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ORIGINAL ARTICLE

# The Significance of Systemic Immune-Inflammatory Index and Platelet-Neutrophil Ratio on Early Mortality in Septic Shock Patients and their Association with Vitamin D and Parathyroid Hormone Ratio

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ABSTRACT

Objectives: Sepsis is a life-threatening organ dysfunction characterized by complex pro-inflammatory and anti-inflammatory processes. Vitamin D deficiency is frequently observed in sepsis and associated with worse outcomes. We aimed to evaluate the effect of vitamin D-to-PTH ratio, systemic immune-inflammatory index (SII), and platelet-to-neutrophil ratio (PNR) on mortality in septic shock patients with vitamin D deficiency and insufficiency.

Material and methods: In this cross-sectional study, vitamin D insufficiency was defined as vitamin D levels between 12-20 ng/ml and vitamin D deficiency as < 12 ng/ml. The SII is calculated by multiplying the neutrophil count with the platelet count and dividing the result by the lymphocyte count (N*P/L), and the PNR is calculated by dividing the platelet count by the neutrophil count (P/N). We used receiver operating curve (ROC) analysis, logistic regression analysis, and Kaplan-Meier survival analysis to determine the association between SII, PNR, vitamin D deficiency, vitamin D-to-PTH ratio and early mortality within 7-days.

Results: This study consisted of 39 patients with septic shock. While 11(28%) of patients had vitamin D insufficiency, 28(72%) had vitamin D deficiency. Vitamin D insufficiency was associated with higher levels of SII and PNR than vitamin D deficiency. ROC analysis showed that 0.077 and 67 are cut-off values with the highest sensitivity and specificity for the vitamin D-to-PTH ratio (AUC: 0.77, p=0.01) and SII (AUC: 0.78, p=0.008) to predict early mortality. Both cut-off values were significantly associated with mortality in logistic regression analysis. SII higher than 67 and vitamin D-to-PTH ratio higher than 0.077 were associated with survival in 7-days (91% vs. 60%, p=0.004, and 91% vs. 63%, p=0.006, respectively).

Conclusion: SII was significantly suppressed in patients with vitamin D deficiency which was associated with increased mortality in septic shock. In addition, the decreased level of vitamin D-to-PTH ratio, which could be an indicator of immune balance, may be associated with early mortality in the intensive care unit.

Keywords: sepsis, vitamin D, parathyroid hormone, mortality, systemic immune-inflammatory index, platelet-to-neutrophil ratio.

## INTRODUCTION

Sepsis results from a complex interaction between the immune response and infecting organism resulting in life-threatening organ dysfunction, and it affects ranging from 19 to 48.9 million cases annually worldwide [1-5]. Potentially, any infected patient could develop sepsis, and the incidence of sepsis is as high as 1-2% of all hospitalized patients [3]. Inflammatory imbalance and immune dysregulation represent the fundamentals of sepsis pathogenesis and occur from both exogenous factors derived from the pathogen and endogenous factors released by injured cells [2,3]. Recent studies have shown that both pro-inflammatory and anti-inflammatory responses occur early and simultaneously in sepsis [3,6].

Vitamin D and parathyroid hormone (PTH) were the primary determinants of calcium metabolism. In the last decade, it was shown that both hormones affect inflammation [7-10]. Vitamin D modulates immune and inflammatory cells' activation, proliferation, and differentiation via the vitamin D receptor [9]. Vitamin D balances pro-inflammatory and anti-inflammatory states [9,10]. In this context, vitamin D may have an important place in a situation that contains cytokine storm such as sepsis. Vitamin D deficiency has been linked to an increase in the risk of development and the progression of infections. Additionally, a higher risk of developing sepsis and intensive care unit (ICU) mortality has been reported in patients with vitamin D deficiency [11]. Also, PTH could contribute to the inflammatory state by stimulating the release of interleukin-6 [8].

It is well known that vitamin D deficiency is frequently observed during course of sepsis and it is associated with worse outcome. However, there are not sufficient data in the literature examining the effect of PTH levels in sepsis. The relationship between low vitamin D levels and poor outcomes in sepsis is well known, and it can be speculated that PTH, the counter hormone of vitamin D, and the ratio of these two hormones may have an effect on the course of sepsis.

