

CLINICAL STUDY

Vitamin D deficiency – a potential risk factor for sepsis development, correlation with inflammatory markers, SOFA score and higher early mortality risk in sepsis

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ABSTRACT

OBJECTIVES: Sepsis is a life-threatening organ dysfunction generated due to the dysregulation of the immune response to infection. The aim of this study was to highlight the role of vitamin D in sepsis and non-infectious SIRS (systemic inflammatory response syndrome) and to find correlation of vitamin D levels with inflammatory markers, severity of the disease, and association with the 7th and 28th survival rate of patients.

METHODS: We investigated 32 patients (21 men, 11 women) admitted to an intensive care unit with both SIRS and sepsis. Blood was taken within 24 hours after admission. Plasma levels of 25(OH)D, sTREM-1, CRP, presepsin and procalcitonin were investigated.

RESULTS: Patients with sepsis had lower levels of 25(OH)D (n = 25) than SIRS patients (n = 7; p = 0.0032). Significantly lower levels of 25(OH)D were found also in patients, who did not survive the 7th (p = 0.0076) and 28th day (p = 0.0338) of hospital care compared to 7th, resp. 28th day survivors. We revealed a negative correlation between the levels of 25(OH)D and inflammatory markers CRP (p = 0.0003), presepsin (p = 0.0032) and sTREM-1 (p = 0.0065) in all SIRS/sepsis patients and clinical condition (SOFA score; p = 0.0385).

CONCLUSION: Our results showed that vitamin D deficiency predisposed to the development of sepsis, negatively correlated with CRP, presepsin, sTREM-1 and SOFA score and their levels associates with both 7th and 28th days survival of patients (*Tab. 5, Ref. 64*). Text in PDF www.elis.sk.

KEY WORDS: procalcitonin, presepsin, sepsis, SIRS, sTREM-1, vitamin D.

Introduction

Sepsis was known since the time of Hippocrates (460-377 BC) without exact information regarding its pathogenesis (1). According to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) in 2016, sepsis was defined as a life-threatening organ dysfunction caused by a dysregulated host's immune response to infection (2). This severe complex clinical syndrome is a major cause of admission to the intensive care unit (ICU) and death from infectious diseases (2, 3). It is estimated, that sepsis is the leading cause of mortality and critical

illness worldwide (4, 5). Even with optimal medical care, mortality rates in severe sepsis increase to around 50 % (6).

A wide range of pathogens could cause such potentially lethal responses leading to single or multiple vital organ failure. The non-infectious systemic inflammatory response syndrome (SIRS) can be triggered by diverse forms of injury including burns, ischemia, autoimmune diseases, surgery and traumatic injuries (7, 8). The prevalence of SIRS is very high, affecting one-third of all in-hospital patients and more than 50% of all ICU patients (6, 9).

The development of systemic inflammation starts with the activation of the mechanisms of natural immunity. Pattern recognition receptors (PRR) on the surface of monocytes/macrophages, vascular endothelial cells and other stromal cells recognize and bind pathogen associated molecular patterns (PAMPs) on the surface of microorganisms or their DNA or RNA during infection. These PRR recognize also danger/damage-associated molecular patterns (DAMPs) – “alarmins” of endogenous origin. These “signals of threatening” – e.g. high-mobility group box 1 (HMGB1) protein, histones, heat shock proteins (HSPs), fibrinogen, heparin - sulfate and other substances are released after trauma, burn, surgical intervention, cell and tissue injury, cell necrosis, etc. The binding of PAMPs and DAMPs on PRR results in the activation of inflammasomes and production of proinflammatory cytokines IL-

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1 β and IL-18 and development of inflammation (10–14). It leads to an auto-amplifying production of numerous pro-inflammatory molecules including TNF- α , IL-1 β , IL-2, IL-6, IL-8, HMGB1, TREM-1 (triggering receptor expressed on myelocytes) and IFN- γ in a so-called “cytokine storm” (15–17). To avoid the negative side effects of an exaggerated inflammatory reaction and hyper-activated Th1 immune response, almost simultaneously (after 24 hours) with the development of the inflammatory response, a counter-regulatory anti-inflammatory reaction develops (compensatory anti-inflammatory response syndrome – CARS) and Th2 immune response is activated (IL-4, IL-10) (8, 9). Loss of control over this balance may lead to organ dysfunction or failure due to SIRS, or immune suppression, even an immune paralysis associated with fatal polymicrobial sepsis may be the result of severe contra regulatory anti-inflammatory response syndrome (previous exaggerated CARS) development (18).

