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Vitamin D supplementation could reduce the risk of acute cellular rejection and infection in vitamin D deficient liver allograft recipients

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ABSTRACT

Background: Vitamin D regulates the immune system and affects the outcome of allografts. We investigated the mechanisms underlying the preventative potential of vitamin D in acute cellular rejection (ACR) and infection, and determined its effects on the induction of both T cells and complement.

Methods: A total of 141 patients who received a liver allograft at our center between 2012 and 2016 were enrolled in the study and divided into a vitamin D supplementation group (case group, n = 71) and a non-vitamin D supplementation group (control group, n = 70). Serum was collected in the hours prior to transplantation and within the first month of transplantation. We evaluated the relationship between the serum levels of 25-hydroxyvitamin D ACR, infection, T cells, complement, and graft function. Follow-up was conducted until patient death or June 30, 2018.

Results: Vitamin D deficiency was an important independent risk factor for ACR. The incidence of ACR, and bacterial and fungal infection was reduced in patients with vitamin D supplementation. The frequency of Treg, Tmemory, T naïve cells and CD8 + CD28+ T cells (CTL) and the level of complement component 3 were related to ACR in the first month after transplantation. This study showed increased numbers of Treg cells and Tmemory cells and decreased numbers of Naïve cells and CTL in the case group. Vitamin D status was significantly associated with mortality.

Conclusions: Vitamin D supplementation is associated with a lower risk of ACR and infection, suggesting that it may promote immune tolerance towards the liver allografts.

1. Introduction

Vitamin D is synthesized in the skin or acquired from dietary sources and is transported to the liver where it is hydroxylated to form 25-hydroxyvitamin D (25(OH)D), which is the single best indicator of a patient's vitamin D status. A second hydroxylation step occurs in the kidney and at multiple sites of local metabolism to produce 1,25-dihydroxyvitamin D, which acts as a steroid hormone that regulates gene expression in multiple tissues, increasing the intestinal absorption of calcium and strengthening bone [1,2]. An association between vitamin D deficiency and all-cause mortality has been demonstrated [3,4]. However, vitamin D has been demonstrated to have many additional effects that may benefit patients undergoing solid organ transplantation, including effects on anti-inflammatory and anti-fibrotic effects [5] and a reduction in rates of acute allograft rejection [6–8]. Some pathways have been elucidated in molecular mechanisms studies, in which vitamin D acted as a regulator of both innate immunity and adaptive immunity [9–11].

The liver is the main site where hydroxylation of vitamin D at position C-25 takes place. Therefore, it is not surprising that the severity of liver dysfunction correlates with calcidiol levels [12]. Individuals with advanced liver disease often have low concentrations of total vitamin D, which is attributed to malabsorption, a failure of liver cells to 25-hydroxylate calciferol to 25(OH) vitamin D [25(OH)D], and decreased hepatic synthesis of albumin [13–17]. Liver transplant recipients experience a high burden of comorbidities associated with vitamin D deficiency, such as fractures, infections and diabetes [18,19]. Furthermore, a recent study suggested that vitamin D deficiency may contribute to cellular rejection of the hepatic allograft [8,20,21]. Therefore, an assessment of changes in vitamin D status among liver transplant

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ACR, acute cellular rejection; C3, complement component 3; CMV, cytomegalovirus; HR, Hazard ratios; IQR, interquartile range

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recipients may provide insights into the possible roles of vitamin D in promoting comorbidities before and after transplantation.

The present study was designed to investigate the association between the vitamin D status and the liver allograft function , and the incidence of acute cellular rejection (ACR) and infection, during the first month of transplantation in 141 liver graft recipients in a single center study. We further investigated the mechanisms underlying the preventative or therapeutic potential of vitamin D in ACR and infection, and determined its effects on the induction of both T cells and complement. Specifically, we examined the effects of vitamin D on total, unfractionated CD4 + T-cell populations, representative of those likely to be encountered in vivo.

2. Materials and methods

2.1. Subjects

This retrospective cohort clinical study was conducted in adults undergoing liver transplantation at Shanghai General Hospital of Shanghai Jiao Tong University-Shanghai Organ Transplantation Medical Center between January 2012 and December 2016. Among the 194 patients who received a liver transplantation, after exclusion of children (< 18 years; n = 13), patients who died in the perioperative period (n = 8), patients who received re-transplantation (n = 3), patients who were diagnosed as acute and chronic liver failure (ACLF) (n = 12), patients who refused to be enrolled in (n = 7), and patients who received vitamin D supplementation before admission or with vitamin D sufficiency prior to transplantation(n = 10). No organs from executed prisoners were used. A total of 141 25(OH)D deficient patients were enrolled in the study and divided into a vitamin D supplementation group (case group, n = 71), and a non-vitamin D supplementation group (control group, n = 70). Patients in the vitamin D supplementation group received vitamin D supplementation to promote the absorption of calcium and prevent osteoporosis. Patient serum was collected in the hours prior to transplantation and within the first month of transplantation. The time-points for serum collection included: enrollment (pre-transplant), 1 week, 2 weeks and 1-month post-transplant. Follow-up was conducted until patient death or June 30, 2018.

Patients who received a liver transplantation due to hepatocellular carcinoma met the traditional Milan criteria (single tumor ≤5 cm, or 2–3 tumors with none exceeding 3 cm, and no vascular invasion and/or extrahepatic spread) [22]. Livers were sourced from donors after cardiac death. Clinical and surgical data for the cases were obtained from the China Liver Transplant Registry (www.cltr.org) and by checking the original patient notes. The clinical and demographical characteristics of the studied population are presented in Table 1A.

This clinical study was approved by the Shanghai Jiao Tong University Institutional Review Board and was performed in accordance with Helsinki Declaration of 1975.

2.2. Surgical procedure

Liver harvesting was performed in accordance with standard techniques, using University of Wisconsin preservation fluid and cold storage solution for solid organ preservation. A conventional orthotropic liver transplantation technique was used for implantation, as described previously, and all had end-to-end biliary anastomosis constructed without T-tube drainage [23,24].

