Nontraditional cardiovascular risk factors in end-stage renal disease : studies on inflammatory markers and thyroid hormones

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Chapter 6

Baseline levels and trimestral variation of triiodothyronine and thyroxine and their association with mortality in maintenance hemodialysis patients

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Abstract

Background: Conflicting evidence exists with regards to the association of thyroid hormone levels and mortality risk in patients with end-stage renal disease (*ESRD*). This study assesses the association between basal and trimestral variation of thyroid stimulating hormone (*TSH*), triiodothyronine (*T3*) and thyroxine (*T4*) and cause-specific mortality in dialysis patients.

Methods: In 210 prevalent hemodialysis patients, serum T3, T4, TSH, and interleukin-6 (*IL-6*)were measured 3 months apart. Cardiovascular and non-cardiovascular deaths were registered during follow-up. Based on fluctuations along tertiles of distribution, four trimestral patterns were defined for each thyroid hormone: persistently low, decrease, increase, and persistently high. The association of baseline levels and trimestral variation with mortality was investigated with Kaplan–Meier curves and Cox proportional hazard models.

Results: During follow-up, 103 deaths occurred. TSH levels did not associate with mortality. Patients with relatively low basal T3 concentrations had higher hazards of dying than patients with high levels. Longitudinally, patients with persistently low levels of T3 during the 3-month period had higher mortality hazards than those having persistently high levels. These associations were mainly attributable to cardiovascular-related mortality. The association between T4 and mortality was not altered after adjustment for T3 levels.

Conclusions: Hemodialysis patients with reduced T3 or T4 levels bear an increased mortality risk, especially due to cardiovascular causes. This was true when considering both baseline measurements and trimestral variation patterns. Our longitudinal design adds observational evidence supporting the hypothesis that the link may underlie a causal effect.

Introduction

A reduction in kidney function encompasses a number of alterations in thyroid hormone metabolism that renders a particular "Nonthyroidal illness" syndrome[1] in patients with chronic kidney disease (*CKD*), where low levels of both Total (*T3*) and free triiodothyroinine (*fT3*) are hallmark findings. Recent reports suggest that as many as 70% of patients with end-stage renal disease (*ESRD*) present with low T3 levels,[2-5] and as many as 20-25% have subclinical hypothyroidism.[6,7] The underlying pathophysiology of these derangements is likely multifactorial, involving iodine retention, altered serum protein binding capacity, systemic inflammation and peripheral deiodinase activity.[8,9]
These abnormalities have traditionally been considered as a physiological mechanism to save energy in response to uremic wasting.[10] However, growing evidence suggests T3 to participate in the pathophysiology of endothelial dysfunction,[11] atherosclerosis[12] and cardiac abnormalities[13] of CKD patients. Some studies, with somewhat conflicting results, have also implicated thyroid alterations in the mortality risk of CKD patients.[14-16] Zoccali et al.[14] were the first to report a positive association between low fT3 levels and all-cause mortality in stable hemodialysis (*HD*) patients, with similar findings in peritoneal dialysis (*PD*) patients.[17] We could further confirm and expand these observations in a cohort of euthyroid incident HD patients, in which the variance of all-cause and cardiovascular (*CV*) mortality was better explained by T3 than by fT3 levels.[16] In contrast, a study in 87 HD patients could not confirm these findings,[15] and a more recent retrospective report found that the association between fT3 and all-cause mortality was confounded by the nutritional status of the patients.[5] Whereas existing evidence on the association between thyroid hormones and mortality is limited to studies considering single measurements of hormone levels, it is presently unknown whether thyroid hormone fluctuation over time impacts on mortality hazards. In apparently healthy individuals, thyroid hormone concentrations show a narrow intra-individual variation,[18] although certain circadian and seasonal (mainly due to seasonal differences in iodine intake) fluctuations have been observed.[19] In diseased individuals, however, thyroid hormone concentrations seem subjected to a higher fluctuation as influenced among others by wasting syndromes, persistent inflammation and cardiac comorbidities.[19] No studies, to the best of our knowledge, have assessed variation of thyroid hormones in CKD patients and their association with mortality outcome. Against this background, the objective of the present study was to evaluate the association between both basal levels and longitudinal (3-month) variation of T3, T4 and thyroid stimulating hormone (*TSH*), and all-cause and cause-specific mortality. We did so in a well-defined cohort of maintenance HD patients.

