REVIEW

A Potential Role of Vitamin D on Platelet Leukocyte Aggregation and Pathological Events in Sepsis: An Updated Review

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Abstract: Vitamin D deficiency and sepsis are both significant global health problems. Insufficient vitamin D is considered to be a pathogenically relevant factor of sepsis-related deaths; however, a causal relationship has not yet been demonstrated. Recently, vitamin D has been an exciting field of research owing to the identification of vitamin D receptors on many extra skeletal tissues and cells, suggesting an unexpected role on body physiology, beyond its effects on bone homeostasis. However, while the role of vitamin D on bone health is widely understood and has been successfully translated into clinical applications and public health policies, recent evidence supporting its role in other physiological and pathological processes has not been fully established. In sepsis, there is an induction of local intracellular vitamin D activity by most immune cells, including lymphocytes, macrophages, neutrophils, and dendritic cells, as well as vascular endothelial cells, to ensure efficient clearance of infective microorganisms and mediate anti-inflammatory and tolerogenic effects. The literature suggests an association between low vitamin D levels and sepsis, but clinical trials have yielded contradictory results. A greater understanding of this role may improve disease management. This article reviews the available knowledge regarding vitamin D in immune function, emerging literature regarding the association between its deficiency and sepsis, as well as presenting its potential effect on platelet leukocyte aggregations (PLAs), a significant pathology in sepsis. It also summarizes clinical trials involving vitamin D supplementation during critical illness and sepsis and addresses the impact of relevant factors of sepsis pathogenesis on the efficacy of vitamin D supplementation, which could contribute to the reported inconsistencies. Looking ahead, further studies are required to uncover the possible modulatory relationship between vitamin D and sepsis to define better cut-offs for its levels, proper timing of its administration, and the optimum dosage for best management.

Keywords: infection, inflammation, cellular interaction, 25(OH)D3 deficiency

Background

Vitamin D (VD), is a steroid hormone and a crucial nutrient that is reported to control a wide range of physiological processes.[1] Several sources of VD are available in the form of D_2, known as ergocalciferol, and D_3, known as cholecalciferol. VD_2 is produced from ergosterol found mainly in fungi and also in some plants upon ultraviolet irradiation. Approximately 80% of VD_3 is synthesized endogenously in the human skin as 7-dehydrocholesterol in response to ultraviolet

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ray exposure from sunlight, and about 20% is provided through diet.[2–4] Inside the body, VD_2 and VD_3 undergo two consecutive steps of hydroxylation in the liver and then kidneys to be converted into their active compounds, 25(OH)D3 calcidiol (a clinical marker of plasmaV D level), and 1,25(OH)2D3 calcitriol, respectively.[5]

In addition to its well-known effects on calcium and phosphate metabolism to ensure bone health, VD has an emerging immune modulatory effect. VD is involved immune system regulation; it regulates the action of suppressor T lymphocytes, the synthesis of cytokines, and acts by modulating the processes of cellular apoptosis.[6] In the 19th century, prior to the development of effective antibiotics, VD was serendipitously used to cure infections, such as tuberculosis, through sunlight exposure and administration of cod liver oil, which are the main sources of VD.[7–11] The Nobel Prize for medicine or physiology in 1903 was awarded to Finsen for his contribution in treating lupus vulgaris, a skin disease caused by *Mycobacterium tuberculosis* with ultraviolet (UV) light.[12–14] Since then, some cross-sectional studies have suggested an inverse correlation between lower levels of VD and increased infections, such as tuberculosis (TB) and upper respiratory tract infections. In 1977, it was reported that children with malnutritional rickets were more prone to lung infections associated with an apparent radiographic pulmonary abnormalities called "rachitic lung."[15] However, little attention has been paid to these studies owing to the subsequent discovery and application of antibiotic therapy for infections.

Over the past three decades, Finsen's work has received renewed attention as a consequence of multiple epidemiological studies showing a strong correlation between VD deficiency and the incidence of different infectious diseases, including pneumonia and sepsis.[16–19] A significantly higher rate of such infections were reported during winter when exposure to sunlight, the major source of VD, is reduced.[20,21] Since then, extensive studies of VD and incidence of infection have been published. Most of them focused on respiratory tract infections and consistently revealed the link between low VD plasma level (25 (OH)D3 and the risk of acute respiratory infections.[22,23] These findings were further confirmed by several randomized clinical trials (RCTs) that reported the protective effect of VD supplementation in reducing the risk of acute respiratory infections by 25% at doses of 400–1000 IU per day for 12 months, particularly in those with a baseline of <25 nmol/l.[24,25]

During sepsis, there is growing evidence that VD deficiency is strongly associated with sepsis risk, pathogenesis, and outcomes as described later,[26–29] but to date, these data could not be applied clinically. Several clinical trials aimed at analyzing the effects of supplementing VD on the outcomes of critical illness including sepsis have reported contradictory results as shown in Table 1.