There are clinical scores, such as the Sequential (Sepsis-related) Organ Failure Assessment (SOFA), bedside quickSOFA (qSOFA) (2, 19) and the Acute Physiology And Chronic Health Evaluation II (APACHE II) (20), used for organ dysfunction representation and SIRS severity and patient mortality risk evaluation.

Vitamin D (VD) is a neuro-hormone regulating bone calcium-phosphate homeostasis, but plays also a major role in extra-skeletal metabolic processes, such as glucose metabolism, and in many aspects of cellular functions and immunomodulation (21–24). It has a direct effect on the function of both innate and adaptive immunity via VD receptor expressed on several immune cells (25–28).

Anti-inflammatory effect of VD in human T cells is partially mediated by inhibitory effect on NF κ B (29). VD also participates in the shifting of T helper (Th) cell response from Th1 (specific cell mediated immunity accompanied by inflammation) to Th2 (specific humoral immunity). Inhibiting the production of Th1 cytokine IFN- γ and increasing production of Th2 cytokines IL-4, IL-5 and IL-10, VD may limit the potential tissue damage associated with excessive Th1 cellular immune responses and hyperinflammation (ongoing also in SIRS or sepsis) (30, 31). Furthermore, VD can suppress IL-17 production by Th17 cells and has been proven to promote self-tolerance (22, 32, 33).

VD deficiency is associated with various disorders such as: diabetes, infections, myocardial infarction, autoimmune disease, chronic obstructive pulmonary disease, tuberculosis, and excess mortality in the general population (34–36).

25-hydroxyvitamin D (25(OH)D) is the major circulating form of VD that has a half-life of approximately 2-3 weeks. It is a summation of both VD intake and VD produced from sun exposure and it is just that VD metabolite used to determine whether a patient is VD deficient, sufficient or intoxicated (37, 38). There is no absolute consensus about the normal range for 25(OH)D, but most experts now agree that VD deficiency should be defined as a 25(OH)D level of < 20 ng/mL (50 nmol/L). VD insufficiency is classified as a serum 25(OH)D level between 20 and 29 ng/mL (50–74 nmol/L). The preferred level for 25(OH)D is now recommended to be > 30 ng/mL (75 nmol/L) (37–40).

The aim of our study was to find the association of VD plasma levels with inflammatory markers, clinical condition of patients

expressed by SOFA and APACHE II score and the 7th and 28th day mortality of SIRS/sepsis patients.

Subjects and methods

We investigated 32 adult patients (21 men, 11 women, mean age 59.313 \pm 13.104 years) admitted to the intensive care unit (ICU) of the 1st Department of Anesthesiology and Intensive Care Medicine, Faculty of Medicine, Comenius University and University Hospital Bratislava, Slovakia. In our study, adult patients with suspected or running systemic inflammatory response (sepsis, septic shock or non-infectious SIRS) and with supposed hospitalization length more than 24 hours were enrolled (Tab. 1). Exclusion criteria contained age below 18 years, diagnosis of primary immunodeficiency disease or immunosuppressive therapy in history. Patients in terminal stage with a presumed death within 24 hours from admission to ICU were also excluded.

All patients were examined by physicians and routine laboratory investigations, including complete and differential blood count and inflammatory markers, blood culture and site specific culture as indicated, were performed. Baseline clinical data collected were sex, age, body mass index (BMI), comorbidities, APACHE II score and SOFA score in the first 24 hours, duration of mechanical ventilation, length of the ICU stay and 7th and 28th day mortality. Patients were followed up until their discharge from the ICU or death.

