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Evaluation of vitamin D level in patients from neurosurgical intensive care unit[☆]

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Abstract

Vitamin D plays an important role in maintaining normal bone metabolism. Recent studies have suggested that vitamin D influences many other physiological processes, including muscle function, cardiovascular homeostasis, nerve function, and immune response. Furthermore, accumulated evidence suggests that vitamin D also mediates the immune system response to infection. Critical neurosurgical patients have higher infection and mortality rates. To correlate vitamin D deficiency to the immunological status of neurosurgical intensive care unit patients, we detected serum vitamin D level in 15 patients with clinically suspected infection and 10 patients with confirmed infection. Serum level of 25-hydroxyvitamin D, the primary circulating form of vitamin D, was significantly decreased in patients with suspected or confirmed infection after a 2-week neurosurgical intensive care unit hospitalization, while serum level of 1,25-dihydroxyvitamin D, the active form of vitamin D, was significantly decreased in patients after a 4-week neurosurgical intensive care unit hospitalization. These findings suggest that vitamin D deficiency is linked to the immunological status of neurosurgical intensive care unit patients and vitamin D supplementation can improve patient's immunological status.

Key Words

neural regeneration; vitamin D; white blood cell; immune deficiency; infectious diseases; intensive care unit; neurosurgery; neuroregeneration

Research Highlights

- (1) Vitamin D deficiency was correlated to the immunological status of neurosurgical intensive care unit patients.
- (2) Serum levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were significantly decreased in patients with clinically suspected infection and confirmed infection after 2- and 4-week neurosurgical intensive care unit hospitalization.
- (3) Vitamin D supplementation can help improve the immunological status of patients in the neurosurgical intensive care unit.

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INTRODUCTION

The effects of vitamin D on calcium and bone metabolism are well known. Recent advances in the understanding of vitamin D

have revolutionized our view of this classic nutritional factor and have suggested that vitamin D influences many other physiological processes, including muscle function, cardiovascular homeostasis, nervous function, and immune response^[1-2].

Vitamin D plays a role in modulation of the innate immune response^[3-6]. Vitamin D affects the activity of several factors, such as Toll-like receptors on macrophages, polymorphic nuclear cells, monocytes, and epithelial cells, all of which are central to the innate immune response^[3].

Vitamin D deficiency has been associated with a myriad of diseases in the general population and is a common disorder associated with excess morbidity and mortality rates in general population studies^[4-18]. Some studies demonstrate that vitamin D deficiency is very common in intensive care unit patients^[4-6], and it is significantly associated with longer time-to-alive intensive care unit discharge^[4], a trend toward increased organ failure, and a non-significant trend toward a higher number of infections^[4].

Furthermore, vitamin D deficiency is correlated with many infectious diseases^[4-6]. Vitamin D deficiency and susceptibility to respiratory infections are well known in the context of infection by *Mycobacterium tuberculosis*^[5-6]. Several clinical reports suggest that poor vitamin D status is a predisposing factor to infections of the upper and lower respiratory tracts^[7], and the incidence of some viral infectious diseases (chronic hepatitis C virus infection, human immunodeficiency virus infection, and influenza) is increased in vitamin D-deficient patients^[6-10]. Therefore, we focused on vitamin D levels in patients from the neurosurgical intensive care unit and tried to elucidate the relationship between vitamin D and other factors such as infection and length of intensive care unit admission.

RESULTS

Baseline patient data

General patient data is shown in Table 1. Clinically suspected infection was found in 15 patients, of which 10 tested positive in bacterial culture studies.

Table 1 Demographic data of patients studied

Variable	Data
Total number of patients	55
Mean age (year)	56.2
Sex (male/female, n)	35/20
Mean initial Glasgow Coma Scale score	11.8
Mean duration of intensive care unit hospitalization (day)	7
Mean white blood cell count (/L)	6.88×10^9
Clinically infection suspected patients (n)	15
Positive culture finding patients (n)	10

White blood cell count and neutrophil percentage in patients

A gradual increase in mean white blood cell count and neutrophil percentage was shown in 55 patients. Mean white blood cell count was increased from $6.88 \times 10^9/L$ at day 1 to $12.14 \times 10^9/L$ at 5 weeks, and neutrophil percentage was increased from 56.2% to 76.6% at the same time (Table 2). No significant findings were observed in routine biochemical studies. Ten patients had positive bacterial cultures, with the presence of eight different bacteria. Some patients had duplicated positive findings (Table 3).