Estimation of inflammation based on complete blood count has become a useful technique to predict outcomes of various diseases in recent years. Both systemic immune-inflammatory index (SII) and platelet-to-neutrophil ratio (PNR) are parameters derived from complete blood count that high levels are associated with inflammation and unfavorable prognosis in a variety of diseases such as malignancy, rheumatologic diseases, and cardiovascular diseases [12-15]. However, the predictive value of these markers for sepsis mortality is not well studied. On the other hand, the presence of simple, cheap, and accessible biomarkers is important for early detection and timely intervention of sepsis. Significant changes in neutrophil, lymphocyte, and platelet counts as a reflection of the immune imbalance in the pathogenesis of sepsis could provide considerable information about the prognosis of the disease

This study aimed to evaluate the effect of vitamin D insufficiency and deficiency and the vitamin D-to-PTH ratio on early mortality in patients with septic shock. Also, we determined the association between vitamin D and the changes in inflammatory biomarkers, including C-reactive protein (CRP), SII, and PNR, during septic shock.

## MATERIALS AND METHODS

### Ethical Approval

The ethics committee approved the design and procedures of the study of the University (Approval date: 23/07/2014, Project No: GO 14/400) in agreement with the principles of the Helsinki Declaration and ethical standards for human experimentation. Written informed consent was obtained from all participants or their first-degree relatives.

### Patient Population

This cross-sectional study was conducted in the intensive care unit at a tertiary center hospital, Ankara, Turkey, from September 2014 to January 2016. The inclusion criteria of the study were age between 18-80 years old, vitamin D level below 20 ng/ml, and diagnosis of septic shock. Septic shock was determined as sepsis-induced hypotension persisting despite fluid resuscitation [11,16]. Patients were excluded if they had a disease affecting calcium, PTH, and vitamin D metabolism (malignancy, chronic kidney disease, parathyroid disorders, pancreatitis, tumor lysis syndrome, rhabdomyolysis, renal tubular disorders, and

 

pregnancy) and unwilling to give informed written consent. A total of 39 patients with septic shock who had vitamin D insufficiency (12-20 ng/ml) or deficiency (<12 ng/ml) were evaluated [17].

### Laboratory Parameters

Routine laboratory measurements and blood samples for biomarkers (hemoglobin, leukocyte, neutrophil, lymphocyte, platelet, albumin, calcium, C-rp) were obtained within the first hour of septic shock. Also, consecutive measurements were performed at the 12th, 24th, 48th, 72nd hours, and day 5 for biomarkers. The arterial blood gas analysis obtained the ionized calcium measurement (reference range 1.15-1.3 mmol/L).

Concurrent Venous blood samples were collected directly into an EDTA-containing tube (vitamin D) on day one of septic shock to measure vitamin D and PTH levels. The samples for vitamin D were immediately stored on ice, and all blood samples were centrifuged (5000 rpm for 10 minutes) and stored at -80 °C until assay. While vitamin D was estimated by liquid chromatograph-mass spectrometer (LC-MS) technique using Shımadzu LCMS-8040 (JAPAN), serum PTH level was measured by Immuno Radio Metric Assay (IRMA) technique using Beckman Coulter (USA).

### Clinical outcomes

Hospital medical records were used for baseline information such as gender, age, comorbidities, need for mechanic ventilation and renal replacement therapy, calculation of Acute Physiology and Chronic Health Evaluation (APACHE) II score, Sequential Organ Failure Assessment (SOFA) score, and Charlson comorbidity index. The 7-days mortality rates and period of hospitalization in the ICU were determined for all patients. Since SII and PNR are dynamic biomarkers, we thought that these biomarkers could provide more accurate information on early mortality since they may be affected by ventilator-associated pneumonia and other secondary infections that may develop during the ICU stay. Therefore, we used the 7-day mortality that used in different patient populations in the intensive care unit in the literature [18,19].

We used SII and PNR as inflammatory biomarkers, and consecutive measurements were performed on the 12th, 24th, 48th,72nd hours, and day 5. The SII is calculated by multiplying the neutrophil count with the platelet count and dividing the result by the lymphocyte count (N*P/L), and the PNR is calculated by dividing the platelet count by the neutrophil count (P/N). We used the patients' vitamin D and PTH levels and calculated the vitamin D-to-PTH levels by dividing serum vitamin D levels by the serum PTH levels (vitamin D/ PTH). We evaluated the relation between inflammatory biomarkers (C-rp, SII, PNR, vitamin D, and vitamin D-to-PTH ratio) with the early (7 days) mortality in septic shock patients'