For purpose of this study, 10 mL of blood was taken within 24 hours from admission to the ICU, 5 mL into a tubes with EDTA (for DNA and plasma isolation) and 5 mL into a tube without anti-coagulants (for isolation of serum). Samples were centrifuged and serum and plasma were stored at –80 °C until the end of the study, when all of the samples were analyzed for each marker as a single batch.

Various pro-inflammatory cytokines and inflammatory markers were investigated, including plasma level of sTREM-1 by Enzyme-linked Immunosorbent Assay (Human sTREM-1 Elisa kit; Cloud-Clone Corp., Houston, USA), all in strict accordance with manufacturer’s instructions. The plasma concentrations of presepsin (sCD14-ST) were investigated by a PATHFAST Presepsin chemiluminescent enzyme immunoassay (CLEIA; Mitsubishi

Tab. 1. Characteristics of investigated groups of patients.

	All patients	SIRS	Sepsis	Septic shock
Number	32	7	11	14
Mean age (years)	59.3 \pm 13.1	59.5 \pm 20.7	58.1 \pm 12.5	60.1 \pm 9.3
Sex (men/women)	21/11	3/4	9/2	9/5
VD severe deficiency	16	1	4	11
VD deficiency	13	4	6	3
VD insufficiency	2	1	1	0
VD sufficiency	1	1	0	0
7th day survival	27	7	10	10
28th day survival	23	5	8	10

SIRS – systemic inflammatory response syndrome; VD – 25(OH) vitamin D; VD severe deficiency – the plasma level of 25(OH)D < 12 ng/mL; VD deficiency – the plasma level of 25(OH)D < 20 ng/mL; VD insufficiency – the plasma level of 25(OH)D between 20–29 ng/mL; VD sufficiency – the plasma level of 25(OH)D \geq 30 ng/mL

Tab. 2. Comparison of plasma 25(OH)D levels between group of patients with sepsis and group of patients with non-infectious SIRS.

Group	n	Mean (µg/L)	SD	p
All patients	32	12.831	6.642	
Septic patients	25	11.084	4.965	0.0032 *
Sepsis	11	14.130	3.590	0.004 **
Septic shock	14	8.690	4.651	
SIRS	7	19.071	8.440	

n – number, Septic patients – patients with sepsis and septic shock; SD – standard deviation, SIRS – systemic inflammatory response syndrome, p – double edged t-test, *sepsis vs SIRS, **sepsis vs septic shock

Chemical Europe GmbH, Dusseldorf, Germany) on an automated immunoassay analyzer. The levels of procalcitonin (PCT) were determined by electrochemiluminescent enzyme immunoassay (ECLIA), and concentrations of C-reactive protein (CRP) were tested immunoturbidimetrically. The plasma levels of presepsin, PCT and CRP and routine biochemical and hematological examinations were performed at the specialized biochemical and immunological hospital laboratory Medirex Ltd., Bratislava, Slovakia. In addition, plasma levels of 25(OH)D were evaluated by electrochemiluminescent binding test (Elecsys Vitamin D total-cobas; Roche Diagnostics GmbH, Mannheim, Germany) in Laboratória Piešťany Ltd., Piešťany, Slovakia. All of the parameters and the results mentioned in this study were obtained from samples taken the next day after admission to ICU (within 24 hours – sample T1).

The study is a part of a larger study and was approved by the Ethical Committee for Research of the Faculty of Medicine, Comenius University in Bratislava and University Hospital Bratislava, Slovakia and an informed consent was obtained from each patient or his/her legally authorized representative.

Statistical analysis

Statistical analysis was performed by the statistical program InStat3.06, GraphPad Software, Inc., San Diego, USA. We compared the values by a two-tailed T-test or non-parametric parametric Mann–Whitney test if the data did not pass the normality test. We also used non-parametric Spearman test for correlation. A significant difference was assumed if p value was < 0.05.

Results

Decreased plasma levels of 25(OH)D in septic patients

Our results from Tab. 1 shows that all sepsis and septic shock patients suffered from 25(OH)D deficiency (plasma levels of

25(OH)D <20 ng/mL). Out of 7 SIRS patients, 1 had severe 25(OH)D deficiency, 4 had 25(OH)D deficiency and 1 had 25(OH)D insufficiency. Only 1 of SIRS and none of septic patients had normal level of 25(OH)D.