Table 2 Changes in white blood cell count and neutrophil percentage in all patients

Time (day)	White blood cell count ($\times 10^9/L$)	Neutrophil (%)
1	6.88 ± 0.77	56.2 ± 0.97
3	7.01 ± 1.12	61.4 ± 0.78
7	7.23 ± 0.78	62.3 ± 0.97
14	8.22 ± 0.96	70.1 ± 1.07
21	10.01 ± 1.37	72.2 ± 1.87
28	12.11 ± 1.98	73.8 ± 1.71
35	12.14 ± 2.21	76.6 ± 1.94

Data were expressed as mean \pm SD.

Table 3 Findings from bacterial culture in 10 patients

Cultured bacteria	Number of patients	Patients (total, %)
<i>Enterococcus faecalis</i>	2	20
<i>Stenotrophomonas maltophilia</i>	1	10
<i>Coagulase negative staphylococcus</i>	2	20
<i>Staphylococcus hominis</i>	2	20
<i>Escherichia coli</i>	1	10
<i>Klebsiella pneumoniae</i>	1	10
<i>Staphylococcus aureus</i>	2	20
<i>Staphylococci</i>	2	20

Vitamin D levels in all patients and those suspected of infection

After more than 2 weeks in the intensive care unit, 25-hydroxyvitamin D [25(OH)D] levels were in the deficient range (< 10 ng/mL). Levels of 1,25-dihydroxyvitamin D [1,25(OH)D], the active form of vitamin D, fell to the deficient range (< 25 ng/mL) after 4 weeks at the average vitamin D level of all patients (Figure 1). In patients with clinically suspected infection, 25(OH)D and 1,25(OH)₂D were in the deficient state after 7 days and 14 days, respectively (Figure 2).

Change in vitamin D levels in patients with positive bacterial cultures

After 7 and 14 days in the intensive care unit, the 25(OH)D and 1,25(OH)₂D levels in patients with positive

bacterial cultures were in the deficient range (Figure 3).

