

Original Article  
Gastroenterology &  
Hepatology



# Differential Impact of Serum 25-Hydroxyvitamin D3 Levels on the Prognosis of Patients with Liver Cirrhosis According to MELD and Child-Pugh Scores

OPEN ACCESS

Received: Nov 12, 2019

Accepted: Mar 3, 2020

Address for Correspondence:

Soon Ho Um, MD, PhD

Department of Internal Medicine, Korea University College of Medicine, 73 Goryeodae-ro, Seongbuk-gu, Seoul 02841, Korea.

E-mail: umsh@korea.ac.kr

\*Present address: Division of Gastroenterology and Hepatology, Department of Internal Medicine, Sanggye Paik Hospital, Inje University College of Medicine, Seoul, Korea

© 2020 The Korean Academy of Medical Sciences.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID iDs

Tae Hyung Kim

<https://orcid.org/0000-0002-7747-4293>

Seung Gyu Yun

<https://orcid.org/0000-0002-9915-9681>

Jimi Choi

<https://orcid.org/0000-0003-2834-2718>

Hyun Gil Goh

<https://orcid.org/0000-0002-5280-4794>

Han Ah Lee

<https://orcid.org/0000-0003-4082-1121>

Sun Young Yim

<https://orcid.org/0000-0001-7346-5974>

Tae Hyung Kim <sup>1</sup>, Seung Gyu Yun <sup>2</sup>, Jimi Choi <sup>3</sup>, Hyun Gil Goh <sup>1</sup>, Han Ah Lee <sup>1</sup>, Sun Young Yim <sup>1</sup>, Seong Ji Choi <sup>1</sup>, Young-Sun Lee <sup>1</sup>, Eileen L. Yoon <sup>1</sup>, Young Kul Jung <sup>1</sup>, Yeon Seok Seo <sup>1</sup>, Ji Hoon Kim <sup>1</sup>, Hyung Joon Yim <sup>1</sup>, Jong Eun Yeon <sup>1</sup>, Kwan Soo Byun <sup>1</sup> and Soon Ho Um <sup>1</sup>

<sup>1</sup>Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea

<sup>2</sup>Department of Laboratory Medicine, Korea University College of Medicine, Seoul, Korea

<sup>3</sup>Department of Biostatistics, Korea University College of Medicine, Seoul, Korea

## ABSTRACT





**Background:** Prognosis of patients with diverse chronic diseases is reportedly associated with 25-hydroxyvitamin D levels. In this study, we investigated the potential role of 25-hydroxyvitamin D3 (25[OH]D3) levels in improving the predictive power of conventional prognostic models for patients with liver cirrhosis.

**Methods:** We investigated clinical findings, including serum 25(OH)D3 levels at admission, of 155 patients with cirrhosis who were followed up for a median of 16.9 months.

**Results:** Median 25(OH)D3 levels were significantly different among patients exhibiting Child-Pugh grades A, B, and C. Mortality, including urgent transplantation, was significantly associated with 25(OH)D3 levels in univariate analysis. Severe vitamin-D deficiency (serum 25[OH]D3 level < 5.0 ng/mL) was significantly related to increased mortality, even after adjusting for Child-Pugh and Model for End-stage Liver Disease (MELD) scores. In particular, the presence of severe vitamin D deficiency clearly defined a subgroup with significantly poorer survival among patients with Child-Pugh scores of 5–10 or MELD scores ≤ 20. A new combination model of MELD score and severe vitamin D deficiency showed significantly more accurate predictive power for short- and long-term mortality than MELD scores alone. Additionally, serum 25(OH)D3 levels and new model scores were significantly associated with the development of spontaneous bacterial peritonitis, overt encephalopathy, and acute kidney injury.

**Conclusion:** Serum 25(OH)D3 level is an independent prognostic factor for patients with liver cirrhosis and has a differential impact on disease outcomes according to MELD and Child-Pugh scores.

**Keywords:** Vitamin D; Cirrhosis; Prognosis; Complication

Seong Ji Choi <https://orcid.org/0000-0002-1969-516X>Young-Sun Lee <https://orcid.org/0000-0001-6396-0859>Eileen L. Yoon <https://orcid.org/0000-0003-0474-048X>Young Kul Jung <https://orcid.org/0000-0002-6566-1382>Yeon Seok Seo <https://orcid.org/0000-0003-1457-5350>Ji Hoon Kim <https://orcid.org/0000-0003-3924-0434>Hyung Joon Yim <https://orcid.org/0000-0002-6036-2754>Jong Eun Yeon <https://orcid.org/0000-0002-0510-7371>Kwan Soo Byun <https://orcid.org/0000-0003-4031-842X>Soon Ho Um <https://orcid.org/0000-0002-4545-7907>**Disclosure**

The authors have no potential conflicts of interest to disclose.

**Author Contributions**

Conceptualization: Yun SG, Um SH. Data curation: Goh HG, Lee HA, Yim SY, Choi SJ, Lee YS. Formal analysis: Choi J. Investigation: Yoon EL, Jung YK, Seo YS, Kim JH. Project administration: Kim TH. Supervision: Um SH. Writing - original draft: Kim TH. Writing - review & editing: Seo YS, Kim JH, Yim HJ, Yeon JE, Byun KS.

**INTRODUCTION**

Liver cirrhosis is an end-stage liver disease, and its progression results in high mortality and occurrence of complications that dramatically lower the quality of life of patients with cirrhosis. The 1- and 2-year mortality of patients with decompensated cirrhosis was estimated to be 40% and 55%, respectively, and accounted for approximately 2% of global deaths.<sup>1</sup> Therefore, many studies have been conducted to determine a useful marker for prognosis and survival prolongation in patients diagnosed with cirrhosis.<sup>2-5</sup> Based on the results of those studies, the Child-Pugh and the Model for End-stage Liver Disease (MELD) scoring systems are now widely used for clinical decision-making in patients with decompensated cirrhosis.

Serum vitamin D levels are associated with the viral status and treatment efficacy in chronic viral hepatitis,<sup>6-8</sup> progression of liver fibrosis,<sup>8-10</sup> and prognosis in patients with chronic liver diseases such as cirrhosis.<sup>10-17</sup> Vitamin D not only acts as an essential molecule that sustains the homeostasis of muscle and bone metabolism but also modulates the immunologic cascade as an anti-inflammatory molecule and suppresses tumorigenesis in many organs.<sup>18,19</sup> Vitamin D3, the major and most effective form of vitamin D in humans, is produced by epidermal cells following sunlight exposure, and is then delivered to the liver by carrier proteins such as vitamin D-binding protein or albumin to undergo 25-hydroxylation. Next, 25-hydroxyvitamin D3 (25[OH]D3) is delivered to the kidney where it is hydroxylated again to calcitriol, the active form of vitamin D.<sup>20</sup> During this process, any functional disorganization of the liver can affect serum levels of 25(OH)D3 since the liver is the main organ responsible for 25-hydroxylation of vitamin D and production of 25(OH)D3 carrier proteins. In fact, 25(OH)D3 might act as an immune modulator and affect the progression of chronic liver disease.<sup>6-8,10</sup>

It remains unclear, however, whether serum vitamin D levels have any significant association with the prognosis of patients with cirrhosis that is independent of conventional prognostic models, such as Child-Pugh or MELD scores. In addition, since most previous studies examining the prognostic impact of vitamin D in the context of chronic liver disease were conducted in Western countries with Caucasians as test populations, the data on Asian patients with chronic liver disease are scarce, despite the fact that serum vitamin D levels can vary by ethnicity and region.<sup>21,22</sup>

Thus, in this study, we elucidated whether serum 25(OH)D3 levels have any significant role in improving the predictive power of conventional prognostic models for end-stage liver diseases, using Korean patients diagnosed with liver cirrhosis.

