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Falsely Elevated Serum Vitamin B_{12} Levels Were Associated with the Severity and Prognosis of Chronic Viral Liver Disease

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ABSTRACT

Background Vitamin B_{12} is stored primarily in the liver, and highly elevated serum vitamin B_{12} levels occur in acute hepatitis and severe alcoholic liver disease. We evaluated the relationship between vitamin B_{12} levels and liver disease severity and long term prognosis in patients with chronic viral hepatitis and cirrhosis.

Methods We enrolled 90 patients (57 men, 33 women) with chronic viral hepatitis and cirrhosis who admitted to our hospital as a prospective cohort study. Overall, 37 patients had chronic hepatitis and 53 had cirrhosis (Child-Pugh A 33, B 13, and C 7); 57 patients had primary liver cancer. Serum vitamin B_{12} concentration and holotranscobalamin (holoTC) II (active form of vitamin B_{12}) were determined and followed prospectively for at least 5 years.

Results Mean total serum vitamin B_{12} concentration was significantly higher in Child-Pugh C (1308 ± 599 pg/mL) compared to those with chronic hepatitis (655 ± 551 pg/mL), Child-Pugh A (784 ± 559 pg/mL), and Child-Pugh B (660 ± 464 pg/mL) ($P = 0.036$) Presence of primary liver cancer also influenced serum vitamin B_{12} levels [657 (167–2956) vs. 432 (189–2956); $P = 0.015$]. Patients were divided into quartiles by vitamin B_{12} level. Patients without primary liver cancer in quartile 4 (≥ 880 pg/mL) demonstrated significantly poorer prognosis than those in quartiles 1–3 (< 880 pg/mL) ($P = 0.023$). The percentage of holohaptocorrin (holoHC) [(total vitamin B_{12} – holoTC II) × 100] was significantly higher in Child-Pugh B and C 86 (80–87)% than chronic hepatitis and Child-Pugh A 77 (31–89)% ($P = 0.006$) Multivariate analysis indicated serum vitamin B_{12} levels (HR = 1.001, $P = 0.029$) as a prognostic factor.

Conclusion Falsely elevated serum vitamin B_{12} levels mainly composed of increased holoHC were associated with severity (Child-Pugh C and primary liver cancer) and prognosis in chronic viral liver disease.

Key words holotranscobalamin; liver cirrhosis; prognosis; viral hepatitis; vitamin B_{12}

Vitamin B_{12} is stored primarily in the liver; this vitamin is essential for one-carbon metabolism and cell division. It acts as a cofactor for two enzymatic reactions, namely, methionine synthesis from homocysteine and succinyl-CoA synthesis from methylmalonyl-CoA, in mammalian systems.[1] Some studies have indicated that elevated serum levels of vitamin B_{12} might be a sign of a serious and life-threatening disease. Such falsely high valued of serum vitamin B_{12} levels are observed in myeloproliferative disease, acute hepatitis, severe alcoholic liver disease, and cirrhosis.[2–6]

Vitamin B_{12} binds with transcobalamin (TC) II. The complex holoTC II, which is the biologically active form of vitamin B_{12}, is recognized by specific receptors on all cell types. The active form of vitamin B_{12} comprises only 6–20% of the total serum vitamin B_{12} level. The remaining major portion (70–90%) is bound to haptocorrin (HC); this is named holohaptocorrin (holoHC) and is the inactive form of vitamin B_{12}, which is stored in the liver.[7, 8] Several reports have reported that the falsely high values are due to increasing the holoHC levels.[3]

In our experience, we have observed highly elevated vitamin B_{12} levels in patients with advanced stages of viral liver cirrhosis. There are only one report that demonstrated the association of vitamin B_{12} level with disease severity and mortality in hepatitis B virus infection with short term.[9] No reports have evaluated the dynamics of vitamin B_{12} in chronic viral hepatitis. Accordingly, the present study aimed to evaluate the relationship of serum vitamin B_{12} between the disease severity and long term prognosis of liver disease in patients with viral hepatitis and cirrhosis.

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Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CD, cluster of differentiation; DCP, des-γ-carboxy prothrombin; HBV, hepatitis B virus; HC, haptocorrin; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; holoHC, holohaptocorrin; holoTC, holotranscobalamin; HR, hazard ratio; NFκB, nuclear factor kappa B; NK, natural killer; TC, transcobalamin; TNF-α, tumor necrosis factor-alpha; TNM, TNM classification of malignant tumours

SUBJECTS AND METHODS

Subjects

From March 2007 to March 2010, consecutive patients with chronic viral hepatitis and viral induced cirrhosis were prospectively enrolled in the present study. Patients who underwent a total gastrectomy, vegetarians and those receiving nutrient supplementation, including vitamin B_{12}, were excluded from the study.