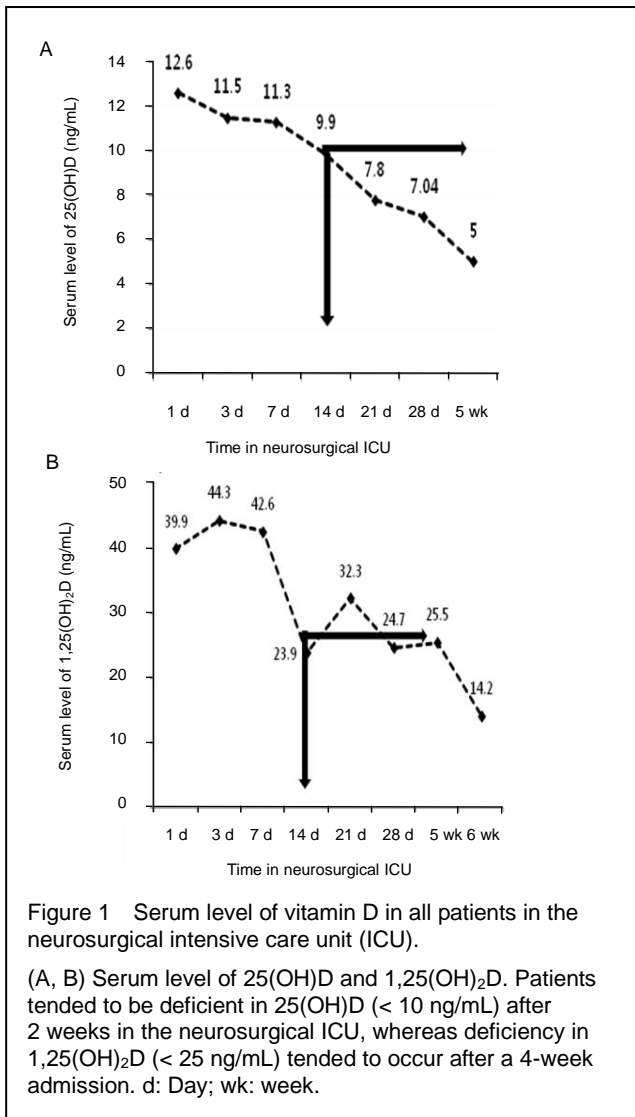


Figure 1 Serum level of vitamin D in all patients in the neurosurgical intensive care unit (ICU). (A, B) Serum level of 25(OH)D and 1,25(OH)₂D. Patients tended to be deficient in 25(OH)D (< 10 ng/mL) after 2 weeks in the neurosurgical ICU, whereas deficiency in 1,25(OH)₂D (< 25 ng/mL) tended to occur after a 4-week admission. d: Day; wk: week.

Vitamin D levels in the clinically suspected infection group versus normal group

The 25(OH)D serum levels were statistically lower in the clinically suspected infection group (*P* < 0.05) (Figure 4) and in patients who had positive bacterial culture findings when compared with the normal group (*P* < 0.05; Figure 5).

DISCUSSION

Vitamin D is a steroid hormone that is produced by the skin after exposure to sunlight (7-dehydrocholesterol is converted to cholecalciferol, vitamin D₃)^[6], and it can also be obtained from foods or supplements. It can be ingested in the form of vitamin D₃ or vitamin D₂ (ergocalciferol)^[11]. Vitamin D₂ is derived from irradiation of the fungal steroid ergosterol^[12]. Vitamin D exists in several forms, including

25(OH)D, the primary circulating form, and 1,25(OH)₂D, the active form. After digestion, vitamin D is processed by 25-hydroxylases that are present in the liver and other tissues to give 25(OH)D^[13-14]. Subsequently, 25(OH)D is converted to 1,25(OH)₂D by the enzyme 25-hydroxyvitamin D-1- α -hydroxylase and CYP27B1 in the kidneys^[1-15].

