Neuroimmunomodulation

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Review Article
Understanding the immune-endocrine effects of vitamin D in SARS-CoV-2 infection: a role in protecting against neurodamage?

Emanuele Gotelli[a], Stefano Soldano[a], Elvis Hysa[a], Andrea Casabella[b], Andrea Cere[a], Carmen Pizzorni[a], Sabrina Paolino[a], Alberto Sulli[a], Vanessa Smith[c,d,e], Maurizio Cutolo[a]

[a] Laboratory of Experimental Rheumatology and Academic Division of Clinical Rheumatology, Department of Internal Medicine and Specialties, University of Genova, IRCCS San Martino Polyclinic Hospital, Genova, Italy
[b] Laboratory of Experimental Rheumatology and Academic Division of Clinical Rheumatology, IRCCS San Martino Polyclinic Hospital, Genova, Italy
[c] Department of Internal Medicine, University of Ghent, Ghent University Hospital, Ghent, Belgium
[d] Department of Rheumatology, University of Ghent, Ghent University Hospital, Ghent, Belgium
[e] Unit for Molecular Immunology and Inflammation, Flemish Institute for Biotechnology, Inflammation Research Center, Ghent, Belgium

Emanuele Gotelli, MD, PhD Student, Rheumatologist (emanuele.gotelli@live.it)
Stefano Soldano, PhD, Biological Scientist (stefano.soldano@unige.it)
Elvis Hysa, MD, Fellow in Rheumatology (elvis.hysa@gmail.com)
Andrea Casabella, PhD, Biological Scientist (andrea.casabella@hsanmartino.it)
Andrea Cere, MD, Fellow in Rheumatology (andrea.cere00@gmail.com)
Carmen Pizzorni, MD, PhD, Associate Professor of Rheumatology (carmen.pizzorni@unige.it)
Sabrina Paolino, MD, Associate Professor of Rheumatology (sabrina.paolino@unige.it)
Alberto Sulli, MD, Associate Professor of Rheumatology (albertosulli@unige.it)
Vanessa Smith, MD, PhD, Full Professor of Rheumatology (vanessa.smith@ugent.be)
Maurizio Cutolo, MD, Full Professor of Rheumatology (mcutolo@unige.it)

Corresponding Author:
Dr. Emanuele Gotelli, MD, PhD Student
Laboratory of Experimental Rheumatology and Academic Division of Clinical Rheumatology, Department of Internal Medicine and Specialties, University of Genova, IRCCS San Martino Polyclinic Hospital
Viale Benedetto XV, 6, Genova, 16132, Italy
Tel: +390103538617
E-mail: emanuele.gotelli@live.it

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Abstract

Calcitriol and hydroxyderivatives of lumisterol and tachisterol are secosteroid hormones with immunomodulatory and anti-inflammatory properties. Since the beginning of the COVID-19 pandemic, several studies have correlated deficient serum concentrations of vitamin D_3 (calcifediol) with increased severity of the course of SARS-CoV-2 infection. Among systemic complications, subjective (anosmia, ageusia, depression, dizziness) and objective (ischemic stroke, meningo-encephalitis, myelitis, seizures, Guillain-Barré syndrome) neurological symptoms have been reported in up to 80% of severe COVID-19 patients. In this narrative review we will resume the pathophysiology of SARS-CoV-2 infection and the mechanisms of acute and chronic neurological damage. SARS-CoV-2 can disrupt the integrity of the endothelial cells of the blood-brain barrier to enter the nervous central system. Invasion of pro-inflammatory cytokines and polarization of astrocytes and microglia cells always in a pro-inflammatory sense together with the pro-coagulative phenotype of cerebral endothelial vessels in response to both SARS-CoV-2 and immune cells invasion (immunothrombosis) are the major drivers of neuro-damage. Calcitriol and hydroxyderivatives of lumisterol and tachisterol could play an adjuvant role in neuroprotection, through mitigation of neuroinflammation and protection of endothelial integrity of the blood-brain barrier. Dedicated studies on this topic are currently lacking and are desirable to confirm the link between vitamin D_3 and neuroprotection in COVID-19 patients.

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Introduction

Vitamin D_3 is a fat-soluble steroid hormone with pleiotropic biologic effects [1]. It derives both from food and from the physiological photoconversion of cutaneous 7-dehydrocholesterol into pre-vitamin D_3 and then into cholecalciferol following exposure to UV-B solar rays. Cholecalciferol binds to circulating vitamin D-binding protein (VBP) and in the liver is hydroxylated to calcifediol [25(OH)D_3] by 25-hydroxylase enzyme (cytochrome CYP2R1). Calcifediol is then further hydroxylated to calcitriol [1,25(OH)$_2D_3$] by 1α-hydroxylase enzyme (cytochrome CYP27B1). Calcitriol is the active hormonal final form of vitamin D_3 and can play both non-genomic and genomic effects, acting on vitamin D-receptor (VDR). Schematically, when hydroxylation in position 1 occurs in the kidney, calcitriol exerts endocrine rapid non-genomic effects on target cells (gut epithelial cells, osteoblasts, osteoclasts, parathyroid cells, tubular renal cells), regulating calcium-phosphorus homeostasis (shown in Fig. 1) [1]. On the other hand, when hydroxylation in position 1 occurs in immune cells, calcitriol exerts paracrine/autocrine slower genomic effects on immune cells themselves, downregulating autoimmune/inflammatory processes (shown in Fig. 1) [1]. Calcitriol is ultimately inactivated by 24-hydroxylase enzyme (cytochrome CYP24A1) into calcitroic acid, which is excreted in the bile and then eliminated in the faeces [1].

In addition to this canonical activation of pre-vitamin D_3, a non-canonical pathway has also been identified [2]. After prolonged sun exposure, pre-vitamin D_3 can be converted by CYP11A1 into two photoisomers, lumisterol (L_3) and tachysterol (T_3). L_3 and T_3 can be further hydroxylated to biologically active forms, such as 20(OH)L_3, 22(OH)L_3, 20,22(OH)$_2L_3$, 24(OH)L_3, 20(OH)T_3, 25(OH)T_3 by CYP27A1 [2]. These hydroxyderivatives interact with the VDR, but also with human aryl hydrocarbon receptor (AhR), liver X receptor (LXR) α and β, peroxisome proliferator-activated receptor γ (PPARγ) and retinoid-related orphan receptors (ROR) α and γ [3]. Of note, they don't have calcemic properties, but they can affect immune function and proinflammatory pathways like calcitriol (Fig.1) [3].

In daily clinical practice, serum 25(OH)D_3 concentrations are indicator of vitamin D_3 status of a person (calcifediol has a long half-life of about three weeks). The ranges of normality of serum 25(OH)D_3 concentrations have been established by The Endocrine Society in 2011: concentrations lower than 20 ng/ml are considered as "deficiency", concentrations between 20 and 29 ng/ml are considered as "insufficiency" while concentrations greater than 29 ng/ml are considered as "normality" [4]. Normal serum 25(OH)D_3 concentrations allow for adequate intestinal absorption of calcium and maintenance of normal serum parathormone values in most people, while a cut-off to ensure an immunomodulating effect has not yet been identified with absolute certainty [4].