We found significantly decreased levels of 25(OH)D in all septic (sepsis and septic shock; n = 25; 11.084 ± 4.965 µg/L) compared to non-infectious SIRS patients (n = 7; 19.071 ± 8.440 µg/L; p = 0.0032) (Tab. 2). We further subdivided the group of septic patients into two subgroups: sepsis (n = 11) and septic shock patients (n = 14) and found that patients with septic shock had significantly decreased levels of vitamin D than patients with sepsis (p = 0.004) (Tab. 2).

Decreased plasma levels of 25(OH)D in patients, who did not survive the 7th or 28th day of hospital care

Significantly lower levels of 25(OH)D were found in the group of patients, who did not survive the 7th day of hospital care (n = 5; 5.200 µg/L; IQR: 7.755) compared to those, who survived the 7th day after admission to ICU (n = 27; 14.100 µg/L; IQR: 7.20; p = 0.0076) (Tab. 3). A significant difference was found also between the levels of vitamin D in patients, who did not survive the 28th day of hospital care (n = 8; 9.200 µg/L; IQR: 9.850) and those, who survived the 28th day of hospital care (n = 23; 13.600 µg/L; IQR: 7.200; p = 0.0338) (Tab. 3).

Plasma levels of inflammatory markers sTREM-1, and plasma levels of C-reactive protein, presepsin, and procalcitonin and values of clinical scores in SIRS, all septic patients, and patients with sepsis and septic shock

Our results showed, that septic patients (patients with sepsis and septic shock) had significantly higher levels of CRP (p < 0.0001), presepsin (p = 0.0017), sTREM-1 (p = 0.0190) and PCT (p = 0.0071) than non-infectious SIRS patients (Tab. 4). Comparing subdivided groups of septic patients, we found, that the patients suffering from septic shock had significantly higher levels of PCT than patients with sepsis. Other investigated parameters were also higher in septic shock patients than in the patients with sepsis, however the differences were not statistically significant.

Interesting differences were found between the groups of patients with SIRS and sepsis. Patients with sepsis had significantly higher levels of CRP (p = 0.0064), sCD14-ST (p = 0.0083) and sTREM-1 (p = 0.0379), than patients with SIRS.

Tab. 3. Comparison of 25(OH)D levels between group of 7th and 28th day survivors and non-survivors in all of patients with both SIRS and sepsis.

Parameter	7th day non-survivors	7th day survivors	28th day non-survivors	28th day survivors
n (sepsis/SIRS)	5 (5/0)	27 (20/7)	8 (7/1)	23 (18/5)
Min	3.000	3.700	3.000	3.700
Max	11.300	35.500	15.700	35.500
Median	5.200	14.100	9.200	13.600
IQR	7.750	7.200	9.850	7.200
Mann-Whitney test		p = 0.0076		p = 0.0338

n – number, IQR – interquartile range, SIRS – systemic inflammatory response syndrome

Tab. 4. Plasma levels of modern inflammatory markers in SIRS, all septic patients and subgroups of sepsis and septic shock patients and values of clinical condition scores.

Parameters	SIRS	Sepsis+Septic shock	Sepsis	Septic shock
Number	7	25	11	14
Mean age (years)	59.571±20.759	58.429±10,525	58.091±12.565	60.143±9.330
Sex (men/women)	3/4	18/7	9/2	9/5
CRP (mg/L) (mean±SD)	53.016 ±34.02	216.624±124.93	171.867±111,82	242.574±138.03
(T-test)		p<0.0001*	p=0.0064**	p=0.1813***
sCD14-ST (ng/L)	323	1355.000	827.000	2034.000
(median /IQR/)	/537/	/2456.5/	/1210/	/2752.5/
(Mann-Whitney)		p=0.0017*	p=0.0083**	p=0.0507***
sTREM-1 (ng/L)	72,839	131.475	163.420	120.543
(median /IQR/)	/36/	/151.8/	/142.0/	/203.8/
(Mann-Whitney)		p=0.0190*	p=0.0379**	p=0.8429***
PCT (ng/L)	5.07	26.021	20.580	95.910
(median /IQR/)	/7.64/	/87.0/	/52.8/	/80.5/
(Mann-Whitney)		p=0.0071*	p=0.2463**	p=0.0198***
SOFA (mean±SD)	11.429±2.573	13.063±2.154	13.182±1.940	13.786±1.762
APACHE II (mean±SD)	22.571±10.937	34.120±7.102	31.818±7.278	35.928±6.662

