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Low Free Triiodothyronine Level as a Predictor of Cardiovascular Events and All-Cause Mortality in Patients Undergoing Hemodialysis: The DREAM Cohort

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Aim: Low T3 syndrome is characterized by low serum triiodothyronine (T3) levels without elevation of thyroid-stimulating hormone (TSH) in patients without apparent thyroid disease, which is known to be associated with worse clinical outcomes in various populations including those with kidney failure. In this study, we examined whether low free T3 (FT3) levels are independent predictor of cardiovascular disease (CVD) events in patients undergoing hemodialysis.

Methods: This was a prospective cohort study of patients with chronic kidney disease undergoing hemodialysis. From the total of 518 patients, we excluded patients with treated or untreated hyperthyroidism or hypothyroidism and those treated with corticosteroids.

Results: We analyzed data from 438 eligible patients. During the 5-year follow-up, 154 new CVD events and 86 all-cause deaths were recorded. Kaplan-Meier analysis showed that lower FT3 levels were associated with higher risks for new cardiovascular events and all-cause death. This inverse association of FT3 and new CVD events remained significant after adjustment for age, sex, duration of hemodialysis, diabetic kidney disease, hypertension, dyslipidemia, and smoking; however, it was no longer significant after further adjustment for prior CVD or N-terminal fragment of probrain natriuretic peptide (NT-proBNP). FT3 did not show an independent association with all-cause mortality.

Conclusions: Our results indicate that low FT3 status is not an independent predictor of new CVD events and that the following factors are closely associated: prior CVD, low FT3 and high NT-proBNP levels at present, and future risk of new CVD events in hemodialysis patients.

Key words: Thyroid hormone, Hemodialysis, Cardiovascular disease, Mortality

Introduction

Patients suffering from kidney failure are at an increased risk for cardiovascular disease (CVD) and all-cause mortality, with extremely elevated risk for cardiovascular mortality as compared with the general population[1)]. The elevated risk for CVD and death has been attributed not only to traditional risk factors but also to nontraditional risk factors[2)], including metabolic and endocrinological alterations due to kidney failure such as insulin resistance[3)], growth hormone-insulin-like growth factor 1 (IGF-1) axis[4)],

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testosterone[5], and adrenal sex hormone[6]. In addition, patients with kidney failure often have abnormal thyroid function tests[7], which are predictive of poor outcomes in this population[8].

Low T3 syndrome is characterized by low triiodothyronine (T3) level without elevation of thyroid-stimulating hormone (TSH)[9]. Thyroxine (T4) level is usually within the normal range in low T3 syndrome, and the conversion from T4 to T3 in peripheral tissues is suppressed in response to severe disease conditions such as heart failure[10] and kidney failure[11] in the absence of apparent thyroid disease. Thus, low T3 syndrome, which is also referred to as "euthyroid sick syndrome" or "nonthyroidal illness syndrome," has been regarded as an important sign of severe illness. Although low T3 syndrome may represent "adaptation" to severe disease conditions in order to avoid wasting[12], it may also play a pathophysiologic role in the worsening of the severe conditions[13]. Since thyroid hormones were shown to have cardioprotective properties[9, 14, 15], a low T3 status itself could further deteriorate cardiac function. In fact, T3 replacement was shown to improve cardiac performance in a randomized controlled study of patients with heart failure and low T3 syndrome[15].

Low T3 or free T3 (FT3) levels were reported to be an independent predictor of all-cause mortality in a cohort of hemodialysis patients[8, 16, 17], and this association was independent of inflammation marker interleukin 6 (IL-6) as shown in some studies[8]. Similar findings were made in patients treated with peritoneal dialysis[18], although such an association between low T3 (FT3) status and long-term mortality risk was not confirmed in other studies[19]. Furthermore, information has remained to be lacking on whether a low FT3 level is an independent factor for cardiovascular events in this population.

Thus, in the present study, we hypothesized that a low FT3 level is an independent predictor of cardiovascular events. We also examined its association with all-cause mortality.

Methods

Study Design

This was a prospective cohort study with cross-sectional analyses. In cross-sectional analyses, we examined associations of FT3 levels with other factors at baseline. In longitudinal analysis, we examined the associations of FT3 levels with cardiovascular events and all-cause mortality.

Selection of Patients for Analysis

This report is a part of a series of analyses derived from our prospective cohort study named DREAM (Dialysis-Related Endocrine And Metabolic changes affecting cardiovascular disease)[6, 20-22]. The DREAM cohort included 518 patients treated with maintenance hemodialysis at Inoue Hospital, Suita, Osaka, Japan, in December 2004. This cohort study adhered to the Declaration of Helsinki, and the protocol of this study was reviewed and approved by the ethics committee at Inoue Hospital (Approval No. 121). The DREAM cohort was registered at UMIN-CTR (ID UMIN000006168, http://www.umin.ac.jp/ctr/index.htm). All the participants gave written informed consent.