**METHODS****Study populations**

We retrospectively analyzed a cohort of patients who were diagnosed with liver cirrhosis and had been hospitalized in the Department of Gastroenterology and Hepatology of Korea University Anam Hospital, Seoul, Korea, between November 2016 to October 2017. We checked serum 25(OH)D3 levels in all the patients admitted. We recruited 328 cirrhosis patients, and patients with malignant diseases (n = 113) and chronic kidney diseases (n = 35). Patients taking medications that affect vitamin D metabolism, such as vitamin D, calcium,

or hormone supplements ( $n = 25$ ), were excluded. The cohort consisted of the remaining 155 hospitalized patients with cirrhosis.

### Measurement of serum 25(OH)D3

Serum 25(OH)D3 levels were assessed by liquid chromatography (LC)-mass spectrometry (MS) using a Triple Quad™ 4500 LC/MS/MS (AB SCIEX, Concord, Canada) because it has adequate sensitivity and provides high accuracy in metabolite identification for assessment of 25(OH)D and its subtypes.<sup>23</sup>

### Diagnostic criteria and definition

The diagnosis of cirrhosis was based on liver histology, gross findings during surgery, or radiological findings of an irregular liver margin with ascites, varices, or thrombocytopenia ( $< 10^5$  cells/mm<sup>3</sup>).<sup>5</sup> Alcohol overuse was defined by a history of daily alcohol intake  $\geq 60$  g for men and  $\geq 30$  g for women.<sup>24</sup> Hepatic encephalopathy (HEP) was defined based on the West Haven Criteria.<sup>25</sup> Acute kidney injury (AKI) was diagnosed as an acute increase in serum creatinine levels by  $\geq 0.3$  mg/dL in  $< 48$  hour or a  $\geq 50\%$  increase in serum creatinine levels from a baseline serum creatinine value in seven days, based on the International Ascites Club criteria.<sup>26</sup> Severe vitamin D deficiency was defined as  $< 5.0$  ng/mL of serum 25(OH)D3. The time for vitamin D measurements was categorized as autumn to winter (October–March) and spring to summer (April–September).<sup>27</sup> The primary outcomes were defined as death and urgent liver transplantation (LT) during follow-up,<sup>5</sup> and secondary outcomes were defined as cirrhosis complications such as spontaneous bacterial peritonitis (SBP), overt HEP, and AKI at baseline and their new occurrences within three months of inclusion. For the analyses of secondary outcomes, patients who died or failed to follow-up within three months of inclusion without the occurrence of each complication were excluded.

### Statistics

We analyzed the data using R programming language (v 3.6.1; R Development Core Team, 2019), and compared continuous variables using the Mann-Whitney U test and Kruskal-Wallis test, whereas categorical variables were analyzed using the  $\chi^2$  test, as appropriate. Pearson's correlation analyses were used to determine a correlation between serum 25(OH)D3 levels and prognostic models of cirrhosis. We conducted Cox proportional hazard regression analyses to investigate factors associated with primary outcomes, which comprised death and urgent LT. Patients lost to follow-up and who received elective transplantation were considered to be censored. The significantly associated factors with  $P < 0.05$  in univariate analyses were included for multivariate analysis. The assumptions of hazards proportionality were confirmed by log-minus-log hazard plots. In addition, we calculated and compared the cumulative rates of primary outcomes using Kaplan–Meier plots and log-rank tests. We compared the discriminatory power of prognostic models for primary outcomes using C-statistics by receiver operating characteristic (ROC) curves. In addition, a calibration plot was drawn to verify the performance of the new model compared with actual outcomes, applying internal validation with bootstrap resampling. A Hosmer–Lemeshow test was used to assess the goodness of fit of the new model. A two tailed  $P < 0.05$  was considered statistically significant.

### Ethics statement

This study protocol was reviewed and approved by the Institutional Review Boards of the Korea University Anam Hospital (2018AN0398) and was performed in accordance with the ethical guidelines of the Declaration of Helsinki. A waiver of informed consent was obtained.

## RESULTS

### Baseline characteristics of patients

For the 155 test subjects, the median age was 56.2 years (interquartile range [IQR], 48.7–69.3 years), and among them, 110 were men (71.0%). Alcohol and chronic hepatitis B accounted for 62.6% and 20.0% of all cirrhosis cases, respectively. The median MELD score was 15 (IQR, 11–21). Ascites was absent, mild/moderate, or severe in 60 (38.7%), 31 (20.0%), and 64 (41.3%) patients, respectively. Severe vitamin D deficiency was confirmed in 72 patients (46.5%). The main reasons for admission were jaundice (23.9%), ascites (21.9%), variceal bleeding (13.5%), HEP (9.7%), infection (9.7%), and AKI (5.2%).

**Table 1** shows baseline characteristics for liver function and accompanying diseases based on Child-Pugh grade. The differences in age, gender, body mass index, and etiology of liver disease were not significant. Patients with a higher Child-Pugh grade exhibited significantly lower serum levels of 25(OH)D3, higher incidence of severe vitamin D deficiency, and higher MELD scores ( $P < 0.001$ ).

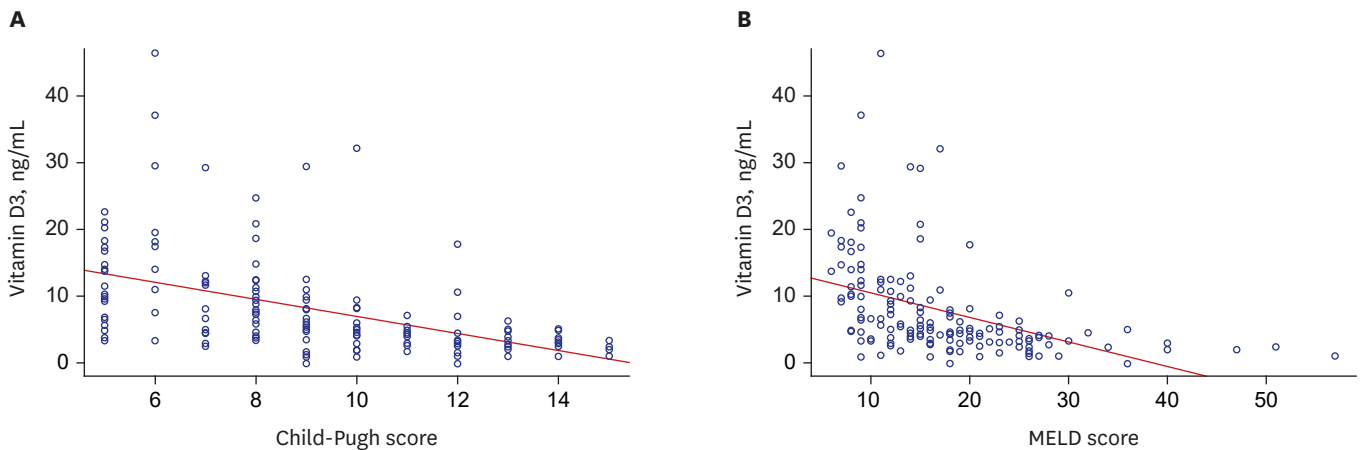
### Serum 25(OH)D3 levels as a representative for liver function

To determine the correlation between serum 25(OH)D3 and liver function in patients with cirrhosis, we conducted correlation analyses using Child-Pugh and MELD scores, as they are well-known representatives of liver function in advanced liver disease. Our results indicated