### Statistical Analysis

Statistical analysis was performed using the IBM© SPSS© Statistics Version 20.0 for Windows (Armonk, NY: IBM Corporation, released 2019). Kolmogorov-Smirnov test was used to determine the data distribution. The homogeneity of variables was determined using the one-way ANOVA homogeneity of variance test. According to data distribution, continuous variables are reported as mean ± standard deviation or median (inter-quartile range). The distribution of inflammatory markers according to vitamin D level was shown in the graphs with mean ± mean of standard error. Mann-Whitney U test was used to compare continuous variables for independent groups. In case of evaluating dependent groups, Wilcoxon test was used for two related samples and Friedman test was used for more than two related samples. Categorical variables were reported by percentages. A Chi-square test was used to compare categorical variables. Receiver operating curve (ROC) analyses were plotted to illustrate the SII, PNR, and vitamin D/PTH ratio cut-off value for estimation of seven-days intensive care unit mortality. Logistic regression analysis was used to determine the association between inflammatory biomarkers and mortality with odds ratio (OR) and 95% confidence interval (95% CI). Survival analysis was performed using Kaplan-Meier curves and log-rank analysis. P values ≤0.05 were considered to indicate statistical significance.

## RESULTS

Thirty-nine patients with a mean age of 61.7±19.2 years old were enrolled in this study, and 51% were female. While 11 (28%) of patients had vitamin D insufficiency, 28 (72%) had vitamin D deficiency.

Demographic characteristics of the study population are shown in Table 1. The most common cause of septic shock was respiratory system infection, causing 69% (n=27) of cases. While 32 (82%) patients needed mechanical ventilation, 16 (41%) patients needed renal replacement therapy. The median ICU stay of length was 16 (3-21) days. The total mortality rate of the study population was 26% (10/39) on day seven. Although patients with vitamin D deficiency had higher mortality rate on day seven compared to the patients with vitamin D insufficiency, the difference was not significant [9 (32%) vs. 1 (9%), p=0.1]. Non-survivors had significantly higher APACHE II and SOFA scores compared to survivors [35 (28-40) vs. 27 (22-31), p=0.005, and 13 (11-16) vs. 10 (6-13), p=0.008, respectively). On the other hand, survivors had significantly higher levels of SII and vitamin D/PTH levels than non-survivors [154.1 (63.2-370.5) 103/mm3 vs. 35 (9.8-64.7) 103/mm3, p=0.008, and 0.14 (0.06-0.28) vs. 0.05 (0.01-0.07), p=0.01, respectively]. (Table 2)

Table 3 demonstrates the laboratory parameters of the patients at the time of ICU admission. Patients with vitamin D insufficiency had similar laboratory parameters to those with vitamin D deficiency at ICU admission. On the other hand, patients with vitamin D insufficiency had a significantly higher level of vitamin D-to-PTH ratio than those with vitamin D deficiency [0.46 (0.16-0.87) vs. 0.08 (0.03-0.14), p=0.002].

Although patients had similar laboratory parameters at the time of ICU admission, patients with vitamin D insufficiency had higher levels of SII and PNR than those with vitamin D deficiency (Figure 1). While SII significantly decreased (p=0.01) and PNR significantly increased (p=0.04) within the five days of ICU admission in patients with vitamin D insufficiency, we did not observe any significant change in SII (p=0.4) and PNR (p=0.9) in patients with vitamin D deficiency. Although patients with vitamin D insufficiency had higher PNR and SII, baseline C-rp levels of patients with vitamin D insufficiency and deficiency were similar (14.63±4.3 vs. 16.47±2.08, p=0.4). The difference between groups tended to be significant at day 5 in terms of C-rp levels (5.7±1.46 vs. 13.6±3.16, p=0.06). Within five days, the decrease in C-rp in patients with vitamin D insufficiency and deficiency was not statistically significant (p=0.07 and p=0.1, respectively). However, eight patients were not included in the analysis as they died during this time.