Statistic tests: T-test and Mann-Whitney test; SIRS – systemic inflammatory response syndrome; CRP-C – reactive protein; sCD14-ST – soluble CD14 subtype-presepsin; sTREM-1 – soluble triggering receptor expressed on myelocytes; PCT – procalcitonin; SOFA – sequential organ failure assessment score; APACHE II – acute physiology and chronic health evaluation II; p* – comparison between SIRS and all septic patients (sepsis+septic shock); p** – comparison between SIRS and sepsis patients; p*** – comparison between sepsis and septic shock patients

Tab. 5. Correlation between the levels of 25(OH)D and inflammatory markers/clinical scores in all of patients with both infectious and non-infectious systemic inflammatory response syndrome (sepsis+noninfectious SIRS).

25(OH)D	CRP	sCD14-ST	sTREM-1	PCT	SOFA	APACHE II
n	32	32	27	32	32	32
SR	-0.6012	-0.5045	-0.5110	-0.3190	-0.3676	-0.2813
95% CI	-0.7894	-0.7307	-0.7514	-0.6077	-0.6413	-0.5810
	-0.3096	-0.1786	-0.1509	0.04425	-0.01084	0.08551
p	0.0003	0.0032	0.0065	0.0752	0.0385	=0.1188

n – number; SR – Spearman r; CI – confidence interval; sTREM-1 – soluble triggering receptor expressed on myeloid cells; sCD14-ST – presepsin; PCT – procalcitonin; SOFA – sequential organ failure assessment score; APACHE II – acute physiology and chronic health evaluation II

Correlation between the levels of 25(OH)D and levels of inflammatory markers sTREM-1, C-reactive protein, presepsin, procalcitonin and values of clinical condition scores

We revealed a very significant negative correlations between the levels of 25(OH)D and inflammatory markers CRP (p = 0.0003), sCD14-ST (p = 0.0032), sTREM-1 (p = 0.0065) and a modest non-significant negative correlation with levels of PCT (p = 0.0752) in all of the patients with systemic inflammatory response of both infectious and non-infectious origin (Tab. 5).

There was also a significant correlation between the 25(OH)D levels and clinical condition of patients represented by SOFA score (p = 0.0385) in all of the patients, but not with APACHE II score (p = 0.1188) (Tab. 5).

Discussion

VD deficiency is common among the general population. It is also observed in up to 76 % of critically ill patients (41). Despite the high prevalence of hypovitaminosis D in critical illness, VD is often overlooked by medical staff as the clinical implications and consequences of VD deficiency in acute contexts remain to be fully understood (42).

Both SIRS and sepsis in their beginnings are predominantly associated with an exaggerated inflammatory response and elevated inflammatory markers. That is why the mortality of sepsis in early phase of this disease is associated with hyperinflammation and failure of vital organs due to cytokine storm. During later phase of sepsis, the contra-regulatory immune response, that downregulate the inflammation, can lead to immune suppression, even immune paralysis, what is very dangerous for patients that can die due to polymicrobial sepsis (18, 43, 44).

To reduce the mortality, the early diagnosis of sepsis (SIRS with proven infectious agent) is essential, and the knowledge about immune status of patients is also very important. Early diagnosis of bacteremia is extremely important for the implementation of antimicrobial therapy. Although blood culture is the “gold standard” for diagnosis of bacteremia, this method has limited usefulness for the early detection of blood-stream infection. Some inflammatory biomarkers – CRP, sTREM-1, HMGB1 (high mobility group box 1) and presepsin are studied as predictors of bacteremia in SIRS/sepsis patients. Also, for SIRS/sepsis monitoring, the state of immunity (Th1 vs Th2) and the levels of inflammatory and anti-inflammatory molecules and cytokines that might be influenced by many factors, are useful to test.