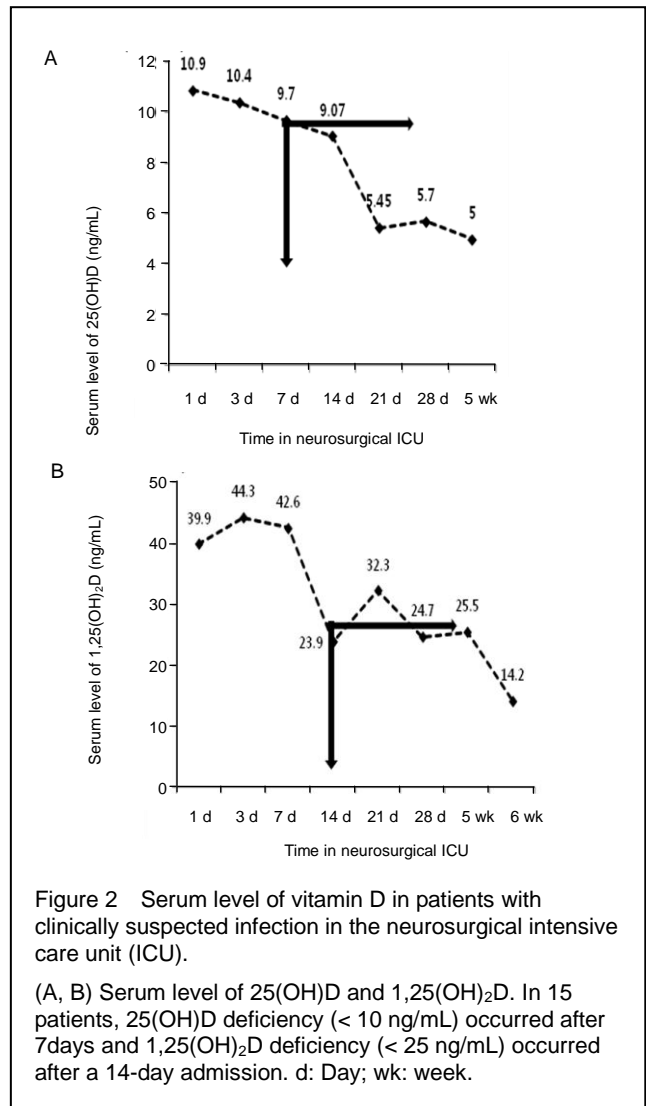


Figure 2 Serum level of vitamin D in patients with clinically suspected infection in the neurosurgical intensive care unit (ICU). (A, B) Serum level of 25(OH)D and 1,25(OH)₂D. In 15 patients, 25(OH)D deficiency (< 10 ng/mL) occurred after 7 days and 1,25(OH)₂D deficiency (< 25 ng/mL) occurred after a 14-day admission. d: Day; wk: week.

The active form of vitamin D is 1,25(OH)₂D. However, its biological half-life is too short to represent the general condition of a patient. Furthermore, it is clinically unstable, so it is not commonly used to diagnose and monitor vitamin D status^[7-16]. Serum 25(OH)D levels correlate with overall vitamin D stores and are the most commonly used biomarkers for assessing vitamin D deficiency^[17]. Unfortunately, its increased biological half-life serves as a disadvantage, with 25(OH)D normally being present in slightly higher concentrations than its active metabolite^[11-17]. We checked the levels of both forms of vitamin D in this study. However, to detect changes in primary vitamin D levels, serum 25(OH)D

levels are more important than the levels of the active form 1,25(OH)₂D^[12-13].

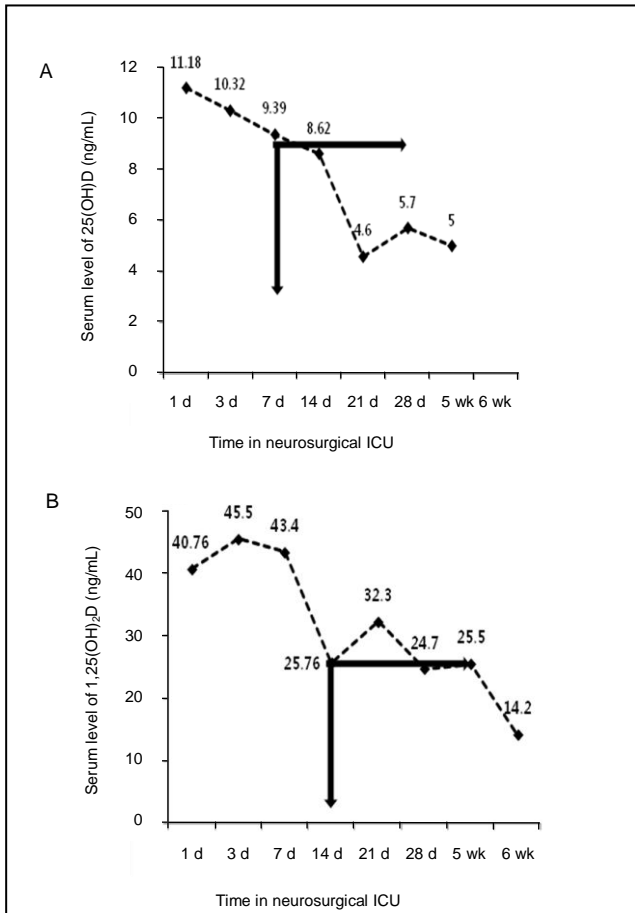


Figure 3 Serum level of vitamin D in patients from the neurosurgical intensive care unit (ICU) who displayed positive culture findings.

(A, B) Serum level of 25(OH)D and 1,25(OH)₂D. 25(OH)D tended to be deficient (< 10 ng/mL) after 7 days and 1,25(OH)₂D tended to be deficient (< 25 ng/mL) after a 14-day admission. d: Day; wk: week.