Vitamin D_3 and respiratory infectious diseases

The correlation between serum 25(OH)D_3 concentrations and paracrine/autocrine anti-inflammatory effects has been extensively investigated in autoimmune and inflammatory conditions, including infectious diseases [1]. Heliotherapy has been the only treatment of tuberculosis for centuries, until the discovery of antibiotics [1]. In recent decades it has been clarified that the benefits of heliotherapy were due to the endogenous production of calcitriol, after the photoconversion of 7-dehydrocholesterol in cholecalciferol. In fact, calcitriol stimulates the synthesis and release of cathelicidin by innate immunity cells (monocytes and neutrophils) of tuberculosis patients [5]. LL-37 residue of cathelicidin is an antimicrobial peptide that damages lipoprotein membranes of Mycobacterium tuberculosis, hindering the formation of surface biofilms [5]. Moreover, LL-37 induces the production of interleukin (IL)-8 by monocytes/macrophages with chemotactic function for neutrophils [5].

Consequently, great interest has developed in the correlation between serum 25(OH)D_3 concentrations and the course of other respiratory infections. A 2017 meta-analysis of 25 randomized clinical trials (RCTs) (11321 participants) reported that vitamin D_3 supplementation was associated with a reduction of the risk of acute respiratory infections, with an adjusted odds ratio of

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0.88 [6]. Protection seemed greater in those subjects with baseline serum $25(OH)D_3$ concentrations < 25 ng/ml [6]. Daily or at most weekly doses of vitamin D_3 were more effective than monthly doses in raising serum $25(OH)D_3$ concentrations, as monthly supplementation boluses activated 24-hydroxylase enzyme and therefore the catabolic pathway of vitamin D_3 [7]. In 2021, an update of the previous meta-analysis included more RCTs (n = 46) and participants (n = 75541) and confirmed the protective effect of vitamin D_3 supplementation against respiratory infections, with an odds ratio of 0.92 [8].

After the spread of COVID-19 pandemic, the role of vitamin D_3 in SARS-CoV-2 infection has been the object of thousands of studies and reports [9]. Deficient/insufficient serum $25(OH)D_3$ concentrations have been correlated with increased susceptibility to infection and more severe disease courses [10]. The biological basis of these observations will therefore be discussed below, first summarizing the pathophysiology of SARS-CoV-2 infection. Then, this narrative review will focus on neurological involvement of COVID-19, speculating on the protective role that vitamin D_3 may exert in neuroprotection.

SARS-CoV-2 pathophysiology

SARS-CoV-2 is an RNA virus that is transmitted from human to human by airborne droplets [11]. Although there are some structural differences due to the different viral variants, SARS-CoV-2 virion is formed by essential proteins, such as nucleocapside proteins (N), membrane proteins (M) and a glycoprotein envelope (E), from which two spike proteins (S_1 and S_2) protrude. S_1 and S_2 adhere to upper respiratory tract cells and nasal olfactory mucosa. S_1 binds to host receptor angiotensin-converting enzyme 2 (ACE-2), while S_2, cleaved and activated by host transmembrane protease serine-protease-2 (TMPRSS-2), fuses viral and host envelopes, integrating viral RNA within the human cells [11].

Subsequently, SARS-CoV-2 replicates and releases double-stranded RNA inside the cells, usually recognized by cytosolic pattern recognition receptors (PRRs), such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5). These PRRs stimulate the production of type I (α and β) and type III (λ) interferons, which have a direct and indirect antiviral function, through the recruitment of the cells of the innate immunity. Neutrophils, monocytes and dendritic cells in turn present surface PRRs, such as Toll-like receptor (TLR)-2 and TLR-4, which recognizes viral glycoproteic envelope. TLR-2 and TLR-4 stimulate nuclear factor kappa b (NF-kB) signaling pathway, with activation of Nod-like receptor protein 3 (NLRP3) inflammasome. NLRP3 releases pro-inflammatory cytokines (IL-1β, IL-18), that drive pyroptosis, the inflammatory form of programmed cell death [11]. Innate immunity cells then activate the more specific adaptive immunity (T and B lymphocytes) and SARS-CoV-2 infection ends in most cases within a few days, with patients reporting only flu-like symptoms (fever, nasopharyngitis, arthralgia) [11].

However, in a minority of cases, depending on different risk factors, SARS-CoV-2 can evade defense mechanisms, downregulating interferons production and blocking autophagy [12,13]. This allows SARS-CoV-2 to spread from the upper to lower respiratory tract, thus reaching the alveoli. The intense but ineffective inflammatory response that is activated in the lungs leads to a progressive worsening of respiratory function. Together with epithelial cell damage, inflammation disrupts integrity of the lung vascular endothelium, promoting a pro-coagulative phenotype [14].

Although not yet fully elucidated, the mechanisms leading to endothelial damage are multiple. The binding between SARS-CoV-2 and ACE-2 receptor unbalances the renin-angiotensin-aldosterone (RAS) system [15]. Under physiological conditions, a reduction in systemic blood pressure stimulates renal renin production, which cuts circulating angiotensinogen produced by the liver into various fragments, including angiotensin I. Angiotensin I is converted into angiotensin II by ACE enzyme and in turn ACE-2 enzyme converts angiotensin II to angiotensin 1-7. Angiotensin 1-7 acts on angiotensin type 2 and Mas receptors, promoting the expression of endothelial nitric oxide synthase and reducing platelet aggregation, with an overall vasodilatory, anti-inflammatory and antifibrotic effect

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(shown in Fig. 2) [15]. Binding between SARS-CoV-2 and ACE-2 dramatically reduces the production of angiotensin 1-7 and conversely leads to the accumulation of angiotensin II, which acts on angiotensin type 1 receptor downregulating the expression of endothelial nitric oxide synthase and promoting platelet aggregation, with an overall vasoconstrictor, pro-inflammatory and pro-fibrotic effect (shown in Fig. 2). At vascular level, there is therefore an oxidative stress with the release of oxygen free radicals following episodes of hypoxia-ischemia which worsens endothelial damage [16]. Moreover, SARS-CoV-2 can induce a thrombotic endothelial damage, mediated by immune cells (immunothrombosis) (shown in Fig. 3). Following the activation of PRRs, monocytes express tissue factor on the surface, a protein that interacts with circulating coagulation factors to activate the extrinsic pathway of coagulation [11]. Furthermore, SARS-CoV-2 is recognized by the complement system via the mannose binding lectin, thus generating C5a fragment [17]. C5a is not only a chemoattractant for neutrophils, but also stimulates neutrophils to express tissue factor on the surface [17]. Activated neutrophils then extrude nuclear material to trap and eliminate viral particles (NETosis) [18]. These traps can in turn activate the coagulation cascade by interacting with factor XII of the intrinsic pathway of coagulation [11]. Finally, SARS-CoV-2 can directly disrupt endothelial tight junctions, causing the exposure by the endothelium itself of the tissue factor [11]. The result of this redundant stimulation of the coagulation system is the formation of the fibrin clot, also favored by a deficit of the fibrinolysis pathway (shown in Fig. 3) [11]. Indeed, COVID-19 patients have high serum concentrations of plasminogen activator inhibitor 1, which inhibits the fibrinolytic activity of tissue plasminogen activator and urokinase [11].

A systemic endothelial damage has been demonstrated in the peripheral skin circulation by nailfold videocapillaroscopy, a non-invasive examination that allows to analyze the morphology and number of capillaries at the level of nailfold beds with a magnification of 40-200 times [19]. A videocapillaroscopic analysis performed on 61 subjects recovered from COVID-19 revealed a significant reduction in skin capillary density compared to healthy population and possibly involved in tissue and organs hypoxia in presence of long-COVID [19,20].