**Table 1.** Baseline characteristics based on Child-Pugh grades

Factors	Child-Pugh grade			P value
	A (n = 32)	B (n = 51)	C (n = 72)	
Age, yr	56.2 (48.4–73.8)	56.6 (49.8–69.4)	55.1 (48.5–66.3)	0.623 <sup>a</sup>
Gender, men	24 (75.0)	38 (74.5)	48 (66.7)	0.546 <sup>b</sup>
DM	15 (46.9)	27 (52.9)	14 (19.4)	< 0.001 <sup>b</sup>
HTN	9 (28.1)	18 (35.3)	13 (18.1)	0.093 <sup>b</sup>
BMI, kg/m <sup>2</sup>	24.2 (20.1–28.2)	23.1 (20.8–25.3)	23.8 (21.6–26.8)	0.515 <sup>a</sup>
Etiology				0.155 <sup>b</sup>
Alcohol	14 (43.8)	34 (66.7)	49 (68.1)	
HBV	9 (28.1)	7 (13.7)	15 (20.8)	
HCV	2 (6.3)	4 (7.8)	3 (4.2)	
Others	7 (21.9)	6 (11.8)	5 (6.9)	
Hb, g/dL	13.0 (10.4–14.8)	11.1 (9.3–12.6)	10.8 (9.4–12.2)	0.009 <sup>a</sup>
Platelet, × 10 <sup>3</sup> /mm <sup>3</sup>	108 (83–169)	89 (64–126)	83 (57–109)	0.004 <sup>a</sup>
AST, IU/L	50 (33–69)	56 (38–128)	78 (53–129)	0.002 <sup>a</sup>
ALT, IU/L	35 (17–43)	30 (21–59)	30 (23–45)	0.726 <sup>a</sup>
Albumin, g/dL	3.9 (3.5–4.2)	3.1 (2.8–3.4)	2.7 (2.3–3.0)	< 0.001 <sup>a</sup>
Bilirubin, mg/dL	1.1 (0.8–1.3)	1.9 (1.2–2.9)	6.1 (3.5–11.0)	< 0.001 <sup>a</sup>
Prothrombin time, INR	1.13 (1.06–1.17)	1.29 (1.23–1.42)	1.80 (1.63–2.02)	< 0.001 <sup>a</sup>
BUN, mg/dL	13.7 (9.9–18.3)	14.4 (9.0–20.5)	17.1 (11.5–35.4)	0.038 <sup>a</sup>
Creatinine, mg/dL	0.8 (0.7–1.0)	0.9 (0.7–1.0)	1.0 (0.7–1.7)	0.092 <sup>a</sup>
Sodium, mmol/L	139 (136–140)	137 (134–139)	132 (129–135)	< 0.001 <sup>a</sup>
Ascites	0	33 (64.7)	62 (86.1)	< 0.001 <sup>b</sup>
Hepatic encephalopathy	0	8 (15.7)	30 (41.7)	< 0.001 <sup>b</sup>
Spleen size, cm	11.3 (10.2–13.8)	13.0 (11.1–14.0)	13.2 (11.9–15.2)	0.040 <sup>a</sup>
Vitamin D measurement in autumn to winter	20 (62.5)	35 (68.6)	49 (68.1)	0.823
25(OH)D3, ng/mL	12.7 (6.8–18.3)	7.7 (4.7–11.5)	4.0 (2.5–5.0)	< 0.001 <sup>a</sup>
Severe vitamin D deficiency	5 (15.6)	15 (29.4)	52 (72.2)	< 0.001 <sup>b</sup>
MELD	8 (8–10)	13 (11–15)	22 (18–27)	< 0.001 <sup>a</sup>

Data are presented as number (%) and median (interquartile range). Severe vitamin D deficiency is defined as serum 25(OH)D3 levels lower than 5.0 ng/mL. DM = diabetes mellitus, HTN = hypertension, BMI = body mass index, HBV = hepatitis B virus, HCV = hepatitis C virus, Hb = hemoglobin, AST = aspartate aminotransferase, ALT = alanine aminotransferase, BUN = blood urea nitrogen, 25(OH)D3 = 25-hydroxyvitamin D3, MELD = Model for End-stage Liver Disease. <sup>a</sup>Kruskal-Wallis test; <sup>b</sup> $\chi^2$  test.



**Fig. 1.** Correlation between serum 25(OH)D3 levels and prognostic models for liver cirrhosis. **(A)** Correlation between serum levels and Child-Pugh scores and **(B)** MELD scores. Serum 25(OH)D3 levels were inversely correlated with Child-Pugh scores (Pearson correlation coefficient,  $-0.493$ ;  $P < 0.001$ ) and MELD scores (Pearson correlation coefficient,  $-0.436$ ;  $P < 0.001$ ).

25(OH)D3 = 25-hydroxyvitamin D3, MELD = Model for End-stage Liver Disease.

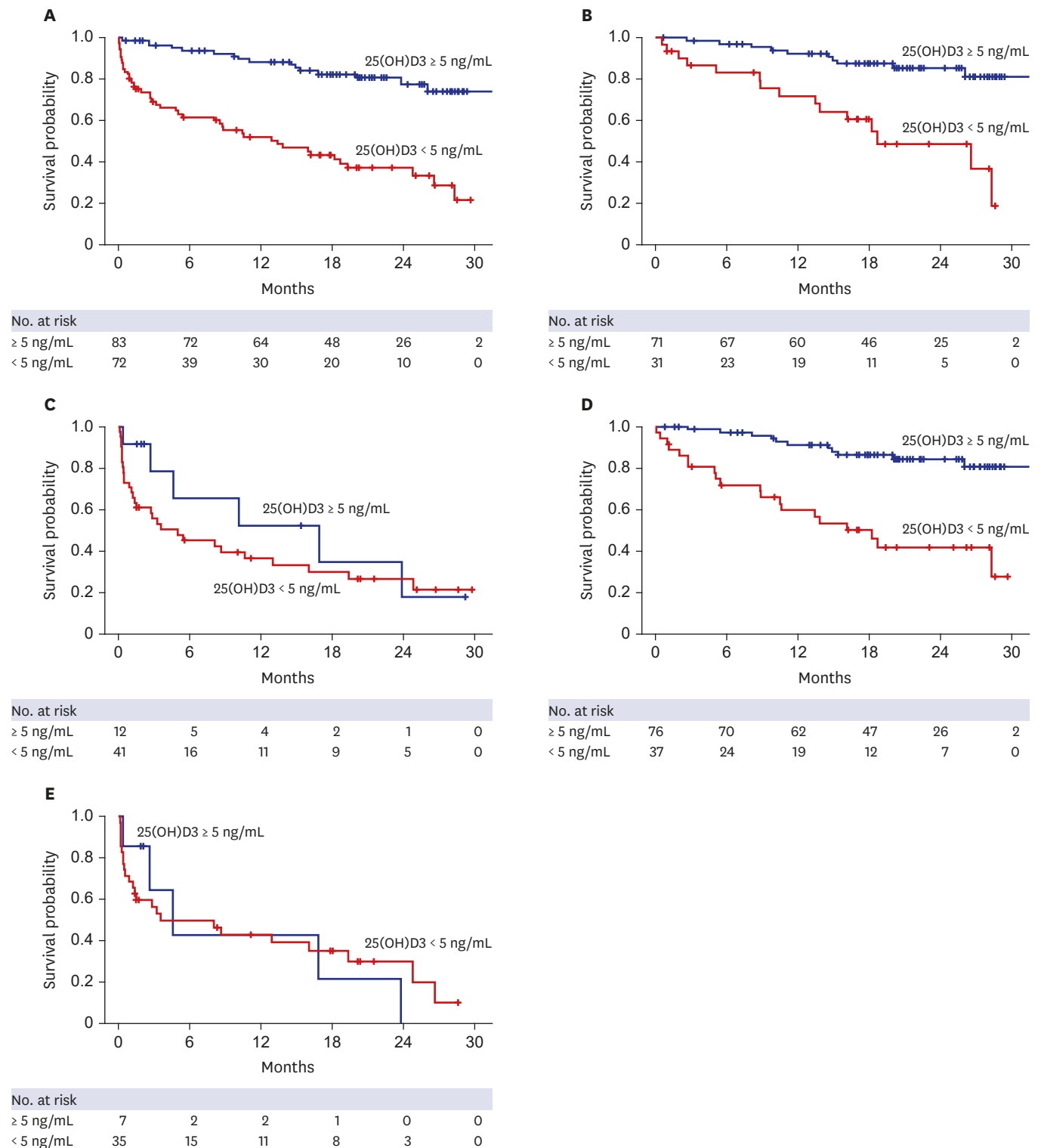
that serum 25(OH)D3 levels were inversely correlated with Child-Pugh scores (Pearson correlation coefficient,  $-0.493$ ;  $P < 0.001$ ) and MELD score (Pearson correlation coefficient,  $-0.436$ ;  $P < 0.001$ ) (Fig. 1).