Methods and patients

The Medical Ethics Committee at Karolinska University Hospital and Uppsala University approved the study protocol, and all patients consented to participate. This is a post-hoc analysis from the observational Mapping of Inflammatory Markers in Chronic Kidney Disease (*MIMICK*) cohort,

consisting of prevalent HD patients in the Stockholm region. The protocol has been described elsewhere in more detail.[20,21] The inclusion period ranged from October 2003 till September 2004. Laboratory measurements and patient characteristics were gathered at baseline and after 3 months. From inclusion onward, patients were followed for the occurrence of fatal events. Out of 224 patients originally included in this cohort, TSH, T3 and T4 were not measured in 6 individuals due to limited plasma volume. The patients were on angiotensin-converting enzyme inhibitors and/or angiotensin II receptor antagonists (n=72), betablockers (n=109), calcium-channel blockers (n=57), diuretics (n=104), statins (n=72), antiplatelet drugs (n=65), erythropoesis-stimulating agents (n=211), and iron substitution (n=144). Prescribed medication possibly influencing thyroid hormone levels was extracted from medical records: 16 patients were receiving levothyroxine and one of these 16 patients additionally used amiodarone. No patients were receiving lithium medication or propranolol. Comorbidity was classified according to Davies et al.[22], on a seven point scale which was simplified into a three risk category scale (low, medium and high comorbidity risk). Nutritional status was evaluated by means of the subjective global assessment (*SGA*).

Causes of death were retrieved from death certificates and classified as cardiovascular (*CV*) or non-CV. CV mortality was defined as death due to myocardial ischemia or infarction, cardiac arrest or unknown sudden death, acute as well as chronic heart failure, cerebrovascular accidents, cerebral hemorrhage, and ruptured aortic aneurysm. Non-CV death was defined as that not attributable to a cardiovascular origin. Individuals with unknown causes of death (n=13) were grouped within the non-CV group.

Biochemical Methods

Venous blood samples were drawn with the patient in a supine position and stored at -70 °C if not analyzed immediately. Concentrations of high-sensitivity C-reactive protein (*CRP*) and serum albumin (bromcresol purple) were determined using routine methods at the Department of Laboratory Medicine, Karolinska University Hospital, Huddinge, Sweden. Serum IL-6 concentrations were quantified in an automated immulite analyzer (Siemens Healthcare Diagnostics, Los Angeles, CA, USA). Thyroid hormones levels were also assessed on an Immulite system, using commercially available immunometric assays for T3 [analytical sensitivity (AS), 0.54 nmol/L; total coefficient of variation (CVs), 13.2% and 5.4% at the levels of 0.95 and 6.02 nmol/L)], T4 [AS, 5 nmol/L; total CVs, 8.4% and 6.3% at the levels of 49 and 167 nmol/L], and TSH [AS, 0.004 mIU/L; total CVs, 12.5%, and 4.6% at the levels of 0.016 and 1.3 mIU L]. Results are expressed as the average of two measurements.