In the most severe cases, the association between hyper-inflammatory cytokine storm and thrombotic events leads to systemic complications, among all acute respiratory distress syndrome, with multiorgan failure and patient's death [11].

Vitamin D$_3$ and SARS-CoV-2 infection
The link between serum 25(OH)D$_3$ concentrations and the course of SARS-CoV-2 infection has been extensively investigated and most studies agree that vitamin D$_3$ deficiency is related to a poorer prognosis of the disease [1,9,21]. Serum 25(OH)D$_3$ concentrations below 25 nmol/l have been associated with a higher risk of severe COVID-19 and systemic complications [22-24]. Potential associations between VDR genetic polymorphisms, which can affect the expression and function of the protein, and the severity and/or mortality for COVID-19 have been also investigated. FokI (rs2228570), TaqI (rs731236), BsmI (rs1544410) and ApaI (rs7975232) are VDR single nucleotide polymorphisms which have been variously associated with different aspects of COVID-19 (susceptibility, severity, mortality). However, the results of observational, retrospective or case-control studies on this topic have been conflicting and do not allow to determine with certainty which polymorphisms contribute most to mitigating or aggravating the disease, also considering the different viral variants [25-27].

Calcitriol and hydroxyderivatives of L$_3$ and T$_3$ could reduce SARS-CoV-2 invasion and replication, inflammation and endothelial damage [3]. In course of COVID-19, inadequate serum 25(OH)D$_3$ concentrations correlate with reduced ACE2 levels and ACE2 mRNA expression and calcitriol supplementation seems to restore ACE2 levels, re-establishing a physiological ratio of angiotensin 1-7/angiotensin II concentrations (shown in Fig. 2) [28]. Calcitriol and hydroxyderivatives of L$_3$ and T$_3$ can also bind to SARS-CoV-2 receptor binding domain of ACE2, hindering the interaction between the virus and the receptor [29]. Moreover, they can cause a conformational change in TMPRSS-2

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structure, further reducing the probability of virus entry into the host cell [29]. Furthermore, *in vitro* experiments have demonstrated that hydroxyderivatives of L_3 and T_3 could block some of the proteases used by SARS-CoV-2 to replicate (3CL-Chymotripsin or Main protease, RNA-dependent RNA Polymerase) and calcitriol stimulates monocyte production of β-defensin 2 and cathelicidin, further reducing viral replication [30,31]. Active vitamin D_3 also promotes the elimination of damaged (infected) cells by autophagy, upregulating the expression of Beclin 1 (activating factor of autophagy) and downregulating mTOR pathway (inhibitor pathway of autophagy) [32].

Regarding immune effects, calcitriol and hydroxyderivatives of L_3 and T_3 can mitigate inflammation, including the pro-inflammatory cytokine storm that can develop in the most severe cases of the disease. In an in vitro study, it has been demonstrated that calcitriol can downregulate NF-kB, a pivotal transcription factor for the activation of pro-inflammatory genes, in particular IL-1, IL-6, IL-8, IL12, IL-17, IL-23 and tumor necrosis factor (TNF) α [33,34]. L_3 and T_3-hydroxyderivatives of provitamin D_3 can downregulate IL-17 production antagonizing not only NF-kB, but also ROR α and γ and AhR [35]. They also upregulate the expression of Nrf2, a transcription factor for several proteins with antioxidant and anti-inflammatory effects (glutamate-cysteine ligase catalytic subunit, glutathione S-transferase, NAD(P)H quinone oxidoreductase 1, heme oxygenase-1) [36,37]. Moreover, calcitriol can reduce neutrophil extracellular traps release in vitro, mitigating both inflammatory and endothelial damage (shown in Fig. 3) [38]. Calcitriol therefore stimulates the shift from T helper 1 (Th1) lymphocytes to Th2 lymphocytes (IL-10 production) with an anti-inflammatory effect, through an autocrine signaling induced by C3b fragment of complement [39].

At last, healthy subjects with serum 25(OH)D_3 concentrations below 26 ng/ml show upregulation of the pro-coagulative platelet-monocyte and monocyte-endothelium interactions [40]. In vitro, calcitriol can also upregulate monocyte expression of thrombomodulin, a protein that reduces the activation of circulating factor VIII (intrinsic pathway of coagulation) and factor V (common pathway of coagulation) and which inhibits plasminogen activator inhibitor 1, with a final fibrinolytic effect and a protective role on the endothelium (shown in Fig. 3) [41].

SARS-CoV-2 neurodamage and the potential protective role of vitamin D_3

Self-reported and/or objectively detectable neurological symptoms are described in more than 80% of hospitalized COVID-19 patients [42]. The most frequent subjective symptom is headache, followed by changes in smell (anosmia) and taste (ageusia), depression and dizziness [42,43]. Objective neurological manifestations have also been reported such as ischemic stroke, meningo-encephalitis, myelitis, seizures, Guillain-Barré syndrome, demyelinating diseases and others [43].

It has been demonstrated that SARS-CoV-2 can infect peripheral nervous system, interacting with ACE-2 and TMPRSS-2 expressed by olfactory epithelial cells [44]. A review of 24 autoptic studies of 149 brains of unvaccinated patients who died from COVID-19, revealed that viral RNA was detectable in brain or olfactory nerve at low levels by targeted quantitative reverse transcriptase polymerase chain reaction [45]. SARS-CoV-2 was also identified by immunohistochemistry in the glossopharyngeal and vagal nerves of another cohort of 43 unvaccinated patients (53% of cases) who died from COVID-19 [46].

SARS-CoV-2 can also invade the central nervous system (CNS), using as receptors not only ACE-2 and TMPRSS-2, but also neuropilin-1, highly expressed by pericytes and astrocytes of the blood-brain barrier (BBB) [47]. In fact, a digital polymerase chain reaction investigation detected SARS-CoV-2 nucleocapsid gene expression in multiple areas of the CNS (cervical spinal cord, olfactory nerve, basal ganglia, cerebral cortex, brainstem, cerebellum, thalamus, hypothalamus, corpus callosum and dura mater) of 44 unvaccinated patients who died from COVID-19 (100% of the study population) [48]. SARS-CoV-2 thus alters the permeability of the BBB, increasing the expression of matrix metalloproteinase-9 that destroys the basement membrane through the degradation of collagen IV and activates RhoA, a small G-protein, which promotes the disassembly of tight junctions through modifications of the cytoskeleton [49]. Moreover, the integrity of the BBB can be disrupted by

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peripheral inflammation [50]. Pro-inflammatory cytokines (i.e., IL-1, IL-6, IL-17) upregulate the expression of adhesion molecules on BBB endothelial cells (E-selectin, VCAM-1, ICAM-1) and enter the CNS [50]. Then, they polarize resting microglial immune cells towards an M1 phenotype, which promotes neurotoxicity via the release of further IL-1β, IL-6, TNF-α, reactive oxygen species (ROS) [51]. M1 microglia induces also a neuroinflammatory reactive astrocyte phenotype, stimulating astrocytes to secrete pro-inflammatory cytokines and vascular endothelial growth factor, further weakening the BBB [50,52]. So, peripheral lymphocytes/cytokines infiltration cause neuroinflammation, that is detrimental to neurons, neurotransmission and neural circuit functions [53].

