ED), and the complement activation. Nevertheless, the molecular mechanisms involved in each pathway will be emphasized in view of the serum VitD levels.

Pathophysiology of coronavirus disease 2019 infection

The pathogenesis of COVID-19 infection encompasses the attachment of the virus to angiotensin converting enzyme-2 receptors (ACE2) on the alveolar macrophages followed by the viral antigen presentation and activation of the innate immunity. The multisystemic clinical

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DOI:10.1097/MBC.0000000000001212

presentations of the COVID-19 infection could be attributed to the widespread expression of ACE2 in the cardiovascular, renal and the gastrointestinal systems [3]. Stimulation of the cells of the innate immune response including the mononuclear phagocytic cells, the neutrophils, and the T-lymphocytes will lead to enhanced continued overproduction of the inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α) [4,5]. The uncontrolled augmented release of the pro-inflammatory mediators will produce a cytokine storm ending up by subsequent necrosis and apoptosis of Type 2 alveolar epithelial cells. This impairs the surfactant secretion and predisposes some patients to the problem of acute respiratory distress syndrome (ARDS) [4,5]. The bilateral extensive lung tissue infiltration by viral particles will result in viral pneumonia due to endothelial injury, local extensive cytokine release and immune dysfunction [4,6]. This will lead to impairment of gas exchange resulting in hypoxemia.

Target therapies of coronavirus disease 2019 (COVID-19) infection

In spite of the fact that significant efforts are being made to create countermeasures for COVID-19 virus infection, the Food and Drug Administration has only authorized the use of convalescent plasma and repurposed medications (such as remdesivir as a direct antiviral and dexamethasone as an immune modulator for patients on ventilators) [7]. Clinical trials are now being conducted to test promising therapeutic monoclonal antibodies [8]. To decrease the acquisition of the virus infection, several vaccine candidates has been developed with various technical platforms, and widespread immunization programs have been implemented in many countries [9]. In fact, suppression of the COVID-19-associated cytokine storm appears to provide the greatest hope for holding the complications of this deadly virus [10]. Here in, clinical trials proposed that vitamins and supplements that exert anti-inflammatory and antioxidant effects may have some potential benefits as a targeted COVID-19 treatment [11]. This suggestion is consistent with the current major organizational standards for COVID-19 treatments that have the potential to be successful [11]. In this regards, vitamin D3 was associated with protective effects against moderate or severe chronic obstructive pulmonary diseases (COPD) exacerbations in one of these clinical trials, but not in people with baseline blood 25-hydroxyvitamin D concentrations more than 50 nmol/l [12].

Role of immuno-nutrition in the prevention and treatment of coronavirus disease 2019 infection

The consumption of a well balanced healthy diet that contains appropriate amounts of micro and macronutrients is essential for the metabolic processes required for the normal growth, development, and maturation of the healthy body [13]. The relationship between healthy nutrition and the appropriate function of the immune system is a focus of current research. Promising findings suggesting a clinical benefit of VitD, poly unsaturated fatty acids (PUFAs), and catechins in autoimmune and inflammatory disorders, and the reduction of viral infections by Vitamins D and E, zinc, and probiotics intake were reported [14]. In this regard, the Mediterranean Food rich in active components such as PUFAs, certain amino acids, trace elements and vitamins is evidenced to have strong immunomodulatory actions [14–16]. The latter intitles the suppression of the proinflammatory cytokines production with the augmented release of the anti-inflammatory mediators resulting in a balanced relationship between the natural and the specific immune responses [14].

In the following parts of this review, we will have a closer look at the physiological functions of VitD on the immune system and the haemostatic parameters, and will highlight the pathophysiological changes associated with hypovitaminosis D in relation to the immune responses and the blood coagulation pathways.

Synthesis of vitamin D and its mechanisms of action in the human body

Vitamin D is a steroid hormone produced by the human skin during the exposure to the ultraviolet rays of the sunlight [17]. The most common form of VitD in the circulation is 25-hydroxycholicalciferal, which is transformed by the enzyme α1-hydroxlase in the kidney, into the physiologically active form 1,25-dihydroxcholicalciferal [$1,25(OH)_2D_3$] [18].