Statistical Methods

Because existing evidence suggests that up to 70% of ESRD and dialysis patients present with low T3 levels,[3,5] in our study, we chose to define low T3 or T4 categories as those below the 66th percentile of distribution (higher tertile as the reference group). For the analysis of trimestral thyroid hormone variation (two time points: at study inclusion and after 3 months), we classified patients

according to their shift along tertile distribution at each time point, a strategy described in more detail elsewhere[23] and illustrated in **Figure 1**. From the nine possible combinations, four groups were created by clustering patients with changes in the same direction: (i) individuals who showed a change to lower tertiles were classified as the 'decrease' group, (ii) individuals showing an increase to upper tertiles were assigned to the 'increase' group, (iii) individuals with both values within the highest tertile of distribution were labeled as a 'persistently high' group and (iv) individuals with both values within the lower or the middle tertile were labelled as a 'persistently low' group. Differences between groups were tested by means of parametric (independent sample t-test, one-way ANOVA), non-parametric (Mann-Whitney U and Kruskal-Wallis test), and Chi-square tests as appropriate. Correlation was assessed by means of Pearson or Spearman correlation coefficients, as appropriate. Intra-patient variability of two measurements is depicted as a coefficient of intra-patient variation for each thyroid hormone. Survival during follow-up was analyzed by the Kaplan Meier method and hazard ratios (HR) were calculated with Cox-proportional hazard models with different degrees of adjustment for potential confounders. Age, sex, comorbidity, protein-energy wasting (PEW), serum albumin levels, smoking history, levothyroxine prescription, dialysis vintage, and IL-6 were considered as possible confounders in the associations. In these models, T3, IL-6, and vintage on dialysis were logarithmically transformed because of a non-normal distribution. In order to test whether the effect of T4 levels at baseline and variation groupings on mortality was due to the effect of T3 (in the causal pathway), we performed an additional adjustment for Log T3 or delta T3, respectively.

As a sensitivity analysis, Cox analyses were repeated in biochemically euthyroid patients only, being defined as presenting with normal TSH (reference range 0.1-4.5 mIU/L) and T4 (reference range 57.9-169.9 nmol/L). Finally, Cox analyses were adjusted by CRP instead of IL-6 as a surrogate of inflammation. HRs with 95% confidence intervals (95% CI) not including one were considered to be statistically significant. For all other tests, differences with p-values below 0.05 were considered to be statistically significant. SPSS version 17.0 (SPSS Inc., Chicago, USA) was used to analyse the study material.

Figure 1. Classification of trimestral variation patterns for each thyroid hormone based on shifts between tertiles of distribution

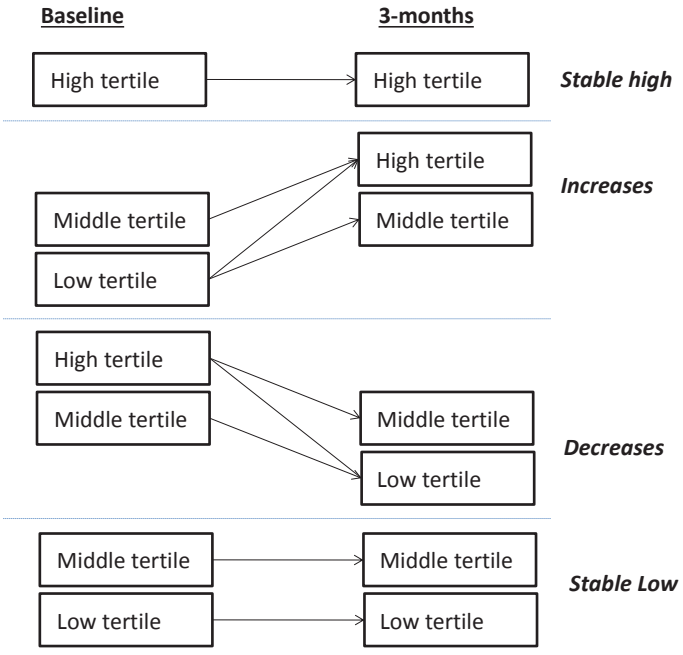


Table 1. Baseline characteristics according to T3 and T4 dichotomization in 218 prevalent hemodialysis patients [1]

