PIVKA-II is associated with liver function, bone metabolism, and muscle function in patients with liver disease

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Abstract. Protein induced by vitamin K (VK) absence-II (PIVKA-II) is a sensitive marker for diagnosing hepatoma but is occasionally detected in patients without hepatoma Here, the clinical significance of serum PIVKA-II levels in patients who were not administered warfarin and did not have hepatoma or liver disease were evaluated. As VK is related to muscle and bone metabolism, PIVKA-II and clinical factors related to bone and muscle were compared. A total of 441 patients with various liver diseases were evaluated. Of these, 236 patients were female. Clinical factors and anthropometric measurements were obtained for each participant during outpatient visits. Among the clinical factors, type I procollagen N-propeptide (P1NP), a low titer of undercarboxylated osteocalcin (ucOC), and 25(OH) vitamin D (VD) were used as bone metabolic markers, and SARC-F and grip strength were used as muscle-related markers. Serum PIVKA-II levels above the upper limit were associated with Child B/C (Child-Pugh score), high titers of total P1NP, and low titers of ucOC in females, and alcohol-related liver disease and low VD in males. The titer of PIVKA-II were associated with immunoglobulin (Ig) A and prothrombin time (PT)-international normalized ratio (INR) in females, and fibrosis-4-4, IgG, total bilirubin, PT-INR, and SARC-F in males. Elevated PIVKA-II levels were associated with abnormal bone physiology in females, weak muscles in males, and severe liver disease in both sexes. Assessing PIVKA-II may assist in evaluating the clinical and bone-muscle metabolic stages in liver disease. Nutrition and supplementation with fat-soluble vitamins, including VK and VD may thus serve as a potential method to alleviate or prevent bone-muscle pathophysiology in patients with liver disease.

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Abbreviations: DAA, direct-acting antiviral; HCV, hepatitis C virus; eGFR, estimated glomerular filtration rate; Cr, creatinine; CysC, cystatin C; CBMM, calculated body muscle mass; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HOD, hepatic osteodystrophy; TRACP-5b, tartrate-resistant acid phosphatase isoform 5b; ucOC, undercarboxylated osteocalcin; LDL, low-density lipoprotein; sdLDL, small dense low-density lipoprotein; TG, triglyceride; HbA1c, hemoglobin A1c; HOMA, homeostatic model assessment; M2BPGi, mac-2 binding protein glycosylation isomer; FIB-4, fibrosis 4; ALBI, albumin-bilirubin grade; BMD, bone mineral density; YAM, young adult mean; P1NP, procollagen type I intact N-terminal propeptide; VD, 25(OH) vitamin D; VK, vitamin K; PIVKA-II, protein induced by vitamin K absence-II; SVR, sustained virologic response; HBV, hepatitis B virus; HBs, hepatitis B surface; HBc, hepatitis B core; SOF/RBV, sofosbuvir/ribavirin; γGTP, γ glutamyl transpeptidase

Key words: PIVKA-II, PT-INR, Child-Pugh score, bone metabolic marker, SARC-F

Introduction

Liver cancer is the sixth most prevalent cancer and the third leading cause of mortality globally (1). Hepatocellular carcinoma (HCC) accounts for 85-90% of primary liver cancers (2). Recently, the incidence of HCCs derived from non-hepatitis B virus and non-hepatitis C virus (NBNC) has been increasing, and protein induced by vitamin K (VK) absence-II (PIVKA-II) is more sensitive than α-fetoprotein for the diagnosis of NBNC-HCC (3,4). Therefore, PIVKA-II is frequently measured for HCC screening in liver diseases of various origins, and a high PIVKA-II value is occasionally observed in patients without HCC.

The precursor of prothrombin (PT) is converted to prothrombin by a VK-dependent enzymatic reaction of

c-glutamyl carboxylase (5). Under physiological conditions, this enzymatic process completely converts all ten glutamic acid (Glu) residues to γ-carboxylated glutamic acid (5). When this reaction is disturbed, PIVKA-II, which carries Glu residues, is produced (5). There is an aberrant increase in PIVKA-II in patients with obstructive jaundice, VK deficiency, or in those consuming warfarin, resulting from a problem with the current methodology for PIVKA-II measurement (5). Patients with alcoholic liver disease (ALD) administered antibiotics have increased serum PIVKA-II levels (6,7). Of note, sex differences in PIVKA-II were not found (8).

PIVKA-II is produced in the liver, but VK-dependent proteins are present in the bone. Osteocalcin is produced by osteoblasts and gains hydroxyapatite-binding ability through g-carboxylated glutamic acid (9). The levels of circulating undercarboxylated osteocalcin (ucOC) are elevated in elder women, and this is predictive of a subsequent risk of hip fractures (10). VK intake was significantly correlated with serum PIVKA-II and ucOC/OC levels but not serum ucOC levels (11). Significantly higher doses of VK are required for the γ-carboxylation of osteocalcin than for blood coagulation factors (12). Recent advances have indicated that ucOC is not only a nutritional biomarker reflective of VK status and an indicator of bone health but also an active hormone that mediates glucose metabolism (13). ucOC showed an inverse correlation with markers of insulin resistance, central obesity, and the presence of metabolic syndrome in postmenopausal women and appeared to protect against metabolic syndrome (14). ucOC levels are inversely associated with glycemic index and insulin resistance in a population of Japanese men (15). Similar to ucOC, OC also showed an inverse correlation with markers of insulin resistance, central obesity, and the presence of metabolic syndrome in postmenopausal women, and osteocalcin levels were inversely associated with glycemic index and insulin resistance in a population of Japanese men, and after adjustment for confounding glucose, lipid, and bone metabolism parameters, the male and female participants within the lowest quartile of OC still exhibited more severe liver steatosis (16). That is OC and ucOC are related to metabolic and nutrition status.

VK deficiency is related to PIVKA-II and is common in cholestatic liver disease (17). VK is naturally present as phylloquinone synthesized by green plants and menaquinones produced by intestinal bacteria. Dietary phylloquinones are the primary source of VK in humans (17). VK deficiency is usually diagnosed by measuring prothrombin time (PT), which is prolonged in different forms of liver disease (18). All cholestatic adults and children with an elevated PT-international normalized ratio (PT-INR) are VK-deficient (17). Approximately 20-40% of patients with cirrhosis have coagulation abnormalities, regardless of cholestatic liver disease (19).

In the present study, the clinical significance of serum PIVKA-II in patients who did not take warfarin and did not have HCC or liver disease was evaluated. The degree of severity of liver disease was compared to PIVKA-II levels. Recently, attention has been paid to the relationship between sarcopenia and liver disease (20). Hepatic osteodystrophy (HOD) has also been reported to be a critical complication of chronic liver disease (21). VK is associated with muscle and bone metabolism (10,13). Therefore, PIVKA-II levels were compared with clinical bone muscle-related factors.