Environmental factors in the intensive care unit are associated with vitamin D deficiency^[12]. The intensive care unit environment is devoid of sunlight as a main source of vitamin D synthesis^[13]. Furthermore, most neurosurgical patients admitted to the intensive care unit are elderly and have many underlying diseases, such as coronary artery disease, hypertension, chronic obstructive pulmonary disease, renal disease, and liver disease^[18-19], which complicates the ability to determine the origin of vitamin D deficiency in these patients in this setting. Additionally, studies have demonstrated that 25(OH)D insufficiency is very common in patients admitted to the intensive care unit and is significantly associated with longer time-to-alive intensive care unit discharge, a trend toward increased organ failure rates, and a non-significant trend toward a higher number of infections^[20]. In addition, these studies report that

25(OH)D levels continue to significantly decrease throughout the duration of a patient's intensive care unit stay. Therefore, patients may be more susceptible to pathological and overactive immune responses to common intensive care unit infections^[21]. Our study demonstrated that white blood cell count and neutrophil percentage showed a tendency to increase, while vitamin D levels were simultaneously decreasing. Vitamin D deficiency was present in neurosurgical intensive care unit patients and was more severe in cases of long-term hospitalization. Vitamin D deficiency also correlated with long-term intensive care unit hospitalization, increasing the infectious disease rate in our research. As such, continuous monitoring and checking of vitamin D levels is very important in patients admitted to the neurosurgical intensive care unit.

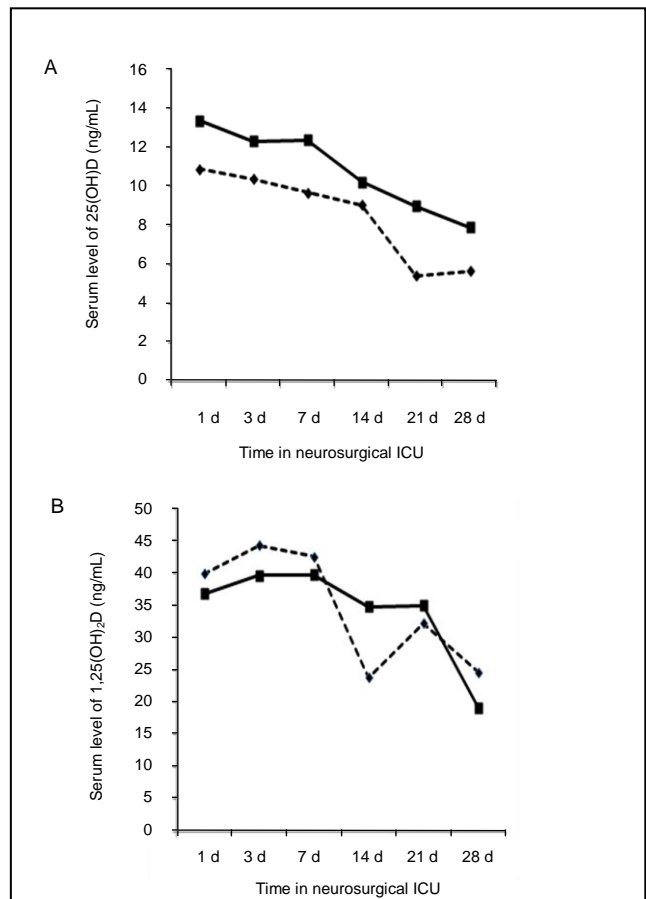
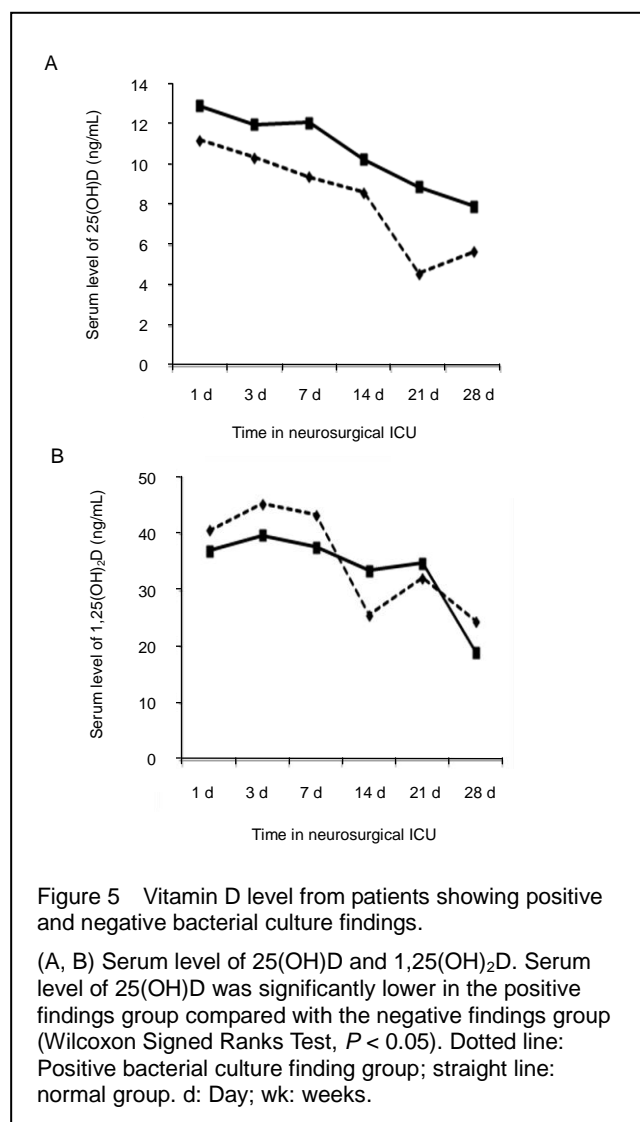