2.3. Postoperative management

All liver transplant recipients were managed in accordance with the protocol of our center. The recipients received IL-2 receptor antibody therapy as induction therapy and received intravenous methylprednisolone at surgery followed by a rapid taper of oral prednisone. Immunosuppressive therapy including tacrolimus and mycophenolate mofetil, without maintenance corticosteroids, was used in patients. They received maintenance immunosuppressive therapy comprised of tacrolimus and mycophenolate mofetil, without maintenance corticosteroids. The tacrolimus dosage was calculated to obtain predose serum levels ranging from 8 to 10 mg/L in the first months after transplant and from 5 to 8 mg/L thereafter. In the event of moderate to severe rejection episodes, three intravenous methylprednisolone boluses of 500 mg/day followed by 4 days of 40 mg oral prednisone were administered [24].

Anti-infective prophylaxis was administered in accordance with local practice. The most common protocol was the administration of antibiotics (cefoperazone sodium and sulbactam sodium/piperacillin sodium and tazobactam sodium) for 5–7 days without antiviral prophylaxis. Antiviral prophylaxis with valganciclovir was administered only to cytomegalovirus (CMV) mismatch patients (CMV IgG-positive donor/CMV IgG-negative recipient).

The postoperative anti-HBV protocol included the administration of entecavir plus low-dose intramuscular HBV immunoglobulin. The recurrence of HBV was monitored by evaluating the presence of HBV surface antigen and HBV DNA in serum. These tests were performed at each follow up visit [25].

2.4. Vitamin D measurement and supplementation

Circulating 25(OH)D levels were measured using a chemo-luminescent immunoassay implemented on a Liaison automatic analyzer (DiaSorin Inc., Stillwater, MN, USA). Sufficiency of 25(OH)D was defined as a level > 50 nmol/L, mild to moderate vitamin D deficiency (insufficiency) as 25 to 50 nmol/L, and a severe deficiency as < 25 nmol/L, based on the Endocrine Society Clinical Practice guidelines [26,27].

During the first postoperative day, the transplant physician would initiate vitamin D supplementation. The vitamin D supplementation group (n = 71) received oral calcitriol (250 ng/day, Rocaltrol, Roche) during the first month after transplantation. The non-vitamin D supplementation group received neither 25(OH)D nor 1,25(OH)2D3.

2.5. Histology

Biopsies were performed when an inspection revealed that there may be an ACR reaction. ACR was confirmed through a biopsy confirmed in each instance. Biopsy specimens were evaluated by two experienced transplant pathologists For controversial specimens, a consensus was reached after discussion between the two pathologists [23].

2.6. Infections

Suspected infections were confirmed through an exhaustive work-up, including full clinical examination, blood culture, urine culture, chest X-ray (sometimes associated with a chest computed tomography-scan), an ascitic fluid white cell count and cultures for all patients with ascites, a stool culture if diarrhea was present, and direct examination and culture of sputum. Multiple samples from the same patient were taken at different time points [28]. In the case of persistent unexplained fever, dental panoramic and sinus X-rays, and chest and abdominal computed tomography scans with intravenous contrast were performed [29].

2.7. Flow cytometry analysis of T cells subsets

Cells were collected from patients' peripheral blood for flow cytometry (BD FACSCalibur) analysis. Most T cell subsets have been tested.

2.8. Complement and immunoglobulin concentrations

In our center, the levels of serum C3, C4, IgE, IgG, IgA, and IgM

Table 1A
Basic characteristics of case and control participants.

Variable	Overall N = 141	Cases N = 71	Controls N = 70	P value
Age at blood draw, year, (mean ± SD)	49.7(10.1)	49.5(8.5)	50.5(11.6)	0.56
Female Gender, N	21	7	14	0.09
Body mass index, kg/m2	22.6(3.5)	23.27(3.6)	22.09(3.3)	0.06
History of HBV	103	50	53	0.48
Diabetes mellitus, N	36	19	17	0.74
History of abdominal surgery	32	20	12	0.12
Alcho; consumption	33	17	16	0.88
Pack-year of smoking	46	22	24	0.68
Current smoker(%)	33	31	34	0.62
Main diagnosis				
Hepatocellular carcinoma	32	12	20	0.1
Hepatitis B	30	17	13	0.4
Hepatitis B and hepatocellular carcinoma	59	31	28	0.66
Alcohol	9	5	4	0.9
Autoimmune	11	6	5	0.77
Laboratory findings				
Serum AST, U/L,(mean ± SD)	75.01(119.51)	79.35(131.42)	67.53(97.41)	0.63
Total bilirubin, mg/dl, (mean ± SD)	70.27(87.73)	74.09(90.24)	69.27(85.39)	0.51
Albumin, (mean ± SD)	35.77(6.71)	35.12(6.70)	36.13(6.74)	0.46
Creatinine, (mean ± SD)	69.24(21.61)	72.03(25.08)	66.41(16.19)	0.12
INR, INR units (mean ± SD)	1.30(0.50)	1.32(0.56)	1.27(0.38)	0.64
GLU, (mean ± SD)	5.25(1.76)	5.31(1.49)	5.15(2.16)	0.67
MELD score	13.28(6.68)	13.49(7.25)	13.07(6.10)	0.71
Plasma 25-hydroxyvitamin, median (interquartile), nmol/L, (mean ± SD)	32.34(8.43)	34.06(6.79)	30.58(9.55)	0.74

AST, aspartate aminotransferase; BMI, body mass index; INR, international normalised ratio; MELD, model for end-stage liver disease score.

were assessed routinely using an automatic biochemistry analyzer (Hitachi 7600, Japan), based on the principles of immunonephelometry.

2.9. Statistical analyses

Analyses were completed using SPSS 22 and EmpowerStats (http://www.empowerstats.com/). Continuous variables are reported as median and interquartile range (IQR). Univariate and multivariable logistic regression analysis were used to analyze the risk factors of severe vitamin D deficiency. The relationship between severe vitamin D deficiency prior to transplantation and ACR and infection was also analyzed through univariate and multivariable Cox regression analyses and demonstrated using Kaplan-Meier curves with a log-rank test. Multivariable Cox regression was used to evaluate the potential association between vitamin D deficiency and ACR in the first month post-transplantation. The multivariable analysis for the identification of independent risk factors associated with ACR included characteristics associated with ACR in a univariate Cox regression analysis ($P < 0.10$) Hazard ratios (HR) are presented with 95% confidence intervals (95% CI). $P < 0.05$ indicated statistical significance. A mixed-effects model repeated-measures analysis was used to detect the differences in T cell subsets and C3 at pre-transplant, and on the first second, and fourth weeks following transplantation between the vitamin D supplementation group and the control group. Box and whisker plots were used to illustrate the relationship between vitamin D status and allograft function and kidney function. Moreover, Kaplan–Meier curves were also used to show 6 months survival in the vitamin D supplementation group and control group. P values were calculated using the Wilcoxon-Rank sum test.