In most cells, the active VitD complexes with nuclear VitD receptor (VDR) and binds to the promoter region of the target genes, and controls their expression [17]. The VDR and the VitD-synthesizing enzymes are found in nearly all cells of the innate and adaptive immune system pathways [19]. The presence of VDR on the circulating mononuclear cells [20] indicates that VitD is important in eliciting immune responses against invading pathogens [21]. The complex of VitD and its VDR may elicit two distinct host response mechanisms: one that inhibits the expression of the proinflammatory cytokines by blocking the TNF-induced nuclear factor of kappa Light polypeptide gene enhancer in B-cells 1 (NFKB1) signaling pathway, and another mechanism that activates the expression of interferon-stimulating genes by activating the interferon gamma (IFN-γ)- induced Jak-STAT signaling pathway [22].

Immunomodulatory functions of vitamin D

Whereas VitD is primarily needed for the normal calcium metabolism; it also either directly or indirectly regulates the expression of numerous genes involved in cellular proliferation, differentiation, apoptosis, and angiogenesis [23]. Moreover, it is recently evidenced that VitD has a complex immunomodulatory role on the host immune response. It influences cytokines production by the

specific cells involved in the natural and definitive immune reactions [24,25] Clinical studies demonstrated that VitD administration improved the vascular endothelial function in patients with stable moderate chronic renal disease [26].This was evidenced by decreased levels of the circulating endothelial function indicators such E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1) [26]. Vitamin D supplementation also decreases the inflammatory cytokines IL-6, TNF-α [27] and increases the anti-inflammatory cytokines production in inflammatory bowel disease patients [28]. This explains its anti-inflammatory properties [29].

Increased risk of upper respiratory tract infections in patients with vitamin D-deficiency

Although approximately 80% of VitD is endogenously produced by the effect of UV rays on the skin; deficiency of VitD is prevalent worldwide [30]. Serum VitD levels less than 50 nmol/l is present in 30–60% of the populations in the European countries and in 80% of the people in the Middle East Region. Moreover, serum VitD levels lower than 30 nmol/l were reported in more than 10% of the European population [31] in association with high rates of COVID -19 infections and mortality [32].

Serum VitD concentration was found to be inversely related to the risk and severity of acute respiratory tract infection, with each 10 nmol/l decrease in VitD levels increasing the odds of acute respiratory tract infection by 1.02 (0.97–1.07) [33]. The recent investigations of the relationship between VitD and viral immunity revealed that hypovitaminosis D is associated with an increased CD4 (helper)/CD8 (cytotoxic) ratio, which would result in the decreased ability of the immune system to produce activated T-lymphocytes ($CD8^+$ T). The latter, is mainly responsible for attacking the virally infected cells [34]. Therefore, patients with hypovitaminosis D are at greater risk of upper respiratory tract viral infections and pneumonia due to their low capacity to produce activated $CD8^+$ T. Additionally, they have high levels of the pro-inflammatory cytokines such as IL-6 [1].

The regulatory T lymphocytes (T-reg) are the main line of defense against uncontrolled inflammation and against viral infection in general. Many COVID-19 patients have been reported having low T-reg levels, which can be raised by VitD supplementation [1]. These studies raise the possibility that having enough VitD may help prevent cytokine storms, which can happen in COVID-19 patients [1].