Given the fact that sepsis is the most common cause of critical illness,[30] all RCTs investigating the effect of VD supplementation for critically ill and septic adult patients controlled with a placebo were retrieved. The databases of Pumped, Scopus, Medline, Embase, Web of Science, and Clinical Trials.gov were used to search for the following words: RCT, administration, supplementation, vitamin D, vitamin D_2, vitamin D_3, cholecalciferol, ergocalciferol, calcitriol, calcidiol, 25-hydroxyvitamin D3, 25(OH)D3, 1,25-dihydroxyvitamin D3, 1,25(OH)2D3, sepsis, critical ill, intensive care unit, septic shock. All critical and septic cases with low VD plasma levels (25(OH)D3 ≤50 nmol/l) at admission were eligible for inclusion.

In some studies, the administration of VD resulted in significant increases in leukocyte mRNA expression of cathelicidin (LL-37, antimicrobial peptide)[31] and plasma cathelicidin, significant reductions in IL-1β and 1L-6 among septic patients,[32] and showed a reduction in 30-day ICU readmission in septic cases, a lower hospital death rate among critically ill patients with severe VD deficiency (25(OH)D3 ≤30 nmol/l),[33] a significant decrease in the duration of hospital stay,[34] a reduction in the duration of respiratory support with mechanical ventilators and hospitalization, and a reduction in mortality rate in the ICU among the critically ill.[35] However, other studies demonstrated the ineffectiveness of VD supplementation on the mortality rate and duration of hospital stay.[31,33,36,37]

VD deficiency and sepsis are very common,[20,26] and both conditions frequently coexist clinically. However, neither the effects of its deficiency on the pathogenesis of the disease and outcomes nor the effects of the disease itself on the correct assessment of VD status have yet been estimated. Such effects may contribute to the confusion about positivity and negativity of VD effects in the reported results so far. For the first time, this paper discusses the role of VD in the pathogenesis of sepsis with particular focus on platelet leukocyte aggregations (PLAs) and how it may reduce aggregate formation as well as their adherence to the endothelium to mitigate sepsis progression. It also addresses the impact of relevant factors of

Table 1 Randomized Clinical Trial of Vitamin D Reinforcement in Critical Ill and Sepsis

Study	Participant's Conditions	Number	Age	Intervention	Changes in Vitamin D Level After Intervention	Changes in Markers of Immune Function	Clinical Outcomes
Amrein et al, 2011,[36] Double-blind	Critical ill patients	Placebo 12 VD group 13	Both placebo and VD group 62± 16 year	Single dose of 540,000 IU Cholecalciferol or matched placebo Orally	25(OH)D3 (nmol/L) **Baseline** Placebo group 35.25 ± 9.25 VD group 32.75 ± 5 **At 7th day** Placebo group 34.25 ± 10.5 VD group 95.5 ± 41.25	Not measured	No significant effect in 28 mortality, Hospital stays and ICU stays.
Leaf et al, 2014[31] Double-blind	Severe sepsis	Placebo group 31 VD group 36	Placebo group 58 (49–69) year VD group 68 (54–70) year	Single dose of 2 µg of (calcitriol) 1,25-dihydroxyvitamin D or matched placebo intravenous	Significant increase in 1.25 D concentration after 6 hours in VD group- versus placebo- (75.7 [52.1–115.5] and 16.9 [9.0–26.9] pg/mL)	Significant increase in Leukocyte mRNA Expression of cathelicidin at 24 h of calcitriol administration No difference between groups inTNF-α, IL-1β, IL-2, IL-10, IL-6, And Cathelicidin (LL-37)	No significant effects in extent of respiratory support with mechanical ventilator, length of hospitalization and 28-day mortality.
Amrein et al, 2014[33] Double blind	Critical ill patients	Placebo group 238 VD group 237	Placebo group 63.9 VD group 65.3	Initial dose of 540 000 IU, following that, 5 doses of 90,000 IU each month or matched placebo Orally	25(OH)D3 (nmol/L) **Baseline** Placebo group 32.75 VD group 32.5 **At day 7th** Placebo group 36.25 VD group 88.75	No significant effect in CRP	No significant effect in duration of hospital stays, hospital death rate 6-month deaths Lower hospital death rate among subgroup of sever vitamin deficiency (25(OH)D3 ≤12 ng/mL)
Quraishi et al, 2015[32] Double blind	Sepsis	Placebo group 10 VD group (200,000 IU) 10 VD group (400,000 IU) 10	Placebo group 65 (58–70) VD group (200,000 IU) 64 (55–66) VD group (400,000 IU) 62 (59–67)	Single dose of 200.000 IU or 400.000 IU of cholecalciferol or matched placebo Orally	25(OH)D3 (nmol/L) **Baseline** placebo group 47.5 (32.5–55) VD group (200,000 IU) 37.5 (30–50) VD group (400.000 IU) 42.5 (32.5–62.5) **At 5th day** Placebo group 47.5 (27.5–57.5) VD group (200.000 IU) 55 (40–62.5) VD group (400.000 IU) 72.5 (80–102.5)	Significant increase in cathelicidin LL-37 in VD group Significant reduction in IL-1β IL-6 increase in Il-10 in VD group No differences in hsCRP levels between groups	30-day ICU readmission rate is 20% in placebo group And 0% In both vitamin D group No difference ICU length of stay.