Neuroinflammation has been confirmed by a single-cell transcriptomic study of the brains of 8 unvaccinated patients who died of COVID-19 [54]. In the choroid plexuses there was an up-regulation of genes (i.e., NQO1 and ZFP36), which caused a pro-inflammatory activation of microglia, through CCL and CXCL chemokines pathways [54]. Immunohistochemistry also showed a significant over-expression of CD68 (marker of macrophage activation) in the choroid plexuses of COVID-19 patients compared to controls [54]. In another cohort, 41 brains of unvaccinated patients who died from COVID-19 were autopsied, and microglial activation (positivity for CD68 at immunohistochemistry) was detected in 81% of cases, with inflammatory infiltrate of T lymphocytes (positivity for CD3) in 93% of cases [55].

Moreover, the viral and inflammatory damage of the BBB promotes the development of immunothrombosis at the level of the brain vessels [56]. In a 2021 meta-analysis, which considered 108571 patients with COVID-19, acute cerebral vascular events were reported in 1.4% of cases, with cerebral ischemia as the main cause of stroke (87.4% of cases) [57]. Ischemic stroke was predominant in the large vessels with a multi-infarct distribution, supporting a thrombotic pathogenesis of the disease [57].

Of note, several studies have demonstrated anti-inflammatory and neuroprotective effects of vitamin D$_3$. Calcitriol acts at multiple levels, first by reducing the expression of adhesion molecules on BBB, thus limiting the entry of inflammatory cells into the brain [58]. In mouse models of vascular diseases (arterial hypertension, ischemic stroke), microglial cells are polarized towards a M1 phenotype (pro-inflammatory). However, they express VDR receptors on their surface and calcitriol promotes the shift from M1 to M2 (anti-inflammatory) phenotype. In fact, vitamin D$_3$ modulates NF-kB pathway, upregulating M2 microglial expression of IL-10 and downregulating production of ROS, interferon γ and TNFα [59-61]. Similarly, in mice in which cerebral oxidative stress has been induced to mimic memory impairment of Alzheimer's disease, calcitriol downregulate NF-kB pathway and upregulate NRF-2 and HO-1 genes in the brain with an antioxidant effect [62].

Furthermore, VDR and CYP27B1 are also expressed by astrocytes and oligodendrocytes. Through an autocrine loop, calcitriol can reduce the release of IL-1, IL-6 and TNFα from reactive astrocytes [63]. Vitamin D$_3$ promotes also oligodendrogenesis, and therefore the production of myelin, inducing oligodendrocyte precursor cells differentiation [64]. It is well known that deficient serum 25(OH)D$_3$ concentrations are a risk factor for the development of demyelinating lesions in course of multiple sclerosis [65]. Vitamin D$_3$ therefore promotes the release of neurotrophic cytokines, including nerve growth factor and brain derived neurotrophic factor, supporting neuronal differentiation, growth and development [66].

The effects of L$_3$ and T$_3$-hydroxyderivatives on neuroimmunological mechanisms have not yet been elucidated, but they can interact with neuroinflammation, stimulating the activation of the hypothalamic-pituitary adrenal axis and therefore the release of glucocorticoids with immunosuppressive function [67].

At last, involvement of the CNS is part of the post-COVID-19 syndrome, known as long-COVID, which is defined as "the condition that occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually three months from the onset of COVID-19, with symptoms that last for at least two months and cannot be explained by an alternative diagnosis" [68]. A recent meta-analysis

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reported fatigue as the most common symptom of long-COVID, followed by memory problems ("brain fog") [68]. Other commonly reported neurological symptoms are persistent changes in taste and smell, anxiety, depression and sleep disorders [69]. The pathophysiology of long-COVID is still partly unknown, but it is reasonable that the mechanisms of neurological damage are superimposable to those described in the acute phase, i.e. the passage of inflammatory cells through a damaged BBB in association with micro-thrombotic vascular disease which maintains chronic hypoxia and brain damage [69]. A very recent investigation reported that low serum $25(OH)D_3$ concentrations at baseline of SARS-CoV-2 infection are correlated to the development of long-COVID symptoms, including neurocognitive ones, with an odds ratio of 1.09 after multiple-regression analyzes [70].

Conclusions

Although nowadays the danger of acute SARS-CoV-2 infection has been mitigated by less aggressive viral variants and by mass vaccinations, adequate serum $25(OH)D_3$ concentrations in COVID-19 patients could be protective against systemic complications, including acute and chronic neurological manifestations (long-COVID) [71,72]. Calcitriol and hydroxyderivatives of L_3 and T_3 show interesting neuroimmunoendocrine effects in course of SARS-CoV-2 infection and can play an adjuvant role in neuroprotection, reducing BBB endothelial damage, antagonizing vascular immunothrombosis and downregulating neuroinflammation. Specific studies in humans are desirable to confirm the evidence collected to date.

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Author Contributions

EG and MC conceptualized the review, collecting data and writing the manuscript. SS created all the figures. SS, A Casabella, EH, A Cere, CP, SP, AS and VS revised the manuscript for important intellectual content. All Authors agreed to the content of the review and are accountable for all aspects of accuracy and integrity. All Authors read and agreed to the current version of the manuscript.

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References

1. Cutolo M, Smith V, Paolino S, Gotelli E. Involvement of the secosteroid vitamin D in autoimmune rheumatic diseases and COVID-19. **Nat Rev Rheumatol**. 2023 May;19(5):265-287.

2. Slominski AT, Kim TK, Li W, Postlethwaite A, Tieu EW, Tang EKY, Tuckey RC. Detection of novel CYP11A1-derived secosteroids in the human epidermis and serum and pig adrenal gland. **Sci Rep**. 2015 Oct;5:14875.

3. Slominski AT, Kim TK, Slominski RM, Song Y, Janjetovic Z, Podgorska E, et al. Metabolic activation of tachysterol$_3$ to biologically active hydroxyderivatives that act on VDR, AhR, LXRs, and PPARγ receptors. **FASEB J**. 2022 Aug;36(8):e22451.

4. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al.; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. **J Clin Endocrinol Metab**. 2011 Jul;96(7):1911-1930.

5. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. **J Immunol**. 2004 Sep;173(5):2909-2912.

6. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. **BMJ**. 2017 Feb; 356:i6583.

7. Ketha H, Thacher TD, Oberhelman SS, Fischer PR, Singh RJ, Kumar R. Comparison of the effect of daily versus bolus dose maternal vitamin D$_3$ supplementation on the 24,25-dihydroxyvitamin D$_3$ to 25-hydroxyvitamin D$_3$ ratio. **Bone**. 2018 May;110:321-325.

8. Jolliffe DA, Camargo CA Jr, Sluyter JD, Aglipay M, Aloia JF, Ganmaa D, et al. Vitamin D supplementation to prevent acute respiratory infections: a systematic review and meta-analysis of aggregate data from randomised controlled trials. **Lancet Diabetes Endocrinol**. 2021 May;9(5):276-292.

9. Gotelli E, Soldano S, Hysa E, Paolino S, Campitiello R, Pizzorni C, et al. Vitamin D and COVID-19: Narrative Review after 3 Years of Pandemic. **Nutrients**. 2022 Nov;14(22):4907.

10. Chiodini I, Gatti D, Soranna D, Merlotti D, Mingiano C, Fassio A, et al. Vitamin D Status and SARS-CoV-2 Infection and COVID-19 Clinical Outcomes. **Front Public Health**. 2021 Dec;9:736665.

11. Lamers MM, Haagmans BL. SARS-CoV-2 pathogenesis. **Nat Rev Microbiol**. 2022 May;20(5):270-284.

12. Miller K, McGrath ME, Hu Z, Ariannejad S, Weston S, Frieman M, et al. Coronavirus interactions with the cellular autophagy machinery. **Autophagy**. 2020 Dec;16(12):2131-2139.