Figure 4 Serum level of vitamin D in patients with clinically suspected infection and those without infection sign.

(A, B) Serum level of 25(OH)D and 1,25(OH)₂D. The serum level of 25(OH)D was significantly lower in the clinically suspected infection group than that in the normal group (Wilcoxon signed-rank test, *P* < 0.05). Dotted line: Clinically suspected infection group and straight line: normal group. d: Day; wk: weeks.

The important role of vitamin D in calcium metabolism and bone health is well accepted^[12]. The discovery that

most human tissues are able to produce and use active vitamin D triggered extensive research into the biological functions of vitamin D beyond bone health^[22-23]. Recent studies have shown a potential physiological role of vitamin D in regulating normal innate and adaptive immunity^[3-24]. A recent report found significantly low levels of the endogenous antimicrobial peptide cathelicidin (LL-37) in critically ill patients who were deficient of vitamin D, suggesting a role for vitamin D in maintaining innate immunity to infection in the intensive care unit^[19]. Furthermore, 1,25(OH)₂D has been shown to have a stimulatory effect on the innate immune system by increasing interleukin-1 production and stimulating monocyte proliferation^[18]. Thus, neurosurgical intensive care unit patients are at a high risk of contracting infectious diseases because of their deteriorating general conditions combined with vitamin D deficiency. In our study, the clinically suspected infection group and the positive culture group had lower levels of vitamin D compared with the normal group.



A relatively small number of participants (*i.e.* small sample size), few statistically significant data, and a short follow-up period are some of the limitations that exist for the present study. Furthermore, this study was not a prospective randomized control study of drug effectiveness, and the effect of vitamin D deficiency on the outcome of critically ill patients remains unclear^[2-25]. Therefore, prior to any large clinical outcome trial, it will be vital to first conduct a well-designed dosing trial to ensure that sufficient doses of vitamin D are administered to correct the deficiencies observed in many intensive care unit patients.

Vitamin D supplementation has been demonstrated to yield good effects for infectious diseases including pneumonia^[26]. Single oral ultra-high dose of cholecalciferol corrects vitamin D deficiency, but further studies into the correct dosage of vitamin D supplementation are required^[27]. In conclusion, the optimal dosage of vitamin D required to both normalize the deficiency observed in the intensive care unit setting and potentially have a favorable effect on infection and other outcomes is currently unknown and requires further study.