(*Continued*)

Table 1 (Continued).

Study	Participant's Conditions	Number	Age	Intervention	Changes in Vitamin D Level After Intervention	Changes in Markers of Immune Function	Clinical Outcomes
Han et al, 2016[34] Double blind	Intensive care unit patients	Placebo group 10 VD group (50,000 IU) 10 VD group (100,000 IU) 11	Placebo group 64.8 VD group (50,000 IU) 56.4 VD group (100,000 IU) 68.1	50,000 IU VD3 or 100,000 IU VD3 every day for 5 days consecutively or matched placebo orally	25(OH)D3 (nmol/L) **Baseline** Placebo group 53.75 VD group (50,000 IU) 58 VD group (100,000 IU) 50 **At day 7th** Placebo group no change VD group (50,000 IU) 112.5 ± 50 VD group (100,000 IU) 137.5 ±35	No significant effect in antimicrobial peptide cathelicidin (LL-37)	Significant reduction in duration of hospital stay in both vitamin D group
National Heart and Network, 2019[37] Double blind	Critical ill	Placebo group 540 VD group 530	Placebo group 56 ±15.9 VD group 54.6±16.7	Single dose of 540,000 IU vitamin D3 or matched placebo Orally	25(OH)D3 (nmol/L) **Baseline** Placebo group 27.5±11.75 VD group 28 ± 12 **At 3rd Day** Placebo group 28.5 ±14 VD group 117.25±58	Not measured	No significant effect in 28 day death rate, 90 day hospital death rate, duration of stay at hospital and other health care facility and duration respiratory support free time
Miri et al, 2019[35] Double blind	Critical ill	Placebo group 18 VD group 22	Placebo group 56 ± 22.1 VD group 52 ± 22.1	Single dose of 300,000 IU vitamin D or matched placebo Intramuscular	25(OH)D3 (ng/dl) **Baseline** placebo group 28.38 ± 18.23 VD group 8.43 ± 6.8 **At 7th day** Placebo group 11.16 ± 18.22 VD group 10.48 ± 9.80	Not measured	Reduction in the duration of respiratory support with mechanical ventilators, the duration of hospitalization, and mortality rate in the ICU

Abbreviations: VD, vitamin D; ICU, intensive care unit; TNF, tumor necrosis factor; CRP, C reactive protein; hsCRP, high sensitivity C reactive protein.

sepsis pathogenesis on the effectiveness of VD supplementation, which could contribute to clarification of the controversy in the reported results.

Association Between Vitamin D Deficiency and Sepsis

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to an infection.[38] It is a fast-growing international health issue that imposes a substantial economic burden. Worldwide, it accounts for 48.9 million cases annually, resulting in more than 11 million deaths.[39] In the UK, around 250,000 cases and 44,000 deaths of sepsis are reported every year.[40,41] In the United States, 1.7 million cases are diagnosed and 270,000 of these cases die from sepsis.[42] Very little is known about the estimates of the incidence and outcome of sepsis from developing countries, especially Saudi Arabia. However, all the available data confirm that sepsis is a serious cause of morbidity and mortality all over the world. Approximately two-thirds of septic patients are treated in intensive care units (ICU) with an annual estimated cost of £15.6 billion in the UK[41] and $24 billion in the United States.[39] The current management guidelines applied for sepsis, including antibiotics and fluid replacement, are crucial and lead to significant improvement in clinical outcomes and reduction in mortality.[43] However, some patients may still die and this maybe owing to the difficulty in assigning a patient's disease course to the relative over- or under inflammation that may occur,[44] or microbial adaptation within the host in the form of acquiring resistance genes to the applied antibiotics (bacterial sepsis).[45] Development of new adjunctive therapy to improve the disease might be helpful in those cases when standard care is not sufficient.