Patients and methods

Patients. A total of 441 patients with liver disease who visited Nagasaki Harbor Medical Center between April 2021 and March 2022 were initially recruited. The median age of the patients was 69 years, and the age range was 16-93 years. Of these, 236 patients were female, and 205 patients were male: 19 patients presented with autoimmune hepatitis (AIH), 30 patients with ALD, 102 patients were treated with naïve hepatitis B virus (HBV), 31 patients were naïve to Tenofovir Alafenamide Fumarate (TAF) treatment (Vemlidy, Gilead Sciences). A total of 102 patients had treatment-naive hepatitis C virus (HCV), 38 patients were judged to have a sustained viral response (SVR) 24 weeks after the end of direct-acting anti-viral treatment, 93 patients had nonalcoholic fatty liver disease (NAFLD), and 15 patients had treatment-naïve primary biliary cholangitis (PBC). PBC was treated with ursodeoxycholic acid (UDCA) in 18 patients. Heavy alcohol consumption was defined as >7 drinks per week for women and >14 drinks per week for men (22). The diagnoses of hypertension and hyperlipidemia were based on the history and use of oral medications. In the present study, diabetes mellitus status was evaluated based on patient history and prescribed medication at recruitment. Proton pump inhibitor users and patients on osteoporosis medication were assessed using medical records. The inclusion criterion was patients with chronic liver disease, and the exclusion criteria were hepatoma complications and warfarin use at entry. However, cancers without hepatomas were included.

The medical records of 441 patients were retrospectively reviewed. All laboratory measurements were obtained from the medical records. Informed consent was obtained from each patient included in the study, and they were guaranteed the right to leave the study if desired. The study protocol conformed to the guidelines of the 1975 Declaration of Helsinki (23), and was approved by the Human Research Ethics Committee of the Nagasaki Harbor Medical Center (approval no. H30-031).

Laboratory measurements. Laboratory data and anthropometric measurements were obtained for each participant during the outpatient visit. The body mass index of each patient was calculated by dividing their weight in kilograms by the square of their height in meters. Grip strength (GS) was measured using a dynamometer (Smedley Dynamo Meter; TTM), with participants standing in an erect position with both arms at their sides. The maximum values of the two tests were used for further analysis. Using the JSH criteria, female patients with a maximum GS of <18 kg and male patients with a maximum GS of <26 kg were categorized into the low-GS group (24). SARC-F (25) was evaluated during outpatient visits. Laboratory examinations included total protein, albumin, PT (%), INR, platelet count, creatinine (Cr), cystatin C (CysC), alanine aminotransferase (ALT), alanine aminotransferase (ALT), α-fetoprotein (AFP), PIVKA-II, immunoglobulin (Ig)G, IgM, IgA, total type I procollagen N-propeptide (P1NP), tartrate-resistant acid phosphatase 5b (TRACP-5b), 25(OH) vitamin D (VD), and ucOC. The Child-Pugh (CP) score (26),

ALBI (27), MELD score (28), and fibrosis-4 (FIB-4) index (29) were calculated as previously reported. The normal ranges of various factors were: AST, 10-40 U/l; ALT, 5-45 U/l; platelet counts, 14.0-37.9x10^4/μl; albumin, 3.7-5.5 g/dl; total bilirubin, 0.3-1.2 mg/dl; total protein, 0.3-1.2 mg/dl; prothrombin time (%), 70-130; international normalized ratio (INR), 0.85-1.15; Cr, 0.65-1.09 (male) and 0.46-0.82 (female) mg/dl; CysC is 0.58-0.87 mg/l (male) and 0.47-0.82 (female) mg/l; AFP levels <10 ng/ml; protein levels induced by PIVKA-II <40 mAU/ml; IgG, 820-1,747 mg/dl; IgM, 31-269 mg/dl; IgA, 90-393 mg/dl; grip strength, mean grip strength of both hands; TRACP-5b levels, 170-590 mU/dl (male) and 120-420 mU/dl (female); total P1NP, 18.1-74.1 ng/ml (male), 16.8-70.1 ng/ml (premenopausal female) and 26.4-98.2 ng/ml (postmenopausal female); ucOC, <4.5 ng/ml; low grip strength, <26 kg (male) and 18 kg (female). The P1NP high (P1NPH) group was above the upper limit of P1NP for each sex. The P1NP-N group was within the upper limit. ucOCH refers to an ucOC value >4.5 ng/ml. The PHUN group was the PIVKA-II high range (above the upper limits of 40 mAU/ml) and the ucOC in the normal range group. Additionally, the PIVKA-II and VD insufficiencies were scored. The PIVKA-II-high and VD <10 ng/ml groups had mean scores of 2. The score was 1 if the patient met one of the following criteria: PIVKA-II high or VD <10 ng/ml. The PIVKA-II normal and VD >10 ng/ml groups had scores of 0.

Cr- and CysC-based estimated GFRs (eGFRs) (ml/min/1.73 m^2) in women and men were calculated using the equations provided by the Japanese Society of Nephrology for Japanese patients (30). The sarcopenia index (SI) was calculated as follows: Cr/CysC x100 (31). dGFR was calculated as follows: Cr-based eGFR, CysC-based eGFR (32). The body muscle mass (CBMM) was calculated as follows: [body weight (kg) x Cr]/[(K x body weight (kg) x CysC)+ Cr], where K=0.00675 for men and 0.01006 for women (33).

Statistical analysis. Data were analyzed using StatFlex (version 6.0; Artech Co., Ltd.) and are presented as the mean ± SD. Laboratory variables were compared using t-tests (for differences between the two groups) and χ^2 tests. A multi-regression analysis was performed. A standardized partial regression coefficient, β, was employed. Univariate and multivariate analyses were performed using logistic regression analysis. Correlations were evaluated using Pearson's correlation coefficient (R). Analysis of the detection level was performed using the receiver operating characteristic curve (ROC) method. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 441 patients with liver disease were analyzed (Table I). The present study included 236 female and 205 male patients. The PIVKA-II high group (above the upper limits of 40 mAU/ml) included 47 cases. VD was categorized as severe deficiency (0-10 ng/ml), deficiency (10-20 ng/ml), insufficiency (20-30 ng/ml), or normal (30 ng/ml). The low VD group had severe deficiency (102 cases). The ucOC high group (158 cases) had levels above the upper limit of ucOC. There were 91 female and 84 male patients aged >65 years (P=0.5803).