	Low T3 n = 146	High T3 n = 72	p-value	Low T4 n = 147	High T4 n = 71	p-value
Age, years [3]	64.0 (13.8)	61.0 (15.1)	0.16	62.2 (14.5)	64.6 (13.8)	0.26
Men, % [4]	53	60	0.33	63	39	0.001
Dialysis vintage [5]	26 (12 – 50)	32.0 (17 – 63)	0.07	26 (12 – 55)	31 (17 – 61)	0.15
Comorbidity, % [2,4]	14/58/28	32/51/17	0.004	16/57/27	27/55/18	0.12
Smoking history, % [4]	17	20	0.60	15	25	0.07
SGA>1, % [4]	49	44	0.57	37	67	<0.0001
Albumin, g/L [3]	34.3 (4.8)	35.3 (4.1)	0.21	35.1 (4.2)	33.7 (5.2)	0.03
IL-6, pg/mL [5]	9.2 (5.5 – 15.7)	7.0 (4.3 – 12.1)	0.03	8.4 (4.8 – 14.6)	8.5 (5.1 – 15.4)	0.34
TSH, mIU/L [5]	1.40 (0.82 – 2.66)	1.40 (0.84 – 2.24)	0.43	1.41 (0.93 – 2.48)	1.30 (0.62 – 2.64)	0.30
T4, nmol/L [3]	64.40 (48.90 – 74.60)	83.70 (68.53 – 97.80)	<0.0001	60.5 (48.9 – 69.5)	94.0 (86.2 – 104.2)	-
T3, nmol/L [5]	0.73 (0.60 – 0.82)	1.11 (1.03 – 1.28)	-	0.76 (0.60 – 0.90)	1.00 (0.82 – 1.20)	<0.0001

1. Low T3 and Low T4 were defined as < 66th percentile (lower and middle tertile).
2. Davies score (see methods) showing the percentage of patients with low/medium/high comorbidity risk.
3. Means (SD). Differences were tested by independent t-test.
4. Percentages. Differences were tested by Chi square test.
5. Medians (IQR). Differences were tested by Mann-Whitney U tests.

Results

Basal thyroid hormone levels and its association with mortality

In all 218 individuals, median (IQR) levels of TSH, and T3 were 1.35 (0.84 – 2.52) uIU/mL, and 0.82 (0.67 – 1.03) nmol/L, respectively. Basal T4 levels were on average (SD) 70.44 (24.65) nmol/L and positively related to T3 levels (rho: 0.529, P<0.001). Thyroid hormone disorders on the basis of circulating hormones were common: 146 patients were classified as having a Low T3 syndrome, 11 patients were classified as having overt hypothyroidism, and additional 4 patients as having subclinical hypothyroidism. General and thyroid specific characteristics of studied patients are presented in **Table 1**, according to low levels of T3 (≤66th percentile; ≤0.94 nmol/L) and T4 (≤ 66th percentile; ≤77.2 nmol/L). Patients with low T3 presented with a higher prevalence of comorbidities, increased IL-6 levels and lower T4 concentrations. Patients with low T4 were more often men, malnourished and presenting with lower T3 levels. In univariate analyses, IL-6 positively associated with TSH (Spearman's rank test rho=0.16, P=0.01) and negatively with T3 (rho= -0.17, P=0.009). CRP levels positively associated with T4 (rho=0.19, P=0.003).

Table 2. Mortality hazard ratios (HR) and 95% confidence intervals (95% CI) according to basal T3 and T4 in 218 prevalent hemodialysis patients

	Crude, HR (95%CI)	Model 1, HR (95%CI)[1]	Model 2 HR (95%CI)[2]	Model 3 HR (95%CI)[3]
	Low T3, < 66th percentile (≤0.94 nmol/L)			
All-cause mortality	1.8 (1.1 – 2.8)	1.5 (0.9 – 2.4)	1.6 (1.0 – 2.6)	-
CV-mortality	2.8 (1.2 – 6.4)	2.5 (1.1 – 5.7)	2.6 (1.1 – 6.0)	-
	Low T4, < 66th percentile (≤77.2 nmol/L)			
All-cause mortality	1.2 (0.8 – 1.9)	1.7 (1.1 – 2.8)	2.1 (1.3 – 3.5)	2.1 (1.1 – 4.0)
CV-mortality	1.8 (0.9 – 3.8)	2.6 (1.2 – 5.9)	3.1 (1.3 – 7.0)	2.6 (1.0 – 6.8)

1. Model 1: Adjusted for age, sex, Davies Comorbidity score, protein-energy wasting (SGA>1), smoking, log-(vintage on dialysis) and serum albumin levels.
2. Model 2: Additionally adjusted for log-IL-6
3. Model 3: Variables included in Model 2 plus baseline log-T3 levels.