3. Results

3.1. Subject baseline characteristics and circulating pre-transplantation levels of 25(OH)D in the 141 liver allograft recipients

The baseline characteristics of patients in case and control group are listed in Table 1A. Variables such as gender, age, prior history of HBV, main diagnosis, laboratory findings and serum 25(OH)D levels were not different between the two groups.

A total of 56 patients had severe vitamin D deficiency and 86 patients had vitamin D insufficiency prior to transplantation. We found that female gender, hepatocellular carcinoma and alcohol consumption were associated with a higher risk of severe vitamin D deficiency compared with vitamin D insufficiency, and the association was statistically significant (Table 1B). The pretransplant circulating levels of 25(OH)D in our study cohort of 141 liver transplant recipients was 32.34 nmol/L (± 0.71) (mean (± SEM)) (Fig. 1).

3.2. Effects of pre-transplantation vitamin D status on ACR and post-transplantation infection

The number of patients with ACR with different circulating 25(OH)D levels is shown in Fig. 2A. In the first month of transplantation, 18 of the 141 vitamin D-deficient patients (13.4%) suffered an episode of ACR. ACR was significantly more frequent in the severe D deficiency group (n = 11, 19.6%) than in the vitamin D insufficiency group (n = 7, 8.1%) ($P = 0.04$ by Pearson Chi-Square). As Kaplan-Meier analysis of the time at which ACR occurred showed that patients with severe vitamin D deficiency were at greater risk of ACR occurrence in the first month post-transplantation than those with insufficiency (Fig. 2B; log rank test: $P = 0.044$). The number of patients with infection with different circulating 25(OH)D levels is shown in Fig. 2C. In the first month of transplantation, 26 of the 56 severe vitamin D deficient recipients (46.4%) experienced an episode of an infection and 22 (25.8%) of the vitamin D insufficient recipients developed an infection. Infection was significantly more frequent in the severe vitamin D deficiency group than in the vitamin D insufficiency group ($P = 0.012$ by Pearson Chi-Square). A Kaplan-Meier analysis of infection risk revealed that patients with severe vitamin D deficiency were at greater risk of infection occurrence in the first month post-transplantation compared with those with vitamin D insufficiency. (Fig. 2D, log rank test $P = 0.01$).

Table 1B
Factors associated with severe vitamin D deficiency prior to liver transplantation.

		Total N = 141	25(OH)D ≤ 25 nmol/L	25(OH)D > 25 nmol/L	OR(95% CI) for vitamin D severe deficiency	P-value*
Gender	Male	120	43(76.8%)	77(90.6%)	3.12(1.17,7.79)	0.024
	Female	21	13(23.2%)	8(9.4%)		
Diabetes Mellitus	Yes	36	16 (28.6%)	20(23.5%)	1.31(0.73,3.05)	0.502
	No	105	40(71.4%)	65(76.5%)		
Hepatocellular carcinoma	Yes	64	17 (30.4%)	47 (55.3%)	3.2(1.4, 7.10)	0.004
	No	78	39(69.6%)	38(44.7%)		
Cirrhosis	Yes	112	48 (85.7%)	64(75.3%)	0.71(0.41,1.70)	0.4
	No	29	8(14.3%)	21(24.7%)		
Pack-year of smoking	Yes	45	14(25.0%)	31(36.5%)	0.76(0.35.1.67)	0.5
	No	96	42(75.0%)	54(63.5%)		
Alcohol consumption	Yes	33	8 (14.3%)	25 (29.4%)	1.98(1.16,2,22)	0.038
	No	108	48(85.7%)	60(70.6%)		
History of abdominal surgery	Yes	45	17(30.4%)	28 (32.9%)	0.74(0.33,1.67)	0.747
	No	96	39(69.6%)	57(67.1%)		
Age(years)	Median(IQR)	N/A	50(45,58)	49(44,57)	1.01(0.98,1.01)	0.51
MELD	Median(IQR)	N/A	14(9.16)	13(8,17)	1.02(0.97,1.08)	0.39
BMI(Kg/m2)	Median(IQR)	N/A	22.76(21.22,24.91)	22.67(20.10,24,48)	0.99(0.90,1.11)	0.9

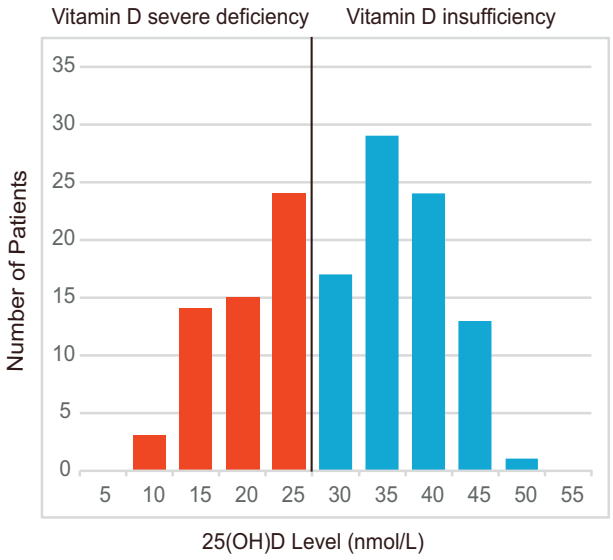

Fig. 1. Distribution of the 25(OH)D levels among the study cohort of 141 liver transplant recipients
Each bar on the X axis represents an interval of 5 nmol/L and ranges from 0 nmol/L to 50 nmol/L. The y-axis depicts the number of subjects corresponding to each bar. A circulating level of 25 nmol/L was used as the threshold to classify the liver graft recipients as demonstrating vitamin D deficiency (red bars, circulating levels of 25(OH)D, ≤25 nmol/L) and vitamin D mild to moderate deficiency; blue bars, circulating levels of 25(OH)D, > 25 nmol/L). The black vertical line in the frequency histogram is the threshold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Effect of vitamin D supplementation on ACR

Vitamin D supplementation was initiated by the attending physician within the first postoperative day, and this cohort of patients received oral calcitriol (250 ng/day) during the first month after transplantation. The non-supplementation group received neither 25(OH)D nor 1,25(OH)2D3.