13. Cutolo M, Smith V, Paolino S. Understanding immune effects of oestrogens to explain the reduced morbidity and mortality in female versus male COVID-19 patients. Comparisons with autoimmunity and vaccination. **Clin Exp Rheumatol**. 2020 May-Jun;38(3):383-386.

14. Bonaventura A, Vecchié A, Dagna L, Martinod K, Dixon DL, Van Tassell BW, et al. Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. **Nat Rev Immunol**. 2021 May;21(5):319-329.

15. Scialo F, Daniele A, Amato F, Pastore L, Matera MG, Cazzola M, et al. ACE2: The Major Cell Entry Receptor for SARS-CoV-2. **Lung**. 2020 Dec;198(6):867-877.

16. Montiel V, Lobysheva I, Gérard L, Vermeersch M, Perez-Morga D, Castelein T, et al. Oxidative stress-induced endothelial dysfunction and decreased vascular nitric oxide in COVID-19 patients. **EBioMedicine**. 2022 Mar;77:103893.

17. Carvelli J, Demaria O, Vély F, Batista L, Chouaki Benmansour N, Fares J, et al. Association of COVID-19 inflammation with activation of the C5a-C5aR1 axis. **Nature**. 2020 Dec;588(7836):146-150.

18. Zhu Y, Chen X, Liu X. NETosis and Neutrophil Extracellular Traps in COVID-19: Immunothrombosis and Beyond. **Front Immunol**. 2022 Mar;13:838011.

19. Sulli A, Gotelli E, Bica PF, Schiavetti I, Pizzorni C, Aloè T, et al. Detailed videocapillaroscopic microvascular changes detectable in adult COVID-19 survivors. **Microvasc Res**. 2022 Jul;142:104361.

20. Cutolo M, Sulli A, Smith V, Gotelli E. Emerging nailfold capillaroscopic patterns in COVID-19: from acute patients to survivors. **Reumatismo**. 2023 Mar;74(4).

21. di Filippo L, Uygur M, Locatelli M, Nannipieri F, Frara S, Giustina A. Low vitamin D levels predict outcomes of COVID-19 in patients with both severe and non-severe disease at hospitalization. **Endocrine**. 2023 Mar;1–15.

22. Nielsen NM, Junker TG, Boelt SG, Cohen AS, Munger KL, Stenager E, et al. Vitamin D status and severity of COVID-19. **Sci Rep**. 2022 Nov;12(1):19823.

23. Pereira M, Dantas Damascena A, Galvão Azevedo LM, de Almeida Oliveira T, da Mota Santana J. Vitamin D deficiency aggravates COVID-19: systematic review and meta-analysis. **Crit Rev Food Sci Nutr**. 2022;62(5):1308-1316.

24. Sulli A, Gotelli E, Casabella A, Paolino S, Pizzorni C, Alessandri E, et al. Vitamin D and Lung Outcomes in Elderly COVID-19 Patients. **Nutrients**. 2021 Feb;13(3):717.

25. Charoenngam N, Jaroenlapnopparat A, Mettler SK, Grover A. Genetic Variations of the Vitamin D Metabolic Pathway and COVID-19 Susceptibility and Severity: Current Understanding and Existing Evidence. **Biomedicines**. 2023 Jan;11(2):400.

26. Dobrijevic Z, Robajac D, Gligorijevic N, Šunderic M, Penezic A, Miljuš G, et al. The association of *ACE1*, *ACE2*, *TMPRSS2*, *IFITM3* and *VDR* polymorphisms with COVID-19 severity: A systematic review and meta-analysis. **EXCLI J**. 2022 Jun;21:818-839.

27. Al-Gharrawi ANR, Anvari E, Fateh A. Association of ApaI rs7975232 and BsmI rs1544410 in clinical outcomes of COVID-19 patients according to different SARS-CoV-2 variants. **Sci Rep**. 2023 Mar;13(1):3612.

28. Ortatatli M, Fatsa T, Mulazimoglu DD, Oren S, Artuk C, Hosbul T, et al. Role of Vitamin D, ACE2 and the Proteases as TMPRSS2 and Furin on SARS-CoV-2 Pathogenesis and COVID-19 Severity. **Arch Med Res**. 2023 Apr;54(3):223-230.

29. Song Y, Qayyum S, Greer RA, Slominski RM, Raman C, Slominski AT, et al. Vitamin D3 and its hydroxyderivatives as promising drugs against COVID-19: a computational study. **J Biomol Struct Dyn**. 2022;40(22):11594-11610.

30. Qayyum S, Mohammad T, Slominski RM, Hassan MI, Tuckey RC, Raman C, et al. Vitamin D and lumisterol novel metabolites can inhibit SARS-CoV-2 replication machinery enzymes. **Am J Physiol Endocrinol Metab**. 2021 Aug;321(2):E246-E251.

31. Gilani SJ, Bin-Jumah MN, Nadeem MS, Kazmi I. Vitamin D attenuates COVID-19 complications via modulation of proinflammatory cytokines, antiviral proteins, and autophagy. **Expert Rev Anti Infect Ther**. 2022 Feb;20(2):231-241.

32. Bhutia SK. Vitamin D in autophagy signaling for health and diseases: Insights on potential mechanisms and future perspectives. **J Nutr Biochem**. 2022 Jan;99:108841.

33. Chen S, Zhu J, Chen G, Zuo S, Zhang J, Chen Z, et al. 1,25-Dihydroxyvitamin D3 preserves intestinal epithelial barrier function from TNF-α induced injury via suppression of NF-kB p65 mediated MLCK-P-MLC signaling pathway. **Biochem Biophys Res Commun**. 2015 May;460(3):873-878.

34. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. **Signal Transduct Target Ther**. 2017;2:17023.

35. Slominski RM, Stefan J, Athar M, Holick MF, Jetten AM, Raman C, et al. COVID-19 and Vitamin D: A lesson from the skin. **Exp Dermatol**. 2020 Sep;29(9):885-890.

36. Buendia I, Michalska P, Navarro E, Gameiro I, Egea J, León R. Nrf2-ARE pathway: An emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases. **Pharmacol Ther**. 2016 Jan;157:84-104.

37. Qayyum S, Slominski RM, Raman C, Slominski AT. Novel CYP11A1-Derived Vitamin D and Lumisterol Biometabolites for the Management of COVID-19. **Nutrients**. 2022 Nov;14(22):4779.

38. Chen C, Weng H, Zhang X, Wang S, Lu C, Jin H, et al. Low-Dose Vitamin D Protects Hyperoxia-Induced Bronchopulmonary Dysplasia by Inhibiting Neutrophil Extracellular Traps. **Front Pediatr**. 2020 Jul;8:335.

39. Chauss D, Freiwald T, McGregor R, Yan B, Wang L, Nova-Lamperti E, et al. Autocrine vitamin D signaling switches off pro-inflammatory programs of T_H1 cells. **Nat Immunol**. 2022 Jan;23(1):62-74.

40. Tay HM, Yeap WH, Dalan R, Wong SC, Hou HW. Increased monocyte-platelet aggregates and monocyte-endothelial adhesion in healthy individuals with vitamin D deficiency. **FASEB J.** 2020 Aug;34(8):11133-11142.

41. Ohsawa M, Koyama T, Yamamoto K, Hirosawa S, Kamei S, Kamiyama R. 1alpha,25-dihydroxyvitamin D(3) and its potent synthetic analogs downregulate tissue factor and upregulate thrombomodulin expression in monocytic cells, counteracting the effects of tumor necrosis factor and oxidized LDL. **Circulation**. 2000 Dec;102(23):2867-2872.