#### Vitamin D deficiency as an independent predictor for survival

During a median follow-up period of 16.9 months (range, 0.1–31.8 months), 47 patients died, and 13 patients underwent urgent LT. The major causes of deaths in the study cohort were liver failure (40.4%), infection (27.7%), variceal bleeding (10.6%), and other causes (21.3%) such as stroke and heart disease. Patients with severe vitamin D deficiency at baseline died of infection (34.4%), liver failure (31.3%), and variceal bleeding (9.4%), whereas those without vitamin D deficiency expired because of infection (13.3%), liver failure (60.0%), and variceal bleeding (13.3%).

LT-free survival rates in the entire cohort were 78.6%, 70.6%, and 58.6% at 6-, 12-, and 24-months, respectively. Kaplan–Meier plots showed that patients with severe vitamin D deficiency had significantly lower LT-free survival rates than those without vitamin D deficiency (cumulative survival probabilities at 6-, 12-, and 24-months were 61.2%, 51.5%, and 36.1% vs. 93.6%, 88.2%, and 77.3%, respectively;  $P < 0.001$ ) (Fig. 2A).

To identify risk factors for primary outcomes (LT-free survival), we conducted a Cox proportional hazards regression analyses. Univariate analysis revealed that death or urgent LT were significantly associated with the presence of diabetes mellitus, ascites, encephalopathy, severe vitamin D deficiency, hemoglobin levels, prothrombin time international normalized ratio (PT INR), serum albumin, bilirubin, creatinine, and sodium levels, Child-Pugh score, and MELD score. The seasonal variability in vitamin D measurement did not significantly affect the primary outcome. Among these variables, serum bilirubin levels (hazard ratio [HR], 1.04; 95% confidence interval [CI], 1.01–1.08;  $P = 0.012$ ), the presence of ascites (HR, 2.95; 95% CI, 1.28–6.83;  $P = 0.011$ ), encephalopathy (HR, 2.01; 95% CI, 1.15–3.51;  $P = 0.014$ ), and severe vitamin D deficiency (HR, 2.90; 95% CI, 1.53–5.51;  $P = 0.001$ ) served as independent predictors for the primary outcome in the multivariate Cox model, excluding Child-Pugh and MELD scores. Alternative Cox models, including Child-Pugh or MELD score,



**Fig. 2.** Kaplan–Meier plots for primary outcomes based on the presence of severe vitamin D deficiency and MELD scores. **(A)** LT-free survival rates based on the presence of severe vitamin D deficiency (median survival time, not reached vs. 12.9 months for patients without and with severe vitamin D deficiency, respectively;  $P < 0.001$ ). **(B)** LT-free survival rates in patients with Child-Pugh scores of 5–10 (median survival time, not reached vs. 18.7 months;  $P < 0.001$ ). **(C)** LT-free survival rates in patients with Child-Pugh scores of 11–15 (median survival time, 16.8 vs. 4.9 months;  $P = 0.320$ ). **(D)** LT-free survival rates in patients with a MELD score of  $\leq 20$  (median survival time, not reached vs. 16.2 months;  $P < 0.001$ ). **(E)** LT-free survival rates in patients with a MELD score of  $> 20$  (median survival time, 9.5 vs. 8.1 months, respectively;  $P = 0.890$ ). LT = liver transplantation, MELD = Model for End-stage Liver Disease, 25(OH)D3 = 25-hydroxyvitamin D3.

**Table 2.** Univariate and multivariate Cox regression analyses for mortality and urgent transplantation

Variables	Univariate analyses		Multivariate analyses					
	HR (95% CI)	P value	Model 1		Model 2		Model 3	
			HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age, yr	1.02 (1.00–1.04)	0.096						
Gender, men	0.63 (0.37–1.07)	0.089						
DM, presence	0.57 (0.32–0.99)	0.048	0.82 (0.44–1.54)	0.532	0.77 (0.42–1.39)	0.381	0.95 (0.49–1.85)	0.889
HTN, presence	1.26 (0.72–2.19)	0.416						
BMI, kg/m <sup>2</sup>	0.97 (0.91–1.02)	0.250						
Etiology, alcohol	1.30 (0.76–2.23)	0.333						
Platelet, ×10 <sup>3</sup> /mm <sup>3</sup>	1.00 (0.99–1.00)	0.778						
Albumin, g/dL	0.30 (0.18–0.48)	< 0.001	0.66 (0.34–1.28)	0.217	Excluded		0.97 (0.51–1.84)	0.932
Bilirubin, mg/dL	1.05 (1.03–1.07)	< 0.001	1.04 (1.01–1.08)	0.012	Excluded		Excluded	
Prothrombin time, INR	1.37 (1.14–1.64)	0.001	0.88 (0.60–1.28)	0.491	Excluded		Excluded	
Creatinine, mg/dL	1.42 (1.24–1.63)	< 0.001	1.08 (0.87–1.34)	0.506	1.22 (1.04–1.43)	0.012	Excluded	
Sodium, mmol/L	0.93 (0.89–0.96)	< 0.001	1.02 (0.97–1.08)	0.395	1.01 (0.96–1.06)	0.754	1.02 (0.97–1.07)	0.468
Ascites, presence	3.92 (2.03–7.55)	< 0.001	2.95 (1.28–6.83)	0.011	Excluded		2.97 (1.16–6.43)	0.012
HEP, presence	2.86 (1.71–4.79)	< 0.001	2.01 (1.15–3.51)	0.014	Excluded		1.79 (1.05–3.05)	0.031
Spleen size, cm	1.00 (0.91–1.09)	0.958						
Vitamin D measurement in autumn to winter	0.87 (0.50–1.52)	0.634						
25(OH)D3, ng/mL	0.77 (0.70–0.85)	< 0.001	Excluded		Excluded		Excluded	
Severe vitamin D deficiency	4.92 (2.77–8.76)	< 0.001	2.95 (1.28–6.83)	0.001	2.45 (1.28–4.69)	0.007	2.50 (1.30–4.81)	0.006
Child-Pugh score	1.43 (1.29–1.58)	< 0.001	Excluded		1.31 (1.16–1.48)	< 0.001	Excluded	
MELD score	1.08 (1.06–1.10)	< 0.001	Excluded		Excluded		1.07 (1.02–1.11)	0.002

Model 1, multivariate Cox model excluding Child-Pugh and MELD scores; Model 2, multivariate Cox model including Child-Pugh scores without its component variables; Model 3, multivariate Cox model including MELD scores without its component variables.

HR = hazard ratio, CI = confidence interval, DM = diabetes mellitus, HTN = hypertension, BMI = body mass index, AST = aspartate aminotransferase, ALT = alanine aminotransferase, INR = international normalized ratio, 25(OH)D3 = 25-hydroxyvitamin D3, MELD = model for end-stage liver disease.