We found that the incidence of the development of ACR was higher in the no vitamin D supplementation group (n = 13) compared with to the vitamin D supplementation group (n = 5) (P = 0.04, Pearson Chi-Square) (Fig. 3A). A Kaplan-Meier analysis of time to ACR development showed that patients in the control group were more inclined to develop ACR within the first month post- transplantation compared with those in the vitamin D supplementation group (Fig. 3B; log rank test: P = 0.044). Then, we take the vitamin supplementation group as a reference, a Cox regression analysis was used to analyze the risk factors for ACR. A univariate and multivariable Cox regression analysis was used to analyze the characteristics and to identify the independent risk factors leading to ACR. The results showed that vitamin D deficiency was an important independent risk factor for ACR. Moreover, this study also found that the frequency of Treg cells, T memory cells, T naïve cells and CD8 + CD28+ T cells CTL played a key role in maintaining the development of ACR (Table 2A).

3.4. Vitamin D supplementation and infection

The incidence of bacterial infection and that of fungal infection were also higher in the non-supplementation group (n = 30) compared with the vitamin D supplementation group (n = 18) (25 vs. 43%; P = 0.001, Pearson Chi-Square) (Fig. 3C). A Kaplan-Meier analysis of time to infection occurrence revealed that patients in the control group were at greater risk of infection in the first month post transplantation compared with those in the vitamin D supplementation group (Fig. 3D, log rank test: P = 0.015). The main bacteria found were Gram-negative bacilli and Gram-positive cocci followed by Gram-negative cocci (Table 2B).

3.5. Vitamin D supplementation and T-cell subsets and complement

A mixed-effects model repeated-measures analysis was used to detect differences in serum Treg cells, T memory cells, T Naïve cells, CTL and C3 at pre-transplant, and on the first, second, and fourth week following transplant between the vitamin D supplementation group and the control group. Serum 25(0H)D levels in the vitamin D supplementation group were higher than in the control group (P = 0.001) (Fig. 4A). Patients in the vitamin D supplementation group had a significantly higher concentration of Treg cells and T memory cells at the second and fourth week after liver transplantation (Fig. 4B, C). Moreover, the proportion of T naïve cells and CTL in control group was higher than that in vitamin D supplementation group from the 2nd week after liver transplantation (Fig. 4D, E). Furthermore, patients in the vitamin D supplementation group had a significantly lower C3 concentration at the fourth week after liver transplantation (Fig. 4F). There were no significant differences in IgE, IgG, IgA, IgM or CH50 concentrations between patients in the vitamin D supplementation group and the control group (data not shown). However, patients in the vitamin D supplementation group had a significantly lower C3 concentration at the fourth week after liver transplantation.