To select patients without apparent thyroid disease, we excluded the following patients: (1) those with treated hyperthyroidism, (2) those with treated hypothyroidism, (3) those treated with corticosteroids as these could affect thyroid function, (4) those with untreated primary hyperthyroidism, (5) those with untreated primary hypothyroidism, (6) those with untreated secondary hyperthyroidism, (7) those with untreated secondary hypothyroidism, and (8) those with missing thyroid function test.

Definitions of Thyroid Hormone Status

We have measured FT3, FT4, and TSH levels using blood taken from blood access just before the first hemodialysis session of the week. Serum FT3, FT4, and TSH levels were measured by specific chemiluminescent immunoassay (CLIA) methods performed by BIO MEDICAL LABORATORIES (BML, Inc.) in Tokyo, Japan. The reference ranges were 2.2–4.1 pg/mL for FT3, 0.8–1.9 ng/dL for FT4, and 0.4–4.0 μIU/mL for TSH. Based on the medications and the reference ranges of TSH and FT4 levels, we classified the patients into the following groups: (1) those with treated hyperthyroidism who were taking anti-thyroid medication (thiamazole, propylthiouracil), (2) those with treated hypothyroidism who were receiving levothyroxine replacement, (3) those treated with corticosteroids at any dosage, (4) those with untreated primary hyperthyroidism with high FT4 and low TSH levels, (5) those with untreated primary hypothyroidism with low FT4 and high TSH levels, (6) those with untreated secondary hyperthyroidism with high FT4 and high TSH levels, (7) those with untreated secondary hypothyroidism with low FT4 and low TSH levels, (8) those with missing thyroid function test, and (9) the others. We considered (9) the others were patients who were eligible for this study.

Definition of Cardiovascular Disease

We defined CVDs in the DREAM cohort as the

composite of ischemic heart disease, ischemic stroke, hemorrhagic stroke, peripheral artery disease, pulmonary edema, and cardiac valve disease. CVDs include incidence and recurrence of CVDs as well as interventions for arterial and/or valve diseases as described elsewhere[6, 20-22]. We used the same definitions for pre-existing CVD as of December 2004 and the new CVD events during the follow-up. Sudden deaths were included as new and fatal CVD events.

Other Variables

Other variables at baseline included age, sex, duration of hemodialysis, clinical diagnosis of underlying renal disease [diabetic kidney disease (DKD) or not], pre-existing CVD, hypertension, dyslipidemia, current smoking status, body mass index (BMI) after dialysis, serum albumin, C-reactive protein (CRP), calcium, phosphate, intact parathyroid hormone (PTH), use of vitamin D receptor activator (VDRA), hematocrit, dose of erythropoiesis-stimulating agent (ESA), and use of intravenous iron injections. In addition, we included N-terminal fragment of probrain natriuretic peptide (NT-proBNP) as a prognostic factor[21]. Hypertension was defined as the use of any antihypertensive medication, systolic blood pressure ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg according to the guidelines by the Japanese Society of Hypertension[23]. Dyslipidemia was defined as the use of statin, non-HDL-C ≥ 150 mg/dL, and/or HDL-C ≤ 40 mg/dL, with these lipid levels being derived from the target levels for patients with chronic kidney disease as recommended by the guidelines by Japan Atherosclerosis Society[24].

Follow-Up of the Cohort

The cohort was followed up for up to 5 years, from 2004 to 2009. The attending physicians reported outcomes using an annual follow-up sheet; this included new onsets of CVDs, dates and causes of death, and censoring due to renal transplantation, switching to peritoneal dialysis, or moving away to other dialysis unit.

Statistics

In the cross-sectional studies at baseline, the distributions of FT3, FT4, and TSH levels were shown by histograms. Also, the number of patients in each category of thyroid function status was summarized in a cross-tabulation table. Unadjusted associations of FT3 levels with other continuous variables were examined using Spearman's rank correlation. Difference in FT3 levels between groups was examined using Mann-Whitney *U* test.

In the cohort study, the patients were divided into tertile of FT3, and the baseline characteristics were then compared. Continuous data were summarized as medians and interquartile ranges (IQR), and these were compared using Kruskal-Wallis test. Categorical data were summarized as numbers and percentages, and these were compared using χ^2 test. Kaplan-Meier curves of CVD events and all-cause mortality were constructed for the participants divided by the tertile of FT3 and were further compared by log-rank test. Unadjusted and multivariable-adjusted Cox proportional hazards analyses were conducted in order to assess the independent associations of FT3 level as a continuous variable with the outcomes. Cox analysis, which calculates the hazard ratio for FT3, was performed without adjustments (model 1) and with adjustment for the four major demographic variables, namely, age, sex, duration of dialysis, and diabetic kidney disease (model 2). In addition to the four variables, model 3 included hypertension, smoking, and dyslipidemia as traditional risk factors. Further adjustment was done for each of the following potential confounders: BMI, serum albumin, CRP (log-transformed), serum calcium, phosphate, intact PTH, use of VDRA, hematocrit, ESA dose, use of IV iron, prior CVD, and NT-proBNP (log-transformed).

A *p*-value less than 0.05 by two-sided test was considered to be statistically significant. All statistical calculations were done with statistical software JMP 12 (SAS Institute Japan, Tokyo, Japan) using Windows personal computer.