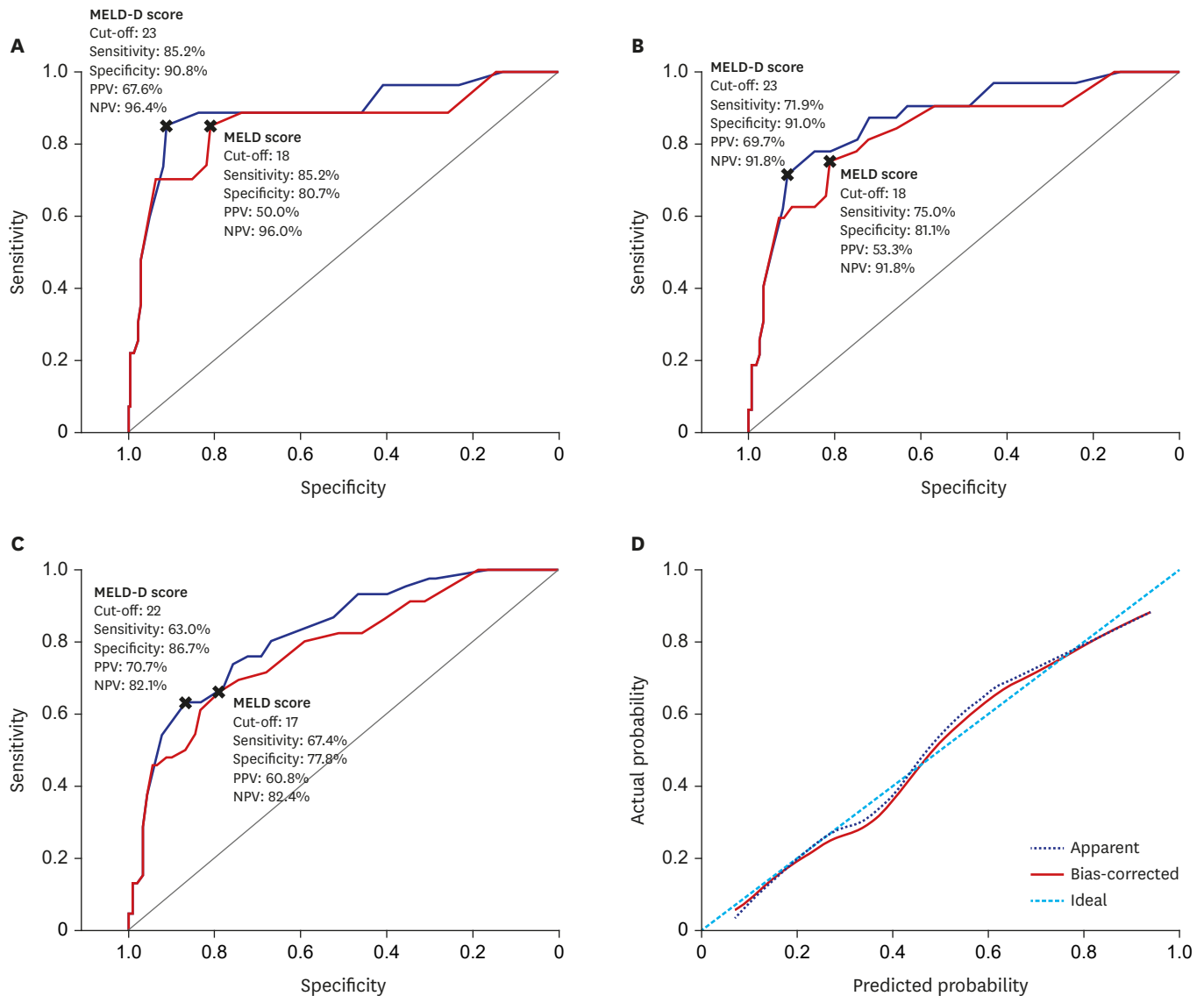
in place of their component variables similarly demonstrated that severe vitamin D deficiency remained a significant independent predictor for primary outcome, even after adjusting for Child-Pugh or MELD scores (Table 2).

### Vitamin D deficiency and survival in relation to Child-Pugh or MELD scores

Next, in subgroup analyses, we investigated whether vitamin D deficiency acted as an independent predictor for survival in all spectrums of patients with cirrhosis. We found that the survival difference between those with and without severe vitamin D deficiency was only recognized in the subgroups corresponding to Child-Pugh scores of 5–10 (Fig. 2B) or MELD scores ≤ 20 (Fig. 2D). There was no significant difference in survival according to the presence or absence of severe vitamin D deficiency in patient groups with Child-Pugh scores of 11–15 (Fig. 2C) or MELD scores > 20 (Fig. 2E). In addition, serum 25(OH)D3 levels in those with and without severe vitamin D deficiency showed large differences in the patient groups corresponding to Child-Pugh scores of 5–10 (3.4 ± 1.3 and 13.0 ± 8.2 ng/mL, respectively) and MELD scores ≤ 20 (3.4 ± 1.3 and 12.7 ± 8.1 ng/mL, respectively). In contrast, in patient groups with high Child-Pugh or MELD scores, serum 25(OH)D3 levels in those with and without severe vitamin D deficiency were 2.9 ± 1.2 and 7.1 ± 3.7 ng/mL, respectively, for the group with Child-Pugh scores of 11–15, and 2.8 ± 1.2 and 6.4 ± 2.0 ng/mL, respectively, for the group with MELD scores > 20.

### Predictive power of a combination model containing MELD score and vitamin D deficiency

To further confirm the usefulness of serum 25(OH)D3 levels in predicting the survival of patients with cirrhosis, we incorporated the presence of severe vitamin D deficiency into the MELD score and constructed a new prognostic model, named the MELD-D score. This score

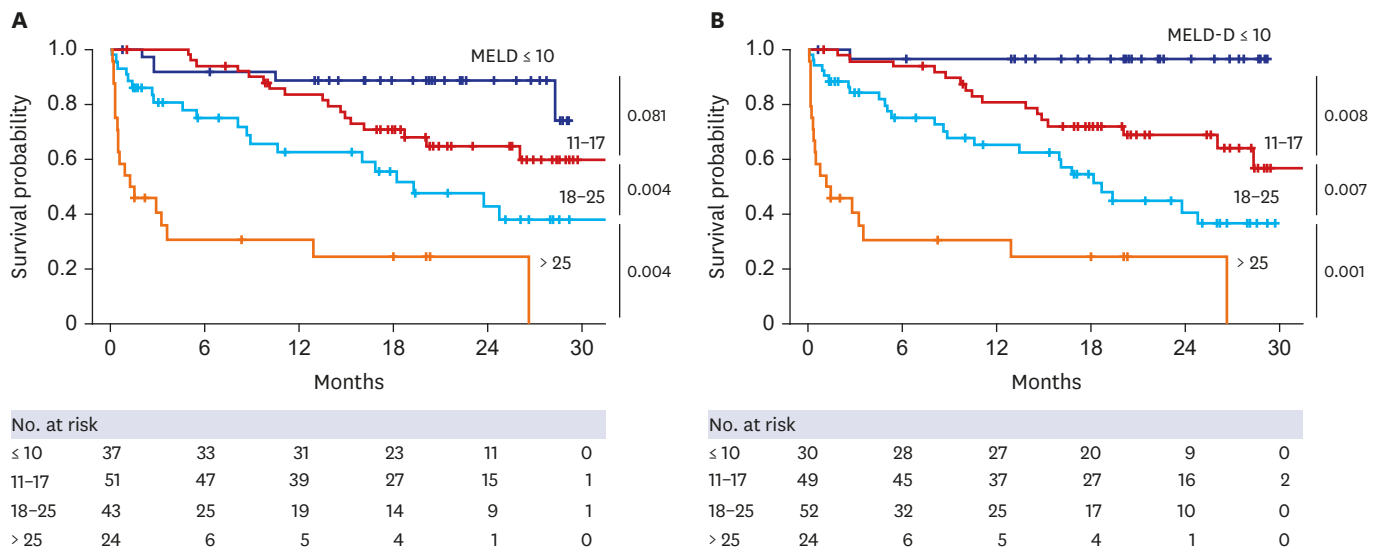


**Fig. 3.** Comparing predictivity of MELD-D and MELD scores for short- and long-term survival. **(A)** AUROC curves for 3-month mortality. **(B)** AUROC curves for 6-month mortality. **(C)** AUROC curves for 12-month mortality. MELD-D scores had significantly higher AUCs than MELD scores through 3-, 6-, 12-months (AUC: 0.889 vs. 0.855, 0.870 vs. 0.833, and 0.823 vs. 0.782;  $P = 0.018, 0.011, \text{ and } 0.009$ , respectively). **(D)** Calibration plot for 12-month mortality. The mean absolute error was 0.019 using 40 repetitions of bootstrap. MELD-D = Model for End-stage Liver Disease with vitamin D, MELD = Model for End-stage Liver Disease, AUROC = area under the receiver operating characteristic curve, AUC = area under the curve, PPV = positive predictive value, NPV = negative predictive value.

was calculated by adding five points to patients with MELD scores  $\leq 20$  and severe vitamin D deficiency, based on a beta regression coefficient of severe vitamin D deficiency after adjusting for MELD score. Patients with a MELD score  $> 20$  or those without severe vitamin D deficiency, had a MELD-D score that was the same as the MELD score.