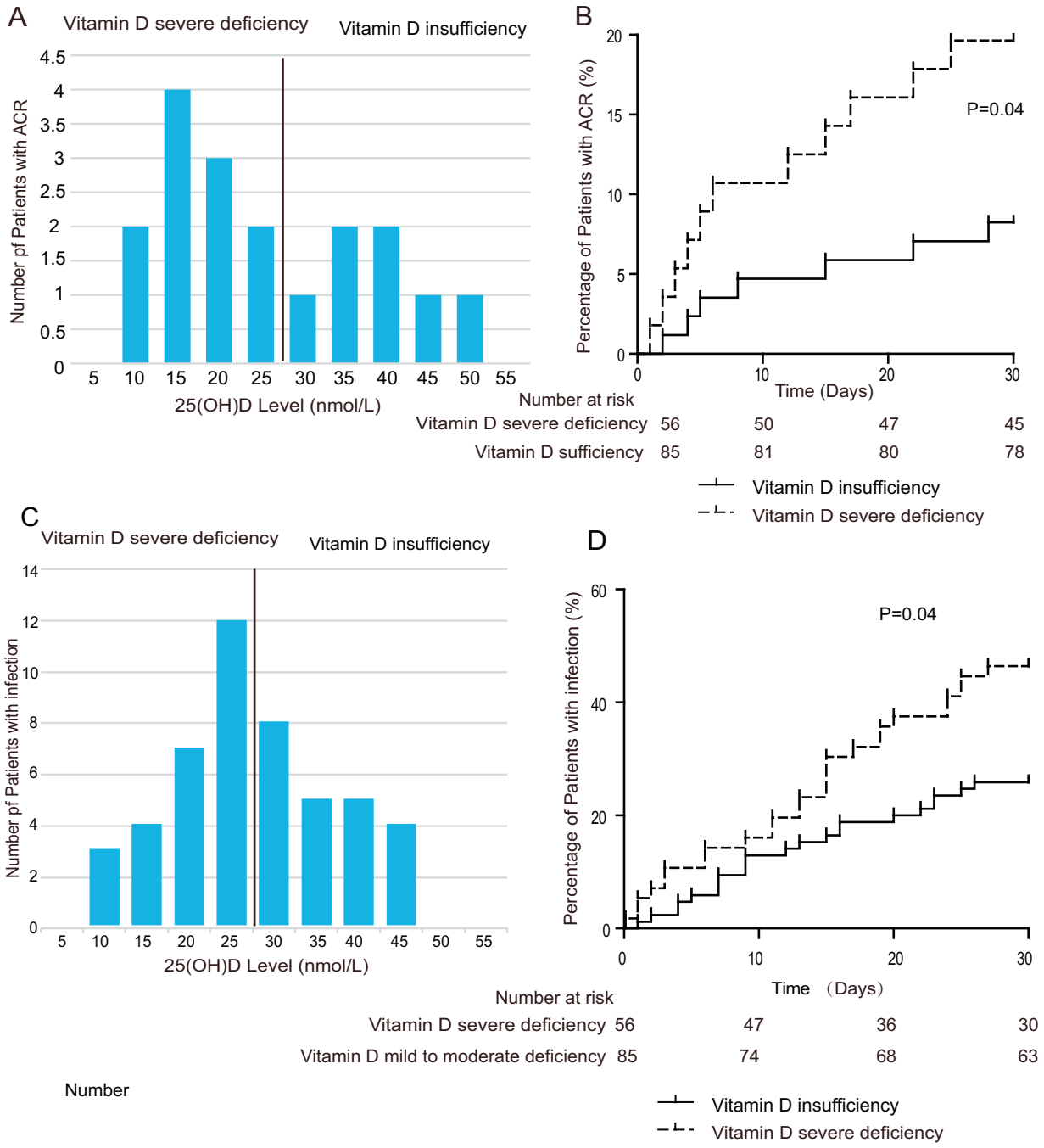

Fig. 2. Vitamin D status and acute cellular rejection and infection.
(A) Each bar on the X-axis represents an interval of 5 nmol/L of circulating levels of 25(OH)D and the levels range from 0 ng/mL to 50 nmol/L. The y-axis depicts the number of patients who developed ACR in each cohort represented by the corresponding interval.
(B) The number of days post-transplantation is represented on the x-axis and the proportion of patients with ACR is represented on the y-axis. The number of liver graft recipients at risk for ACR is provided below the corresponding time points. The Kaplan-Meier curves were compared using a log-rank test.
(C) Each bar on the X-axis represents an interval of 5 nmol/L of circulating levels of 25(OH)D and the levels range from 0 nmol/L to 50 nmol/L. The y-axis depicts the number of recipients who developed an infection represented by the corresponding interval.
(D) The number of days post-transplantation is represented on the x-axis and the proportion of patients with infection is represented on the y-axis. The number of liver graft recipients at risk for ACR is provided below the corresponding time points. The Kaplan-Meier curves were compared using a log-rank test.

3.6. Vitamin D status and allograft function and kidney function after transplantation

There were no significant differences in allograft function or kidney function between recipients in the vitamin D supplementation group and the control group (Fig. 5). The Fig. 5C shows data for 67 liver transplant recipients who received vitamin D supplementation after transplantation and 56 liver transplant recipients without vitamin D supplementation after transplantation, excluding ACR patients. eGFR at 4 weeks post-transplantation was calculated using CKD-EPI equation.

3.7. Kaplan–Meier curves

Follow-up was conducted until death or June 2018. This study

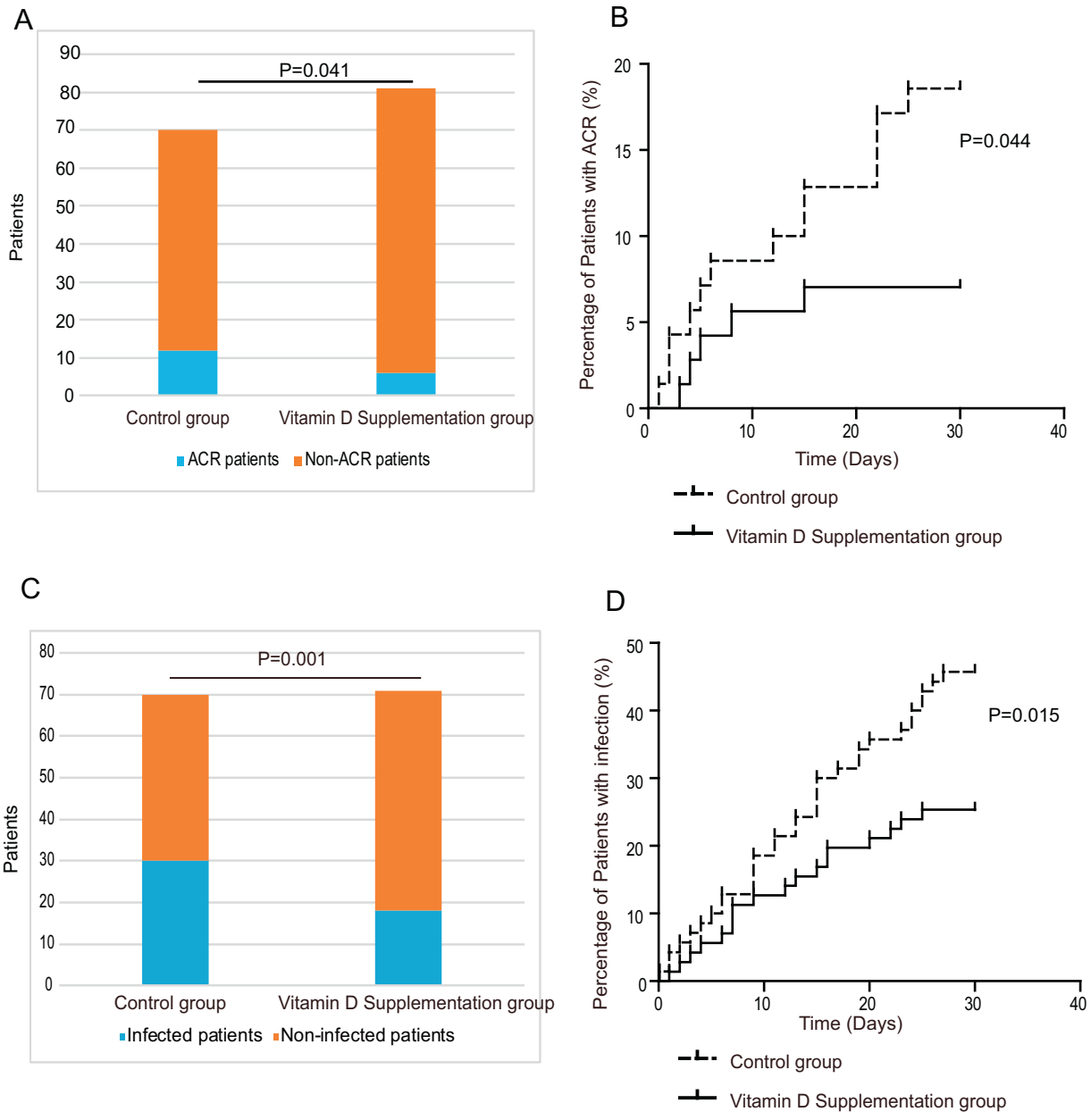

Fig. 3. Effectes of vitamin D supplementation on ACR and infection
(A) Proportion of patients with ACR between the control group and the vitamin D supplementation group
(B) Kaplan-Meier analysis of time to development of ACR between the control group and the vitamin D supplementation group
(C) Proportion of infected patients between the control group and the vitamin D supplementation group.
(D) Kaplan-Meier analysis of time to development of infection between the control group and the vitamin D supplementation group.

found that vitamin D supplementation can effectively improve the survival rate at 18 months post-transplantation. (Fig. 6; log rank test: P = 0.037).