Results

Selection of Patients for Analysis

Fig. 1 shows the selection of patients eligible for the current analysis. We excluded 80 patients from the entire cohort of 518 participants, and the remaining 438 patients were analyzed for this report.

Distributions of FT3, FT4, and TSH Levels

Fig. 2 provides the histograms of FT3, FT4, and TSH levels. **Table 1** shows that the study population included 307 (70.0%) patients with normal FT4 and normal TSH levels (euthyroidism), 101 (23.1%) patients with normal FT4 and high TSH levels (subclinical primary hypothyroidism), 5 (1.1%) patients with normal FT4 and low TSH levels (subclinical primary hyperthyroidism), and 25 (5.7%) patients with low FT4 and normal TSH levels. Among the 438 patients analyzed, 223 (50.9%) patients showed low FT3 levels, and the proportions of such patients were similar among patients with euthyroidism (50.5%), subclinical hypothyroidism

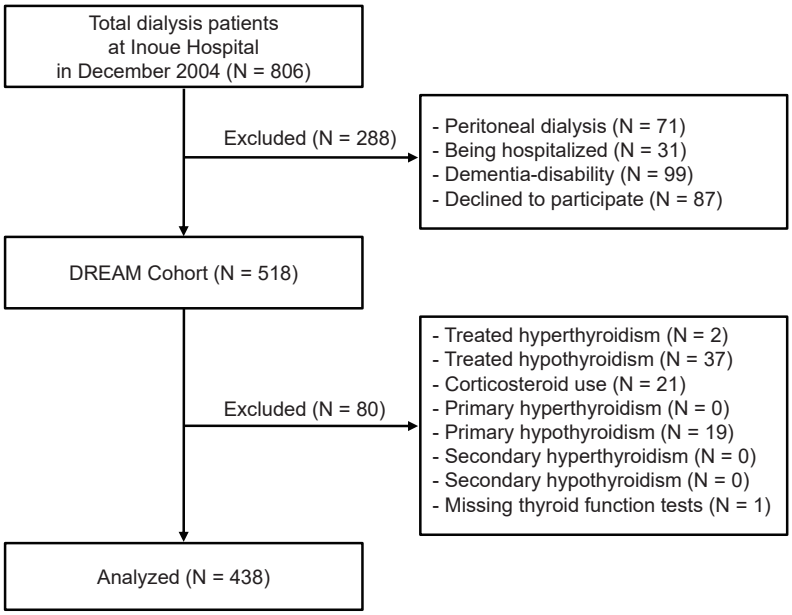

Fig. 1. Selection of patients for this analysis

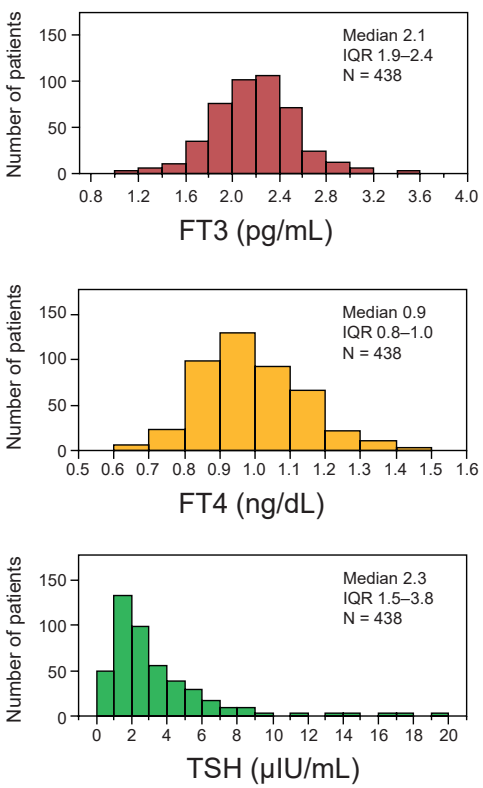

Fig. 2. Histograms of FT3, FT4, and TSH levels

Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; IQR, interquartile range

(48.5%), subclinical hyperthyroidism (60.0%), and others (64.0%).

Relationship between FT3 and other Variables

Table 2 shows the correlations between FT3 and other variables and the comparison of FT3 levels between subgroups of the patients. FT3 correlated positively with FT4 and inversely with TSH. FT3 showed a positive correlation with BMI, whereas it showed inverse associations with age, CRP, and NT-proBNP levels. FT3 level was lower in women than in men, in patients with DKD, in patients using intravenous iron, and in patients with prior CVD.

Baseline Characteristics of the Cohort

Table 3 gives the baseline characteristics of the cohort by tertile of FT3. Significant difference across tertile was observed in FT4, age, DKD or not, BMI, CRP, ESA dose, and NT-proBNP.