12

42. Chou SH, Beghi E, Helbok R, Moro E, Sampson J, Altamirano V, et al.; GCS-NeuroCOVID Consortium and ENERGY Consortium. Global Incidence of Neurological Manifestations Among Patients Hospitalized With COVID-19-A Report for the GCS-NeuroCOVID Consortium and the ENERGY Consortium. **JAMA Netw Open**. 2021 May;4(5):e2112131.

43. Harapan BN, Yoo HJ. Neurological symptoms, manifestations, and complications associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease 19 (COVID-19). **J Neurol**. 2021 Sep;268(9):3059-3071.

44. Aghagoli G, Gallo Marin B, Katchur NJ, Chaves-Sell F, Asaad WF, Murphy SA. Neurological Involvement in COVID-19 and Potential Mechanisms: A Review. **Neurocrit Care**. 2021 Jun;34(3):1062-1071.

45. Mukerji SS, Solomon IH. What can we learn from brain autopsies in COVID-19? **Neurosci Lett**. 2021 Jan;742:135528.

46. Matschke J, Lütgehetmann M, Hagel C, Sperhake JP, Schröder AS, Edler C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. **Lancet Neurol**. 2020 Nov;19(11):919-929.

47. Malik JR, Acharya A, Avedissian SN, Byrareddy SN, Fletcher CV, Podany AT, et al. ACE-2, TMPRSS2, and Neuropilin-1 Receptor Expression on Human Brain Astrocytes and Pericytes and SARS-CoV-2 Infection Kinetics. **Int J Mol Sci**. 2023 May;24(10):8622.

48. Stein SR, Ramelli SC, Grazioli A, Chung JY, Singh M, Yinda CK, et al. SARS-CoV-2 infection and persistence in the human body and brain at autopsy. **Nature**. 2022 Dec;612(7941):758-763.

49. Hernández-Parra H, Reyes-Hernández OD, Figueroa-González G, González-Del Carmen M, González-Torres M, Peña-Corona SI, et al. Alteration of the blood-brain barrier by COVID-19 and its implication in the permeation of drugs into the brain. **Front Cell Neurosci**. 2023 Mar;17:1125109.

50. Huang X, Hussain B, Chang J. Peripheral inflammation and blood-brain barrier disruption: effects and mechanisms. **CNS Neurosci Ther**. 2021 Jan;27(1):36-47.

51. Guo S, Wang H, Yin Y. Microglia Polarization From M1 to M2 in Neurodegenerative Diseases. **Front Aging Neurosci**. 2022 Feb;14:815347.

52. Lawrence JM, Schardien K, Wigdahl B, Nonnemacher MR. Roles of neuropathology-associated reactive astrocytes: a systematic review. **Acta Neuropathol Commun**. 2023 Mar;11(1):42.

53. Vanderheiden A, Klein RS. Neuroinflammation and COVID-19. **Curr Opin Neurobiol**. 2022 Oct;76:102608.

54. Yang AC, Kern F, Losada PM, Agam MR, Maat CA, Schmartz GP, et al. Dysregulation of brain and choroid plexus cell types in severe COVID-19. **Nature**. 2021 Jul;595(7868):565-571.

55. Thakur KT, Miller EH, Glendinning MD, Al-Dalahmah O, Banu MA, Boehme AK, et al. COVID-19 neuropathology at Columbia University Irving Medical Center/New York Presbyterian Hospital. **Brain**. 2021 Oct;144(9):2696-2708.

56. Erickson MA, Rhea EM, Knopp RC, Banks WA. Interactions of SARS-CoV-2 with the Blood-Brain Barrier. **Int J Mol Sci**. 2021 Mar;22(5):2681.

57. Nannoni S, de Groot R, Bell S, Markus HS. Stroke in COVID-19: A systematic review and meta-analysis. **Int J Stroke**. 2021 Feb;16(2):137-149.

58. Cimmino G, Morello A, Conte S, Pellegrino G, Marra L, Golino P, et al. Vitamin D inhibits Tissue Factor and CAMs expression in oxidized low-density lipoproteins-treated human endothelial cells by modulating NF-κB pathway. **Eur J Pharmacol**. 2020 Oct;885:173422.

59. Cui C, Xu P, Li G, Qiao Y, Han W, Geng C, et al. Vitamin D receptor activation regulates microglia polarization and oxidative stress in spontaneously hypertensive rats and angiotensin II-exposed microglial cells: Role of renin-angiotensin system. **Redox Biol**. 2019 Sep;26:101295.

60. Jiang H, Yang X, Wang Y, Zhou C. Vitamin D Protects against Traumatic Brain Injury via Modulating TLR4/MyD88/NF-κB Pathway-Mediated Microglial Polarization and Neuroinflammation. **Biomed Res Int**. 2022 Jul;2022:3363036.

61. Cui P, Lu W, Wang J, Wang F, Zhang X, Hou X, et al. Microglia/macrophages require vitamin D signaling to restrain neuroinflammation and brain injury in a murine ischemic stroke model. **J Neuroinflammation**. 2023 Mar;20(1):63.

13

62. Ali A, Shah SA, Zaman N, Uddin MN, Khan W, Ali A, Riaz M, Kamil A. Vitamin D exerts neuroprotection via SIRT1/nrf-2/NF-kB signaling pathways against D-galactose-induced memory impairment in adult mice. **Neurochem Int**. 2021 Jan;142:104893.

63. Jiao KP, Li SM, Lv WY, Jv ML, He HY. Vitamin D3 repressed astrocyte activation following lipopolysaccharide stimulation in vitro and in neonatal rats. **Neuroreport**. 2017 Jun;28(9):492-497.

64. Li N, Yao M, Liu J, Zhu Z, Lam TL, Zhang P, et al. Vitamin D Promotes Remyelination by Suppressing c-Myc and Inducing Oligodendrocyte Precursor Cell Differentiation after Traumatic Spinal Cord Injury. **Int J Biol Sci**. 2022 Aug;18(14):5391-5404.

65. Feige J, Moser T, Bieler L, Schwenker K, Hauer L, Sellner J. Vitamin D Supplementation in Multiple Sclerosis: A Critical Analysis of Potentials and Threats. **Nutrients**. 2020 Mar;12(3):783.

66. Groves NJ, Burne THJ. The impact of vitamin D deficiency on neurogenesis in the adult brain. **Neural Regen Res**. 2017 Mar;12(3):393-394.

67. Reichrath J, März W, DE Gruijl FR, Vieth R, Grant WB, Slominski AT, et al. An Appraisal to Address Health Consequences of Vitamin D Deficiency With Food Fortification and Supplements: Time to Act! **Anticancer Res**. 2022 Oct;42(10):5009-5015.

68. Chen C, Haupert SR, Zimmermann L, Shi X, Fritsche LG, Mukherjee B. Global Prevalence of Post-Coronavirus Disease 2019 (COVID-19) Condition or Long COVID: A Meta-Analysis and Systematic Review. **J Infect Dis**. 2022 Nov;226(9):1593-1607.

69. Monje M, Iwasaki A. The neurobiology of long COVID. **Neuron**. 2022 Nov;110(21):3484-3496.

70. di Filippo L, Frara S, Nannipieri F, Cotellessa A, Locatelli M, Rovere Querini P, et al. Low vitamin D levels are associated with Long COVID syndrome in COVID-19 survivors. **J Clin Endocrinol Metab**. 2023 Apr:dgad207.