Receiving operating curve showed that 0.077, 67 and 0.05 are sensitive and specific cut off value for the vitamin D-to-PTH ratio (AUC: 0.77(95% CI:0.61-0.92), sensitivity: 72%, specificity: 80%, and p=0.01),

**Table 1.** Demographic characteristics of study population

| | Total patients n:39 | Vitamin D insufficiency n:11 (28%) | Vitamin D deficiency n:28 (72%) | P value |
|---|---|---|---|---|
| **Gender**, n (%) | | | | |
| Female | 20(51%) | 7(64%) | 13(46%) | |
| **Age**, (mean±SD) | 61.7±19.2 | 58.3±21.9 | 63.1±18.3 | 0.3 |
| **APACHE II score**, median (IQR) | 29(24-34) | 27(18-34) | 29(24-35) | 0.6 |
| **SOFA score**, median (IQR) | 11(8-13) | 11(5-14) | 11(8-13) | 0.3 |
| **Charlson co-morbidity score**, median (IQR) | 5(3-7) | 4(1-7) | 5.5(3.5-6.5) | 0.9 |
| **Cause of sepsis**, n (%) | | | | 0.5 |
| Respiratory system | 27(69%) | 9(82%) | 18(64%) | |
| Urinary tract | 2(5%) | 0 | 2(7%) | |
| Soft tissue | 2(5%) | 0 | 2(7%) | |
| Other | 8(21%) | 2(18%) | 6(22%) | |
| **Mechanical Ventilation**, n (%) | 32(82%) | 10(91%) | 22(79%) | 0.4 |
| **Need for RRT**, n (%) | 16(41%) | 5(46%) | 11(39%) | 0.5 |
| **Length of ICU stay**, days median (IQR) | 13(3-21) | 15(7-21) | 10(3-21.5) | 0.3 |
| **Mortality**, n (%) | | | | |
| 7 days | 10(26%) | 1(9%) | 9(32%) | 0.1 |

IQR: Inter quartile range, APACHE II: Acute Physiology and Chronic Health Evaluation II, SOFA:Sequential Organ Failure Assessment score, RRT: Renal replacement therapy, ICU: Intensive care unit

 

SII (AUC:0.78 (95% CI:0.62-0.96), sensitivity:73%, specificity:80%, and p=0.008), and PNR (AUC: 0.63 (95% CI:0.75--1.16), sensitivity: 62%, specificity: 70%, and p=0.2) to predict early mortality. We showed that Vitamin D-to-PTH ratio below 0.077 was a biomarker for early mortality in all logistic regression models. We also found that the SOFA score, vitamin D-to-PTH ratio below 0.077 and SII

**Table 2.** Demographic and laboratory parameters of survivor and non-survivor patients

| | Survivor n=29 (74%) | Non-survivor n=10 (26%) | P value |
|---|---|---|---|
| **Gender**, n (%) | | | |
| Female | 15(52%) | 5(50%) | 0.9 |
| **Age**, (mean±SD) | 62.1±18.8 | 60.7±21.2 | 0.8 |
| **APACHE II score**, median (IQR) | 27(22-31) | 35(28-40) | 0.005 |
| **SOFA score**, median (IQR) | 10(6-13) | 13(11-16) | 0.008 |
| **Charlson co-morbidity score**, median (IQR) | 6(4-8) | 4.5(3-6) | |
| **Cause of sepsis**, n (%) | | | 0.2 |
| Respiratory system | 18(62%) | 9(90%) | |
| Urinary tract | 2(7%) | 0 | |
| Soft tissue | 2(7%) | 0 | |
| Other | 7(24%) | 1(10%) | |
| **Mechanical Ventilation**, n (%) | 22(76%) | 10(100%) | 0.2 |
| **Need for RRT**, n (%) | 12(41%) | 4(40%) | 0.9 |
| **SII (baseline)** ($10^3$/$mm^3$) | 154.1(63.2-370.5) | 35(9.8-64.7) | 0.008 |
| **PNR (baseline)** | 0.77(0.37-1.36) | 0.34(0.42-0.82) | 0.1 |
| **C-reactive protein (mg/dl)** | 12.3(6.5-18) | 23(2.2-9) | 0.3 |
| **Procalcitonin (ng/ml)** | 2.85(1.3-9.1) | 3.8(1.99-27.9) | 0.4 |
| **Vitamin D (ng/ml)** | 9.4±5.6 | 6.47±4.6 | 0.1 |
| **PTH, (ng/L)** | 49.8(27.4-95.8) | 194.4(42-361) | 0.08 |
| **Vitamin D/PTH ratio** | 0.14(0.06-0.28) | 0.05(0.01-0.07) | 0.01 |

APACHE II: Acute Physiology and Chronic Health Evaluation II, SOFA:Sequential Organ Failure Assessment score, RRT: Renal replacement therapy, SII: Systemic immune-inflammatory index, PNR: platelet-to-neutrophil ratio PTH: Parathyroid hormone