New CVD Events and All-Cause Mortality

In total, 154 new CVD events and 86 all-cause deaths were recorded during the 5-year follow-up. For these 154 new CVD events, the breakdown of the first events during follow-up was as follows: pulmonary edema (N=47), ischemic heart disease (N=39), ischemic stroke (N=25), hemorrhagic stroke (N=16), valve disease (N=11), PAD (N=8), and sudden death (N=8). Among the 86 cases of all-cause deaths recorded, the direct cause of death was adjudicated to cardiovascular (N=30) and non-cardiovascular (N=40) causes, whereas the cause of death was

Table 1. Distribution of thyroid function tests in 438 hemodialysis patients analyzed

Patients analyzed *N*=438 (Low FT3=223)		TSH		
		Low	Normal	High
FT4	High	0	0	0
	Normal	5 (Low FT3=3)	307 (Low FT3=155)	101 (Low FT3=49)
	Low	0	25 (Low FT3=16)	0

The analyzed patients were first classified by the combination of TSH and FT4, and the number of patients in each category was shown in the table with the number of patients with low FT3 in parenthesis. No patient had FT3 level higher than normal range. The classification was based on the following reference ranges: 2.2–4.1 pg/mL for FT3, 0.8–1.9 ng/dL for FT4, and 0.4–4.0 μIU/mL for TSH.
Abbreviations: TSH, thyroid stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine.

Table 2. Relationship of FT3 and other variables at baseline

Continuous Variable	Spearman's rank correlation coefficient			*P* value
TSH	-0.102			0.034
FT4	0.205			<0.001
Age	-0.251			<0.001
Duration of dialysis	0.050			0.296
Body mass index	0.200			<0.001
Serum albumin	0.113			0.018
C-reactive protein	-0.156			0.001
Calcium	0.038			0.423
Phosphate	-0.000			0.999
Intact PTH	0.094			0.049
Hematocrit	0.103			0.031
ESA dose	-0.109			0.022
NTproBNP	-0.327			<0.001
Categorical Variable	**Category**	***N***	**FT3 (pg/mL)**	***P* value**
Sex	Male	279	2.2 (1.9–2.4)	0.044
	Female	159	2.1 (1.9–2.3)	
DKD	Yes	100	2.1 (1.8–2.3)	0.006
	No	338	2.2 (1.9–2.4)	
Smoking	Yes	187	2.2 (1.9–2.3)	0.537
	No	251	2.1 (1.9–2.4)	
Hypertension	Yes	381	2.1 (1.9–2.3)	0.545
	No	57	2.2 (2.0–2.4)	
Dyslipidemia	Yes	213	2.2 (1.9–2.4)	0.190
	No	225	2.1 (1.9–2.3)	
Use of VDRA	Yes	165	2.1 (1.9–2.4)	0.536
	No	273	2.2 (1.9–2.3)	
Use of IV iron	Yes	253	2.1 (1.9–2.3)	0.027
	No	185	2.2 (2.0–2.4)	
Prior CVD	Yes	146	2.1 (1.9–2.3)	0.017
	No	292	2.2 (2.0–2.4)	

The upper part of the table shows Spearman's rank correlation between FT3 and other continuous variables, and the lower part of the table compares FT3 levels between each category. FT3 values are medians (IQR). *P* values were by Mann-Whitney *U*-test.
Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid stimulating hormone; PTH, parathyroid hormone; ESA, erythropoiesis stimulating agent; NT-proBNP, N-terminal fragment of probrain natriuretic peptide; DKD, diabetic kidney disease; VDRA, vitamin D receptor activator; IV, intravenous; CVD, cardiovascular disease; N, number of patients; P, level of significance.

Table 3. Characteristics of the participants by free T3 level

Variables	Tertile of FT3			
	T1 (lowest) *N*=171	T2 (middle) *N*=103	T3 (highest) *N*=164	*P* value
FT3 (pg/mL)	1.9 (1.7–2.0)	2.1 (2.1–2.2)	2.4 (2.3–2.5)	<0.001
FT4 (ng/dL)	0.9 (0.8–1.0)	0.9 (0.8–1.0)	1.0 (0.9–1.1)	<0.001
TSH (μIU/mL)	2.4 (1.5–4.0)	2.3 (1.5–3.8)	2.2 (1.3–3.5)	0.206
Age (year)	63 (57–70)	63 (57–68)	57 (50–64)	<0.001
Male Sex [*N*, (%)]	97 (56.7)	70 (68.0)	112 (68.3)	0.052
HD period (year)	7.9 (3.3–13.3)	8.7 (4.0–17.3)	9.6 (3.8–17.1)	0.165
DKD [*N*, (%)]	49 (28.7)	24 (23.3)	27 (16.5)	0.029
Smoking [*N*, (%)]	69 (40.4)	42 (40.8)	76 (46.3)	0.489
Hypertension [*N*, (%)]	152 (88.9)	88 (85.4)	141 (86.0)	0.633
Dyslipidemia [*N*, (%)]	79, (46.2)	52, (50.5)	82, (50.0)	0.715
BMI (kg/m^2)	21.0 (19.2–23.1)	22.2 (20.3–23.5)	22.1 (20.4–23.9)	0.001
Albumin (g/dL)	3.7 (3.5–3.9)	3.7 (3.6–3.9)	3.8 (3.6–4.0)	0.100
CRP (mg/dL)	0.17 (0.05–0.62)	0.15 (0.06–0.41)	0.11 (0.04–0.25)	0.010
Calcium (mg/dL)	9.1 (8.5–9.8)	9.1 (8.5–9.8)	9.2 (8.6–9.9)	0.711
Phosphate (mg/dL)	5.8 (4.9–6.6)	5.8 (5.0–6.7)	5.7 (5.1–6.7)	0.764
Intact PTH (pg/mL)	96 (37–196)	130 (38–242)	130 (60–244)	0.175
Use of VDRA [*N*, (%)]	67 (39.2)	35 (34.0)	63 (38.4)	0.670
Hematocrit (%)	30.1 (27.7–32.3)	30.3 (28.2–32.5)	31.0 (29.3–32.5)	0.555
ESA dose (x1000 U/week)	9.0 (9.0–9.0)	9.0 (6.0–9.0)	9.0 (6.0–9.0)	0.035
Use of IV iron [*N*, (%)]	110 (64.3)	58 (56.3)	85 (51.8)	0.065
Prior CVD [*N*, (%)]	65 (38.0)	36 (35.0)	45 (27.4)	0.113
NT-proBNP (pg/mL)	9390 (4040–21300)	5650 (2400–17600)	3790 (1870–10100)	<0.001