Methods

Cirrhosis was diagnosed according to clinical, biochemical, and/or imaging findings. The severity of cirrhosis was categorized using the Child-Pugh classification.[10] The presence of primary liver cancer was also recorded. The stages of hepatocellular carcinoma (HCC) were categorized by TNM staging. Blood samples were collected at the date of inclusion, and hemoglobin, blood chemistry [albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT)], prothrombin activity percentage, alpha-fetoprotein (AFP), and des-γ-carboxy prothrombin (DCP), and serum vitamin B_{12} levels were determined. Total serum vitamin B_{12} concentration was determined by chemiluminescent immunoassay (CLIA: Access Vitamin B_{12}; beckman coulter, Tokyo, Japan). Levels of holoTC II were also measured by microparticle enzyme immunoassay (AxSYM Active-B_{12}; abbott diagnostics, Abbott Park, IL). The percentage of holoTC II was expressed as the ratio of holoTC II to total serum vitamin B_{12}. The percentage of holoHC was calculated as (total vitamin B_{12} – holoTC II) × 100. All the patients were followed prospectively for at least 5 years and evaluated for prognosis.

Ethical Considerations

The study protocol confirmed to the ethical guidelines of the Helsinki Declaration of 1975, as revised in 2000, and was approved by the ethics committee of the Tottori University (No. 1231). Patients were enrolled after giving their written informed consent.

Statistical analysis

Data are expressed as median (range) or mean ± SD. Statistical analyses for significant differences among the groups were performed using the chi-square test, Student's *t* test, Mann-Whitney's *U* test, ANOVA, and Kruskal-Wallis Test. *F* test and Bartlett's test was used to assess the equality of variances. Correlations were calculated using Pearson's product-moment correlation coefficient. Cumulative survival rate was calculated by the Kaplan-Meier method, and significant differences between two groups were calculated using the Log rank test. Multivariate analyses were performed using the Cox proportional hazards model. Statistical significance was set at $P < 0.05$.

RESULTS

Patient background

Ninety patients [57 men, 33 women; median age, 69 (range, 30–88) years] were enrolled this study. Of these patients, 60 (67%) had hepatitis C virus (HCV) and 30 patients (33%) had hepatitis B virus (HBV). Twenty patients (22%) habitually consumed ≥ 20 g/day alcohol. Seven patients (8%) had heart disease (e.g. ischemic heart disease, valvular heart disease, and arrhythmia). Nine patients (10%) underwent distal gastrectomy. Underlying liver disease was chronic hepatitis in 37 patients (41%) and cirrhosis in 53 (59%) (Table 1).

Table 1. Clinical findings in study subjects

Patients		*n* = 90
Sex (Male:Female)		57:33
Age (years)		69 (30–88)
Etiology		
HBV infection		30 (33%)
HCV infection		60 (67%)
Alcohol consumption (≥ 20 g/day)		20 (22%)
Comorbidity		
Heart disease		7 (8%)
Gastrectomy (distal)		9 (10%)
Atrophic gastritis		33 (37%)
The severity of underlying liver disease		
Chronic hepatitis		37 (41%)
Cirrhosis		53 (59%)
Child-Pugh classification (A:B:C)		33:13:7
Primary Liver Cancer (HCC:ICC)		56:1 (62%)
TNM Stage (I:II:II:IVA:IVB:unknown)		18:11:17:4:5:2
Laboratory values		
Hemoglobin	(g/dL)	12.3 (6.5–16.9)
MCV	(fL)	95.8 (62.4–112.9)
MCHC	(%)	34.4 (31.4–36)
Albumin	(g/dL)	3.6 (2.1–5.1)
AST	(IU/L)	47 (14–847)
ALT	(IU/L)	37 (7–1073)
Total bililubin	(mg/mL)	0.8 (0.3–15.1)
Prothrombin percent activity	(%)	86.9 (5–117.1)
Total serum vitamin B_{12}	(pg/mL)	584.5 (160–2956)
AFP	(ng/mL)	8.7 (1.2–501570)
DCP	(mAU/mL)	24 (10–84980)

Data are expressed as median (range).
AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; DCP, Des-gamma-carboxy prothrombin; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocellular carcinoma; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; TNM, TNM classification of malignant tumours.