Association of PIVKA-II with clinical factors. First, the clinical factors between the PIVKA-II-high and normal groups were compared (Table II). In females and males, AST, MELD, CPS, AFP, IgM and IgA in the PIVKA-II high group were higher than in the normal group and albumin, PT (%), PT-INR, ALBI, and VD in the PIVKA-II high group were lower than in the normal group. In females, FIB-4-high in the PIVKA-II high group was lower than in the normal group, and ucOC in the the PIVKA-II high group was lower than in the normal group. In males, total bilirubin, FIB-4, deGFR, PINP, and IgG in the PIVKA-II high group were higher than in the normal group, and sarcopenia index and SARC-F high in the PIVKA=II high group was lower than in the normal group. FIB-4 and PIVKA-II exhibited a positive relationship in males but not in females (Fig. S1A). Total bilirubin levels were also positively correlated with PIVKA-II in males but not in females (Fig. S1B). CP score (Fig. S1C), PT-INR (Fig. S1D), IgG (Fig. S2A) and IgA (Fig. S2B) were positively correlated with PIVKA-II in both sexes. Second, the factors contributing to high PIVKA-II using logistic regression analysis were analyzed (Table II). In females, the univariate analysis identified CPG, ALBIG, ALD, FIB-4, P1NP, VD, ucOC, and IgA as contributing factors. Since CPG had a higher P-value than ALBIG in the index of hepatic reserves, CPG was used in the multivariate analysis. CPG, P1NP, and ucOC were contributing factors in the multivariate analysis. Conversely, CPG, ALBIG, deGFR, SI, SARC-F, AFP, PT%, VD, IgG, and IgA were the contributing factors in the univariate analysis in males. Alcohol consumption and VD were found to be contributing factors in the multivariate analysis. Third, the clinical factors contributing to the PIVKA-II value were evaluated (Table III). In the univariate regression analysis, the significant factors identified in Table II were used. CPS, ALBI, IgG, IgA, albumin, and PT-INR were contributing factors in females. As CPS included PT-INR, and ALBI included albumin, IgG, IgA, albumin, and PT-INR were used for the multivariate analysis. IgA and PT-INR were associated with PIVKA-II in females. In males, CPS, ALBI, FIB-4, SI, deGFR, IgG, IgA, total bilirubin (TB), albumin, PT-INR, TRAC-5b, and SARC-F were contributing factors. FIB-4, IgG, TB, PT-INR, and SARC-F levels were associated with PIVKA-II in the multivariate analysis (Table IV). The ROC curves of PIVKA-II were analyzed for the detection of abnormal muscle (SARC-F 4 High, Fig. S3C) and bone metabolism (P1NP high, Fig. S3D). Since SARC-F in males (Table IV) and PINP in females (Table III) significantly were associated with elevated PIVKA-II levels, the association between PIVKA-II and SAC-F and P1NP was analyzed using ROC curves. ucOC was omitted for ROC as ucOC and PIVKA-II are VK-dependent proteins, CPGA/BC, ALD/others, and IgA were not biomarkers for sarcopenia or osteoporosis. The AUC of PIVKA-II for SARC-F high was <0.6 (low), but for P1NP it was moderate (0.6267) in females. In particular, in the low level of VD (1-10), the PIVKA-II detection level for P1NP high was an AUC of 0.7187 in females. However, there was no significant difference between PIVKA-II and VD for the detection of SARC-F high (Fig. S3E-H) and P1NP high (S3H-L) in both low (S3E, G, I, and K) and normal VD (S3F, H, J, and L). As a result, PIVKA-II plus VD could be used for the diagnosis of high SARC-F4 (Fig. S3E-H) and high P1NP (S3H-L) but they were no more effective than PIVKA-II alone.

Table I. Clinical characteristics.

Factor	Female, n=237	Male, n=205
Disease[a]		
AIH naïve	8 (3.37)	1 (0.488)
AIH with PSL	6 (2.53)	4 (1.95)
ALD	6 (2.53)	24 (11.7)
HBV naïve	29 (12.2)	35 (17.1)
HBV with TAF	7 (2.95)	14 (6.83)
HCV naïve	50 (21.1)	52 (25.4)
HCV after SVR	21 (8.86)	17 (8.29)
NAFLD	60 (25.3)	34 (16.6)
PBC naïve	10 (4.22)	1 (0.488)
PBC with UDCA	18 (7.59)	4 (1.95)
Others	22 (9.28)	19 (9.27)
Age, years[b]	69 (18-91)	68 (16-93)
≥65	146 (61.2)	121 (59)
<65	75 (38.8)	
Total bilirubin, mg/dl[b]	0.7 (0.2-17.7)	0.9 (0.2-8.4)
Total protein, g/dl[b]	7.3 (5.6-10.7)	7.3 (5.2-9.2)
Albumin, g/dl[b]	4.1 (2.4-5.1)	4.1 (2-5)
PT (%)[b]	98.4 (45.1-147)	94.4 (34.9-139)
PT-INR[b]	1.01 (0.84-1.51)	1.03 (0.87-1.77)
Child Pugh score[b]	5 (5-10)	5 (5-12)
Grade A[a]	229 (96.6)	185 (90.2)
Grade B[a]	6 (2.53)	17 (82.9)
Grade C[a]	2 (0.844)	2 (0.975)
MELD[b]	7 (6-20)	7 (6-44)
Creatine, mg/dl[b]	0.69 (0.41-8.02)	0.89 (0.42-10.25)
Cr-eGFR, ml/min/1.73 m^{2b}	65.9 (4.5-133.9)	65.4 (4.5-176.4)
Cystatin C, mg/l[b]	0.96 (0.51-10.91)	1.13 (0.62-10.81)
CysC-eGFR, ml/min/1.73 m^{2b}	68.3 (-1.5-156.1)	62.3 (-1.1-137.4)
Body mass index, kg/m^{2b}	22.9 (12.87-37.77)	23.25 (15.9-41)
BMI grade[a]		
Lean, <18.5	30 (12.6)	14 (6.83)
Normal, 18.5-25	127 (53.6)	129 (62.9)
Obesity I, 25-30	62 (26.2)	51 (24.9)
Obesity II, 30-35	14 (5.9)	9 (4.39)
Obesity III, 35-40	4 (1.69)	1 (0.488)
Obesity IV, ≥40	0	1 (0.488)
Platelet, $x10^4/\mu l$[b]	18.9 (5.5-91)	16.5 (2.5-34.5)
AST, U/l[b]	29 (2-745)	31 (10-265)
ALT, U/l[b]	23 (6-1,460)	31 (5-579)
FIB-4[b]	2.261 (0.277-15.558)	2.493 (0.42-22.743)
FIB-4 ≥3.25[a]	70 (29.5)	69 (29.1)
ALBI[b]	-2.773 (-3.585-0.664)	-2.764 (-3.53-0.862)

Table I. Continued.

Factor	Female, n=237	Male, n=205
ALBIG[a]		
1	167 (70.5)	132 (64.4)
2	68 (28.7)	67 (32.7)
3	2 (0.843)	6 (2.93)
AFP, ng/ml[b]	4.6 (0.9-49.6)	3.9 (1-413.4)
PIVKA-II, mAU/ml[b]	21 (11-2,957)	23 (9-798)
PIVKA-II, ≥40[a]	22 (9.28)	25 (12.2)
IgG, mg/dl[b]	1,436 (456-5,318)	1,427 (727-4,157)
IgM, mg/dl[b]	97.5 (7-1,187)	83 (117-722)
IgA, mg/dl[b]	251 (43-967)	292.5 (62-1,476)
Grip strength, kg[b]	13.75 (1.75-40.5)	25.875 (0.25-51.75)
Grip strength, low[a]	171 (72.2)	100 (48.8)
deGFR[b]	0.5 (-45-51.3)	4.6 (-76-39)
Sarcopenia index[b]	68.44 (35.66-114.1)	78.48 (48.3-234.72)
CBMM[b]	29.75 (17.19-48.74)	41.74 (29.29-61.51)
CBMM Low[a]	76 (32.1)	82 (40)
SARC-F[b]	1 (0-9)	0 (0-10)
SARC-F, ≥4[a]	48 (20.3)	20 (9.76)
TRACP-5b, mU/dl[b]	371 (45.7-1,501)	363 (133-1,501)
P1NP, ng/ml[b]	51.6 (11.2-1,090)	44.9 (8.7-872)
VD, ng/ml[b]	12.7 (4.1-34.8)	15.1 (1.83-50.4)
VD grade[a]		
1-10	67 (28.3)	35 (17.1)
10-20	126 (53.2)	119 (58)
20-30	32 (13.5)	40 (19.5)
≥30	4 (1.69)	8 (3.9)
ucOC, ng/ml[b]	3.885 (0.38-96.8)	2.86 (0.38-32.9)
ucOC high[a]	103 (43.5)	55 (26.8)

[a]Mean ± SD; [b]Median (range). AIH is an autoimmune hepatitis that occurs prior to treatment. At entry, AIH with PSL is AIH treated with prednisolone.