71. Cutolo M, Paolino S, Smith V. Evidences for a protective role of vitamin D in COVID-19. **RMD Open**. 2020 Dec;6(3):e001454.

72. Ceolin G, Mano GPR, Hames NS, Antunes LDC, Brietzke E, Rieger DK, Moreira JD. Vitamin D, Depressive Symptoms, and Covid-19 Pandemic. **Front Neurosci**. 2021 May;15:670879.

Figure Legends

Fig. 1.   **Vitamin D$_3$ biosynthesis and neuroimmunoendocrine effects**.
Cutaneous 7-dehydrocholesterol is converted to pre-vitamin D$_3$ and further converted to cholecalciferol under the effects of UV-B rays. Cholecalciferol is then converted to calcifediol in the liver. Depending on the site where the hydroxylation of calcifediol to calcitriol occurs, the latter exerts rapid non-genomic actions (with effect on bone metabolism) or slower genomic actions (with effect on immune and inflammatory response). In case of overexposure to UV-B rays, pre-vitamin D$_3$ is converted to tachisterol and lumisterol in the skin, the hydroxyderivatives of which seems to have similar neuroendocrine functions of calcitriol, without effects on bone metabolism (original figure drawn by co-author Dr Stefano Soldano with www.biorender.com).
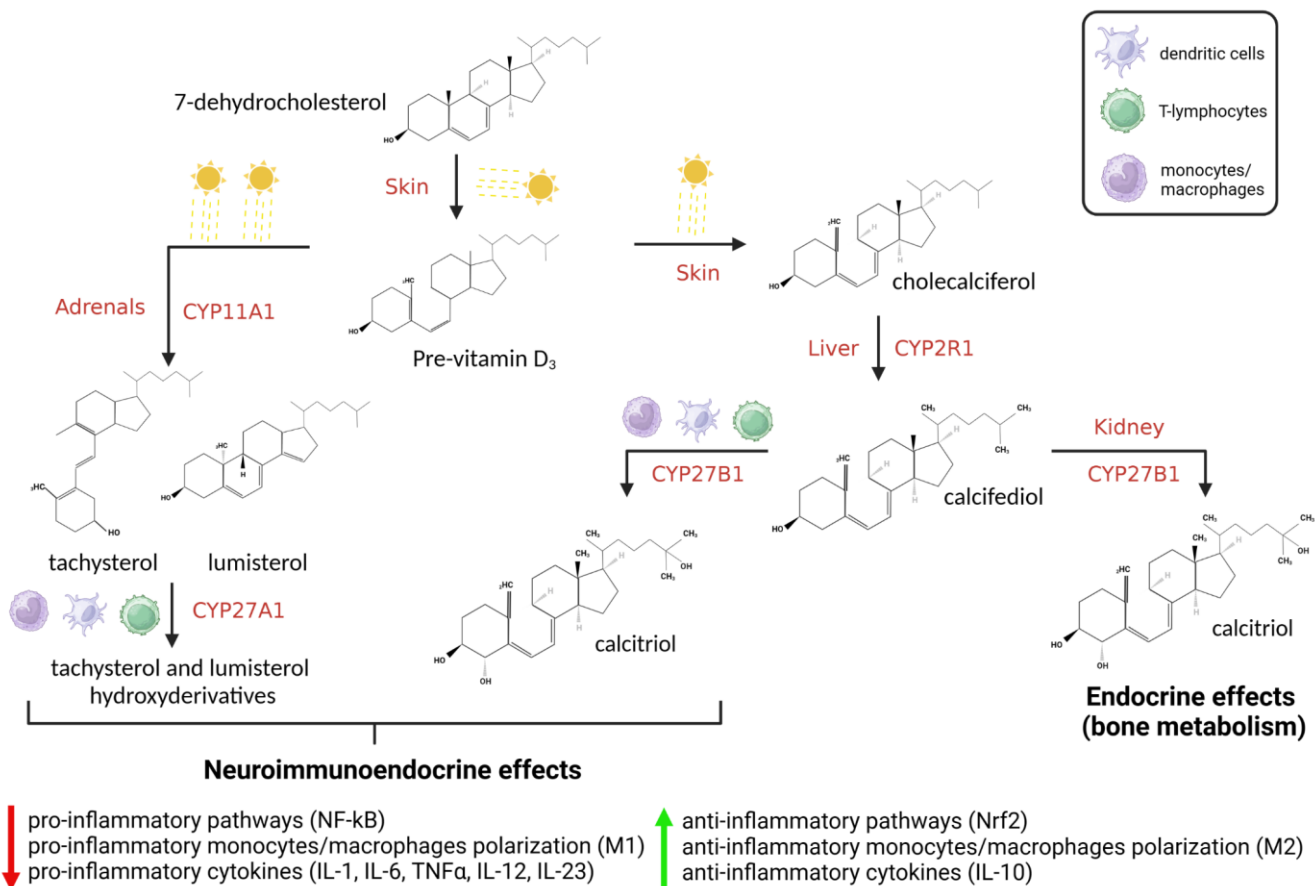
Fig. 2.   **Endothelial cell and ACE-2 in physiological conditions, during SARS-CoV-2 infection and under the effect of vitamin D$_3$**. Under physiological conditions, the expression of ACE-2 by endothelial cells allows the formation of angiotensin 1-7, which, by acting on AT2 receptor, has vasodilatory and antithrombotic effects. In course of SARS-CoV-2 infection, the activity of ACE-2 is perturbed and the accumulation of angiotensin II causes vasoconstriction and promotes platelet aggregation and inflammation. Vitamin D$_3$ can help to restore ACE2 expression on the surface of endothelial cell, decreasing accumulation of angiotensin II.
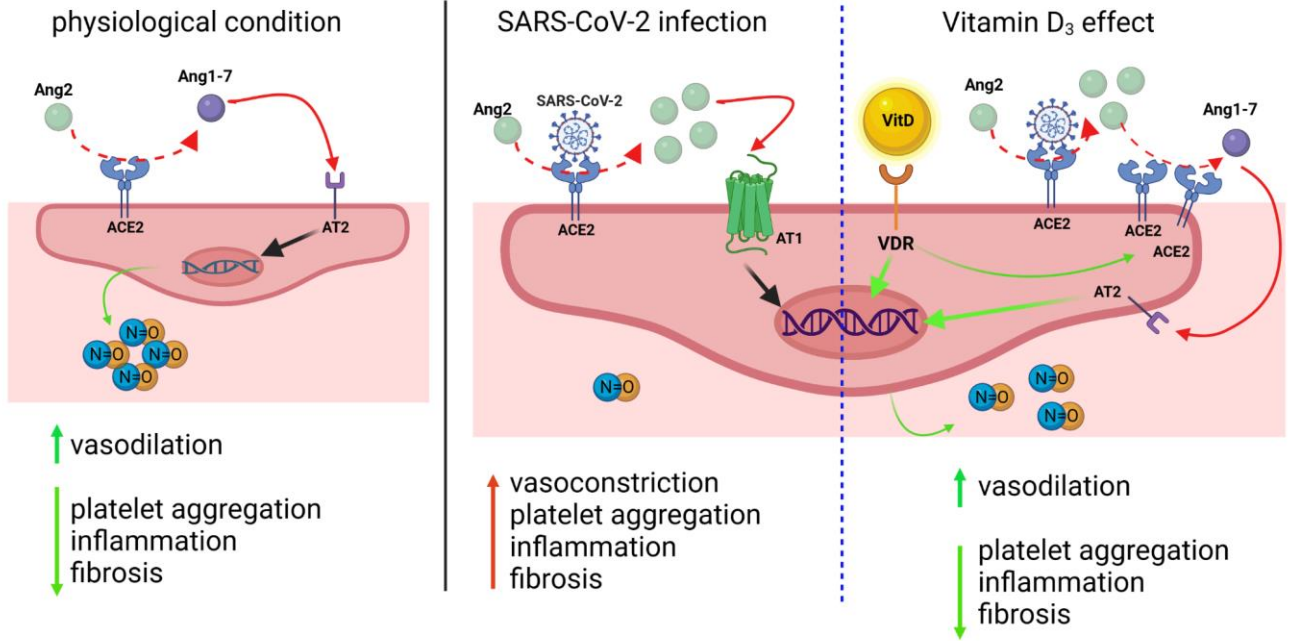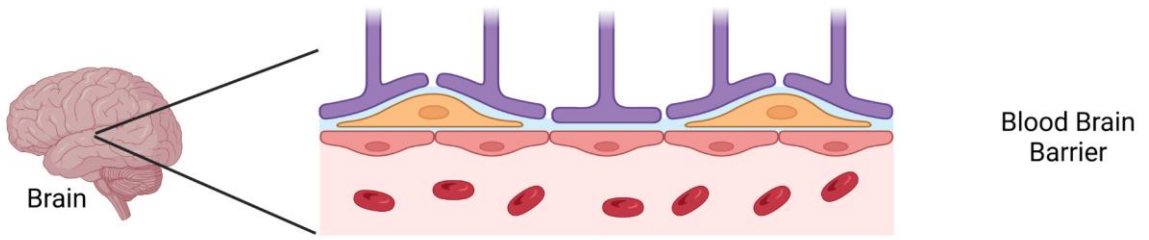Abbreviations: ACE-2: angiotensin-converting enzyme 2; Ang 1-7: angiotensin 1-7; Ang2: angiotensin II; AT1: angiotensin type 1 receptor; AT2: angiotensin type 2 receptor; NO: nitric oxide; VDR: vitamin D receptor (original figure drawn by co-author Dr Stefano Soldano with www.biorender.com).