**Table 3.** Laboratory parameters of the patients

| | Total patients n:39 | Vitamin D insufficiency n:11 (28%) | Vitamin D deficiency n:28 (72%) | P value |
|---|---|---|---|---|
| **ICU admission** | | | | |
| Hemoglobin (g/dl) | 10.09±2.1 | 10.06±1.62 | 10.1±2.31 | 0.9 |
| Lymhocyte (x$10^3$ $mm^3$) | 1.2(0.5-2.6) | 1.2(0.5-2.5) | 1.15(0.5-2.7) | 0.9 |
| Neutrophil (x$10^3$ $mm^3$) | 11(6.1-17.3) | 17.3(6.8-19.5) | 9.75(5.9-13.05) | 0.06 |
| Platelet ( x$10^4$ $mm^3$) | 15.5(7.8-25.4) | 16.7(12.8-28.1) | 14.5(6.4-22.5) | 0.1 |
| Creatinine (mg/dl) | 1.76(1.11-2.7) | 2.1(0.8-2.4) | 1.76(1.2-3.09) | 0.9 |
| Albumin (g/dl) | 2.58±0.6 | 2.77±0.69 | 2.51±0.53 | 0.2 |
| iCalcium (mmol/l) | 1.06±0.09 | 1.09±0.07 | 1.05±0.1 | 0.2 |
| **SII (baseline)** ($10^3$/$mm^3$) | 253.4(126-380) | 426.2(268-583) | 185.6(126-244) | 0.08 |
| PNR (baseline) | 1.32(0.8-2.9) | 2.13(1.06-3.18) | 1.02(0.7-1.34) | 0.07 |
| C-reactive protein (mg/dl) | 12.4(5.9-24) | 7.3(2.58-29) | 14.9(9.3-21.7) | 0.3 |
| Procalcitonin (ng/ml) | 3.04(1.7-10) | 2.8(0.3-37.7) | 3.2(1.9-9.5) | 0.5 |
| Vitamin D (ng/ml) | 8.64±5.5 | 16.4±3.17 | 5.6±2.12 | <0.001 |
| PTH, (ng/L) | 51(31.2-128) | 47(20-123) | 65.3(43.4-133.7) | 0.2 |
| Vitamin D/PTH ratio | 0.11(0.04-0.2) | 0.46(0.16-0.87) | 0.08(0.03-0.14) | 0.002 |

ICU: Intensive care unit, SII: Systemic immune-inflammatory index, PNR: platelet-to-neutrophil ratio PTH: Parathyroid hormone

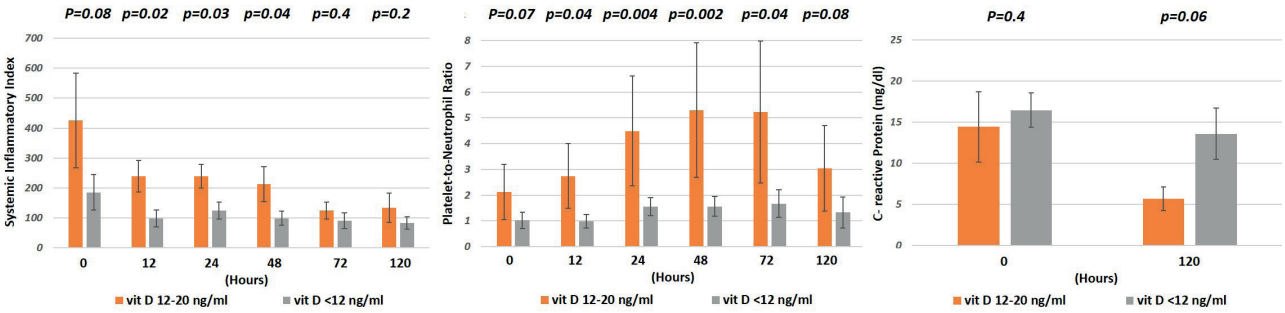


**Figure 1.** Change of inflammatory markers over time according to Vitamin D levels of patients in intensive care follow-up.

<67 in the model 3 are independent risk factors for early mortality (OR:1.613 (95%CI:1.032-2.624), p=0.05, OR:12.012 (95% CI:1.591-46.008), p=0.03, and OR:6.408 (95%CI:1.264-16.241), p=0.04, respectively) (Table 4).