Although there is no consensus in the literature about 25(OH)D plasma concentrations used to define VD deficiency, it is a highly prevalent condition worldwide.[46–48] The minimum agreement to date is that maintaining a plasma level of 25(OH)D above 30 nmol/l shields against VD deficiency-associated bone disorders; a lower 25(OH)D level could be used to define VD deficiency and should be prevented and treated.[46] Based on the 25(OH)D cutoff of <30 nmol/l, VD deficiency is very common worldwide[49] with a reported prevalence of 13% in Europe, 5.9% in the United States, 7.4% in Canada, and more than 20% in many developing countries.[50] The reported worldwide prevalence is much higher when VD deficiency is defined as <50 nmol/l. It is estimated as 40% in Europe, 24% in the United States, 37% in Canada,[50] 34–22% in Africa,[48] and 60% in Saudi Arabia.[51]

Low VD plasma levels have been observed in 79% to 98% of critical care unit patients, involving septic cases.[52–54] The risk of sepsis and its consequential outcomes such as death rate, length of hospital stay, and organ failure are positively correlated with VD deficiency.[52,55] Trongtrakul and Feemuchang found that three-quarters of patients who are diagnosed with severe sepsis had a low plasma level of VD and a higher death rate, especially in cases where VD plasma levels were severely deficient (25(OH)D <30 nmol/l).[29] In the developed world, the number of new cases of sepsis as well as its related deaths are elevated during winter when low plasma levels of VD are detected,[17] albeit that various seasonal factors are also implicated.[56] Thus, restoring the VD to optimal plasma levels could have a valuable impact on sepsis development and outcomes. To date, the suggested recommended plasma concentrations of 25(OH)D that are considered sufficient or optimal vary in different settings. Concentrations of >50 nmol/l are sufficient for bone health maintenance, although concentrations of 60–75 nmol/l are suggested for optimal beneficial effects on bone health. However, several studies note that higher levels of above 75 nmol/l are needed for optimal function of the immune system.[57,58] Thus, it is very important to conduct further studies to determine optimal plasma levels for VD to exert its extra skeletal functions. In the developing world, data regarding the link between VD deficiency and sepsis is very limited. However, with the high reported prevalence of VD deficiency, increasing hospital admissions of septic patients with VD deficiency are to be expected. Future studies to estimate the VD levels among patients with sepsis and to correlate plasma level with various inflammatory mediators and disease outcomes are required.

Vitamin D Mechanisms to Interfere with Sepsis Development

In (bacterial) sepsis, damage and stress signals resulting from invading microorganisms and associated inflammatory responses stimulate intracellular VD activity locally to ensure efficient clearance of the microorganisms and mediate anti-inflammatory and tolerogenic effects.[59] Stimulation of the TLR 2/1 pathway by binding to various pathogen-associated molecular patterns (PAMPs) results in induction of VDR and 1-α-hydroxylase (CYP27B1) genes.

Inflammatory mediators such as IFNγ, IL-15, and IL-17A contribute to 1-α-hydroxylase (CYP27B1) gene activation.[60,61] The majority of immune cells, such as lymphocytes, macrophages, neutrophils, and dendritic cells, as well as vascular endothelial cells have 25-hydroxyvitamin D-1α-hydroxylase (1α-OHase), which acts locally to convert 25-hydroxy-vitamin D (circulating form of VD), to its active compound (1,25(OH)2D).[62] Unlike the conventional renal activation of VD, this conversion is regulated by 25-hydroxy-vitamin D levels; thus, low concentration of 25-hydroxy-vitamin D interferes with its extra-skeletal actions.[63,64] After activation, VD is translocated intracellularly, in association with a protein called VD binding protein and attach to its nuclear receptors (VDR) forming a complex. Subsequently, this complex attaches to the VD response element on DNA to regulate target gene transcription.[60,65,66]