The study population was divided into tertile of FT3 level (33th and 67th percentile levels were 2.0 and 2.2 pg/mL), and their characteristics were compared.
P-values were by Kruscal-Wallis test or by χ^2 test.
Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid stimulating hormone; HD, hemodialysis; DKD, diabetic kidney disease; BMI, body mass index; CVD, cardiovascular disease; CRP, C-reactive protein; and NT-proBNP, N-terminal fragment of probrain natriuretic peptide.

unknown in 16 cases as they died after hospital transfer with no detailed information available. The breakdown of cardiovascular deaths was as follows: sudden death (*N*=14), pulmonary edema (*N*=10), ischemic heart disease (*N*=2), ischemic stroke (*N*=2), hemorrhagic stroke (*N*=1), and unspecified stroke (*N*=1). The breakdown of the non-cardiovascular deaths was as follows: infection (*N*=21), malignancy (*N*=8), respiratory failure (*N*=3), hepatic cirrhosis (*N*=1), pancreatitis (*N*=1), multiple organ failure (*N*=1), uremia (*N*=1), natural death of the old (*N*=2), and suicide/refusal of dialysis treatment (*N*=3).

Unadjusted Associations of FT3 with New CVD Events and All-Cause Mortality

Kaplan-Meier curves show that the unadjusted risks for new CVD events and all-cause deaths were significantly different across the tertiles of FT3 **(Fig. 3)**.

Independent Associations of FT3 with New CVD Events

Table 4 shows the association between FT3 and the risk for new CVD events. The association was deemed significant in an unadjusted model (model 1). It remained significant when adjustments were made for the four major demographic factors including age, sex, duration of hemodialysis, and DKD or not (model 2). The association remained significant after further adjustment for traditional risk factors such as hypertension, dyslipidemia, and smoking (model 3). The inverse association between FT3 and risk of new CVD events has remained significant when further adjustments for each of the following potential confounders were made: BMI, serum albumin, CRP (log-transformed), serum calcium, phosphate, intact PTH, use of VDRA, hematocrit, ESA dose, and use of IV iron.

However, it was no longer significant when adjustments were done for prior CVD (model 14) or log-transformed NT-proBNP (model 15), whereas

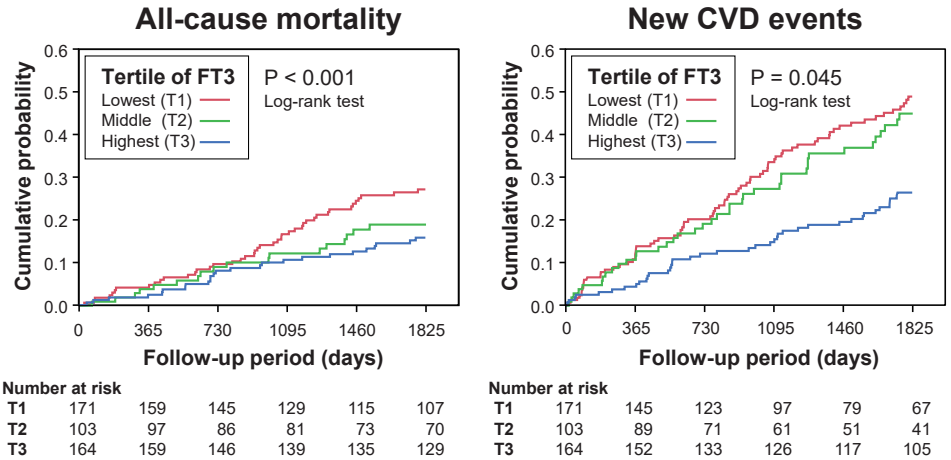

Fig. 3. Kaplan-Meier curves showing associations of FT3 with two clinical outcomes

Abbreviations: FT3, free triiodothyronine; CVD, cardiovascular disease.