Vitamin B_{12} analysis

Serum vitamin B_{12} levels were significantly higher in patients with cirrhosis than chronic hepatitis [647 (160–2956) vs. 461 (189–2956) pg/mL (P = 0.029)], and it was particularly in patients categorized as Child-Pugh C (1308 ± 599 pg/mL) than those with chronic hepatitis (655 ± 551pg/mL), Child-Pugh A (784 ± 559 pg/mL), and Child-Pugh B (660 ± 464 pg/mL) (P = 0.036) (Fig.1). The vitamin B_{12} levels were also higher in patients with primary liver cancer than those without [657 (167–2956) vs. 432 (189–2956) pg/mL, P = 0.015] (Table 2).

Then we divided the patients with and without primary liver cancer. Among the patients with primary liver cancer, the vitamin B_{12} levels were found to be significantly higher in drinkers (alcohol consumption ≥ 20 g/day) than non-drinkers [885 (160–2956) vs. 625 (263–2265) pg/mL, P = 0.025]. On the other hand, among the patients without primary liver cancer, it was significantly higher in patients with hepatitis B virus infection than hepatitis C virus infection [655 (272–2956) vs. 392 (189–1083) pg/mL, P = 0.018], and the patients with cirrhosis than chronic hepatitis [655 (379–2956) vs. 386 (189–1200) pg/mL, P = 0.004] (Table 3).

Moreover, we also divided the patients with chronic hepatitis and cirrhosis to separate the influence of liver function on vitamin B_{12} for each parameter. Vitamin B_{12} levels were significantly higher in primary liver cancer only in patients with chronic hepatitis [731 (196–2956) vs. 386 (189–1200) pg/mL, P = 0.009] (Table 4).

To evaluate the relationship of serum vitamin B_{12} with the patients' clinical and biochemical parameters, the correlations between serum vitamin B_{12} levels and those parameters (age, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, albumin, AST, ALT, total bilirubin, prothrombin activity percentage, AFP, and DCP) were estimated using Pearson's product-moment correlation coefficient. Serum vitamin B_{12} levels were positively correlated with AFP (r = 0.231, P = 0.04), negatively prothrombin activity percentage (r = –0.248, P = 0.017) (Table 5).

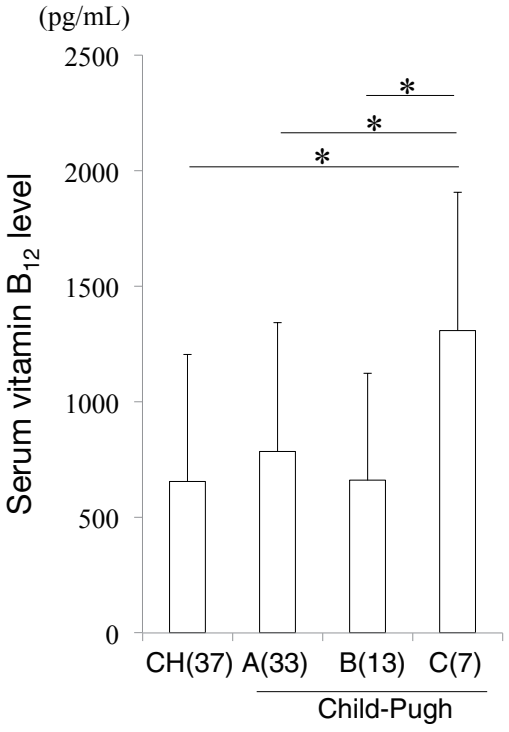

Fig. 1. Serum vitamin B_{12} levels in patients with chronic viral hepatitis. Serum vitamin B_{12} levels were significantly higher in patients categorized as Child-Pugh C (1308 ± 599 pg/mL) than those in CH (655 ± 551 pg/mL), Child-Pugh A (784 ± 559 pg/mL), and Child-Pugh B (660 ± 464 pg/mL) (P = 0.036). *P < 0.05. CH, chronic hepatitis.

Table 2. Influence of clinical factors on serum vitamin B_{12} levels

		Serum vitamin B_{12} (pg/mL)	
		All patients (n = 90)	P value
Sex	(Male:Female)	581 (196–2956):640 (160–2956)	n.s.
Etiology	(HCV:HBV)	513 (189–2956):656 (160–2956)	n.s.
Alcohol ≥ 20g/day	(Yes:No)	660 (160–2956):520 (263–2265)	n.s.
Heart disease	(Yes:No)	431 (320–1298):410 (160–2956)	n.s.
Gastrectomy*	(Yes:No)	607 (160–2956):367 (263–1494)	n.s.
Cirrhosis	(Yes:No)	647 (160–2956):461 (189–2956)	0.029
Primary liver cancer	(Yes:No)	657 (160–2956):432 (189–2956)	0.015
TNM Stage	(I/II:III/IVA/IVB)	549 (233–2956):728 (160–2265)	n.s.