VD receptor is expressed by most immune system cells. Thus, signaling through the VD receptor intensifies the local innate immune response by augmenting the release of antimicrobial peptides (AMPs) such as cathelicidin and LL-37, the active form of cathelicidin.[67] Such AMPs induce a broad spectrum of antimicrobial activity mediated by cytokine release, including chemotaxis, phagocytosis, and programmed cell death.[68] VD reduces antigen presentation processes by decreasing the expression level of major histocompatibility complex (MHC) class II and co-stimulatory molecules, CD40, CD80, CD86, on antigen presenting cells (APCs) such as dendritic cells (DC), resulting in a more tolerogenic, immature state.[69] It also has various effects on the activation status of cells mediating adaptive immunity.[70–72] VD suppresses T lymphocytes (Th1) and their release of IL-2 and interferon gamma (IFNγ).[73,74] Furthermore, the intracellular downstream signaling initiated by the VD-VDR complex in the vascular endothelium reduces cell activity and inflammatory response.[75] Moreover, VD has an anti-inflammatory effect. Four hours pre-incubation of immune cells extracted from blood of normal individuals with 100 nM 1,25(OH)2D3, reduced the production of several proinflammatory mediators (such as IL-1β, TNF-α, and IFN-γ and IL-8), a 53-fold in response to their 24 h-treatment with bacterial stimulus [heat-killed pneumococcal serotype 19F (HK19F)].[76] Another study found that activating blood immune cells isolated from VD deficient samples with TLR stimuli released a wide range of proinflammatory mediators, which were significantly decreased after VD treatment.[77]

VD exerts its anti-inflammatory actions by suppressing the gene expression of Toll-like receptor-2 and Toll-like receptor-4, reducing p38 and p42/42 phosphorylation and its downstream signaling, as well as decreasing the release of reactive oxygen species.[78] In addition, VD acts as a transcription factor to regulate the gene expression of several biological processes controlling immune response, such as cellular proliferation, differentiation, apoptosis, and angiogenesis.[79] Furthermore, VD deficiency results in disturbance of the gut microbiome,[80] which has an emerging role in sepsis pathogenesis as it increases the susceptibility to sepsis and enhances subsequent multiorgan failure.[81,82] VD and VDR both play a significant role in maintaining the normal balance of gut microbiota,[83] which in turn boosts immunity against enteric and systemic pathogens.[84] Currently, multiple sepsis interventional approaches aimed at restoration of a balanced gut microbiota are under investigation.[82] Thus, VD could be used as an adjuvant to enhance their therapeutic effects.

Effect of Vitamin D on Platelet Leukocyte Aggregation

Platelet leukocyte aggregates are liberally generated during sepsis and correlate significantly with diseases severity.[85] The engagement of white blood cells (WBCs) with activated platelets producing cellular aggregates (moving freely in blood or attached to endothelial cells) contributed substantially to provoking and exacerbating organ dysfunction in septic cases.[86] Thus, targeting this pathology at a specific time point in disease duration may limit the severity of sepsis outcomes such as vascular occlusion, decreased blood supply, and organ failure. PLA formation and their attachment to the endothelial lining of the vascular system may be triggered and propagated by a broad spectrum of activated mediators, such as adhesion molecules, proinflammatory cytokines, chemokines, complements, and procoagulant factors, as illustrated in Figure 1. This leads to sustained endothelial dysfunction, increased platelet and leukocyte reactivity, and activation of coagulation.[87]