Table 4. Cox analysis of association of FT3 and new CVD event

Model	Outcome	New CVD events		All-cause mortality	
	Adjustment	HR (95% CI) for FT3 (per 1 pg/mL higher)	*P*	HR (95% CI) for FT3 (per 1 pg/mL higher)	*P*
1	Unadjusted	0.35 (0.22–0.57)	<0.001	0.42 (0.22–0.79)	0.008
2	Adjusted for age, sex, DKD, and duration of HD	0.52 (0.32–0.86)	0.011	0.74 (0.38–1.43)	0.372
3	Model 2 + Smoking, Hypertension, and Dyslipidemia	0.51 (0.31–0.85)	0.009	0.72 (0.37–1.39)	0.322
4	Model 3 + BMI	0.54 (0.32–0.90)	0.017	0.79 (0.40–1.55)	0.497
5	Model 3 + Albumin	0.52 (0.31–0.85)	0.010	0.79 (0.41–1.51)	0.471
6	Model 3 + Log CRP	0.56 (0.34–0.93)	0.026	0.85 (0.43–1.66)	0.645
7	Model 3 + Calcium	0.51 (0.31–0.84)	0.009	0.72 (0.37–1.39)	0.325
8	Model 3 + Phosphate	0.52 (0.31–0.86)	0.012	0.72 (0.37–1.39)	0.323
9	Model 3 + intact PTH	0.51 (0.31–0.85)	0.009	0.71 (0.37–1.38)	0.317
10	Model 3 + Use of VDRA	0.51 (0.31–0.85)	0.009	0.73 (0.38–1.41)	0.351
11	Model 3 + Hematocrit	0.51 (0.31–0.85)	0.009	0.74 (0.38–1.42)	0.363
12	Model 3 + ESA dose	0.51 (0.31–0.84)	0.009	0.68 (0.35–1.33)	0.266
13	Model 3 + Use of IV iron	0.51 (0.31–0.85)	0.010	0.74 (0.38–1.42)	0.363
14	Model 3 + Prior CVD	0.62 (0.38–1.02)	0.059	0.80 (0.41–1.54)	0.503
15	Model 3 + Log NT-proBNP	0.85 (0.50–1.42)	0.534	1.14 (0.57–2.25)	0.716

The table gives HRs (95% CIs) for new CVD events and all-cause mortality per 1 pg/mL higher FT4 in unadjusted and multi-variable adjusted Cox proportional hazard models.

Abbreviations: FT3, free triiodothyronine; CVD, cardiovascular disease; HR, hazard ratio; CI, confidence interval; P, level of significance; BMI, body mass index; CRP, C-reactive protein; PTH, parathyroid hormone; VDRA, vitamin D receptor activator; ESA, erythropoiesis stimulating agent; IV, intravenous; NT-proBNP, N-terminal fragment of probrain natriuretic peptide.

prior CVD (HR, 2.42; 95% CI, 1.73–3.38; $P<0.001$; Model 14) and log-transformed NT-proBNP (HR, 1.53; 95%CI, 1.32–1.76; $P<0.001$; Model 15) were identified to be significant factors independent of the eight variables including FT3.

When we inserted the interaction term between FT3 and prior CVD or between FT3 and NT-proBNP in the above models, no significant effect modification was noted by prior CVD ($P=0.10$) or by NT-proBNP ($P=0.46$) in the association between FT3 and new CVD events.

Independent Associations of FT3 with All-Cause Mortality

The association of FT3 with all-cause mortality was not found to be significant in the Cox analysis

adjusted for the four major demographic factors including age, sex, duration of hemodialysis, and DKD or not **(Table 4)**. FT3 did not significantly associated with all-cause mortality with further adjustment for other variables.

Discussion

This study has examined whether low FT3 levels predict new cardiovascular events independent of other factors in a cohort of hemodialysis patients without apparent thyroid disease. Based on our findings, a lower FT3 level was associated with a higher CVD risk, and this association was determined to be independent of various traditional and nontraditional risk factors. However, it was no longer significant when the model was further adjusted for prior CVDs or NT-proBNP level. These results indicate that low FT3 has no direct association with new CVD events independent of prior CVD and NT-proBNP level in hemodialysis patients on one hand. On the other hand, the results suggest a close relationship among prior CVD, a low FT3 level and a high NT-proBNP level at present, and future risk of new CVD events.

Some previous studies examined the predictive value of thyroid hormone tests in patients with kidney failure. Zoccali *et al.*[8] revealed that low FT3 level was associated with all-cause mortality in a cohort of 200 patients treated with maintenance hemodialysis and that this association was independent of interleukin 6, which serves as the marker of inflammation. Meuwese *et al.*[17] have confirmed the association between baseline T3 (not FT3) and all-cause mortality and further added that patients with decrease in T3 levels during the initial 3 months showed a higher risk for all-cause mortality as compared to those with persistently high T3 levels. According to Chang *et al.*[18], a low T3 was an independent predictor of all-cause death and cardiovascular death in 447 patients starting peritoneal dialysis. These studies are consistent with the inverse association between FT3 (or T3) and all-cause mortality in patients with kidney failure undergoing dialysis. However, none of these studies examined the association of FT3 with new CVD events. Therefore, our study in 438 patients on maintenance hemodialysis is the first one reporting on such association in patients with kidney failure.