Data are expressed as median (range). *Distal gastrectomy.
HBV, hepatitis B virus; HCV, hepatitis C virus; TNM, TNM classification of malignant tumours; n.s., not significant.

Table 3. Influence of clinical factors on serum vitamin B_{12} levels between patients with and without primary liver cancer

	Serum vitamin B_{12} (pg/mL)			
	Patients with primary liver cancer (n = 57)	P value	Patients without primary liver cancer (n = 33)	P value
Sex (Male:Female)	632 (196–2956):686 (160–1957)	n.s.	461 (277–1200):410 (189–2956)	n.s.
Etiology (HCV:HBV)	664 (205–2956):657 (160–2029)	n.s.	392 (189–1083):655 (272–2956)	0.018
Alcohol ≥ 20 g/day (Yes:No)	885 (160–2956):625 (263–2265)	0.025	432 (299–1083):410 (189–2956)	n.s.
Heart disease (Yes:No)	819 (160–2956):647 (320–1298)	n.s.	581 (581–581):424 (189–2956)	n.s.
Gastrectomy* (Yes:No)	320 (263–1494):686 (160–2956)	n.s.	283 (367–379):461 (189–2956)	n.s.
Cirrhosis (Yes:No)	645 (160–2265):731 (196–2956)	n.s.	655 (379–2956):386 (189–1200)	0.004
TNM Stage (I/II:III/IVA/IVB)	549 (233–2956):728 (160–2265)	n.s.	—	—

Data are expressed as median (range). *Distal gastrectomy.
HBV, hepatitis B virus; HCV, hepatitis C virus; TNM, TNM classification of malignant tumours; n.s., not significant.

Table 4. Influence of clinical factors on serum vitamin B_{12} levels between patients with chronic hepatitis and cirrhosis

	Serum vitamin B_{12} (pg/mL)			
	Patients with chronic hepatitis (n = 37)	P value	Patients with cirrhosis (n = 53)	P value
Sex (Male:Female)	509 (196–2956):189 (189–1298)	n.s.	631 (205–2265):647 (160–2956)	n.s.
Etiology (HCV:HBV)	432 (189–2956):634 (196–1298)	n.s.	640 (205–2265):656 (160–2956)	n.s.
Alcohol ≥ 20 g/day (Yes:No)	461 (299–1298):449 (189–2956)	n.s.	1083 (263–2265):624 (160–2956)	n.s.
Heart disease (Yes:No)	634 (320–1298):432 (189–2956)	n.s.	952 (367–1292):645 (160–2956)	n.s.
Gastrectomy* (Yes:No)	344 (320–367):483 (189–2956)	n.s.	263 (160–2956):379 (263–1494)	n.s.
Primary liver cancer (Yes:No)	731 (196–2956):386 (189–1200)	0.009	645 (160–2265):655 (379–2956)	n.s.
TNM Stage (I/II:III/IVA/IVB)	526 (233–2956):731 (196–1417)	n.s.	751 (263–1957):472 (160–2965)	n.s.

Data are expressed as median (range). *Distal gastrectomy.
HBV, hepatitis B virus; HCV, hepatitis C virus; TNM, TNM classification of malignant tumours; n.s., not significant.

HoloTC II analysis

In 43 of 90 patients, levels of holoTC II were evaluated. We found that the percentage of holoTC II tended to decrease with progression of liver disease. The median percentages of holoTC II were 22(11–69)% in patients with chronic hepatitis (n = 25), 24 (19–37) % in Child-Pugh A (n = 13), 16 (13–20)% in Child-Pugh B (n = 4), and 13% in Child-Pugh C (n = 1), and the statistical significance was indicated between Child-Pugh A and B (P = 0.031) (Fig. 2A). The percentage of holoHC was significantly higher in Child-Pugh B and C 86 (80–87)% compared with chronic hepatitis and Child-Pugh A 77 (31–89)% (P = 0.006) (Fig. 2B).

Survival analysis

During the observation period (median, 54.3 months; range, 0.6–101.7months), the overall survival rates were 89% at 1 year, 81% at 2 years, and 67% at 5 years. Cirrhotic patients had significantly poorer prognosis compared with non-cirrhotic patients [33.5 (0.6–101.7) vs.