4. Discussion

In the current study, vitamin D deficiency was defined using a cutoff of 25 nmol/L of 25(OH)D, in keeping with the Institute of Medicine's determination that the health risks of vitamin D deficiency increase when 25(OH)D levels fall below this threshold [2]. We found that most of the recipients had vitamin D deficiency in the pre-transplantation and post-transplantation period. A previous study found that those who were treated using calcitriol supplementation initiated by an attending physician within the first month of transplantation had a lower incidence of both ACR and infection compared with those who did not received calcitriol supplementation [30]. That study also showed an increase in Treg cells and T memory cells in the calcitriol-treated group. The current study demonstrated decreased costimulatory molecule expression (HLA-DR, CD28) and decreased expansions of T naïve cells and CTL. The reduction rate of C3 in the vitamin D treatment group was significantly higher than that in the non-supplementation group.

Studies of the general population have provided mounting evidence that vitamin D deficiency is associated with diabetes, fractures, infections, malignancies and autoimmune diseases [13,31]. These comorbidities also commonly complicate liver disease and liver transplantation [18,19,32]. Many centers advise recommend vitamin D supplementation post-transplantation as indicated, but not usually on a routine basis.

Table 2A
Multivariable cox regression analysis for development of ACR with Vitamin D deficient patients stratified by treatment with or without calcitriol.

Characteristic	N(%)	Univariate analysis			Multivariate analysis		
		HR	95%CI	P-value	HR	95%CI	P-value
Vit D deficient and calcitriol (reference)[a]	71	1.0			1.0		
Vit D deficient and no calcitriol[b]	70	1.68	1.01–4.07	0.002	2.594	1.03–6.56	0.003
Age (per year)	141(100%)	0.98	0.94–1.02	0.2625			
Female gender	21(14.8%)	0.53	0.17–1.63	0. 0963	0.86	0.54–2.34	0.15
infection	38(27%)	1.7	0.65–4.44	0.2823			
Corticosteroid maintenance	57(40%)	1.97	0.79–4.94	0.1464			
Laboratory findings of post liver transplantation							
WBC		1.04	0.96–1.13	0.3612			
TBIL		0.96	0.91–1.01	0.1395			
NK		0.94	0.87–1.02	0.1125			
Treg		0.63	0.60–1.10	0.0422	0.60	0.59–1.23	0.02
Tnaive		2.04	1.01–3.08	0.0068	2.2	1.51–3.47	0.036
Tmemory		0.73	0.71–1.0	0.0265	0.75	0.72–1.04	0.04
C3		1.91	1.38–2.04	0.021	2.01	1.99–3.14	0.032
CTL		1.87	1.50–3.11	0.0400	2.01	1.93–4.17	0.047

[a] Vit D Deficient and calcitriolc represents the graft recipients in vitamin D supplementation group.
[b] Vit D Deficient and no calcitriol represents the graft recipients in control group.

A previous study including 133 liver transplant recipients revealed that the recipients with pre-transplantation circulating levels of 25(OH)D of < 12.5 nmol/L were at greater risk of moderate to-severe ACR episodes within first 2 months of transplantation. [8]. The findings from these studies indicate that vitamin D deficiency could be independent risk factor for ACR, and the clinical effects of vitamin D supplementation on clinical liver transplantation remains to be elucidated [30]. The current shows that low concentrations of 25(OH)D are common among liver transplant candidates [17,18]. The current study also found that severe vitamin deficiency (< 25 nmol/L) was associated with ACR. Moreover, our demonstration that vitamin D supplementation could reduce the risk of ACR in vitamin D deficient liver allograft recipients confirms and extends the results of earlier reports [17,18].

Earlier studies have also demonstrated a relationship between 25(OH)D deficiency and infections in the general population [4,33]. Another potentially beneficial effect of vitamin D is its hypothesized protective effects against infections in kidney transplantation. As indirect support for this hypothesis, in a retrospective evaluation of 89 kidney transplant recipients, the authors found that severe vitamin D deficiency (< 25 nmol/L) was associated with an increased incidence of opportunistic infections [34]. The current study also found that severe vitamin D deficiency was associated with infection. Moreover, vitamin D supplementation could reduce the risk of ACR in vitamin D deficient liver allograft recipients.

Together with the results of previous mentioned above, the present results provide evidence that vitamin D supplementation may represent a useful and inexpensive contribution to improved immune tolerance for successful transplantation and the ability of the host to fight infection in the liver transplantation setting [8]. Most prior studies reported on cohorts of < 50 patients and most did not investigate report East Asian recipients. The samples analyzed in the current study were collected at Shanghai General Hospital of Shanghai Jiao Tong University-Shanghai Organ Transplantation Medical Center in China and extend the findings of smaller studies. To the best of our knowledge, the current study is the first to examine 25(OH)D levels in Chinese liver allograft recipients pre-transplantation and post-transplantation.

1,25(OH)2D3 acts on the vitamin D receptor (VDR), which belongs to the nuclear hormone receptor superfamily [35]. When interacting with 1,25(OH)2D3, VDR heterodimerizes with retinoid X receptor (RXR) and binds target DNA sequences called vitamin D response elements (VDRE) to regulate the expression of interrelated downstream target genes. VDR is ubiquitously expressed in immune cells, including activated T lymphocytes, and cells of the innate immune system, such as macrophages and dendritic cells (DCs). Immune cells not only express the VDR but may contain the machinery for producing biologically active 1,25(OH)2D3 [35]. The protective role of 1,25(OH)2D3 for the allografts has been studied and possible mechanisms are listed as follows: increases inflammatory mediators secreted by T cells such as IL-2 and IL-17, and decreases in interferon-γ [36]; downregulation of DC immunogenic activity [37]; upregulation of the immunoprotective activity of Treg cells [38]. The current study also showed an increase in Treg cells and T memory cells in the vitamin D treatment group. Together, these data support the concept that vitamin D status may control Treg cells frequencies in vivo. Experimentally, vitamin D has multiple effects on immunoregulatory [39]. These findings, along with strong epidemiological evidence linking vitamin D deficiency to multiple autoimmune diseases [39] suggest that 1,25(OH)2D3 could decrease the occurrence of ACR after liver transplantation and regulate

Table 2B
Characteristics of posttransplantation infected patients.