Factors associated with PIVKA-II in females. Among females, the high PIVKA-II group were associated with the P1NP high group and low ucOC groups (Fig. S2C). PINP is a metabolic marker of bone formation; thus the association between TRACP-5b (a bone resorption marker) and P1NP was evaluated. The TRACP-5b levels in the PINP high group did not differ significantly from those in the normal group (Fig. 1A). However, TRACP-5b levels in the PIVKA-II-high and P1NPH groups were higher than those in the normal

Table II. Differences in the clinical factors between the PIVKA-II high and normal groups.

	Female			Male		
Factors	PIVKA-II high	PIVKA-II normal	P-value	PIVKA-II high	PIVKA-II normal	P-value
Age, years[d]	65 (18-884)	69 (24-94)	0.2655	68 (49-8)	68 (16-93)	0.5539
≥65[e]	11 (50)	133 (62.7)	0.2496	18 (72)	103 (57.2)	0.1592
<65[e]	11 (50)	79 (37.3)		7 (28)	77 (43.8)	
Total bilirubin, mg/dl[f]	0.85 (0.3-17.7)	0.7 (0.2-7.1)	0.0646	1.1 (0.5-8,4)	0.9 (0.2-2.8)	0.0287[a]
Total protein, g/dl[d]	7.2 (5.6-8.4)	7.3 (5.9-10.7)	0.1549	7.25 (6.1-8.3)	7.3 (5.2-9.2)	0.7777
Albumin, g/dl[d]	3.75 (2.5-4.9)	4.2 (2.4-5.1)	0.0005[c]	3.6 (2.3-4.8)	4.2 82-5)	0.0008[c]
PT (%)[d]	86.25 (49.8-108.9)	100.4 (45.1-147)	0.0003[c]	87.7 (34.9-123.7)	94.6 (39.3-139)	0.0382[a]
PT-INR[d]	1.07 (0.96-1.49)	1.0 (0.84-1.51)	0.0003[c]	1.06 (0.92-1.77)	1.02 80.87-1.64)	0.0353[a]
Child-Pugh score[d]	5 (5-10)	5 (5-9)	0.0306[a]	5 (5-12)	5 (5-8)	0.0101[a]
CPG A/BC			<0.0001[d]			<0.0001[d]
A[f]	16 (72.7)	210 (99)		15 (60)	170 (94.4)	
BC[f]	6 (27.3)	2 (81)		10 (40)	9 (5.6)	
MELD[d]	8 (6-15)	7 (6-22)	0.0195[a]	8 (6-23)	8 (6-44)	0.1006
Creatine, mg/dl[d]	0.66 (0.41-1.18	0.69 (0.41-8.02)	0.2684	0.88 (0.56-3.42)	0.895 (0.42-10.25)	0.2601
Cr-eGFR, ml/min/1.73 m^{2}[d]	74.75 (44.8-133.9)	65.9 (4.5-127.5)	0.0618	67.9 (15.3-104.8)	65.2 (4.5-176.4)	0.3542
Cystatin C, mg/l[d]	1.005 (0.57-1.74)	0.95 (0.51-10.91)	0.5169	1.14 (0.77-6.17)	1.12 (0.62-10.81)	0.2466
CysC-eGFR, ml/min/1.73 m^{2}[d]	68.1 (34.6-127.2)	69.1 (-1.5-156.1)	0.7737	61.1 (4.5-98.7)	62.55 (-1.1-137.4)	0.2125
BMI, kg/m^{2}[d]	24.53 (14.38-34.67)	22.62 (12.87-37.77)	0.7775	23 (16.85-29.84)	23.2 (15.9-41)	0.6426
BMI			0.1471			0.274
Low[f]	5 (22.7)	25 (11.8)		3 (12)	11 (6.1)	
High[f]	17 (77.3)	187 (88.2)		22 (88)	169 (93.9)	
Platelet, x10^{4}/μl[d]	17.8 (7.1-91)	19 (5.5-44.5)	0.1842	15.9 (3.7-29.1)	16.55 (2.5-34.5)	0.9155
AST, U/l[d]	39.5 (15-418)	29 (2-745)	0.0232[a]	56 (15-265)	28 (15-265)	0.0002[c]
ALT, U/l[d]	30 (10-607)	23 (6-1,460)	0.48	44 (12-579)	29.5 (5-404)	0.0527
FIB-4[d]	3.152 (0.68-10.53)	2.2158 (0.277-22.74)	0.0543	3.071 (1.105-15.84)	2.292 (0.42-22.74)	0.006[b]
≥3.25[f]	11 (50)	59 (27.8)	0.0319[a]	12 (48)	57 (31.7)	0.1054
<3.25[f]	11 (50)	153 72.2)		13 (52)	123 (68.3)	
ALBI[d]	-2.46 (-3.3- -1.365)	-2.803 (-3.585- -0.664)	0.0002[c]	-2.146 (-3.33- -0.862)	-2.789 (-3.53- -0.916)	0.0003[c]
ALBIG 1/23			0.0071[b]			0.002[b]
1[f]	10 (45.5)	155 (73.1)		3 (12)	123 (68.3)	
2/3[f]	12 (54.5)	57 (26.9)		16 (88)	57 (31.7)	
AFP, ng/ml[d]	5.8 (1.7-36.3)	4.5 (0.9-49.6)	0.0152[a]	6.6 (2.3-138.1)	3.7 (1-413.4)	0.0005[c]
Grip strength, kg[d]	12.63 (4-31	14.5 (1.75-40.5)	0.4546	25.25 (11.25-38.75)	26 (0.25-51.75)	0.1148
Grip strength			0.146			0.2622
Low[f]	15 (68.2)	153 (72.2)		15 (60)	85 (47.2)	
Normal[f]	7 (31.8)	7 (7.8)		10 (40)	92 (52.8)	
deGFR[d]	3.15 (-38.1-51.3)	0.35 (-45-38)	0.0622	11 (-25.2-33.8)	4.4 (-76.2-39)	0.0029[a]

Table II. Continued.