In this part of our study, we decided to analyze the plasma levels of 25(OH)D in SIRS, sepsis and septic shock patients, and find an association with 7th and 28th days mortality. Moreover, we were interested also in correlation of the plasma levels of vitamin D with levels of CRP and modern inflammatory markers - presepsin, sTREM-1 and PCT, their difference between SIRS and sepsis and finally the correlation between the levels of 25(OH)D with tested inflammatory markers.

Presepsin, the soluble CD14 subtype, is a cleavage product of the 55 kDa membrane CD14 (mCD14), that colocalizes with toll like receptor 4 (TLR4) and upon binding of the lipopolysaccharide – lipopolysaccharide binding protein complex (LPBP) to this receptor, CD14 activates the TLR4/MD2 – specific pro-inflammatory signaling cascade, thereby starting the inflammatory reaction of the host against infectious agents (45). The complex of LPBP-CD14 is released into circulation by shedding of CD14 from the cell membrane yielding soluble CD14 (sCD14). Another soluble sCD14-ST – presepsin, a 13kDa fragment derived from cleavage of mCD14 can be released during phagocytosis and cleavage with plasma proteases, lysosomal enzymes and cathepsin D (46). Presepsin is used as an aid in the diagnosis and prognosis of sepsis, in the assessment of the degree of septic severity and to aid in the risk stratification of critically ill septic patients. It may contribute to rule out the diagnosis of bacteremia in SIRS patients admitted to the Emergency Department (47) and seems to provide better early diagnostic value than procalcitonin in early diagnosis of neonatal sepsis (48).

TREM-1 belongs to one of the newer PRRs, however its ligand is still unknown. It was first described in 2000 on the surface of myeloid cells (49). It is expressed on the surface of neutrophils, mature monocytes, macrophages and non-myeloid cells, such as epithelial and endothelial cells (50, 51), has pro-inflammatory activity (49, 52) and the presence of extracellular bacteria, fungi and their products increase its expression. The extracellular domain of TREM-1 can be cleaved and released in the body fluids as a soluble TREM-1 (sTREM-1) (53), moreover, it can be also produced by cells (mainly activated monocytes and macrophages) and functions as a decoy receptor present in different body fluids. The levels of sTREM-1 can be measured by ELISA and can serve as a diagnostic inflammatory marker (54-57). Both TREM-1 and sTREM-1 play a great role in the inflammatory response regulation. Increased TREM-1 expression and increased levels of sTREM-1 accompany both infectious and non-infectious inflammatory processes (54, 56), but more intensively infectious processes.

The mean level of 25(OH)D (12.831 ± 6.642 µg/L) in the examined cohort of 32 adult patients admitted to the ICU because of systemic inflammatory response syndrome of both infectious and non-infectious origin is deeply below the normal range of 25(OH)D level (> 30 µg/L) (37, 39, 40). It is even approaching the boundary of severe vitamin D deficiency (25(OH)D level < 12 µg/L) (58, 59).

The finding of significantly decreased levels of 25(OH)D in the group of septic patients (11.084 ± 4.965 µg/L) compared to group of non-infectious SIRS patients ($p = 0.0032$) corresponds with the results of mentioned studies supporting the role of VD in

both the innate and adaptive immune responses to viral and bacterial infections (25, 26) and the association between low vitamin D levels and risk of sepsis (60, 61).

The significantly lower levels of 25(OH)D ranking in severe VD deficiency range in the group of patients, who did not survive the 7th day of hospital care compared to those, who survived the 7th day after admission to ICU ($p = 0.0076$), point out the potential impact of VD level on early mortality risk in patients with systemic inflammatory response syndrome of both SIRS and sepsis. This finding consents with the results of other authors who declare, that VD deficiency at the time of critical care initiation is a significant predictor of overall cause patient mortality in a critically ill patient population, independently of other comorbidities (59, 62). This also correspond with our significant correlation with SOFA score, but non-significant correlation with APACHE II - a scoring system of clinical condition of patients, that takes into account also other comorbidities, not only acute state.