Site of infection	Type of infections								
	Gram-positive bacillus	Gram-negative bacillus	Gram-negative cocc	Gram-positive cocci	*C. difficile*	Bacteriaundocumented	Candida	Aspergillus	Total
Sepsis	1	4	1	3	0	0	0	0	9
Pulmonary infection	2	7	5	6	2	2	1	1	26
Urinary tract infection	0	1	0	0	0	0	2	0	3
Cutaneous infection	0	1	0	2	1	0	0	1	5
Spontaneous bacterial peritonitis	0	1	1	2	0	0	0	0	4
Chronic bone infection	0	0	0	1	0	0	0	0	1
Total	3	14	7	14	3	2	3	2	48

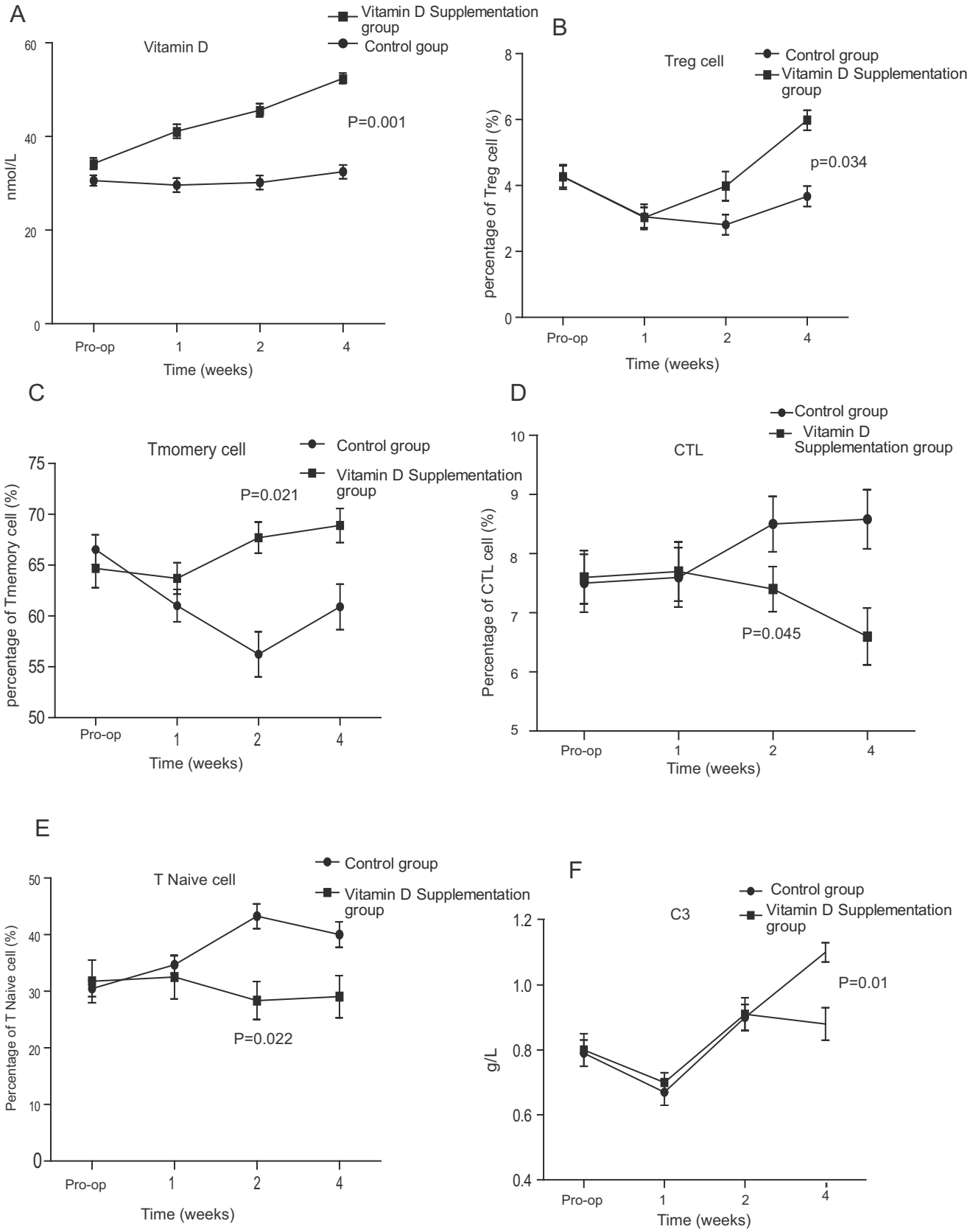

Fig. 4. The frequency of Treg cells, Tmemory cells, T naïve cells, CTL and the levels of C3 Changed in the vitamin D supplementation group compared with the control group

A Mixed-effects model repeated-measures analysis was used to detect the difference in serum Treg cells, Tmemory cells, T naïve cells, CTL and C3 at pre-transplantation, and on the first, second, fourth weeks following transplantation between the vitamin D supplementation group and the control group. (A) The serum 25(OH)D levels in control and vitamin D supplementation group. (B, C) Treg and Tmemory cell levels in the control group and vitamin D supplementation group. (D, E) The proportion of T naïve cells and CTL in the control group and the vitamin D supplementation group. (F) C3 concentration in the control group and vitamin D supplementation group.