Then, we calculated the area under the ROC curve (AUROC) for 3-, 6-, and 12-month mortality to compare the performance of the MELD and MELD-D scores. AUROC of the MELD-D score was significantly higher than those of the MELD score, as shown in **Fig. 3A–C** (0.889 vs. 0.855, 0.870 vs. 0.833, and 0.823 vs. 0.782;  $P = 0.018, 0.011, \text{ and } 0.009$ , respectively).





**Fig. 4.** Kaplan–Meier plot for primary outcome according to four categories of MELD and MELD-D scores. (A) MELD and (B) MELD-D scores were stratified into four categories: ≤ 10, 11–17, 18–25, and > 25. These categories resulted from the optimization process of MELD categorization. Cumulative survival rates during the follow-up period were globally more differentiated according to categories in MELD-D scores than MELD scores. Especially, between categories of ≤ 10 and 11–17, MELD-D scores significantly discriminated survival, but MELD scores did not ( $P = 0.008$  vs.  $0.081$ ). The numbers mentioned to the right of each graph indicate  $P$  values between groups.

MELD = Model for End-stage Liver Disease, MELD-D = Model for End-stage Liver Disease with vitamin D.

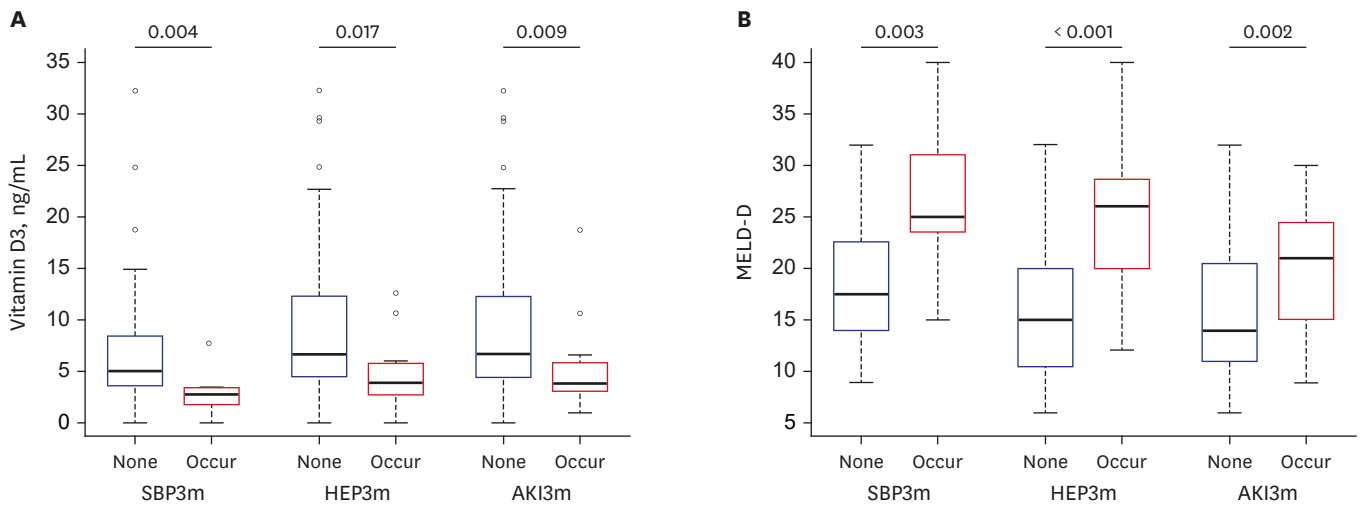
The MELD–D model showed a good overall calibration for 12-month mortality, even with a bias-corrected plot by internal validation (Fig. 3D). Hosmer-Lemeshow test also showed no significant difference between the predicted and actual probability ( $P = 0.525$ ).

In addition, the MELD-D score could differentiate the survival of cirrhotic patients better than the MELD score when the entire cohort was divided into four optimal sections (6–10, 11–17, 18–25, > 25;  $P = 0.006$  vs.  $0.061$ ) (Fig. 4). The cumulative survival probabilities at 3-, 6-, and 12-months were 96.6%, 96.6%, and 96.6% for MELD-D 6–10; 95.8%, 93.7%, and 80.8% for MELD-D 11–17; 84.1%, 75.0%, and 63.2% for MELD-D 18–25; and 40.7%, 30.6%, and 30.6% for MELD-D > 25 sections, respectively.

### Serum 25(OH)D3 level as a risk factor for cirrhosis complications

We investigated new occurrences of SBP, overt encephalopathy attack, and AKI in patients without each complication at baseline. Among 72 patients who had uninfected ascites at baseline and were followed over 3 months, serum 25(OH)D3 levels were significantly lower in those who experienced SBP ( $n = 8$ ) than in those who did not (median [IQR], 2.8 [1.8–3.4] vs. 5.0 [3.7–8.4] ng/mL;  $P = 0.004$ ) (Fig. 5A). Among the 110 patients who had no overt hepatic encephalopathy at baseline and were followed-up, serum 25(OH)D3 levels were significantly lower in those who newly developed overt encephalopathy within 3 months of inclusion ( $n=11$ ) than in those who did not (3.9 [2.8–5.8] vs. 6.7 [4.5–12.3] ng/mL;  $P = 0.017$ ). Of the 114 patients without AKI at baseline, serum 25(OH)D3 levels were significantly lower in those who suffered AKI within 3 months of inclusion ( $n = 15$ ) than in those who did not (3.8 [3.1–5.8] vs. 6.7 [4.4–12.3] ng/mL;  $P = 0.009$ ).

Furthermore, MELD-D score was significantly increased in patients who experienced new episodes of complications within 3 months of inclusion than in those who did not (Fig. 5B). The score was 25.0 (23.5–31.0) vs. 17.5 (14.0–22.5) ( $P = 0.003$ ) for SBP, 26.0 (20.0–28.5) vs. 15.0



**Fig. 5.** Levels of serum 25(OH)D3 and MELD-D based on the new occurrence of cirrhotic complications at baseline and within three months. **(A)** Levels of serum 25(OH)D3 according to new occurrences of cirrhotic complications, including SBP, HEP, and AKI. **(B)** MELD-D scores according to new occurrences of cirrhotic complications. The numbers above the thick lines at the top of graphs indicate *P* values between the patients with and without each complication, respectively. Lower 25(OH)D3 and higher MELD-D scores were associated with new occurrences of cirrhotic complications within three months. 25(OH)D3 = 25-hydroxyvitamin D3, MELD-D = Model for End-stage Liver Disease with vitamin D, SBP = spontaneous bacterial peritonitis, HEP = overt hepatic encephalopathy, AKI = acute kidney injury.

(10.5–20.0) ( $P < 0.001$ ) for overt encephalopathy, and 21.0 (15.0–24.5) vs. 14.0 (11.0–20.5) ( $P = 0.002$ ), respectively.

## DISCUSSION

Recently, vitamin D has attracted attention as a pro-survival molecule. Many researchers have revealed that this molecule has pleiotropic effects in relation to cell differentiation, proliferation, and anti-inflammatory processes.<sup>28</sup> Moreover, many studies have indicated that the deficiency of this vitamin is related to the progression and severity of various pathologic conditions, such as infection, cardiovascular disease, degenerative disease, and cancer.<sup>29,30</sup> In particular, close correlations were demonstrated between vitamin D levels and the status of chronic liver disease, possibly because of the importance of the liver's role in the activation of vitamin D. Several studies focused on the association of serum 25(OH)D levels and the severity and prognosis of patients diagnosed with cirrhosis.<sup>11-17</sup> In most studies, except one,<sup>13</sup> vitamin D levels were significantly correlated with the conventional liver function scoring systems, such as Child-Pugh and MELD scores. In the present study, we have clearly shown that serum 25(OH)D3 levels were inversely correlated with Child-Pugh and MELD scores.