Our results showed, that septic patients had significantly higher levels of CRP ($p < 0.0001$), presepsin ($p = 0.0017$), sTREM-1 ($p = 0.0190$) and PCT ($p = 0.0071$), than non-infectious SIRS patients (Tab. 4). Comparing subdivided group of septic patients, we found, that patients suffering from septic shock had significantly higher levels of PCT than patients with sepsis. Interesting differences were found between the groups of patients with SIRS and sepsis. Patients with sepsis had significantly higher levels of CRP ($p = 0.0064$), sCD14-ST ($p = 0.0083$) and sTREM-1 ($p = 0.0379$), than patients with SIRS, so these markers might serve as diagnostic markers between SIRS and sepsis, that we try to prove in a larger cohort of patients.

Besides the reduced immunological defense against infection in VD deficient septic patients, insufficient regulatory and anti-inflammatory mechanisms of the immune system due to VD deficiency in both patient groups (sepsis and SIRS) can cause more intense and stormy early pro-inflammatory Th1 response of systemic inflammatory response syndrome with a higher lethality. This hypothesis is also supported by our findings of significant negative correlation between the levels of 25(OH)D and inflammatory markers like CRP ($p = 0.0003$), sCD14-ST ($p = 0.0032$) and modest non-significant negative correlation with PCT levels ($p = 0.0752$) in all SIRS/sepsis patients. Very significant negative correlation was also detected between the levels of 25(OH)D and sTREM-1 ($p = 0.0065$) in all of the patients.

VD status is associated with adverse outcomes in the critically ill. Despite, there are only a few clinical studies that evaluated VD supplementation in critically ill patients. Amrein et al (58) conducted the largest randomized controlled trial to date investigating the influence of a high-dose bolus enteral vitamin D3 supplementation on outcomes of 475 critically ill medical and surgical adult patients with VD deficiency (≤ 20 ng/mL). The authors concluded that a high-dose vitamin D3 did not reduce hospital length of stay, hospital mortality, or 6-month mortality. However, they observed a lower hospital mortality in patients with severe VD deficiency (≤ 12 ng/mL) at baseline. A systematic review and meta-analysis of 7 randomized controlled trials (716 patients) concluded that VD administration was associated with a decreased mortality in

critically ill patients without serious adverse events (63). Clinical studies demonstrate that 1,25-dihydroxyvitamin D (1,25(OH)2D3) protects patients from adverse outcome of sepsis, but clinical treatment with 1,25(OH)2D3 is rare.

In 2018 Rao et al performed a clinical study on animal model and found that 1,25(OH)2D3 treatment had beneficial effects and improved the survival rate in LPS-induced mouse model of sepsis by blocking the secretion of HMGB1, the key late regulator of sepsis. LPS-induced HMGB1 secretion was attenuated by 1,25(OH)2D3 via blocking HMGB1 translocation from the nucleus to the cytoplasm in macrophages. 1,25(OH)2D3 can induce the expression of hemeoxygenase-1 (HO-1), which is essential for blocking HMGB1 nuclear translocation and its secretion. 1,25(OH)2D3 is recognized as the key mediator of inflammatory diseases, including sepsis (64). The authors provide evidence that 1,25(OH)2D3 attenuates LPS-induced HMGB1 secretion via the Nrf2 (nuclear factor erythroid 2-related factor 2)/HO-1 pathway in macrophages.

Conclusion

Our results showed that except one all tested ICU (SIRS/sepsis) patients had decreased or insufficient levels of plasma VD with the lowest levels in septic shock patients. The lower levels of 25(OH)D in the group of 7th and 28th day non-survivors point out the potential impact of VD level on early mortality risk and support the assumption that VD deficiency at the time of critical care initiation is a significant predictor of mortality. The levels of inflammatory markers CRP, presepsin, sTREM-1 negatively correlated with the plasma levels of VD, what indicates, that VD deficiency can cause more intense and stormy early pro-inflammatory Th1 response with a higher lethality. This hypothesis is also supported by our findings of a negative correlation between the levels of 25(OH)D and SOFA score.

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