Kaplan Meier survival analysis showed that patients with SII higher than 67 and vitamin D-to-PTH ratio higher than 0.077 had significantly better seven days ICU survival (91% vs. 60%, p=0.004, and 91% vs. 63%, p=0.006, respectively) (Figure 2B and 2D). On the other hand, patients who had PNR higher than 0.05 and vitamin D higher than 12 ng/ml had better seven days ICU survival, but the differences were not significant (85% vs. 67%, p=0.07, and 91% vs. 75%, p=0.2, respectively) (Figure 2A and 2C).

## DISCUSSION

Our findings support that suppression in PNR and SII may be associated with early mortality in septic shock patients in contrast to an increase in well-known inflammatory biomarkers. Also, vitamin D deficiency is significantly associated with a decreased level of SII and PNR, especially in the first 48 hours of septic shock. Besides, a high level of vitamin D, which has an immune-modulator effect, and/or low pro-inflammatory PTH (increased vitamin D to PTH ratio) seem to be associated with improved survival. Our results may illustrate the importance of inflammatory imbalance, perhaps more than increased inflammation.

Sepsis is defined as a life-threatening organ dysfunction caused by the host's uncontrolled response to infections [3]. The pathogenesis of sepsis is extremely complex and includes an imbalance in the inflammatory response and immune dysfunction, mitochondrial damage, coagulopathy, neuroendocrine network abnormality, and endoplasmic reticulum stress [2,3]. The inflammatory imbalance represents the most crucial basis of sepsis pathogenesis and persists throughout sepsis. As a part of inflammatory imbalance, uncontrolled immune response results

**Table 4.** Logistic regression analysis to determine associated factors with early-mortality

| | Odds ratio (95% Confidence Interval) | P value |
|---|---|---|
| Model 1 | | |
| **SOFA score** | 1.546(1.038-1.489) | 0.02 |
| **Vitamin D-to-PTH ratio** (≤ 0.077) | 12.760(1.588-56.494) | 0.02 |
| Model 2 | | |
| **SOFA score** | 1.609(0.990-2.616) | 0.06 |
| **Vitamin D-to-PTH ratio** (≤ 0.077) | 10.854(1.731-34.080) | 0.02 |
| **SII** (0. hour ≤ 67) | 6.468(1.287-14.428) | 0.03 |
| Model 3 | | |
| **SOFA score** | 1.613(1.032-2.624) | 0.05 |
| **Vitamin D-to-PTH ratio** (≤ 0.077) | 12.012(1.591-46.008) | 0.03 |
| **SII** (0. hour ≤ 67) | 6.408(1.264-16.241) | 0.04 |
| **PNR** (0. hour ≤ 0.05) | 1.273(0.114-14.284) | 0.8 |

SOFA: Sequential Organ Failure Assessment score, SII: Systemic immune-inflammatory index, PNR: platelet-to-neutrophil ratio PTH: Parathyroid hormone,

 