66.9 (3.1–72.1) months, $P = 0.003$]. Patients with primary liver cancer had also significantly poorer prognosis compared to those without them [28.5 (0.6–101.7) vs. 67 (15.6–71.7) months, $P < 0.001$]. Ninety patients were divided into quartiles Q1 to Q4, according to serum vitamin B_{12} level: Q1, < 403.25; Q2, 403.25–582; Q3, 583–879; and Q4, ≥ 880 pg/mL (Fig. 3A). Among all the patients, Q4 had markedly low cumulative survival rate than those Q 1-3 ($P = 0.058$) (Fig. 3B). On the other hand, among the patients without primary liver cancer, Q4 had significantly low cumulative survival rate than Q 1-3 ($P = 0.003$) (Fig. 3C). Univariate analysis revealed that serum albumin, total bilirubin, AFP > 10 ng/mL, DCP > 40 AU/mL, and serum vitamin B_{12} level were significant prognostic factors for the overall survival rate. The stepwise multivariate Cox proportional hazards model showed that the independent factors contributing to cumulative survival rate were serum albumin, DCP > 40 AU/mL and serum vitamin B_{12} levels (Table 6).

Table 5. Correlations between serum vitamin B_{12} levels and clinical/biochemical parameters

Patients ($n = 90$)	r	P value
Sex	–0.038	n.s.
Age (year)	–0.005	n.s.
Hemoglobin (g/dL)	–0.053	n.s
MCV (fL)	–0.018	n.s
MCHC (%)	0.001	n.s
Albumin (g/dL)	–0.203	n.s.
AST (IU/L)	0.168	n.s.
ALT (IU/L)	0.114	n.s.
Total bilirubin (mg/mL)	0.113	n.s.
Prothrombin percent activity	–0.248	0.017
AFP	0.231	0.040
DCP	0.084	n.s.

AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BTR, branched-chain amino acids to tyrosine ratio; DCP, des-γ-carboxy prothrombin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; n.s., not significant.

DISCUSSION

Our study demonstrated two important findings. First, serum vitamin B_{12} levels were significantly elevated in patients categorized as Child-Pugh C and with primary liver cancer. Second, serum vitamin B_{12} level was a significant independent predictor for overall survival in patients with chronic viral liver disease.

We found that serum vitamin B_{12} levels were significantly higher in cirrhotic patients with Child-Pugh C than in patients with chronic hepatitis or Child-Pugh A/B cirrhosis. It has been previously reported that highly elevated vitamin B_{12} levels in plasma were indicated in acute hepatitis, severe alcoholic liver disease, and cirrhosis.[2–6, 11, 12] Our data indicate that serum vitamin B_{12} levels were falsely elevated with the severity also in chronic viral liver disease. Total serum vitamin B_{12} levels were

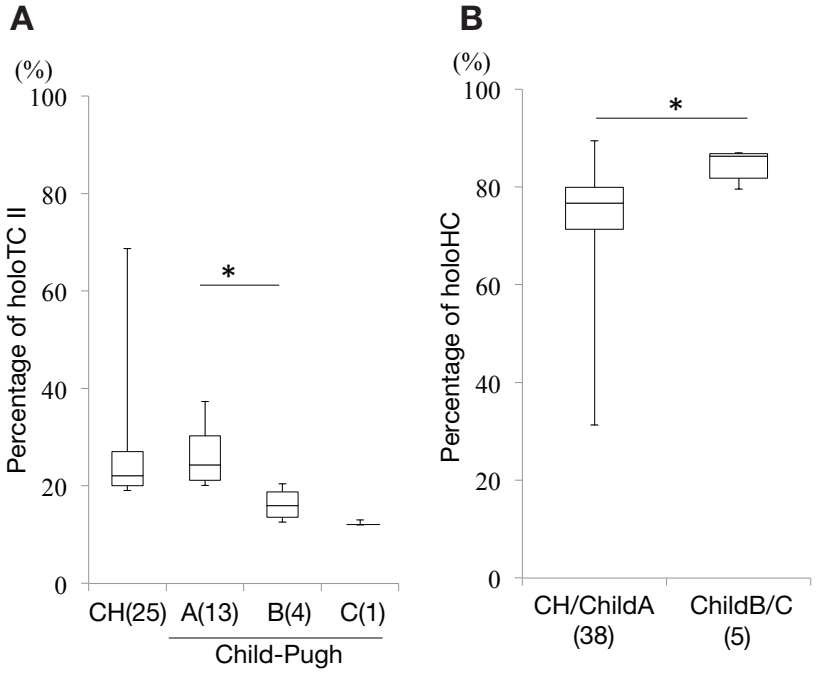

Fig. 2. Percentage of holoTC II and holoHC.
A: Percentage of holoTC II was significantly different between Chid-Pugh A and B ($P = 0.031$). *$P < 0.05$. **B**: The percentage of holoHC [(total vitamin B_{12} – holoTC II) × 100] was significantly higher in Child-Pugh B and C 86 (80–87)% than chronic hepatitis (CH) and Child-Pugh A 77 (31–89)% ($P = 0.006$). **$P < 0.01$. holoHC, holohaptocorrin; holoTC, holotranscobalamin.