The role of vitamin D in the protection and treatment of coronavirus disease 2019 infection

Numerous studies have shown that VitD can reduce the risk of acute viral respiratory tract infections and pneumonia. However, the significance of VitD in the treatment of the acquired infections, such as COVID-19, needs to be further investigated particularly in people with low VitD levels at baseline [35]. VitD opposes the attachment of the virus to the ACE-2 receptors in the pulmonary epithelium [36]. It causes direct inhibition of the viral replication rates and decreases the proinflammatory cytokine concentrations in the lungs lining, which are linked to inflammation and injury [37,38]. Furthermore, during viral infections VitD exerts anti-inflammatory and immunomodulatory mechanisms and it helps to decrease adaptive immune responses in the respiratory system [32]. Specifically, VitD functions to regulate cytokine response to the pathogens, helps to minimize the hyper-inflammatory responses, and speeds up the healing process in the damaged tissues especially in the lungs [36]. Additionally, it has been shown that VitD plays an intrinsic role in chemotaxis activity, indicating its ability to regulate cell movement [39]. Chemotaxis was shown to decrease as VitD concentrations increased [40]. These findings support VitD chemotaxis effect in COVID-19 patients via VDR as an interplaying agent [41].

Effective dosage of vitamin D against upper respiratory tract infection

Due to its potential toxicity; supplementation of higher than the usual daily requirements of VitD should be with great caution. Long term treatment with low doses of VitD was more effective in the prevention of acute respiratory tract infection than short term administration of high doses [42]. Subjects receiving daily or weekly VitD supplementation without additional bolus doses showed protective effects, which were stronger in those with baseline 25-VitD levels of 25 nmol/l [12]. Furthermore, administration of a single large bolus doses of VitD to the intensive care unit (ICU)- patients who have deficiency of VitD, failed to protect against the complications of COVID-19 infection such as pneumonia, ARDS, sepsis, shock, or respiratory failure [43].

Role of vitamin D in hemostasis

Low VitD levels have been linked to the development of deep venous thrombosis (DVT) in patients with ischemic strokes [44]. Clinical studies relating increased thrombotic events to VitD deficiency suggest that VitD and its associated molecules play a role in the control of antithrombotic pathways [23]. In the subsequent parts of this review, we will highlight the interrelationship between VitD and the components of the blood coagulation system including the platelets, the tissue factors, the coagulation proteins, and the natural anticoagulation molecules. An emphasis will be directed to the antithrombotic mechanisms of VitD, through its interaction with the vascular endothelium to enhance endothelial repair and prevent atherosclerosis. likewise, the crosstalk between VitD levels and the components of the complement pathway, that crossbridge inflammation and immunity, will be elaborated in the contest of keeping the vascular wall integrity and homeostasis.

Vitamin D and platelets

Vitamin D and platelets (PLs) have overlapping and distinct roles in coagulation and thrombosis, as well as in inflammation, ED, and immunological response. In addition, their role in mineral metabolism and bone health is another common thread [45]. A large number of researchers have looked into the relationship between VitD levels, platelets count (PC), and mean platelet volume (MPV) in a variety of diseases during the last few decades. Increased PLs indices and VitD insufficiency are linked to an increased rate and/or risk of numerous disorders including cardiovascular diseases and the metabolic syndrome [46]. Additionally, VitD deficiency enhances the release of the inflammatory cytokines, which regulate thrombopoiesis and inflammation, resulting in increased MPV and PC. The latter were shown to be inversely associated to VitD levels [46]. The inverse relationship between MPV and VitD could be explained in a number of ways: MPV links thrombosis with inflammation, while VitD possesses antithrombogenic, anti-inflammatory, and anticoagulant properties [46].

The antithrombotic functions of vitamin D–vitamin D receptor complex

The protective actions of $1,25(OH)_2D_3$ against thromboses is due to upregulation of the anticoagulant glycoprotein (thrombomodulin) and downregulation of the expression of certain coagulation and tissue factors [47]. The effects of VitD on these thrombogenic and antithrombogenic coagulation system components have been well characterized [48]. Antithrombotic factors (ATs) and thrombomodulin gene expression are upregulated by VitD in monocytic cells, while thrombogenic factor (TF) gene expression is downregulated. The opposite was observed with VDR knock out (VDRKO) mice, as a result, the VitD-VDR system promotes ATs expression while suppressing TF expression [49].