* The group with thyroid hormone levels in the highest tertile served as the reference group.

During a median (inter quartile range; IQR) follow-up period of 38.2 (17.6 – 45.1) months, 103 deaths occurred (40 CV and 63 non-CV) of which 77 occurred in the Low T3 group and 26 in the High T3 group. As shown in **Table 2**, the crude and adjusted hazards of dying (by all causes) were 1.8 and 1.6 times higher, respectively, in patients with low T3 levels than in those with high T3 levels. This was mainly due to CV deaths, where HRs were considerably higher in magnitude (crude HR [95%CI]: 2.8 [1.2 – 6.4]) and remained so after adjustment for confounders (adjusted HR: 2.7 [1.2 – 6.3]). A similar analysis was performed for T4. A statistically significant association between low T4 levels and an increased all-cause and CV mortality was observed after adjustment for confounders (adjusted HR: 3.0 [1.3 – 6.8]). Further adjustment for log-T3 levels did not materially alter the strength of these associations, although a 16% reduction in magnitude was

observed for CV mortality. Neither low T3, nor low T4 levels were significantly associated with non-CV mortality. TSH levels did not associate with any outcome measure (data not shown).

Table 3. General characteristics according to T3 trimestral variation in 210 hemodialysis patients[1]

	Persistently low n = 77	Decrease n = 41	Increase n = 44	Persistently High n = 48	p-value
Age, years [3]	65.7 (12.1)	63.2 (15.6)	62.6 (15.3)	58.4 (14.8)	0.06
Men, % [4]	52	61	61	54	0.68
Dialysis Vintage [5]	24 (11–48)	26 (13–49)	34 (16–63)	44 (20–71)	0.05
Comorbidities, % [2,4]	14/55/31	24/59/17	16/59/25	31/50/19	0.21
Smoking history, % [4]	18	18	9	24	0.30
SGA>1, % [4]	49	44	46	48	0.96
Albumin, g/L [3]	35.0 (4.5)	35.1 (4.6)	33.7 (4.1)	35.7 (4.2)	0.16
IL-6 pg/mL [5]	9.3 (5.8–16.6)	7.8 (4.2–14.8)	9.1 (5.5–11.4)	6.2 (4.3–10.1)	0.05
TSH, mIU/L[5]	1.51 (0.85–2.75)	1.66 (1.15–2.64)	1.20 (0.73–2.38)	1.12 (0.80–2.18)	0.20
T4, nmol/L[3]	59.38 (21.45)	78.18 (21.14)	66.48 (25.23)	85.46 (21.66)	<0.0001
T3, nmol/L [4]	0.67 (0.60–0.82)	0.96 (0.80–1.06)	0.72 (0.60–0.84)	1.19 (1.03–1.30)	-

1. Groups defined according to basal and 3-month variation in tertile distribution (See Methods).
2. Davies score (see methods) showing the percentage of patients with low/medium/high comorbidity risk.
3. Means (SD). Differences were tested by one-way ANOVA.
4. Percentages. Differences were tested by Chi square test.
5. Medians (IQR). Differences were tested by Kruskal Wallis tests.