Factors	Female			Male		
	PIVKA-II high	PIVKA-II normal	P-value	PIVKA-II high	PIVKA-II normal	P-value
SARC-F[d]	1 (0-8)	1 (0-9)	0.7138	1 (0-7)	0 (0-10)	0.0692
SARC-F High/normal			0.8416			0.0163[a]
≥4[f]	4 (18.2)	42 (19.8)		6 (24)	14 (7.78)	
<4[f]	15 (81.8)	141 (80.2)		17 (76)	140 (82.2)	
Sarcopenia index[d]	67.9 (35.66-101.43)	69.46 (38.75-114.1)	0.2529	69 (48.3-107.79)	78.79 (52.56-234.72)	0.0007[c]
CBMM[d]	29.16 (21.7-47.33)	29.9 (17.19-48.74)	0.5519	39.58 (29.93-48.13)	41.99 (29.19-61.51)	0.0822
CBMM Low/normal			0.604			0.1912
Low[f]	6 (27.3)	69 (32.5)		13 (52)	69 (38.3)	
Normal[f]	16 (72.7)	143 (67.5)		12 (48)	111 (61.7)	
TRACP-5b, mU/dl[d]	432 (168-1,501)	370 (45.7-1,450)	0.1651	388 (138-997)	363 (133-1501)	0.2702
P1NP, ng/ml[d]	62.9 (20.1-243)	50.85 (11.2-1090)	0.2057	51.1 (9.4-494)	44.1 (8.7-872)	0.0353[a]
VD, ng/ml[d]	9.4 (4.1-21.7)	12.95 (4.6-34.8)	0.0017[b]	12.8 (1.83-26.7)	15.3 (4.2-50.4)	0.0082[b]
VD			0.0152[a]			<0.0001[d]
<10 ng/ml[f]	11 (50)	56 (26.4)		11 (44)	24 (13.3)	
≥10 ng/ml[f]	10 (50)	152 (73.6)		13 (56)	154 (86.7)	
ucOC, ng/ml[d]	2.21 (0.41-32.77)	4.12 (0.38-96.8)	0.0239[a]	2.45 (0.41-16.56)	2.87 (0.38-32.9)	0.4085
IgG, mg/dl[d]	1,593 (796-2,822)	1,427 (456-5,318)	0.0798	1675 (933-4157)	1394 (8727-3668)	0.0186[a]
IgM, mg/dl[d]	132.5 (50-219)	95 (7-1,187)	0.0304[a]	137 (22-305)	82 (17-722)	0.0408[a]
IgA, mg/dl[d]	311.5 (122-684)	245.5 (43-967)	0.062	360 (107-1476)	290 (62-747)	0.0785
Liver disease			0.0006[c]			<0.0001[d]
ALD[f]	3 (13.6)	3 (1.41)		10 (40)	14 (77.8)	
Other[f]	19 (86.4)	209 (98.6)		15 (60)	166 (22.2)	
Cholestasis			0.6515			0.3988
Naïve PBC[f]	2 (9)	26 (12.3)		0 (0)	5 (27.8)	
PBC with UDCA[f]	20 (81)	186 (87.7)		25 (100)	175 (72.2)	
PPI			0.4674			0.409
Yes[f]	5 (22.7)	35 (16.5)		6 (24)	31 (17.2)	
No[f]	17 (77.3)	177 (83.5)		19 (76)	149 (82.8)	
Hypertension			0.228			0.7059
Yes[f]	7 (31.8)	78 (36.8)		9 (36)	58 (32.2)	
No[f]	15 (68.2)	134 (63.2)		16 (64)	122 (67.8)	
Statin			0.5736			0.7522
Yes[f]	3 (13.6)	40 (18.9)		2 (8)	18 (10)	
No[f]	19 (86.4)	172 (81.1)		23 (92)	162 (90)	
Diabetes			0.1248			0.1211
Yes[f]	5 (22.7)	24 (11.3)		2 (8)	38 (21.1)	
No[f]	17 (77.3)	188 (88.7)		23 (92)	142 (78.9)	
Cancer[g]			0.1811			0.5328
Yes[f]	2 (9)	7 (33)		2 (8)	9 (5)	
No[f]	20 (91)	205 (67)		23 (92)	171 (95)	

Table II. Continued.

Factors	Female			Male		
	PIVKA-II high	PIVKA-II normal	P-value	PIVKA-II high	PIVKA-II normal	P-value
Osteoporosis medication-all			0.4784			0.4491
Yes[f]	3 (13.6)	43 (20.2)		1 (4)	15 (8.3)	
No[f]	19 (86.4)	169 (79.8)		24 (96)	165 (91.7)	
Osteoporosis medication-VD			0.4774			0.9786
Yes[f]	1 (45.5)	20 (9.4)		1 (4)	7 (3.9)	
No[f]	21 (54.5)	192 (90.6)		24 (96)	173 (96.1)	

[a]P<0.05, [b]P<0.01, [c]P<0.001, [d]P<0.001. [e]Median (range), [f]n (%), [g]Mean ± SD. [g]Cancer without hepatoma at entry.

group (Fig. 1B). The difference was particularly significant in females (P<0.0001 in females; P=0.001 in males). In contrast, the TRACP-5b levels in the ucOC high group were higher than in the normal group (Fig. 1C). It is hypothesized that high PIVKA-II and low ucOC were related to factors other than bone metabolism. The CPS in the PIVKA-II high and ucOC normal (<4.5 ng/ml; PHUN group, 16 women and 18 men) was higher than that in the other groups (Fig. 1D). Total bilirubin (Fig. 1E) and albumin (Fig. 1F) levels are factors that include CPS (26). Albumin levels in the PHUN patients were lower than than those in the other groups. The titer of albumin in the PHUN group was positively correlated with the titer of ucOC (Fig. 1G-1), but this was not observed in the other groups (Fig. 1G).

Factors associated with PIVKA-II in males. In males, high SARC-F and low VD were the contributing factors for the PIVKA-II high group. The VD concentration in the PIVK-II high group were lower than that in the PIVKA-II normal group in both sexes (Fig. S2D). Total SARC-F was positively correlated with PIVKA-II in males (Fig. 2A), and VD was negatively correlated with PIVKA-II (Fig. 2C). The VD value tended to positively correlate with GS (Fig. 2D). In males, SARC-F was higher in the PIVKA-II-high group than in the PIVKA-II-normal group (Fig. 2B). SARC-F was higher in the VD-low group than in the normal group (Fig. 2E). GS in males was also higher in the VD-normal group than in the VD-low group (Fig. 2F). Additionally, the PIVKA-II and VD insufficiencies were scored. The PIVKA-II high and VD low groups scored 2, the PIVKA-II high or VD low group scored 1, and the normal range in both was 0. In addition, SARC-F (Fig. 2G) significantly increased and GS (Fig. 2H) significantly decreased, in males.

PIVKA-II and muscle markers. Finally, the relationship between PIVKA-II, muscle markers, SARC-F and GS, and bone metabolic markers; TRACP-5b, P1NP, and VD was assessed (Fig. S3). In females, SARC-F was positively correlated with TRACP-5b (R=0.164) and PINP (R=0.184). In males, SARC-F was positively correlated with PIVKA-II (R=0.317) and TRACP-5b (R=0.247), and GS was negatively correlated with TRACP-5b (R=-0.242).

Discussion

In patients who did not use warfarin and did not suffer from HCC with liver disease, serum PIVKA-II levels above 40 mAU/ml were associated with Child B/C (Child-Pugh score), a high titer of PINP and a low titer of ucOC in females, and ALD and low VD in males. The titer of PIVKA-II were associated with IgA and PT-INR in females and FIB-4, IgG, total bilirubin, PT-INR, and SARC-F in males.