Vitamin D plays a role in endothelium repair and the suppression of macrophage transformation into foam cells, which prevents atherosclerosis [50]. The VDRKO mice showed enhanced multiple-organ thrombi development after exogenous lipopolysaccharide injection, regardless of the calcemic circumstances, as observed by Aihara *et al.* [49].

Several studies concluded that the oxidative stress causes thrombocytosis in mice [50]. In the general population, there is a link between oxidative stress and chronic low-grade inflammation index, which includes PLs count [51]. Earlier research has linked increased antioxidant levels to decreased inflammation and suppression of vascular endothelial growth factor release [52]. The antioxidant properties of VitD are well established and could participate in its antithrombotic actions [53]. Furthermore, antioxidation has been linked to a reduced PLs count in a population-based cohort research [54].

Vascular complications of severe coronavirus disease 2019 (COVID-19) infection

Nearly 20–50% of the hospitalized COVID-19 patients have aberrant coagulation profiles including prolonged prothrombin time, thrombocytopenia, high D-dimer and low fibrinogen levels [55]. The increased D-dimer has been identified as a biomarker for thrombotic problems correlated with the disease severity and mortality of these patients [56]. All of these changes are indicative of the hypercoagulable state seen in severe COVID-19 individuals, which may lead to thrombosis in the lungs and other organs [57–59]. Venous and arterial thromboembolism, disseminated intravascular coagulation (DIC), and high levels of the coagulation profiles are all vascular consequences of severe COVID-19 infection [55]. According to Gomez-Mesa *et al.*, DIC is responsible for 71.4% of COVID-19-related mortality. The increased risk of thrombosis in COVID-19 patients is directly related to an enhanced pro-coagulant response to acute phase reactants (factor VIII, Von Willebrand factor, and fibrinogen). In addition, it was reported that the presence of a cytokine storm (IL-6) in severe COVID-19 patients induces tissue factor expression in macrophages, resulting in thrombin activation [55]. A recent study showed that platelet hyperactivation has been linked to increased thromboxane production in COVID-19 patients [60]. This could be related to the active pro-inflammatory and antifibrinolytic state [61–63].

The relationship between hypovitaminosis D and thrombosis in coronavirus disease 2019 (COVID-19)

The significance of VitD in the inflammatory and coagulation pathways, as well as its role in endothelial activation, were described by Mohammad *et al.* [23]. Thrombogenic and antithrombogenic coagulation system components are in a delicate equilibrium that can be disrupted by large changes in VitD levels. Definitely, thrombotic events are common in patients with VitD deficiency especially in the obese and diabetic patients who are prone to greater mortality rate when subjected to COVID-19 infection [1]. In deceased COVID-19 patients, VitD insufficiency was linked to higher blood glucose levels and inflammatory indicators, D-dimer levels, as well as lower levels of serum calcium, albumin, PLs, and lymphocytes according to a recent prospective study [64]. These data point to the possible importance of VitD status in relation to thrombotic disorders, which are a catastrophic COVID-19 consequence; normalizing VitD values may help lower the incidence of thrombotic events, although this claim needs more support [65].

The relationship of vitamin D and the renin–angiotensin–aldosterone system in coronavirus disease 2019-induced thrombosis

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects cells through attaching to the ACE2, which are found in Type 2 alveolar cells, cardiac cells,

kidney proximal tubule cells, bladder urothelial cells, and other organs. The ACE2 is a component of the renin–angiotensin–aldosterone system (RAAS), which comprises several proteins that play multiple roles in regulating blood pressure [66]. Angiotensin-converting enzyme (ACE) is an enzyme expressed by different types of tissues and can convert angiotensin I (ATI) to angiotensin II (ATII), which is known to induce inflammation [67].

The inflammatory ATII is converted to the anti-inflammatory ATI by ACE2. The infection by SARS-CoV-2 depleted the ACE2, which raises the level of ATII. This causes RAAS failure and a number of inflammatory responses, including the activation of Toll-like receptor 4 (TLR4), which stimulates an innate immune response and increases inflammation [68,69]. Because ACE2 is also expressed in blood vessels endothelium, its downregulation as a result of SARS-CoV-2 infection could trigger a proinflammatory response and compromise the integrity of the vascular endothelium. As a result, vascular permeability will increase and coagulation will be activated [70].