As VDR is expressed genetically by most of the cells implicated in PLA formation, such as lymphocytes, neutrophils, macrophages, and vascular endothelial cells,[88] VD may affect the formation of these aggregates. Recently, it was shown that healthy individuals with deficient VD have a high level of circulating PLAs and leukocyte endothelial adhesion.[89] However, there is no
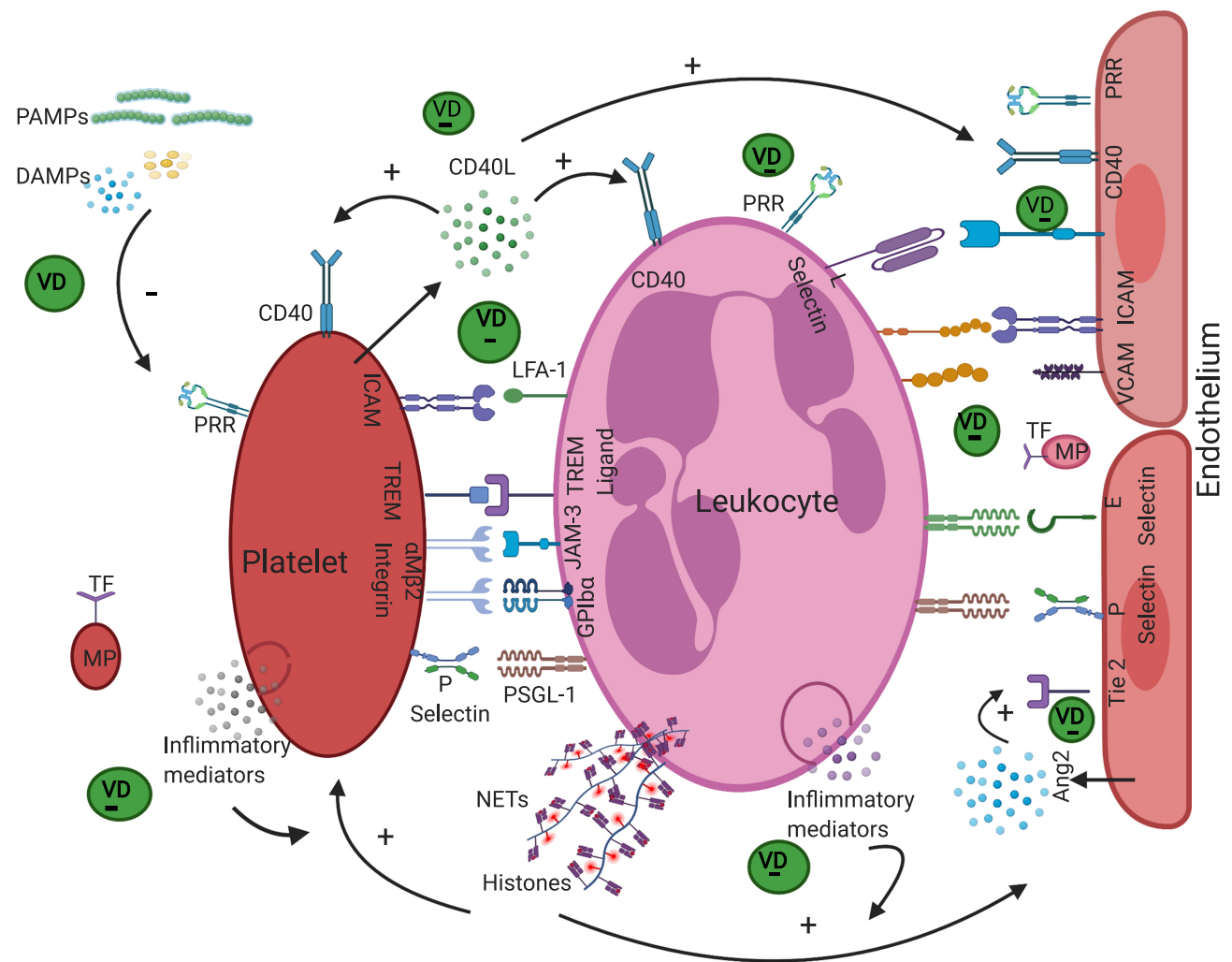
Figure 1 Vitamin D effects on the cellular interaction of platelets, leukocytes and endothelium. Stimulation of platelets, leukocytes and endothelium by PAMPs, DAMPs and the released mediators in response to such stimulation results in the attachment of platelets to leukocytes and generating platelet leukocyte aggregates moving freely in the circulatory system or fixed to the stimulated endothelium. Several molecular interactions, which could be interfered by the action of VD, mediate this pathological phenomenon including P-Selectin with PSGL-1 and αMβ2 integrin with GPIbα, (TREM-1) with its ligand on neutrophil, platelet JAM-3 with neutrophil αMβ2 integrin, and LFA-1 to its legend on platelet. These interactions induce further cell activation resulting in increased surface expression of adhesion receptors, degranulation, release of CD40L, Angiopoietin 2 (Ang2), inflammatory mediators, reactive oxygen species, tissue factor expression, microparticles release, thrombus and neutrophil extracellular trap formation (NET). Sites of Vitamin D (VD) effects are indicated in green. The figure was created using Biorender.com.

reported clinical data of the effect of VD administration on the extent of PLAs. It has also been found that VD reduces the expression of adhesion molecules required for platelet activation[90] and decreases homotypic platelet aggregation and platelet–platelet complexes.[91] Therefore, it becomes crucial to consider the impact of VD and VDR in the formation of PLAs.