In both sexes, the PIVKA-II titer were associated with the PT-INR. PT includes CPS and MELD. PT-INR is dependent on VK, and PIVKA-II is dependent on VK deficiency. According to these results, the degree of severe liver disease depends on VK deficiency. PBC, which represents cholestatic liver disease, was not associated with PIVKA-II in this study (Table II). It is hypothesized that VK deficiency may occur in all liver diseases rather than just in cholestatic diseases. VK is a fat-soluble vitamin, and dietary VK is the most significant supply; however, VK, produced by gut microbiota, is also substantial when consumption is low (34). Changes in the microbiota induce VK deficiency and this results in altered bone metabolism (35). The association between liver disease and gut microbiota has been widely recognized in previous studies (36-38). Advanced liver diseases, such as Child B/C and ALD, are associated with the microbiota (36). Increasing IgA levels in females and IgG levels in males were associated with elevation of PIVKA-II. Advancing cirrhosis, irrespective of the underlying etiology or hepatocellular carcinoma, has been reported to increase serum IgG and IgA levels progressively (39). IgA is associated with dysbiosis in liver diseases (37). It was speculated that the elevation of PIVKA-II was influenced by VK deficiency due to the presence of an advanced liver disease.

In females, high PINP levels contributed to a higher PIVKA-II value. As bone mineral density was not evaluated, TRACP-5b was compared with P1NP. TRACP-5b is a metabolic marker of bone formation and is elevated in osteoporosis and hepatic osteodystrophy (21). In the high P1NP group, TRACP-5b was higher compared with the normal group; the PINP-high and PIVKA-II-high groups had the highest TRACP-5b levels in females. Patients in the high PIVKA-II group tended to have osteoporosis. However, the ucOC-normal

Table III. Logistics regression analysis of the clinical factors associated with the PIVKA-II high group.

	Female				Male			
	Univariate		Multivariate		Univariate		Multivariate	
Factor	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
CPG A/B, C	0.026 (0.005-0.136)	<0.0001[d]	0.03 (0.003-0.349)	0.005	0.079 (0.028-0.226)	<0.0001[d]	0.239 (0.053-1.068)	0.0608
ALBI 1/2, 3	0.308 (0.126-0.748)	0.0098[b]			0.261 (0.109-0.625)	0.0026[b]		
ALD/other liver disease	10.947 (2.075-58.315)	0.0049[b]	0.773 (0.031-20.867)	0.8776	7.905 (3.001-20.821)	<0.0001[d]	11.496 (2.673-49.435)	0.001[b]
FIB-4 high/normal	2.576 (1.067-6.303)	0.0368[a]	1.031 (0.371-3.584)	0.9607	1.992 (0.855-4.638)	0.11		
deGFR high/normal[e]	1.779 (0.741-4.336)	0.2012			3.2 (1.15-8.903)	0.0259[a]	1.832 (0.388-8.662)	0.4447
Sarcopenia index, high/normal[f]	0.706 (0.278-1.818)	0.4664			0.333 (0.141-0.786)	0.0121[a]	1.892 (0.409-8.763)	0.4147
SARC-F, high/normal	0.889 (0.282-2.843)	0.8417			3.529 (1.198-10.402)	0.0222[a]	2.21 (0.451-10.833)	0.3283
AFP, ≥10 ng/ml	1.696 (0.435-5.919)	0.492			2.907 (1.086-7.784)	0.0336[a]	2.32 (0.58-9.274)	0.2338
P1NP high[g]	3.46 (1.213-9.983)	0.021[a]	9.968 (1.448-73.812)	0.0212	1.601 (0.546-4.694)	0.3912		
VD, <10 ng/ml	0.337 (0.135-0.832)	0.0191[a]	0.679 (0.21-2.055)	0.5076	0.184 (0.074-0.458)	0.0003[c]	0.183 (0.05-0.679)	0.0111[a]
ucOC high[g]	0.348 (0.123-0.984)	0.0465[a]	0.119 (0.019-0.678)	0.019 (0.319-2.269)	0.85	0.7461		
IgG high[g]	1.674 (0.648-4.376)	0.2903			2.429 (1.028-5.738)	0.0431[a]	4.143 (0.938-18.311)	0.0608
IgM high[g]	0	0.9751			2.137 (0.418-10.913)	0.3615		
IgA high[g]	3.18 (1.195-8.553)	0.0212[a]	1.759 (0.469-6.816)	0.4102	3.233 (1.352-7.731)	0.0083[b]	0.827 (0.236-2.894)	0.7658

[a]P<0.05, [b]P<0.01, [c]P<0.001, [d]P<0.001. [e]High, ≥2.5; normal, <2.5. [f]High, ≥74.6; normal, <74.6. [g]Above the upper limit.

Table IV. Regression analysis for clinical factors that contribute to an elevated PIVKA-II value.

	Female				Male			
	Univariate		Multivariate		Univariate		Multivariate	
Factor	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value
CPS	0.239 (36.136-115.993)	0.0002[c]			0.677 (44.249-59.972)	<0.0001		
ALBI	0.183 (29.43-154.61)	0.0052[b]			0.386 (37.6-74.72)	<0.0001		
FIB-4	0.094 (3.354-21.847)	0.1515			0.2 (1.723-9.012)	0.0041[b]	0.269 (-10.74-3.531)	0.0001[c]
SI	-0.048 (-2.424-1.113)	0.4666			-0.174 (-1.11-0.136)	0.0125[a]	-0.064 (-1.018-0.599)	0.6093
dGFR	0.05 (0.763-0.4461)	0.447			0.187 (0.241-1.512)	0.0071	-0.096 (-1.469-0.643)	0.4414
IgG	0.162 (0.014-0.118)	0.013[a]	-0.008 (-0.062-0.055)	0.9087	0.374 (0.033-0.068)	<0.0001	0.22 (0.01-0.046)	0.0022[b]
IgA	0.246 (0.188-0.579)	0.0001[c]	0.155 (0.017-0.468)	0.03547	0.354 (0.097-0.208)	<0.0001	0.105 (-0.013-0.095)	0.1365
TB	0.043 (-14.083-28.355)	0.51			0.373 (23.721-48.591)	<0.0001	0.274 (13.233-35.074)	<0.0001[d]
AST	0.054 (-0.202-0.494)	0.4122			0.113 (-0.044-0.457)	0.1057		
Alb	-0.164 (-143.1-17.87)	0.0121[a]	-0.068 (-97.237-30.333)	0.3047	-0.331 (-63.02- -27.39)	<0.0001	0.007 (-19.736-21.508)	0.9325
PT, %	-0.246 (-5.123-1.661)	0.0002[c]			-0.36 (-2.096- -0.987)	<0.0001		
PTINR	0.295 (413.32-1008.896)	<0.0001[d]	0.225 (214.976-870.38)	0.0013[b]	0.507 (224.34-363.7)	<0.0001	0.297 (85.155-251.07)	<0.0001[d]
TRACP-5b	0.073 (-0.056-0.2)	0.2729			0.196 (0.01-0.11)	0.0187	0.108 (-0.008-0.085)	0.1041
P1NP	0.07 (-0.166-0.523)	0.3109			0.072 (-0.073-0.213)	0.32		
SARC-F	0.037 (-10.468-18.049)	0.604			0.313 (6.36-16.865)	<0.0001	0.237 (4.142-14.758)	0.0006[c]

[a]P<0.05, [b]P<0.01, [c]P<0.001, [d]P<0.001.