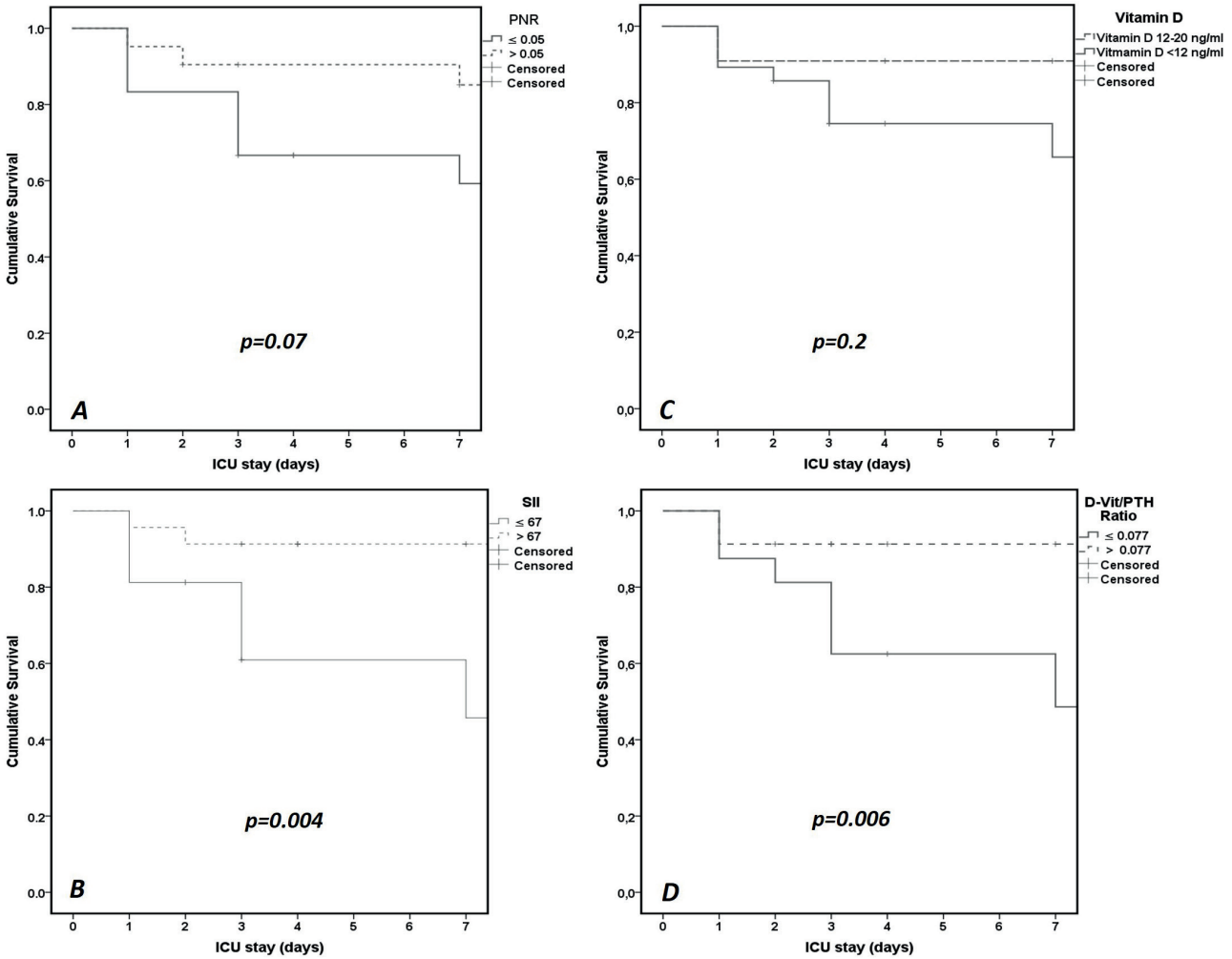


**Figure 2.** Kaplan-Meier survival analysis.

in cytokine storm and hyper-inflammation, and sepsis induced immune-suppression [2,3,6]. Some patients could develop immunosuppression and may die due to secondary opportunistic infections. As a result, the mechanisms of the initiation, maintenance, and termination of sepsis have not yet been fully elucidated, and there is a need for cost-effective and easily-accessible biomarkers that reflect the immune balance.

SII and PNR have commonly reported parameters associated with inflammation and unfavorable prognosis in various diseases such as malignancy, rheumatologic, and cardiovascular diseases [12-14]. However, the relationship of both parameters with sepsis has not been well studied. There is only one study in the literature investigating the relationship between SII and sepsis. However, there is no data on sepsis outcomes in this study [20]. Although increased levels of SII and PNR are associated with worse outcomes and increased mortality in cardiovascular diseases, auto-inflammatory diseases, and malignancy, the association between sepsis may be more complicated. First, platelet count alterations are commonly encountered in the ICU setting, especially in sepsis, and thrombocytopenia often occurs during the course of sepsis [21]. Second, platelets are indispensable for coagulation and likely contribute to disseminated intravascular coagulation. Third, it is well known that platelets are one of the essential actors of immunity, reacting to infection and contributing to pathogen killing and tissue repair as a part of the innate immune response [21]. It is suggested that thrombocytopenia or the non-resolution of thrombocytopenia is linked with mortality [21,22]. During sepsis, neutrophils and lymphocytes rapidly respond to infection. While neutrophil count rises dramatically, lymphocyte counts decrease due to the immunosuppression mentioned above at the late phase of sepsis [23]. Due to the aforementioned mechanisms, the relationship of SII and PNR ratios with sepsis may differ from these biomarkers' relationship with other diseases. Our results support that suppressed

levels of SII are associated with early mortality in septic shock patients. However, the association between early mortality and suppressed PNR level did not reach statistically significance.