Interestingly, the RAAS appears to play a role in the relationship between VitD status and COVID-19 infection severity [71]. Preclinical research has linked RAAS overexpression to VDR depletion, resulting in an increase in thrombogenic events. As a result, low VitD levels could exacerbate the progression of COVID-19 illness, which exploits the ACE2 receptor, a well known essential player in the RAAS system [72].

Inflammatory cytokines- induced hypercoagulability in coronavirus disease 2019 patients

Patients with COVID-19 have hypercoagulability due to elevated inflammatory cytokine levels, such as IL-1, IL-6, and TNF-α [73]. Cytokines encourage neutrophil extracellular traps (NETs) formation, which sets off the intrinsic and extrinsic coagulation pathways and causes the production of thrombin. Equally, NETs also encourage the release of inflammatory cytokines, which results in cytokine storms [74]. These cytokines stimulate the release of tissue factor (TF), which activates the extrinsic coagulation pathway. Additionally, they inhibit the fibrinolysis system through increased plasminogen activator inhibitor-1 (PAI-1) production [75]. Further activation of the coagulation system occurs, in this inflammatory environment, by decreasing the antithrombin and TF pathway inhibitors [76]. The inflammatory cytokines will trigger the expression of phosphatidylserine (PS) on the outer membranes of the blood erythrocytes, leukocytes, and lymphocytes, which results in a hypercoagulable state [77].

Relation between cytokines and platelet function

Platelets, the plasma coagulation cascade, physiologic anticoagulants, fibrinolytic activity, and proinflammatory cytokines and chemokines are all parts of the feedback loop coupling inflammation, coagulation, and VitD. These mediators also cause PLs overactivation, aggregation, and retention, as well as the formation of thrombus at the damaged site, which may deplete PLs and megakaryocytes, resulting in lower PLs production and higher consumption [78].

In Kaur *et al.* study, the findings showed that increased plasma cytokines in severely ill COVID-19 patients. The activation of IL-6 and TNF-α signaling pathways in PLs, may play a role in PLs activation and the development of associated problems such as, thrombophilia [79]. Furthermore, enhanced reactive oxygen species (ROS)-associated metabolic processes in PLs co-existed with a substantial elevation of pathways related to hemostasis, thrombosis, and inflammation. This strongly suggests PLs hyperactivation in ICU patients [79].

Effect of coronavirus disease 2019 infection on the endothelial function

One of the main causes of the microvascular problems is ED which disrupts the vascular system and favors vasoconstriction [80].The latter predisposes to organ ischemia, inflammation with accompanying tissue edema and a pro-coagulant condition. The COVID-19-induced endothelial cell injury could exacerbate ED, which is a hallmark of aging, hypertension, and obesity leading to further complications [81].

Okada *et al.* hypothesize that thrombosis may be directly linked both to the start and worsening of the COVID-19 infection (such as ARDS, heart failure, and cerebral infarction) via the endothelial glycocalyx [80]. The authors reported that the pulmonary capillaries were more susceptible to damage than the cardiac and cerebral capillaries, and this difference was related to the lungs somewhat thinner endothelial glycocalyx [80].

The recent findings by Nicosia *et al.* imply that COVID-19 vasculopathy is caused by an aggressive immune response to viral infection of the nonendothelial cells [81]. This leads to indirect injury of the vascular endothelium, loss of endothelial antithrombogenic properties, endothelial release of prothrombogenic factors, PLs hyperactivation, and coagulopathy ending up by thrombotic occlusion of the blood vessels [81]. The pathophysiology of ED and injury offers insights into COVID-19 associated mortality. The molecular foundation of SARS-CoV-2 infection, the functions of ACE2 and RAAS signaling, and a potential connection between preexisting ED and SARS-CoV-2 produced endothelial injury in COVID-19 linked mortality were all reviewed by Amraei *et al.* [82]. Additionally, they investigated the functions of the cell adhesion molecules (CAMs), and the cell receptors such as CD209L (L-SIGN) and CD209 (DC-SIGN) in facilitating the entrance of the SARS-CoV-2 virus into the infected cells [82].