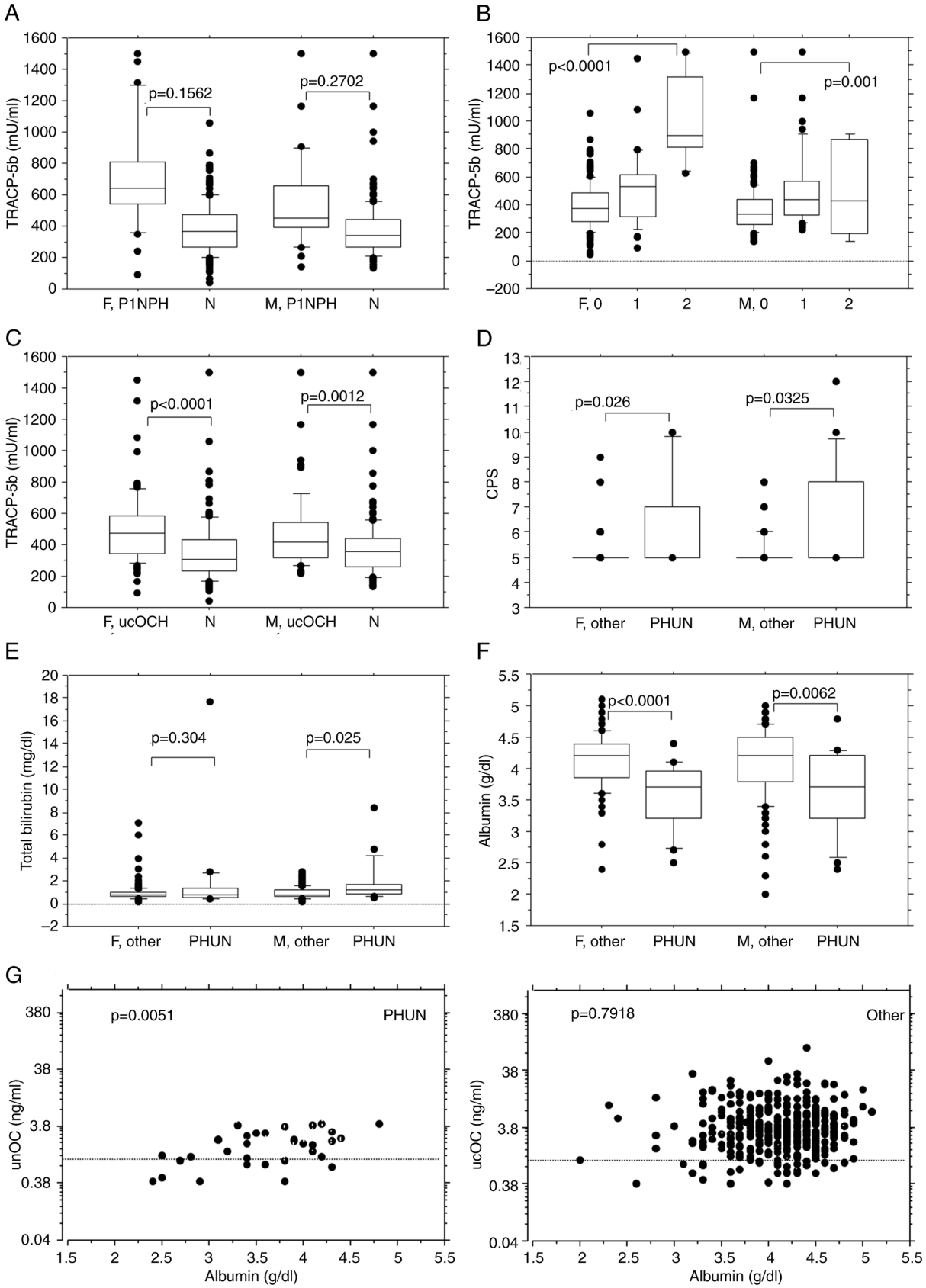

Figure 1. Relationship between PIVKA-II and bone metabolism. The P1NPH group was above the upper limit of P1NP for each sex. The P1NP-N group was within the upper limit. (A) No differences were observed between the PINPH and N groups. (B) PIVKA-II high (>40 mAU/ml) and PINPH groups had a score of 2, PIVKA-II high and P1NP normal or P1NPH and PIVKA-II normal had a score of 1, and both normal (N) scores were 0. TRACP-5b was higher than the other groups, with a score of 2. ucOCH refers to a ucOC value >4.5 ng/ml. (C) TRACP-5b in the ucOCH group was higher than in the N group. (D) The PHUN group had a higher CPS than the rest of the cohort. (E) Total bilirubin (mg/dl) in PHUN did not differ from that in females but was higher than that in males. (F) Albumin levels (g/dl) in the PHUN group were higher than that in the other groups. (G) Correlation analysis of albumin with ucOC levels. P1NPH, P1NP-high; N, normal; F, female; M, male; ucOC, undercarboxylated osteocalcin; ucOCH, ucOC-high; PHUN, PIVKA-II high and ucOC normal; other, patients not included in the PHUN group; CPS, Child-Pugh Score; PIVKA-II, protein induced by vitamin K absence-II.