However, it is controversial whether serum vitamin D levels have any potential role in predicting the mortality of cirrhosis patients independently of conventional prognostic models. Putz-Bankuti et al.<sup>12</sup> found a significant association of serum 25(OH)D levels with hepatic decompensation and mortality in patients with chronic liver failure; however, these associations were largely attenuated towards non-significant trends after adjustments for Child-Pugh or MELD scores. Furthermore, a French study showed no significant association between vitamin D deficiency and mortality in patients with cirrhosis, although those with vitamin deficiency were more susceptible to bacterial infection.<sup>13</sup> In contrast, two studies from Germany and Belgium demonstrated that low vitamin D levels had independent associations with survival even after adjusting for MELD scores.<sup>11,14</sup> Largely consistent with

these latter two studies, the present study demonstrated that serum 25(OH)D3 levels could be an independent prognostic factor for survival, as shown in **Table 2**.

Unlike these two studies, however, our study clarified that the independent prognostic role of serum 25(OH)D3 levels were not relevant in all spectra of patients with liver cirrhosis but were limited only to those with mild to moderate degrees of hepatic decompensation. This could be explained as follows. First, serum vitamin D levels might reflect the deterioration of liver function earlier than Child-Pugh or MELD scores. Levels of transporters of vitamin D (vitamin D-binding protein and albumin) and enzymatic activity of the cytochrome P450 family for hydroxylation could decrease in the early phase of liver dysfunction.<sup>31-34</sup> These changes may be directly reflected in the reduced serum 25(OH)D3 levels, but might not yet affect Child-Pugh or MELD scores.

Second, the actual differences in serum 25(OH)D3 levels between those with and without severe vitamin D deficiency might not be so prominent as to affect survival in severely decompensated patients with a high Child-Pugh score > 10 or MELD score > 20, compared with those without these scores.

Third, vitamin D deficiency itself appears to render patients with mild hepatic decompensation more susceptible to increased hepatic decompensation. According to recent studies, decompensation of cirrhosis is frequently caused by gut microbiota invasion, endotoxemia, and systemic inflammation developing with the progression of portal hypertension and impaired liver function.<sup>35,36</sup> Vitamin D appears to modulate T-cell immune response and exert anti-inflammatory and anti-bacterial effects.<sup>18,36</sup> In particular, vitamin D-dependent LL-37 activation pathway is one of the major antibacterial mechanisms in humans.<sup>37</sup> Furthermore, vitamin D has been demonstrated to regulate tight junctions, epithelial barriers, and gut microbiota of the intestine, suggesting a protective effect against endotoxemia from the gut.<sup>38</sup> Indeed, many clinical studies have shown an association between low 25(OH)D levels and hepatic decompensation, such as SBP and HEP, in patients with cirrhosis.<sup>11,12,15,17</sup> The present study also showed that the risks of impending SBP, overt encephalopathy, and AKI had significant associations with lower levels of serum 25(OH)D3. High susceptibility to decompensation events ultimately affects survival of patients with cirrhosis.

Serum 25(OH)D levels in the present study were slightly lower in the overall population and in each Child-Pugh grades, as compared to the study based on European participants.<sup>12</sup> Most of the previous studies on vitamin D in chronic liver diseases have been performed on Western populations. Therefore, the data in the present study would provide clinically meaningful information for patients in Asian countries because vitamin D levels can vary according to ethnicity and region.<sup>21</sup> Besides, unlike in previous studies, the present study focused on serum 25(OH)D3 levels instead of 25(OH)D levels. Serum 25(OH)D includes 25(OH)D2, which is less effective than 25(OH)D3 and exists in the body only after the intake of dietary supplements.<sup>20</sup> Thus, serum 25(OH)D3 levels might serve as a more reliable marker than 25(OH)D levels without the influence of external factors.

The present study also has certain limitations. The results were analyzed based on a retrospective design and included only hospitalized patients with cirrhosis, although all the cirrhotic patients hospitalized during the study period were included. Therefore, selection bias might have occurred, and all confounding factors related to vitamin D metabolism, such as genetic polymorphism, could not be implicated in the analyses. Moreover, the relatively

small sized cohort limited further statistical analysis and disallowed internal cross-validation of MELD-D scores. Thus, a validation study must be performed using a large cohort of patients with cirrhosis.

Our results suggest that serum 25(OH)D3 level testing would be helpful in managing patients with cirrhosis. The MELD-D score, combined with MELD score and 25(OH)D3 levels, had a better predictivity for short-term and long-term prognosis, than the conventional MELD score. In addition, low serum 25(OH)D3 levels and high MELD-D scores appear to be closely associated with a significantly high risk of impending SBP, overt HEP, and AKI, ultimately leading to death. Thus, careful observation of these patients and the avoidance of aggravating factors, such as hypovolemia, infection, and hepatotoxic agents are required. Although controversy remains regarding the effect of sunlight exposure and vitamin D3 supplementation, these factors could be considered in cirrhotic patients with severe vitamin D3 deficiency.

In conclusion, serum 25(OH)D3 level is a significant independent prognostic factor for patients with liver cirrhosis and has a differential impact on disease outcomes according to MELD and Child-Pugh scores. Therefore, serum 25(OH)D3 levels should be measured in patients with cirrhosis. Additionally, our results warrant a validation of MELD-D model for diverse large cohorts of patients with cirrhosis.

## REFERENCES

1. Mokdad AA, Lopez AD, Shahrzaz S, Lozano R, Mokdad AH, Stanaway J, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC Med* 2014;12(1):145.  
[PUBMED](#) | [CROSSREF](#)
2. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60(8):646-9.  
[PUBMED](#) | [CROSSREF](#)
3. Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000;31(4):864-71.  
[PUBMED](#) | [CROSSREF](#)
4. Kim WR, Biggins SW, Kremers WK, Wiesner RH, Kamath PS, Benson JT, et al. Hyponatremia and mortality among patients on the liver-transplant waiting list. *N Engl J Med* 2008;359(10):1018-26.  
[PUBMED](#) | [CROSSREF](#)
5. Kim TH, Ku DH, Um SH, Lee HA, Park SW, Chang JM, et al. How can we improve the performance of Model for End-Stage Liver Disease sodium score in patients with hepatitis B virus-related decompensated liver cirrhosis commencing antiviral treatment? *J Gastroenterol Hepatol* 2018;33(9):1641-8.  
[PUBMED](#) | [CROSSREF](#)
6. Matsumura T, Kato T, Sugiyama N, Tasaka-Fujita M, Murayama A, Masaki T, et al. 25-hydroxyvitamin D3 suppresses hepatitis C virus production. *Hepatology* 2012;56(4):1231-9.  
[PUBMED](#) | [CROSSREF](#)
7. Chan HL, Elkhatab M, Trinh H, Tak WY, Ma X, Chuang WL, et al. Association of baseline vitamin D levels with clinical parameters and treatment outcomes in chronic hepatitis B. *J Hepatol* 2015;63(5):1086-92.  
[PUBMED](#) | [CROSSREF](#)
8. García-Álvarez M, Pineda-Tenor D, Jiménez-Sousa MA, Fernández-Rodríguez A, Guzmán-Fulgencio M, Resino S. Relationship of vitamin D status with advanced liver fibrosis and response to hepatitis C virus therapy: a meta-analysis. *Hepatology* 2014;60(5):1541-50.  
[PUBMED](#) | [CROSSREF](#)
9. Arai T, Atsukawa M, Tsubota A, Koeda M, Yoshida Y, Okubo T, et al. Association of vitamin D levels and vitamin D-related gene polymorphisms with liver fibrosis in patients with biopsy-proven nonalcoholic fatty liver disease. *Dig Liver Dis* 2019;51(7):1036-42.  
[PUBMED](#) | [CROSSREF](#)