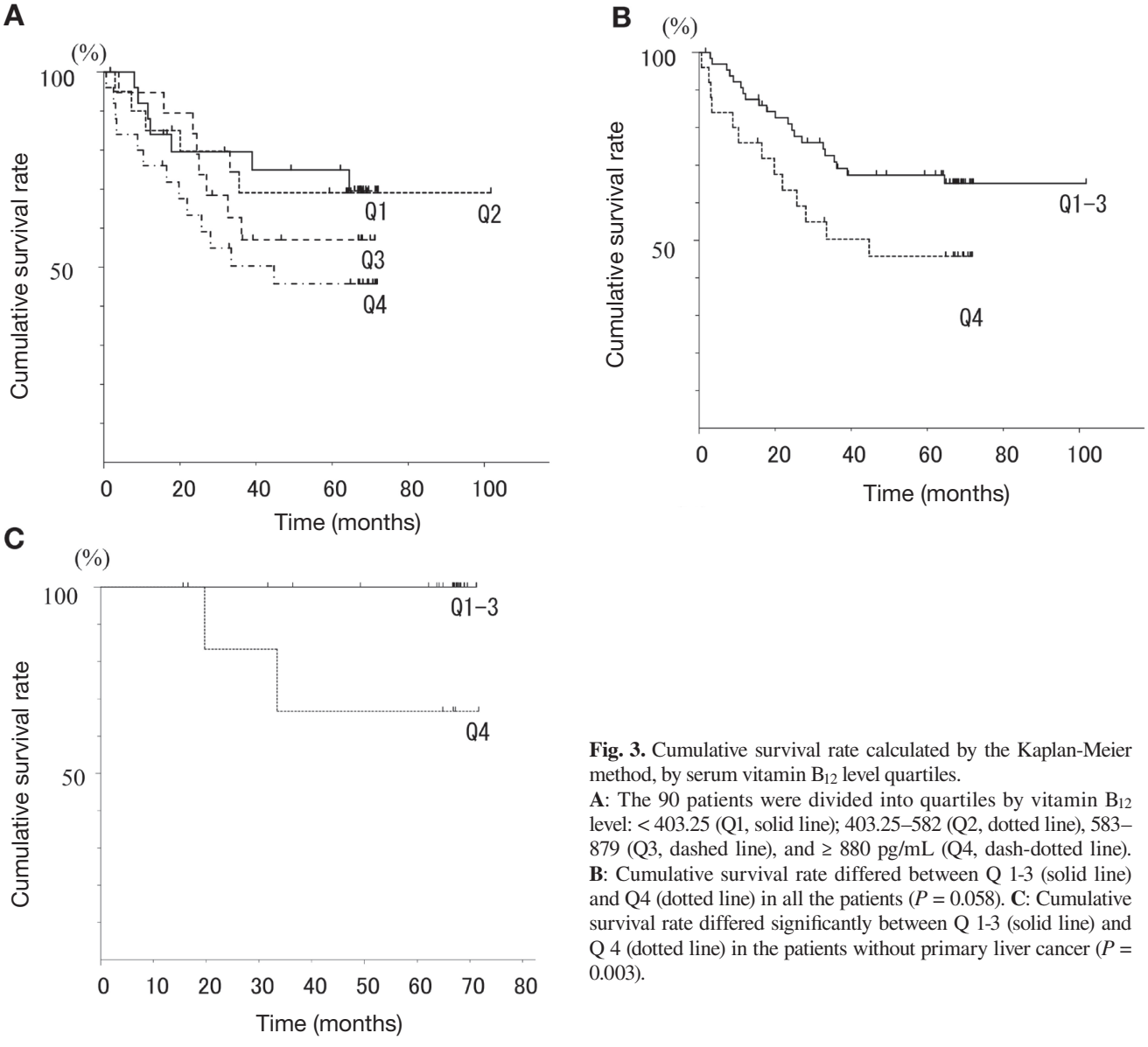

Fig. 3. Cumulative survival rate calculated by the Kaplan-Meier method, by serum vitamin B_{12} level quartiles.
A: The 90 patients were divided into quartiles by vitamin B_{12} level: < 403.25 (Q1, solid line); 403.25–582 (Q2, dotted line), 583–879 (Q3, dashed line), and ≥ 880 pg/mL (Q4, dash-dotted line). **B**: Cumulative survival rate differed between Q 1-3 (solid line) and Q4 (dotted line) in all the patients ($P = 0.058$). **C**: Cumulative survival rate differed significantly between Q 1-3 (solid line) and Q 4 (dotted line) in the patients without primary liver cancer ($P = 0.003$).