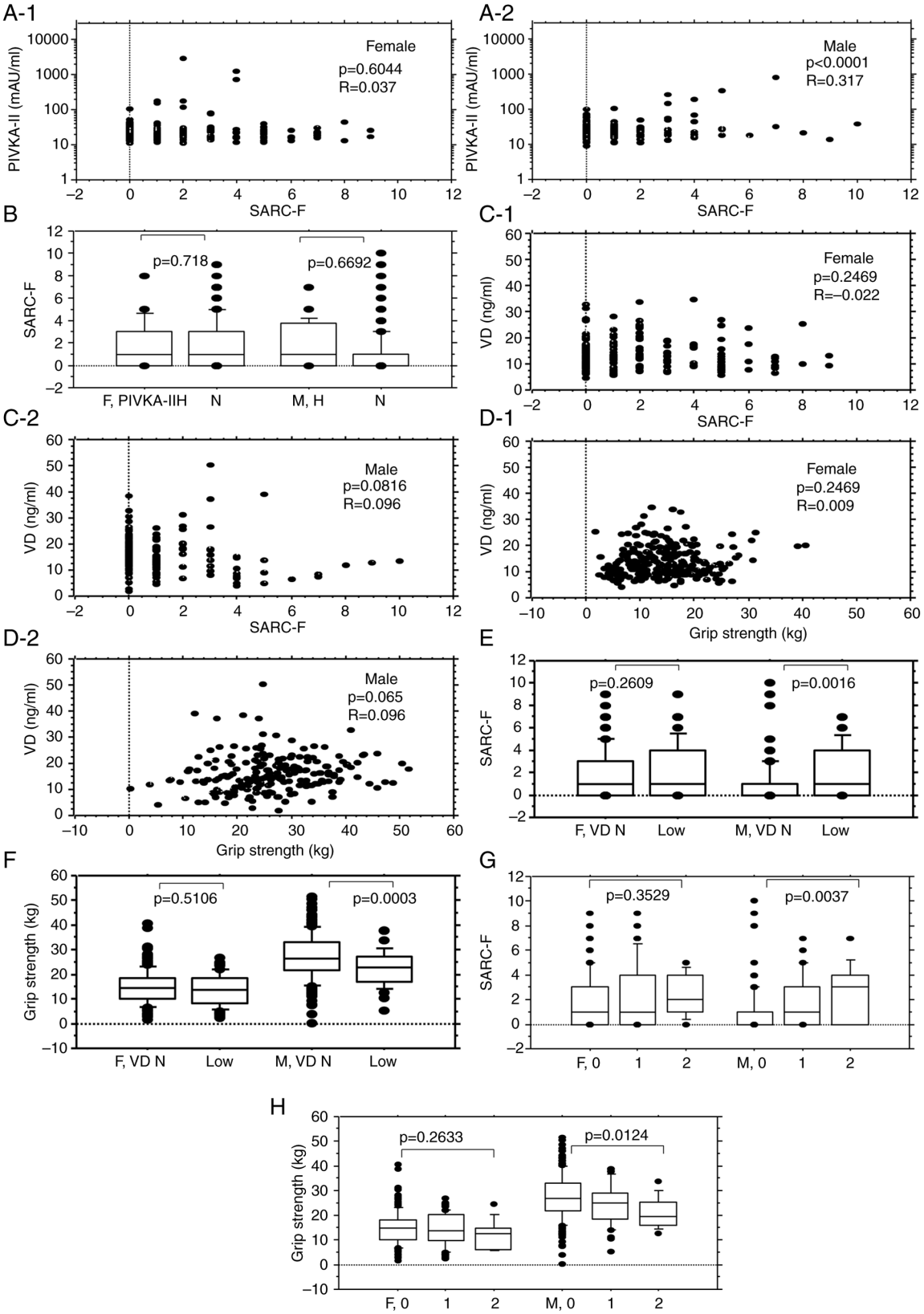

Figure 2. Relationship between SARC-F, PIVKA-II, and VD. (A) Relationship between PIVKA-II and SARC-F. (B) There was a significant positive relationship between PIVKA-II and SARC-F in the males, but no relationship in the females. SARC-F in the PIVKA-II-high group was relatively higher than that in the normal group in males but did not differ from that of the normal group in females (C). There was a negative association between VD and SARC-F in females, but no association was observed in the males. (D) The association between VD and grip strength was relatively positive in males, but no significant association was observed in the females. (E) In males, SARC-F in the VD Low group was higher than that in the VD N group and did not differ significantly in the females. (F) In males, grip strength in the VD Low group was higher than that in the VD N group, but did not differ significantly in females. (G) The PIVKA-II-high and VD Low groups had mean scores of 2. The patients were scored as follows: 1, a patient met one of the following criteria: PIVKA-II high or VD low; 0, PIVKA-II normal and VD low. In females, SARC-F did not differ between patients who scored 0-2, but there was a significant increase in males. (H) Grip strength did not differ between patients who scored 0-2 in females, but there was a significant decrease in males. PIVKA-II, protein induced by vitamin K absence-II; VD, vitamin D; F, female; M, male; VD Low, VD levels <10 ng/ml; VD-10, VD levels >10 ng/ml.

group was associated with the PIVKA-II-high group. The ucOC-high group had higher TRACP-5b levels than the normal group. VK deficiency was associated with elevated ucOC levels and bone fractures (13). The PHUN group had a higher CPS and lower albumin levels. In the PHUN group, ucOC was positively correlated with albumin, and more advanced liver disease than the other groups. ucOC acts as a hormone, associated with metabolic syndrome (14), rather than a component of bone metabolism. SARC-F and GS did not differ in the PHUN group; however, glucose metabolism was not evaluated in the present study. Based on these findings, the role of ucOC in liver disease should thus be assessed in future studies.

In males, low VD and high SARC-F contributed to high PIVKA-II. VD is also a fat-soluble vitamin, similar to VK. VD is produced in the liver, is a key factor in HOD (21), and is related to GS (40). In the present study, low VD was associated with a high SARC-F score and low GS. The PIVKA-II high and VD low groups had significantly higher SARC-F scores and lower GS. High PIVKA-II levels and low VD were associated with sarcopenia. A combination of low VD and VK levels is associated with an increase in mortality risk (41) and fracture risk (42). In the present study, it was speculated that a combination of low VK and VD levels may be associated with sarcopenia.

In the present study, the PIVKA-II levels showed sex-based differences in its pathophysiological effects. Female patients with high PIVKA-II tended to develop osteodystrophy, while male patients developed sarcopenia. Bone and muscle metabolism differ according to sex hormones and other factors (43), but common factors include age, VD levels, and level of physical activity (43). It is reported that liver disease is also a common risk factor for osteoporosis and sarcopenia (44,45). Sarcopenia is related to mortality (20), and bone fracture is associated with a worse prognosis (46). It is reported that studying osteocalcin and ucOC in humans is further complicated due to numerous confounding factors such as sex differences, menopausal status, VK status, physical activity level, body mass index, and insulin sensitivity, among other factors (47). Further mechanistic studies are required to (a) clarify causality, (b) explore the mechanisms involved and (c) define the magnitude of this effect and its clinical importance (47). Estrogens and androgens influence the growth and maintenance of bones and muscles, and are responsible for their sex-based differences (48). The actions of estrogens and androgens on bone and muscle result from the binding of the ligands to classical nuclear hormone receptors; Estrogen receptor (ER) α and β and androgen receptor (AR), respectively, and the effects of sex steroids on bone and muscle result from a complex interplay of actions on different cell types (48). Further study for the relationship between sex differences and PIVKA-II (or VK) is thus required. Several natural products contain VK and there is an unmet need for the study of the use of these substances for the management of NAFLD (49). The fermented soybean product natto, a traditional Japanese delicacy, is a major source of VK2 in Japan (15).

The findings of the present study are limited due to the inclusion of only a few severe stages of cirrhosis, the inclusion of several causes of liver disease, the inclusion of patients with mixed treated/naïve disease, and the retrospective nature. Additionally, bone mineral density and muscle volume were not evaluated. However, it was found that high PIVKA-II was related to abnormal bone metabolism in females, weak muscles in males, and severe liver disease in both sexes. The evaluation of PIVKA-II may thus be useful for evaluating the clinical stages of liver disease. From the perspective of VK deficiency, nutrition and supplementation with fat-soluble vitamins should form the subject of study of future research. Additionally, the ability of PIVKA-II combined with other factors to diagnose muscle and bone metabolism abnormalities in liver disease must be examined.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

TaH wrote the manuscript, analyzed the data, and designed the study. TaI, MY, SY, MK, YN, TeH, HY, OM, YK, ToI, TO, KNAGATA and KNAKAO collected the data. TaI and TaH confirmed the authenticity of the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol conformed to the guidelines of the 1975 Declaration of Helsinki, which was approved by the Human Research Ethics Committee of the Nagasaki Harbor Medical Center (approval no. H30-031). Informed consent was obtained from each patient included in the study, and they were guaranteed the right to leave the study if desired.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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