Finally, vitamin D deficiency in neurosurgical intensive care unit patients is an important issue. In this study, we found that vitamin D deficiency is linked to immunological status. Future studies should clarify whether vitamin D supplementation can improve survival in patients admitted to the neurosurgical intensive care unit.

SUBJECTS AND METHODS

Design

A prospective study.

Time and setting

We collected the clinical radiological data of 55 neurosurgical intensive care unit patients who received treatment in the Department of Neurosurgery, Hangeang Sacred Heart Hospital, College of Medicine, Hallym University, Seoul, Korea from September 2011 to March 2012.

Subjects

We defined suspected infection patients according to high white blood cell count ($> 10.0 \times 10^3/\mu\text{L}$) and fever (body temperature $> 37.8^\circ\text{C}$). Culture-positive patients were diagnosed using sputum, urine or blood cultures. Bacteria were defined as a large domain of prokaryotic microorganisms, typically a few micrometers in length,

having a wide range of shapes, ranging from spheres to rods and spirals. Viruses and fungi were excluded^[28].

Methods

Evaluation of vitamin D levels

To evaluate serum vitamin D levels, we evaluated 25(OH)D levels, the primary circulating form of vitamin D, and 1,25(OH)₂D, the active form of the vitamin^[3-6]. Enzyme-linked immunosorbent assay was used to detect vitamin D, and 25(OH)D was evaluated using chemiluminescent immunoassay. Specific antibodies (DiaSorin Co., Ltd., Stillwater, MN, USA) to vitamin D were used to coat magnetic particles (solid phase), and vitamin D was linked to an isoluminol (Liaison Co., Ltd., Washington, MA, USA) derivative. During incubation, 25(OH)D dissociated from its binding protein and competed with labeled vitamin D for binding sites on the antibody (Cambridge Isotope Laboratories, Andover, MA, USA). The starter reagents were subsequently added and a flash chemiluminescent reaction was initiated. The light signal was measured as relative light units using a photomultiplier (DiaSorin Co., Ltd) and was inversely proportional to the concentration of 25(OH)D present in the samples^[29]. Another factor, 1,25(OH)₂D, was detected by radioimmunoassay. Serum 25-hydroxyvitamin D2 and D3 were measured at a research laboratory in Uppsala using Gamma-B (DiaSorin Co., Ltd)^[30] or competitive binding assay (Deqas Co., Ltd., Fulham, London, UK). Radioimmunoassay isotope dilution-liquid chromatography-mass spectrometry (Deqas Co., Ltd) was used for the measurement of both 25(OH)D and 1,25(OH)₂D in human serum. Results of the assays were compared by means of radioimmunoassay^[30].

The normal value of vitamin D is often defined by checking circulating 25(OH)D levels. Relative insufficiency of the circulating 25(OH)D is defined as < 30 ng/mL (75 nmol/L)^[31-32], while insufficiency is defined as circulating 25(OH)D levels < 20 ng/mL (50 nmol/L)^[1, 21-33]. However, levels ≤ 40 ng/mL (100 nmol/L) are sometimes advocated as normal for specific patients^[11, 34-35]. In our study, we defined 25(OH)D deficiency as < 10 ng/mL. 1,25(OH)₂D was defined as within the normal range at 25–66 ng/mL and < 25 ng/mL was considered a deficiency.

Statistical analysis

All data were statistically analyzed using SPSS 12.0 software (SPSS, Chicago, IL, USA) and were expressed as mean ± SD. The Wilcoxon signed-rank test was used to analyze the differences in vitamin D level between the

clinically suspected infection and confirmed infection groups. This method was also used to analyze the vitamin D level differences between the positive and negative bacterial culture finding groups at different time points. A level of $P < 0.05$ was considered statistically significant.

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Author contributions: All authors participated in experimental design, conduction, data analysis, and approved the final version of the paper.

Conflicts of interest: None declared.

Ethical approval: All study protocols received full approval from Local Ethical Committee, Hangeang Sacred Heart Hospital, College of Medicine, Hallym University, Korea (IRB No. 2012-231).

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