Table 6. Univariate and multivariate analyses of factors predicting the overall survival rate

Factors		Univariate *P* value	Multivariate analysis		
			HR	95% CI	*P* value
Sex		0.374			
Age	year	0.369			
Albumin	g/dL	< 0.001	0.368	0.176–0.767	0.008
Total bilirubin	mg/dL	0.006	1.231	0.915–1.658	0.170
PT	%	0.086	1.010	0.984–1.036	0.457
AFP	> 10 ng/mL	0.003	1.546	0.632–3.781	0.340
DCP	> 40 AU/mL	< 0.001	2.682	1.084–6.636	0.033
Serum vitamin B_{12}	pg/mL	0.034	1.001	1.000–1.001	0.029

CH,chronic hepatitis; AFP, alpha fetoprotein; CI, confidence interval; DCP, des-γ-carboxy prothrombin; HR, hazard ratio; PT, prothrombin percent activity.

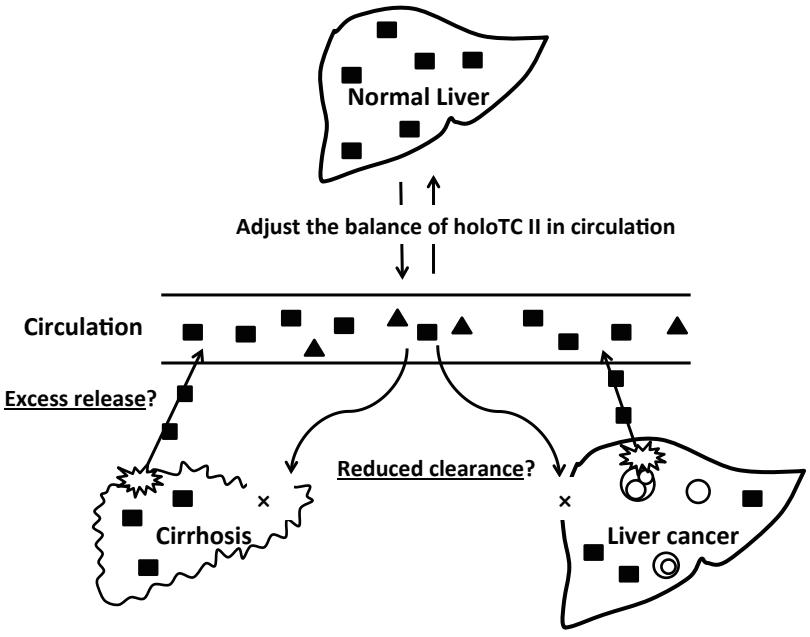

Fig. 4. Scheme of the hypothesis for falsely elevated vitamin B_{12} in liver disease.
HoloHC (■), the inactive form of vitamin B_{12}, is stored in the liver. The stored holoHC adjusts holoTC II(▲), the inactive form of vitamin B_{12}, in circulation. There are possible two mechanisms responsible for falsely elevated serum vitamin B_{12} levels in liver diseases. First, holoHC leaks into the circulation due to the destruction of hepatocytes (excess release hypothesis). Second, the reduced uptake of holoHC by the injured liver (reduced clearance hypothesis). holoHC, holohaptocorrin; holoTC, holotranscobalamin.

significantly higher in cirrhotic patients. The biomarker of hepatic reserve, such as prothrombin activity percentage, was found to be weakly correlated with the total serum vitamin B_{12} levels in the present study.

Among the patients with primary liver cancer, the vitamin B_{12} level was higher in alcohol consumption ≥ 20 g/day group than in those were not. Alcoholic liver disease have already shown to have the falsely high vitamin B_{12} levels associated with liver dysfunction.[4, 5] On the other hand, among the patients without primary liver cancer, serum vitamin B_{12} levels were significantly higher in HBV than those in HCV patients. The reason was the number of patients classified into Child-Pugh B and C was significantly higher in HBV patients without primary liver cancer (data not shown).

Total serum vitamin B_{12} levels were also significantly higher in patients with primary liver cancer than those without. Some reports have also indicated that serum vitamin B_{12} levels are elevated in patients with HCC.[13–15] However, primary liver cancer is usually complicated with viral cirrhosis, the elevation should be distinguished from the association with cirrhosis. In present study, the difference was demonstrated only in patients with chronic hepatitis. Therefore, it could be concluded that primary liver cancer directly associated with serum vitamin B_{12} levels.