The effect of vitamin D on the endothelial function

Vitamin D is recognized to have a vasoprotective effect and hypovitaminosis D increases the likelihood of ED a crucial prognostic indicator for cardiovascular diseases (CVD), which predisposes the blood vessels to the development of atherosclerosis [83]. Recent observational studies have suggested a possible connection between VitD levels and endothelial function. According to Zhang *et al*., in people with nondialysis chronic renal disease, circulating 25-hydroxyvitamin D concentrations were negatively correlated with ED as measured by brachial artery flow-mediated dilation (FMD) [84]. Vitamin D controls the production of nitric oxide (NO), a powerful endothelium dependent vasodilator, in the endothelial cells via modulating the activity of the endothelial NO synthase (eNOS) [85]. Deficiency of VitD is associated with a lower NO bioavailability predisposing to atherosclerosis a serious risk factor for CVD [86]. The oxidative stress caused by the excessive production of reactive oxygen species (ROS) in different pathological conditions enhances NO degradation and suppresses its synthesis leading to lack of NO bioavailability [87]. However, the antioxidant properties of VitD counteracts the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which produces ROS, and improves antioxidant capacity by enhancing the activity of antioxidative enzymes such as superoxide dismutase [85]. In addition to the oxidative stress, the proinflammatory cytokines such as TNF-α and IL-6 are risk factors for ED. They hinder NO and eNOS bioactivity and stimulate different atherosclerotic factors through the nuclear factor kappa B (NF-κB) pathway. Vitamin D exerts anti-inflammatory action by suppressing the signaling of NF-κB and the production of the proinflammatory cytokines [85].

The role of complement activation in coronavirus disease 2019 infection

The complement system, which is an essential component of the innate immune response to viruses and a proinflammatory mediator, can also cause collateral tissue injury in cases of excessive or unregulated activation [88,89]. Patients with COVID-19 have soluble indicators of complement activation in the plasma and showed complement fragment accumulation in several organs [90]. For instance, substantial deposits of C5b-9, C4d, and MASP-2 in the microvasculature are present alongside septal capillary damage in the lungs of people who died of respiratory failure [91].

It was recently reported that the activation of complement component C3 worsens the SARS-CoV-2 associated ARDS [92]. Similarly, widespread complement activation was evidenced in the lung tissue biopsy of patients with severe COVID-19 by increased C5a and C3a generation and C3-fragment deposition. Importantly, anti-C5a antibody treatment resulted in immediate clinical improvement, as measured by increased lung oxygenation and decreased systemic inflammation [93].

Despite equivalent viral loads in the lungs, C3-deficient mice showed less respiratory dysfunction, which was associated with decreased lung infiltration of neutrophils and inflammatory monocytes and lower levels of cytokines and chemokines in both the lungs and sera [92]. Inhibition of C3 could block both C3a and C5a production, as well as intrapulmonary C3 activation and IL-6 release from alveolar macrophages or other cells expressing C3a receptors (C3aRs) and/or C5a receptors (C5aRs). As a result, Angus *et al*. concluded that C3 inhibition may alleviate the inflammatory lung injury and the respiratory complications of SARS-CoV-2 infection [88].

The relationship between vitamin D levels and complement activation in coronavirus disease 2019-patients

Increased C3 and C4 levels are frequently observed in tissue damage, autoimmune dysfunction, and associated inflammatory disorders. Regarding VitD, it regulates innate and adaptive immune function, which is also one of the physiological pathways to support physical function, and can improve the people capacity to fight illness [94,95]. Immune variables and VitD concentrations are linked together as well. According to earlier research, VitD boosts the release of some anti-inflammatory cytokines while decreasing the release of some proinflammatory cytokines [96,97]. Thus, there may be conflicting effect between VitD and the levels of C3 and C4 in immunological and inflammatory pathways [98].