The first immune defense after infection is initiated by recognizing pathogen associated molecular patterns (PAMPs) and the released damage associated molecular pattern from tissue (DAMPs) by recognition receptors (RRs) such as toll-like receptors (TLRs). RRs are expressed by leukocytes and to a certain degree by

endothelial cells and platelets, and result in the stimulation of intracellular signaling pathways and release of inflammatory cytokines such as IL-1, TNFα, IL-6, IL-8, and IL-12.[92–94] VD downregulates the gene expression of TLRs, thus reducing inflammatory responses.[77,95] Moreover, VD decreases many proinflammatory mediators such as IL-6, IL-8, and TNFα,[96] which themselves induce the stimulation of leukocytes, platelets, and endothelial cells and lead to the formation of PLAs.

The plasma level of VD is inversely correlated with mean platelet volume (MPV), a marker for platelet activity, noting that platelets with larger size are more active.[97,98] Activated platelets secrete CD40 ligand

(CD40-L) into the blood, which interacts with CD40 receptors present on platelets to enhance its activation,[99] and on leukocytes to augment its activation and generation of reactive oxygen species, ROS.[100] Furthermore, it may bind to CD40 receptors expressed on vascular endothelial cells amplifying its activation and its expression of several adhesion molecules, such as ICAM and VCAM, on their surface and its production of the chemokine CCL2, thus mediating leukocyte recruitment.

Recently, it has been found that the administration of VD reduces gene expression of the CD40 ligand by blood cells,[101] and serum levels of ICAM and VCAM that are secreted by stimulated endothelium.[102] Angiopoietin 2 (Ang-2) is produced immediately from endothelial Weibel–Palade bodies upon their activation. Ang-2 interacts with Tie 2 receptors in a competitive manner to inhibit the protective effect of Ang-1,[103,104] strengthen the activation of endothelium, and exaggerate the inflammation.[105] Additionally, it interacts synergistically with other inflammatory cytokines to boost their actions; for example, sensitizing the vascular endothelium to stimulation with TNFα.[106,107] It also induces the direct activation of polymorphonuclear cells towards the proinflammatory state.[108] VD administration causes a considerable reduction in the serum level of angiopoietin-2.[109] The interaction of triggering receptors expressed on myeloid cells (TREM-1) on platelets with their ligand on leukocytes is another mediator of PLAs. VD has been reported to inhibit the induced expression of TREM-1 in vitro.[110]

Tissue factor is expressed by activated endothelium, platelets, leukocytes,[111–113] and their released microparticles, bearing procoagulant and proinflammatory properties, upon activation.[114,115] It stimulates the extrinsic pathway of coagulation and generates thrombin and fibrinogen. These are important for the following reasons: Thrombin enhances adhesion molecule expression on the surface of the vascular endothelium such as E and P selection and production of von Willebrand factor (VWF) as well as several soluble secretory products, including platelet activating factor, Il-8, and angiopoietin 2.[116–120] Fibrinogen stabilizes platelet leukocyte endothelial cell interaction by binding to Mac-1 on leukocytic cells and GPIIb/IIIa (αIIbβ3) on platelets and it also binds to CD11b/CD18 on leukocytic cells and intracellular adhesion molecule −1 (ICAM-1) on endothelial cells.[121]

VD downregulates the expression of tissue factor in vitro. Treatment of an activated (inflamed) endothelial cell line with VD suppresses gene expression for tissue

factors and adhesion molecules.[122] Furthermore, treatment with VD in patients with chronic kidney disease who have endothelial dysfunction with high serum levels of circulating microparticles leads to a significant reduction in the level of microparticles;[123] it has also been found to inhibit microparticle release from a human endothelial cell line after their exposure to oxidative stress.[124] Moreover, supplementing high doses of VD decreases thrombin production in severely VD deficient patients.[125]

Potential Confounders of Vitamin D Supplementation in Sepsis

The current in vitro and observational data, discussed in this review, argue for the usefulness of VD supplementation in sepsis. Several RCTs, as summarized in Table 1, have been conducted to evaluate the effect of VD supplementation on the clinical outcome of critical illnesses including sepsis, but their results are contradictory, and the correlation has not been confirmed in all studies.

The results obtained to date warrant further in-depth studies to determine the underlying mechanisms or factors that interfere with yielding the expected protective influences of VD on the progression and outcomes from sepsis. Herein, this part of the review addresses several factors which may confound the effectiveness of VD supplementation.

First, variability in the applied intervention methods regarding the dose, form, route, and duration of VD supplementation as well as heterogeneity of the population and sample size seen across the published RCTs could largely contribute to the reported mixed results. Second, there is a lack of clarity and consistency regarding definitions of VD deficiency and sufficiency in the literature, which produce a significant variation in the criteria of VD deficiency among RCTs.[50,126,127] Thus, non-VD deficient cases could be involved in RCTs and have influenced the reported results.