10. Ebadi M, Bhanji RA, Mazurak VC, Lytvyak E, Mason A, Czaja AJ, et al. Severe vitamin D deficiency is a prognostic biomarker in autoimmune hepatitis. *Aliment Pharmacol Ther* 2019;49(2):173-82.  
[PUBMED](#) | [CROSSREF](#)
11. Trépo E, Ouziel R, Pradat P, Momozawa Y, Quertinmont E, Gervy C, et al. Marked 25-hydroxyvitamin D deficiency is associated with poor prognosis in patients with alcoholic liver disease. *J Hepatol* 2013;59(2):344-50.  
[PUBMED](#) | [CROSSREF](#)
12. Putz-Bankuti C, Pilz S, Stojakovic T, Scharnagl H, Pieber TR, Trauner M, et al. Association of 25-hydroxyvitamin D levels with liver dysfunction and mortality in chronic liver disease. *Liver Int* 2012;32(5):845-51.  
[PUBMED](#) | [CROSSREF](#)
13. Anty R, Tonohouan M, Ferrari-Panaia P, Piche T, Pariente A, Anstee QM, et al. Low levels of 25-hydroxy vitamin D are independently associated with the risk of bacterial infection in cirrhotic patients. *Clin Transl Gastroenterol* 2014;5(5):e56.  
[PUBMED](#) | [CROSSREF](#)
14. Stokes CS, Krawczyk M, Reichel C, Lammert F, Grünhage F. Vitamin D deficiency is associated with mortality in patients with advanced liver cirrhosis. *Eur J Clin Invest* 2014;44(2):176-83.  
[PUBMED](#) | [CROSSREF](#)
15. Kubesch A, Quenstedt L, Saleh M, Rüschenbaum S, Schwarzkopf K, Martinez Y, et al. Vitamin D deficiency is associated with hepatic decompensation and inflammation in patients with liver cirrhosis: a prospective cohort study. *PLoS One* 2018;13(11):e0207162.  
[PUBMED](#) | [CROSSREF](#)
16. Ramadan HK, Makhlof NA, Mahmoud AA, Abd Elrhman M, El-Masry MA. Role of vitamin D deficiency as a risk factor for infections in cirrhotic patients. *Clin Res Hepatol Gastroenterol* 2019;43(1):51-7.  
[PUBMED](#) | [CROSSREF](#)
17. Yousif MM, Sadek AM, Farrag HA, Selim FO, Hamed EF, Salama RI. Associated vitamin D deficiency is a risk factor for the complication of HCV-related liver cirrhosis including hepatic encephalopathy and spontaneous bacterial peritonitis. *Intern Emerg Med* 2019;14(5):753-61.  
[PUBMED](#) | [CROSSREF](#)
18. Chirumbolo S, Björklund G, Sboarina A, Vella A. The role of vitamin D in the immune system as a pro-survival molecule. *Clin Ther* 2017;39(5):894-916.  
[PUBMED](#) | [CROSSREF](#)
19. Budhathoki S, Hidaka A, Yamaji T, Sawada N, Tanaka-Mizuno S, Kuchiba A, et al. Plasma 25-hydroxyvitamin D concentration and subsequent risk of total and site specific cancers in Japanese population: large case-cohort study within Japan Public Health Center-based Prospective Study cohort. *BMJ* 2018;360:k671.  
[PUBMED](#) | [CROSSREF](#)
20. Konstantakis C, Tselekouni P, Kalafateli M, Triantos C. Vitamin D deficiency in patients with liver cirrhosis. *Ann Gastroenterol* 2016;29(3):297-306.  
[PUBMED](#) | [CROSSREF](#)
21. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med* 2013;369(21):1991-2000.  
[PUBMED](#) | [CROSSREF](#)
22. Robinson-Cohen C, Hoofnagle AN, Ix JH, Sachs MC, Tracy RP, Siscovick DS, et al. Racial differences in the association of serum 25-hydroxyvitamin D concentration with coronary heart disease events. *JAMA* 2013;310(2):179-88.  
[PUBMED](#) | [CROSSREF](#)
23. Shah I, Akhtar MK, Hisaindee S, Rauf MA, Sadig M, Ashraf SS. Clinical diagnostic tools for vitamin D assessment. *J Steroid Biochem Mol Biol* 2018;180:105-17.  
[PUBMED](#) | [CROSSREF](#)
24. O'Shea RS, Dasarathy S, McCullough AJ; Practice Guideline Committee of the American Association for the Study of Liver Diseases, Practice Parameters Committee of the American College of Gastroenterology. Alcoholic liver disease. *Hepatology* 2010;51(1):307-28.  
[PUBMED](#) | [CROSSREF](#)
25. Córdoba J. New assessment of hepatic encephalopathy. *J Hepatol* 2011;54(5):1030-40.  
[PUBMED](#) | [CROSSREF](#)
26. Wong F. Acute kidney injury in liver cirrhosis: new definition and application. *Clin Mol Hepatol* 2016;22(4):415-22.  
[PUBMED](#) | [CROSSREF](#)

27. Ko BJ, Kim YS, Kim SG, Park JH, Lee SH, Jeong SW, et al. Relationship between 25-hydroxyvitamin D levels and liver fibrosis as assessed by transient elastography in patients with chronic liver disease. *Gut Liver* 2016;10(5):818-25.  
[PUBMED](#) | [CROSSREF](#)
28. Kwok RM, Torres DM, Harrison SA. Vitamin D and nonalcoholic fatty liver disease (NAFLD): is it more than just an association? *Hepatology* 2013;58(3):1166-74.  
[PUBMED](#) | [CROSSREF](#)
29. Kitson MT, Roberts SK. D-livering the message: the importance of vitamin D status in chronic liver disease. *J Hepatol* 2012;57(4):897-909.  
[PUBMED](#) | [CROSSREF](#)
30. Zhao G, Ford ES, Li C, Croft JB. Serum 25-hydroxyvitamin D levels and all-cause and cardiovascular disease mortality among US adults with hypertension: the NHANES linked mortality study. *J Hypertens* 2012;30(2):284-9.  
[PUBMED](#) | [CROSSREF](#)
31. Masuda S, Okano T, Osawa K, Shinjo M, Suematsu T, Kobayashi T. Concentrations of vitamin D-binding protein and vitamin D metabolites in plasma of patients with liver cirrhosis. *J Nutr Sci Vitaminol (Tokyo)* 1989;35(4):225-34.  
[PUBMED](#) | [CROSSREF](#)
32. Schwartz JB, Lai J, Lizaola B, Kane L, Markova S, Weyland P, et al. A comparison of measured and calculated free 25(OH) vitamin D levels in clinical populations. *J Clin Endocrinol Metab* 2014;99(5):1631-7.  
[PUBMED](#) | [CROSSREF](#)
33. Lai JC, Bikle DD, Lizaola B, Hayssen H, Terrault NA, Schwartz JB. Total 25(OH) vitamin D, free 25(OH) vitamin D and markers of bone turnover in cirrhotics with and without synthetic dysfunction. *Liver Int* 2015;35(10):2294-300.  
[PUBMED](#) | [CROSSREF](#)
34. Petta S, Cammà C, Scazzone C, Tripodo C, Di Marco V, Bono A, et al. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology* 2010;51(4):1158-67.  
[PUBMED](#) | [CROSSREF](#)
35. Piano S, Tonon M, Angeli P. Management of ascites and hepatorenal syndrome. *Hepatol Int* 2018;12(Suppl 1):122-34.  
[PUBMED](#) | [CROSSREF](#)
36. Fiati Kenston SS, Song X, Li Z, Zhao J. Mechanistic insight, diagnosis, and treatment of ammonia-induced hepatic encephalopathy. *J Gastroenterol Hepatol* 2019;34(1):31-9.  
[PUBMED](#) | [CROSSREF](#)
37. Vandamme D, Landuyt B, Luyten W, Schoofs L. A comprehensive summary of LL-37, the factotum human cathelicidin peptide. *Cell Immunol* 2012;280(1):22-35.  
[PUBMED](#) | [CROSSREF](#)
38. Gubatan J, Moss AC. Vitamin D in inflammatory bowel disease: more than just a supplement. *Curr Opin Gastroenterol* 2018;34(4):217-25.  
[PUBMED](#) | [CROSSREF](#)