Small *et al*. presented evidence that 1,25VitD promotes the development of human macrophages by increasing complement receptor immunoglobulin (CRIg) expression which plays an important role in innate immunity. Vit D increases CRIg levels at the mRNA, protein, and cell surface levels, which was associated with increased bacterial and fungal phagocytosis. It was also demonstrated that innate immunity is important in promoting VitD effects [99].

The interplay between the complement system and the neutrophils in determining the tissue damage during coronavirus disease 2019 infection

The key sentinels of innate immunity, neutrophils and complement work in concert to protect the host from invasive infections and to preserve homeostasis [100].

Complement is an important player in pathogen defense, but excessive or unregulated activation can cause collateral tissue injury [101]. Complement opsonization, for instance, promotes the formation of NETs, whereas blocking CR1 and CR3 prevents NETosis (a sort of

programmed cell death) in response to specific infections [100].

Patients with COVID-19 who have SARS-CoV-2 experience an exacerbated host response centered on abnormal neutrophil activation, notably in the lung [102]. This neutrophilia predicts bad outcomes, and the neutrophil-to-lymphocyte ratio is a separate risk factor for serious disease [103]. Additionally, serum samples from COVID-19 patients showed higher levels of the NET-specific markers myeloperoxidase DNA and citrullinated histone H3 [104]. Neutrophil infiltration in pulmonary capillaries, acute inflammation of the capillaries with fibrin deposition, neutrophil extravasation into the alveolar space, and neutrophilic mucositis were all seen in autopsy reports from COVID-19 patients [104].

Role of complement and neutrophils in thrombosis

In the first week of COVID-19 infection complement functions as a 'friend,' and tries to overcome the invading virus particles. However, it turns into a 'foe' during the second or third weeks [105]. Complement activation is related to ARDS, pulmonary inflammation, and thrombotic events, which lead to long-term multiorgan damage. It also interacts with neutrophilia and dysregulated NET formation.

The heightened complement and neutrophil activation jointly contribute to the development of severe COVID-19 symptoms by generating microthrombi in a coagulopathic environment, which results in acute lung, kidney, and heart injury [100]. By turning on the contact pathway of coagulation and increasing other prothrombotic pathways, NETs start arterial and venous thrombosis. This causes excessive thrombin generation and subsequent C5a formation [100]. This leads some researchers to hypothesize the presence of a feedback loop in which complement activation causes NETosis, which then results in procoagulant activity (such as that of thrombin), which then causes continued complement activation to further promote NET formation [105].

In summary, thromboembolism is a major complication of the COVID-19 infection that worsen the patient's prognosis, especially those with comorbidities such as atherosclerosis, obesity, and diabetes mellitus. The incidence of DVT, cerebrovascular accidents and pulmonary embolism in the COVID-19 infected patients is associated with a prolonged hospital stay, increased ICU admission, long life disabilities, and escalating mortality rate. Although multiple components are believed to activate the coagulation pathways during COVID-19 infection resulting in the arteriovenous thromboembolism including: inflammatory cytokines overproduction, dysfunctional endothelium, complement activation, LPs hyperactivity, formation of NETs, tissue hypoxia, and oxidative stress. Nevertheless, it is currently recognized that hypovitaminosis D plays an important role in nurturing quite a lot of the mechanisms of COVID-19-associated coagulopathy. Normalizing VitD levels is essential for balanced immune system function and maintaining the health of the pulmonary epithelium. Thus, correction of hypovitaminosis D with once daily dose seems to protect against upper respiratory tract infections, decreases the complications of COVID-19 infections [106]. Understanding the role of VitD and its associated molecules in protecting against the coagulopathy, vasculopathy, inflammation, oxidative stress and ED in COVID-19 infection could lead to new therapeutic strategies to prevent, treat, and limit the complications of this deadly virus infection.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

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