Another study by Drechsler *et al.*[19] compared the association of different categories of thyroid hormone status, rather than serum thyroid hormone levels, with clinical outcomes in a cohort of 1000 diabetic patients undergoing hemodialysis. They excluded those with overt hypothyroidism ($N=2$) and overt hyperthyroidism ($N=10$) and compared the hazard ratios for all-cause mortality and cardiovascular outcomes among the following four groups: euthyroidism ($N=781$), subclinical hypothyroidism ($N=137$), subclinical hyperthyroidism ($N=16$), and euthyroid sick syndrome ($N=54$). Patients with euthyroid sick syndrome showed higher risks for sudden death ($P=0.09$), myocardial infarction ($P=0.09$), and all-cause mortality ($P=0.09$), but these associations were not significant after adjustments were made for potential confounders including NT-proBNP. The results for euthyroid sick patients are similar to our study, showing no significant association between low FT3 levels and new composite CVD events or all-cause mortality after adjustment for potential confounders such as NT-proBNP, although there are several differences between the study by Drechsler and ours. First, their study participants were all patients with type 2 diabetes. Second, they included patients treated with levothyroxine, whereas we excluded patients treated for thyroid dysfunction. Third, they did not examine the association between FT3 level and clinical outcomes.

Of note, Drechsler *et al.*[19] showed that the elevated risk associated with euthyroid sick syndrome was only significant in the initial 1-year period, and such associations disappeared during the second year and thereafter. This may be somewhat different from the studies by Zoccali *et al.*[8], Meuwese *et al.*[17], and Chang *et al.*[18], whereas it was consistent with our results as it showed no significant association between low FT3 levels and all-cause mortality during the 5-year period.

Similar to kidney failure patients undergoing dialysis, patients with heart failure or those with dilated cardiomyopathy often present with low T3 syndrome, and low total and free T3 levels were reported to predict higher mortality risk in these populations[25-28]. In some studies, the mortality risk associated with low T3 or FT3 was independent of brain natriuretic peptide (BNP)[26] or NT-proBNP[27]. In contrast to these studies on patients with cardiac disease, we found that the association between FT3 level and new composite CVD events was not independent of NT-proBNP. The apparent discrepancy may derived from the difference in populations studied, study endpoints, and measurements of T3 and FT3 levels.

It is still a matter of debate whether low T3 syndrome should be treated or not[9, 12, 14, 29]. Low T3 syndrome (nonthyroidal illness, euthyroid sick syndrome) has been regarded as "adaptation" to wasting caused by severe illness such as cardiac and

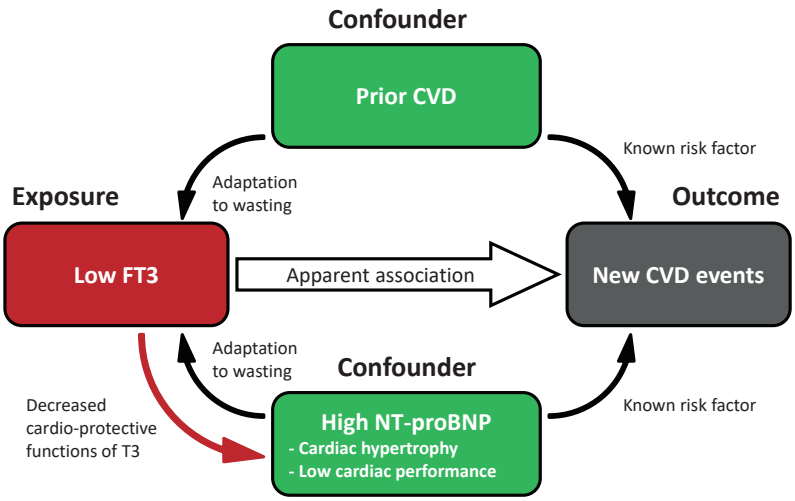

Fig. 4. Interpretation of the results

Prior CVD could reduce FT3 levels by "adaptation" to wasting, and prior CVD has been identified as a well-known risk factor for new CVD events at the same time. Thus, prior CVD can be considered as a confounder. Similarly, a high level of NT-proBNP, which represents cardiac hypertrophy and/or low cardiac performance, can be regarded as a confounder. The results of this cohort study can be explained by the confounding effects of prior CVD and high NT-proBNP levels. Based on the results of basic researches and a randomized clinical trial of T3 replacement[9, 14, 15], a high NT-proBNP may play another role as a mediator between a low T3 level and new CVD events (red arrow). Although this study observed an inverse cross-sectional association between FT3 and NT-proBNP, causality is unknown. Abbreviations: T3, free triiodothyronine; FT3, free triiodothyronine; CVD, cardiovascular disease; NT-proBNP, N-terminal fragment of probrain natriuretic peptide.