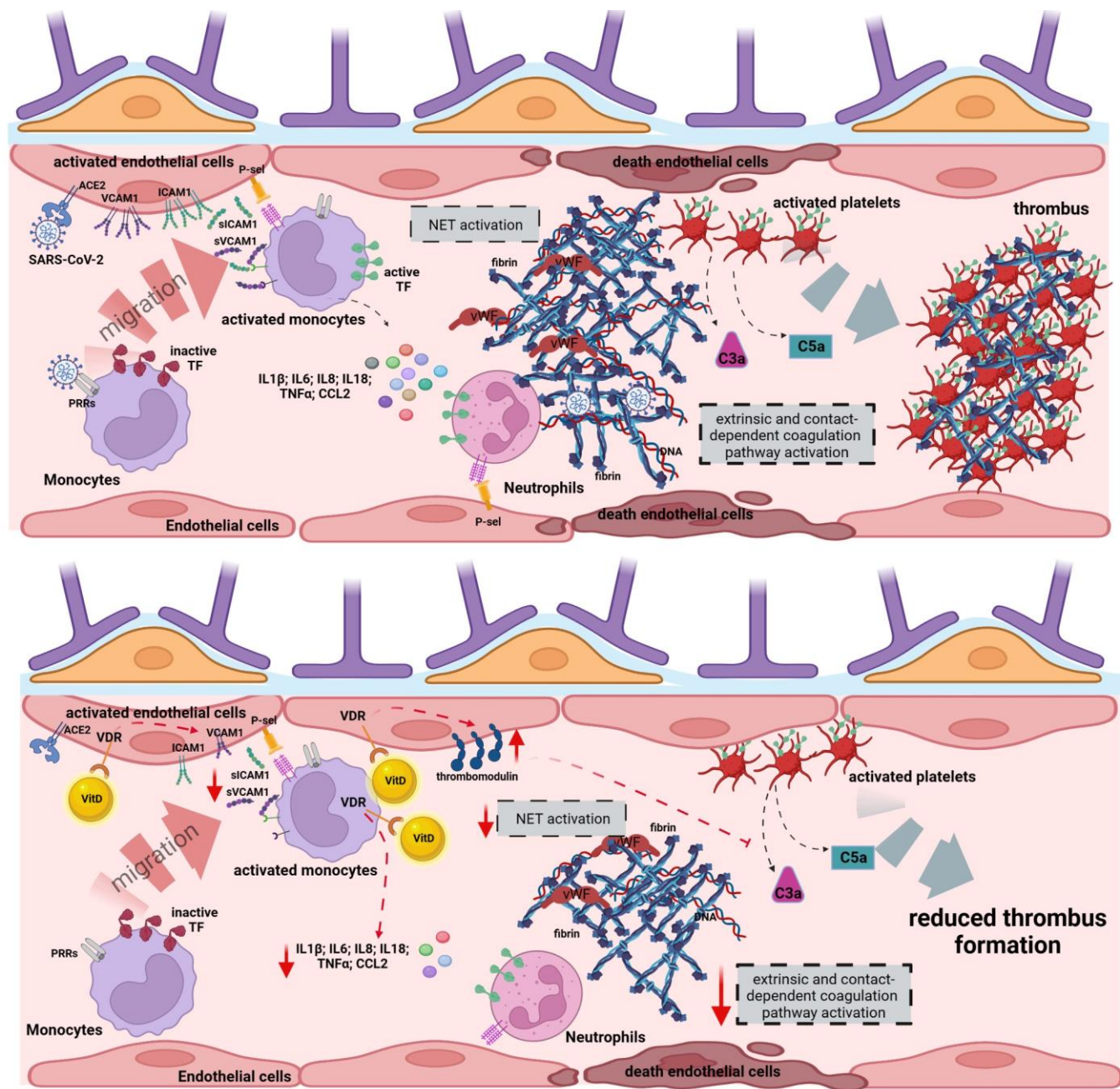Fig. 3 **Interplay between immune cells, inflammation and coagulation factors (immunothrombosis).** The binding of SARS-CoV-2 with ACE-2 causes the increase of adhesion molecules on endothelial cells, promoting the passage of innate immunity cells into the endothelium. Monocytes recognize viral RNA fragments through their pattern recognition receptors (PRRs), expose tissue factor on their surface, and release pro-inflammatory cytokines, which damage the endothelium and attract additional monocytes and neutrophils. Neutrophils release their extracellular traps which further damage the endothelium and activate coagulation factor XII. The combination of damaged endothelium, platelet activation, complement activation, and the coagulation cascade leads to fibrin thrombus formation. The antithrombotic mechanisms proposed for vitamin D$_3$ are reduction of nuclear extracellular traps and adhesion molecules by endothelial cells, increasing also thrombomodulin expression with an overall anti-coagulant effect (original figure drawn by co-author Dr Stefano Soldano with www.biorender.com).

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Neuroimmunoendocrine effects

↓ pro-inflammatory pathways (NF-kB)
pro-inflammatory monocytes/macrophages polarization (M1)
pro-inflammatory cytokines (IL-1, IL-6, TNFα, IL-12, IL-23)

↑ anti-inflammatory pathways (Nrf2)
anti-inflammatory monocytes/macrophages polarization (M2)
anti-inflammatory cytokines (IL-10)

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