The excessive immune response caused by cytokines is a well-known situation in sepsis. However, as mentioned above, maintaining the immune balance is crucial. Therefore, biomarkers reflecting immune balance may provide more critical information about the early prognosis of sepsis. Vitamin D and PTH play a vital role in maintaining calcium and phosphorus metabolism. PTH mediates the renal tubular calcium reabsorption, calcium release from bones, and calcium absorption from the intestine via inducing the activation of 25-OD vitamin D [8,9]. Both vitamin D and PTH affect immunity beyond the bone-mineral metabolism. Vitamin D modulates activation, proliferation, and differentiation of immune and inflammatory cells via the vitamin D receptor and prevents overexpression of inflammatory cytokines [9,11]. Vitamin D modulates the innate immune system by enhancing the phagocytic activity of immune cells and by stimulating monocyte proliferation [24,25]. Adaptive immunity activates in case of pathogen surpasses innate immunity and vitamin D act as an inhibitor factor on adaptive immunity in contrast to innate immunity [24]. Consequently, vitamin D ensures the balance between innate and adaptive immunity. On the other hand, PTH stimulates the release of interleukin-6, which is a significant determinant of inflammation, from osteoblast and the liver [8].

Vitamin D deficiency incidence ranged from 38% to 93% in critically ill patients, and most of the studies support that it is associated with unfavorable prognosis and increased mortality [11,26]. In our study, patients with vitamin D deficiency had higher mortality rates than those with vitamin D insufficiency, but the difference was insignificant. Also, vitamin D deficiency may not be a contributor factor to mortality, according to the Kaplan-Meier analysis. Interestingly, vitamin D deficiency was significantly associated with the low level of SII and PNR, especially in the first 48 hours of septic shock, despite similar C-reactive protein levels. It is known that platelets have their own vitamin D receptor and vitamin D plays a pivotal role in antithrombogenicity [27]. Also, platelet-leukocyte aggregates are liberally generated during sepsis in the circulation and tissues, and they are related with worse outcomes, as mentioned above [27]. Vitamin D deficiency may lead to platelet consumption and may result in a decrease in SII and PNR.

For the comprehensive assessment of any hormone, it is crucial to study the effect of functional regulators of the hormone in conjunction with each other. Also, increased PTH levels may be associated with increased pro-inflammatory cytokines; even the exact mechanism remains unclear [28]. While several pro-inflammatory cytokines, including Interleukin-8, tumor necrosis factor-α, have been shown to enhance PTH secretion in vitro studies, a significant positive association between dietary inflammation index with PTH and hyperparathyroidism was observed in a cross-sectional study with 7,679 adults [28,29]. Consequently, the ratio of these two opposite hormones can provide more profound information about the course of a disease in which the immune balance is impaired, such as sepsis. According to our results, vitamin D-to-PTH ratio of less than 0.077 was associated seven-day ICU mortality in patients with septic shock even though vitamin D and PTH alone were not associated with mortality.

Our study has several limitations. First, the study is a single center study with limited number of patients. This may lead to difficulty interpreting statistical analysis and a decrease in power. For example, although the mortality of vitamin D deficiency is higher than vitamin D insufficiency statistical significance could not be achieved, or PNR could not be determined as an indicator of mortality. On the other hand, excluding diseases such as malignancy and chronic kidney disease, which affect vitamin D and PTH metabolism, increases the strength of the study. Second, the absence of a group with normal vitamin D levels also causes a limitation in evaluating the relationship between vitamin D insufficiency and deficiency with mortality, SII, and PNR. However, the incidence of vitamin D deficiency is over 90% in some studies, and it is difficult to find sufficient number of patients with septic shock and normal vitamin D levels. Therefore, studies addressing these limitations with larger sample sizes and multicenter trials are needed to confirm the crucial association between vitamin D-to-PTH ratio, SII, and PNR with mortality.

 

In conclusion, suppressed levels of SII are associated with increased mortality in patients with septic shock even though it is an inflammatory biomarker, and SII is significantly suppressed in the first 48 hours of septic shock in patients with vitamin D deficiency. In addition, the decreased level of vitamin D-to-PTH ratio, which could be an indicator of immune balance, may be associated with early mortality in the intensive care unit.

## Author contribution

Study conception and design: HHY and AT; data collection: HHY; analysis and interpretation of results: HHY; draft manuscript preparation: HHY, AT. All authors reviewed the results and approved the final version of the manuscript.

## Ethical approval

The study was approved by the ethics committee of Hacettepe University (Protocol no: 14/400 / 23.07.2014)

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## Conflict of interest

The authors declare that there is no conflict of interest.

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