Third, there is an inadequacy of prospective studies to determine the optimal VD dose for extra skeletal tissue functions.[128] Moreover, the exact plasma concentration of VD to replace an insufficiency is controversial in patients with sepsis and its measurement must be interpreted cautiously. VD is physiologically distributed as free (0.3%), or bound to either VD binding protein (DBP; 85%) or albumin (15%). Both DBP level and polymorphic variants affect the bioavailability of VD (free and bound to albumin) and this could affect the response to supplementation.

In some patients, DBP is low due to protein catabolism[129] or leakage to the extracellular matrix owing to increased vascular permeability.[130] In the laboratory, VD sufficiency is commonly determined by measuring the plasma level of total vitamin 25(OH)D.[131] Thus, the bioavailability of free VD (active form) is increased and it can diffuse into most body cells.[132,133] Additionally, the higher rate of polymorphism in the gene encoding VDBP (GC gene) results in DBP isoforms with different binding affinities to VD,[134,135] which affect the bioavailability of VD. Additional VD supplementation therefore requires attention because it may lead to higher risk of hypercalcemia.[63] Approximately 1% of mild hypercalcemia not requiring clinical intervention was reported among septic patients following VD administration.[54] At the molecular level, any increase in extracellular (serum) calcium ions affects the inflammatory response. Extracellular Ca^{2+} act as a danger signal (DAMPs), and amplify inflammation.[136] Moreover, septic patients may be classified as VD deficient based on their plasma level of total vitamin 25(OH)D, even if the bioactive free form of VD is within normal values.[130] Consequently, supplementation of VD may not produce the expected positive effects.

VD levels are important in the maintenance of circulating immunoglobulins and complement. A large study has documented, for low levels of VD, a positive association with IgG2 and C4 and a negative association for IgA (complement binding), IgG1, and C3.[137] These changes are of particular relevance in cases of systemic inflammatory reactions, which draw initially on intact mucosal barriers and a humoral component of the immune response. Depletion of complement components following overactivation and deposition in tissue (consumption in the blood phase) may be a determinant of overall outcome.[138,139] Sepsis associated multiple-organ dysfunction, in which complement activation products and complement dysregulation have an undisputed pathogenic role, may well be influenced by specifically targeting pathways or components thereof. However, the timing of such an intervention for it to be effective and efficient may be difficult to judge.[140] Septic patients suffer initially from an uncontrolled excessive inflammatory response followed by immune suppression.[141] Therefore, proper timing of VD supplementation could significantly influence its supplementation outcomes. Modes of action may include pleiotropic effects on lipid metabolism, which are altered in the context of sepsis.[142] VD deficiency is correlated with high levels of cholesterol, triglycerides, and low-density lipoproteins (LDL).[143] VD supplementation leads to a reduction in lipid parameters, total cholesterol, very-low-density lipoproteins (VLDL), LDL, and triglycerides,[144] which could be falsely interpreted as sepsis, because low lipid profiles are positively associated with poor sepsis outcome.[145,146] Thus, it appears that timing and dose of VD supplementation in sepsis influence its effectiveness and this could be a contributing factor to the controversy in the reported results of interventional studies. Detailed understanding of the molecular basis of VD metabolism and the regulation of its bioavailability during the course of sepsis is required to determine its effectiveness.

Conclusion

VD deficiency is prevalent in cases with sepsis;[26] thus, its deficiency may affect disease pathogenesis and aggravate the condition. VD has immunomodulatory effects. Restoration of VD plasma levels may limit disease progression through decreasing the abundance of DAMPs and PAMPs, inflammatory mediators, extent of PLAs formation and their adhesion to endothelia, and balancing the gut microbiota to fight systemic and enteric pathogens. However, data available from the literature regarding the influence of VD supplementation are conflicting in patients with sepsis and disagree with current evidence from in vitro and observational studies. Several factors may underestimate the expected positive effects. Further studies are warranted to elucidate the molecular interactions of VD with different players involved in the pathogenesis of sepsis (immune homeostasis). Thus, proper timing of VD supplementation, dose, form, and its sufficient plasma levels to be effective, as well as identifying patients who may benefit the most from supplementation with VD, need to be accurately determined in future clinical trials. Targeting the population with severe VD deficiency below 30 nmol/l is highly suggested for future trials.

Acknowledgments

Disclosure

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