According to these findings, we could demonstrate that severe liver dysfunction (Child-Pugh C) and primary liver cancer affected the falsely elevated vitamin B_{12} levels.

In this study, we analyzed holoTC II additionally in some patients to clarify the mechanisms of falsely elevation. Unfortunately, we could evaluate only one patient in Child-Pugh C. We observed significantly lower level (within normal range) of the holoTC II percentage in patients of Child-Pugh B and C. It also means the percentage of holoHC increased in Child-Pugh B and C.

Thus, we propose two mechanisms responsible for elevated serum vitamin B_{12} levels in these patients (Fig. 4). First, holoHC possibly leaks into the circulation due to the destruction of hepatocytes (excess release hypothesis). This is supported by the observation that plasma vitamin B_{12} levels are elevated in 25–40% cases of acute hepatitis.[2, 11, 16, 17] However, in our study, serum vitamin B_{12} levels did not correlate with transaminase levels, this hypothesis could not explain high serum vitamin B_{12} in chronic viral hepatitis. Alternatively, the reduced uptake of holoHC by the injured liver may contribute to elevated levels of serum vitamin B_{12} (reduced clearance hypothesis). The asialoglycoprotein receptors, which bind holoHC for uptake into the liver, are expressed on normal hepatocytes. Since the number of receptors decreases

with the destruction of hepatocytes or the progression of liver cancer, holoHC remains in the circulation.[14, 15] The study using liver biopsy specimens have shown that the loss of vitamin B_{12} storage in the liver is associated with increased serum vitamin B_{12} levels.[16, 18–21]

In our study, elevated levels of serum vitamin B_{12} were also associated with poor prognosis. Some reports have already indicated that vitamin B_{12} levels are associated with increased mortality in critically ill patients of other diseases.[22, 23] The mechanisms that affect the prognosis of such patients remain unknown; however, based on the known aspects of hepatic inflammation and oxidative stress, it is possible to speculate on the mechanism. Previous reports have also shown that vitamin B_{12} modulates inflammation. The relationship between vitamin B_{12} and cytokine levels was demonstrated in humans.[24] In the report, vitamin B_{12} deficiency was related to high levels of tumor necrosis factor-alpha (TNF-α), and vitamin B_{12} supplementation normalized these levels. TNF-α is a well-known cytokine that plays a crucial role in chronic inflammation as well as in chronic hepatitis.[25, 26] Another report has indicated that vitamin B_{12} may regulate nuclear factor kappa B (NFκB), which is transcription regulator activated by cytokines like TNF-α, and it determines cell survival and apoptosis.[27, 28] These reports suggest that vitamin B_{12} plays the role of a modulator for cytokine expression in the injured liver where the vitamin B_{12} storage has diminished.

Tamura et al. also indicated the role of vitamin B_{12} as an immunomodulator.[29] demonstrating that vitamin B_{12} administration increased the percentages of cluster of differentiation (CD) 8^+ cells and natural killer (NK) cells in vitamin B_{12}-deficient patients. Moreover, Birch et al. reported thiolatocobalamin (a vitamin B_{12} derivative) has a protective effect on oxidant-damaged cells.[30] Furthermore, the hepatoprotective effect of vitamin B_{12} has been demonstrated using dimethylnitrosamine-induced liver injury in a mouse model.[31] This hepatoprotective effect of vitamin B_{12} may be achieved by the maintenance of sulfhydryl levels under oxidative conditions.

Taken all things together, the association of falsely elevated vitamin B_{12} with prognosis is considered to be due to diminished hepatic storage (excess release or reduced clearance) and loss of its hepatoprotective effect.

However, it remains unclear whether the hepatic vitamin B_{12} storage has any effect on maintain hepatic function in normal liver. The efficacy of vitamin B_{12} supplementation for cirrhosis also remains unknown. Further studies are needed to clarify the role of vitamin B_{12} in hepatic diseases.

A limitation of our study is the small number of patients. The prognosis of Q4 (vitamin $B_{12} \geq 880$ pg/mL) was significantly poor only in the patients without primary liver cancer; however, primary liver cancer is usually complicated with cirrhosis. To predict the prognosis of viral liver disease, appropriate cut-off level should be determined in all the patients. Large-scale study should be conducted to clarify the cut-off level of serum vitamin B_{12} for viral liver disease.

In conclusion, falsely elevated serum vitamin B_{12} levels mainly composed of its increased holoHC were associated with severity and prognosis in viral liver disease.

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