renal disease; therefore, no replacement was recommended for it[12, 29]. Suppressed conversion of T4 to T3 could be protective against wasting as it reduces energy expenditure in tissues. In contrast, since T3 has cardioprotective functions[9, 14], low T3 status may induce hypofunction of the heart and further deteriorate the cardiovascular health. As recently reviewed in detail[14], T3 regulates the expression of genes which are relevant to cell growth, differentiation, and metabolism by binding to thyroid hormone receptors α and ß in the nucleus. In addition to these genomic actions, T3 exerts its non-genomic actions through binding to membrane-bound and cytoplasmic thyroid hormone receptors. T3 also regulates inotropic, chronotropic, and dromotropic properties of the heart by changing the membrane transport of ions, glucose, and amino acids and by affecting the collagen content in the interstitial tissue of myocardium. Other T3 effects include protection from oxidative stress and inflammation, anti-apoptotic effects, epigenetic modifications, and mitochondrial protection. These beneficial properties of T3 are potentially in favor of cardioprotection at least in a short term. Then, a compensatory reduction of T3 due to cardiac disease may result in the further deterioration of cardiac function, falling into a vicious circle. A randomized controlled trial in patients with chronic heart failure and low T3 syndrome showed an improved cardiac performance via a short-term T3 replacement therapy[15].

Fig. 4 summarizes our results and interpretation. In our study, the apparent association of low FT3 levels and new CVD events was no longer significant when adjustments were done for prior CVD or NT-proBNP. Because prior CVD could affect both FT3 (exposure) and new CVD (outcome), prior CVD can be considered as a confounder. Similarly, a higher NT-proBNP can be regarded as a confounder, since NT-proBNP is considered a biomarker of left ventricular hypertrophy and impaired cardiac function[30, 31]. Thus, our results can be explained by the confounding effects of prior CVD and NT-proBNP. Considering the effect of T3 on cardiac function[9, 14, 15], the observed cross-sectional inverse association between FT3 and NT-proBNP may indicate the causal effect of a lower FT3 on a higher NT-proBNP. If so, low FT3 status might play a causative role in the pathogenesis of increased CVD risk to some extent. However, because of the observational nature of this study, our results are far from the evidence to avoid or recommend thyroid hormone replacement in hemodialysis patients with low FT3. To examine the potential benefit of T3 replacement in hemodialysis patients, interventional

studies are needed in this population, as shown in cardiac disease[15].

When low T3 syndrome was defined as FT3 lower than the reference range in patients without apparent thyroid disease, and the 438 patients in this analysis were deemed to have no apparent thyroid disease, the number of patients with low T3 syndrome was 223 (50.9%), which corresponds to 43.1% of the entire DREAM cohort. When low T3 syndrome was defined as low FT3 and/or FT4 without TSH elevation, the number of patients with low T3 syndrome was 183 (41.8%), which corresponds to 35.3% of the entire DREAM participants. These numbers of low T3 syndrome were lower than that in a previous study by Ozen *et al.* (71.7%)[32] but higher than that reported by Drechsler *et al.* (5.4%)[19]. The difference in prevalence between studies may be attributable to differences in definition, hormone measurements, nutritional status, dialysis duration, ethnicity, and inclusion/exclusion of patients treated with levothyroxine.

There are some limitations in this study. First, since we excluded patients with apparent thyroid disease and those treated with corticosteroids, the association of FT3 and clinical outcomes may differ between the patient groups, included and excluded. Second, because we did not perform serial thyroid hormone measurements during follow-up, we could not examine whether a decrease or increase in thyroid hormones could predict outcomes. Third, the sample size was too small to address CVD risk according to thyroid hormone status. Fourth, we have no definitive diagnosis for thyroid disease, because we did not collect information on the presence of goiter, clinical symptoms, or thyroid-related autoantibodies. Fifth, we used composite of CVD events, which combined cardiac and non-cardiac events and atherosclerotic and non-atherosclerotic CVD events in mixture. Due to the limited number of outcomes of this relatively small cohort, organ-specific and etiology-specific interpretations of the results were difficult. Sixth, although some cohort studies reported a long-term follow-up for 10 years in hemodialysis patients[33], our cohort was followed up for only 5 years. A longer follow-up period merits a larger number of outcomes, whereas it would analyze the association of the exposure with remote outcomes. Thus, a longer follow-up may give different results. Seventh, we did not obtain echocardiographic data or novel serum biomarkers of specific etiology of CVD such as amino terminal propeptide of type III procollagen for cardiac fibrosis[34] at baseline or follow-up. These data, if present, would have provided more detailed information relevant to this analysis. And eighth, the results of this observational study, like others, do not indicate causality. Therefore, no recommendation regarding treatment can be mentioned based on our results.

In conclusion, in this cohort of hemodialysis patients without apparent thyroid disease, a lower FT3 level was associated with a higher risk for new cardiovascular events in an unadjusted model. This association was independent of wide range of traditional and nontraditional risk factors including CRP. However, this association was not independent of the presence of prior CVD or high NT-proBNP levels. These results indicate that the apparent association of low FT3 levels with new CVD events can be explained by the confounding effects of prior CVD and high NT-proBNP levels. Although our results may be used for risk stratification, further study is needed to examine whether thyroid hormone replacement is beneficial or not in hemodialysis patients with low FT3 levels.

Conflict of Interest

None declared conflict of interest relevant to this study.

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