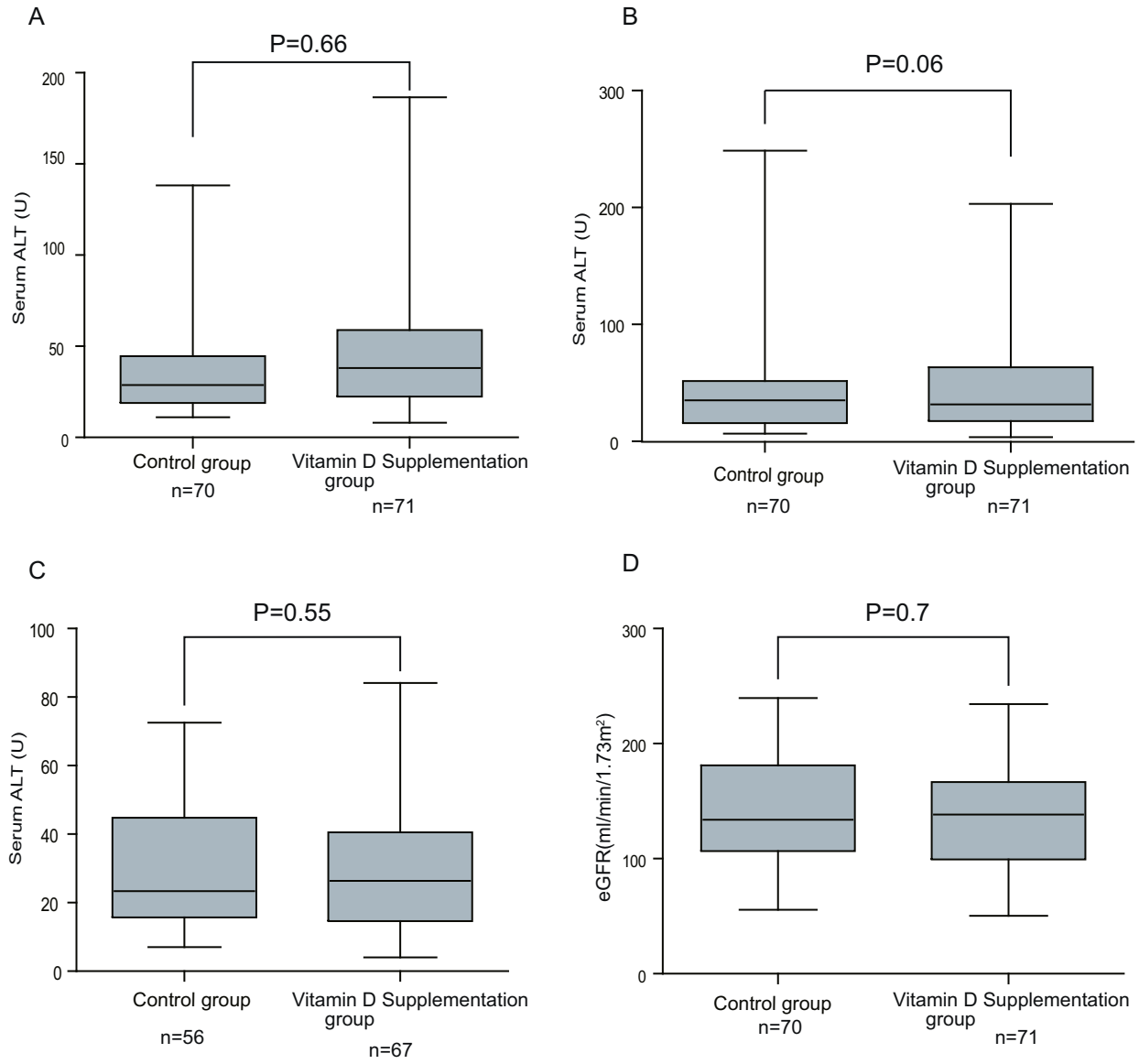

Fig. 5. Vitamin D status and allograft function and kidney function
(A) Box and whisker plots of serum ALT(U) measurements for the vitamin D supplementation group and control group before transplantation. (B) Box and whisker plots of serum ALT(U) measurements for the vitamin D supplementation group and control group at the 4th week after transplantation. (C) Box and whisker plots of serum ALT(U) measurement for the vitamin D supplementation group and control group at the fourth week after transplantation without the development of ACR. (D) Box and whisker plots of eGFR (mL/min/1.73 m2) at the fourth week after transplantation, between the vitamin D supplementation group and control group. In each box plot, the horizontal line represents the median with the edges of the box representing the 25th and 75th percentiles. The top whisker represents the 75th percentile value plus 1.5 times the interquartile range and the bottom whisker represents the 25th percentile value minus 1.5 times the interquartile range. P values were calculated using the Wilcoxon-Rank sum test.

immune homeostasis mainly through inducing Treg cell differentiation.

However, vitamin D supplementation in patients with multiple sclerosis produced no increase in the frequency of peripheral blood Treg cells [40]. Clearly further studies in patients are required to fully understand the impact of vitamin D on T cell subsets [41].

Our study has several strengths, including both clinical outcomes data and complete data concerning 25(OH)D levels in the first month post transplantation, and the availability of at least 18 months of follow up data for all patients [2]. We are fully aware that the current study has several limitations. These limitations include a lack of information about the level of parathyroid hormone and bone density [34], and a lack of data on the level of 25(OH)D levels, T cell subsets and complement components after the first month of transplantation. This was a single-center study including Chinese patients and the results may not be generalizable to other countries.

5. Conclusion

Vitamin D severe deficiency was an important independent risk factor for ACR and infection for liver allograft recipients. Vitamin D supplementation is associated with a lower risk of ACR and infection, suggesting that it may promote immune tolerance towards the liver allografts and likely through inducing Treg cell differentiation.

Authors' contributions

Tonghai Xing was involved in drafting the manuscript, designing of the study, acquiring and analyzing the data, and approving of the final version to be published. Ying Chen, Jinyan Zhang, Lin Zhong and Zhihai Pen contributed to the conception and design of the study, and the acquisition of the data. Qiang Zhou and Lixing Li were responsible

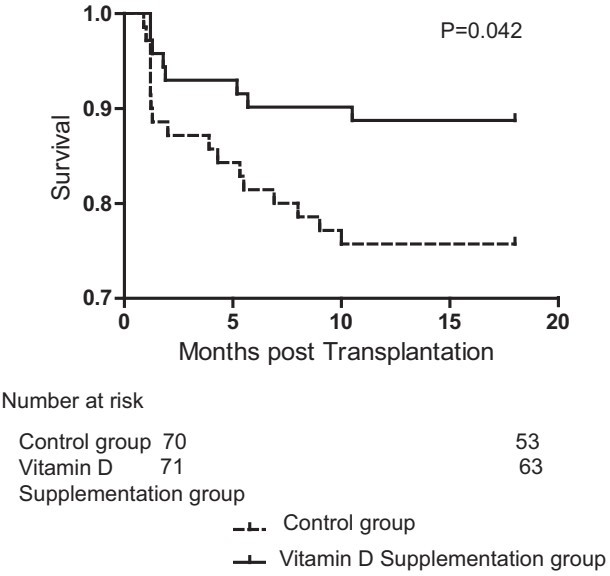

Fig. 6. The presence of vitamin D supplementation predicted mortality. Kaplan–Meier curves for 18-months survival in patients with or without vitamin D supplementation.

for analysis and interpretation of the data. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare no potential conflicts of interest.

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