Trimestral thyroid hormone variation and its association with mortality

Thyroid hormones were assessed again after three months in 210 patients. Median (IQR) coefficients of intra-patient variation were for T3: 7.8 (2.6 – 12.5)%, T4: 7.1 (4.0 – 12.0)%, and TSH: 14.0 (5.4 – 24.8)%. In **Table 3**, general and thyroid specific characteristics are depicted across four different T3 variation groups (see Methods), not observing major differences among these groups. The same was true for T4 variation groups (data not shown). In univariate correlation analyses, delta TSH associated with delta IL-6 (rho= 0.17, P=0.01), while delta T3 associated with delta serum albumin (rho=0.22, P=0.01). **Figure 2** shows the Kaplan Meier curves for T3 and T4 trimestral variation, both being clearly associated with patient outcome. **Table 4** shows crude and adjusted Cox models. Patients with persistently low T3 levels presented the highest hazard for all-cause mortality (HR 2.7 [1.5 –5.0)]) as compared with subjects having persistently high levels. This was particularly true for the association with CV mortality (HR 4.0 [1.3 – 11.7]), while no association between T3 variation and non-CV mortality was observed (data not shown). Adjustment for confounders did neither alter the magnitude of the association nor the statistical significance. Of note, elevated HRs, albeit statistically non-significant, were observed for patients showing increases or decreases of T3 levels during the 3-month follow-up versus those having persistently high concentrations.

Patients with persistently low T4 levels showed elevated hazards for all-cause mortality as compared with patients having persistently high T4 levels, an association that reached statistical significance after adjustment for confounders. Higher hazards were observed for prediction of CV-mortality (crude HR 2.6 [1.0 – 7.0]). In both cases, further adjustment for trimestral T3 variation (as a continuous variable) did not significantly affect the results. Of note, a decrease in T4 was also associated with elevated hazards for CV-mortality. No association was observed between T4 variation patterns and non-CV mortality. Trimestral TSH variation patterns were not associated with outcome. In a sensitivity analysis, all baseline and longitudinal Cox analyses were repeated in biochemically euthyroid patients only (n=210 at baseline, n=202 with available data at both time points). Additionally, Cox adjustment was done with CRP instead of IL-6. In both cases, results were not different (data not shown).

Table 4. Mortality hazard ratios (HR) and 95% confidence intervals (95% CI) according to T3 (Panel A) and T4 (Panel B) trimestral variation in 210 prevalent hemodialysis patients [1]

Panel A	Crude, HR (95%CI)	Model 1, HR (95%CI)[2]	Model 2, HR (95%CI)[2]	
All-cause mortality				
T3 Increase	1.4 (0.7 – 2.8)	1.1 (0.6 – 2.3)	1.3 (0.6 – 2.6)	-
T3 Decrease	1.5 (0.7 – 3.0)	1.3 (0.6 – 2.7)	1.4 (0.7 – 2.9)	-
Persistently low T3	2.7 (1.5 –5.0)	2.2 (1.2 – 4.1)	2.4 (1.3 – 4.5)	-
CV-mortality				
T3 Increase	2.4 (0.7 – 7.8)	2.1 (0.7 – 7.3)	2.3 (0.7 – 7.6)	-
T3 Decrease	1.8 (0.5 – 6.5)	1.8 (0.5 – 6.5)	1.8 (0.5 – 6.7)	-
Persistently low T3	4.0 (1.3 – 11.7)	3.5 (1.2– 10.5)	3.6 (1.2 – 10.9)	-
Panel B	Crude, HR (95%CI)	Model 1, HR (95%CI)[2]	Model 2, HR (95%CI)[3]	Model 3, HR (95%CI)[4]
All-cause mortality				
T4 Increase	0.9 (0.4 – 1.7)	1.5 (0.7 – 3.3)	1.9 (0.9 – 3.4)	2.0 (0.9 – 4.4)
T4 Decrease	1.8 (0.9 – 3.4)	2.0 (1.0 – 3.9)	1.7 (0.9 – 3.6)	1.6 (0.8 – 3.1)
Persistently low T4	1.5 (0.8 – 2.7)	1.9 (1.0 – 3.6)	2.0 (1.1 – 3.8)	2.1 (1.1 – 4.0)
CV-mortality				
T4 Increase	0.9 (0.3 – 3.5)	1.8 (0.5 – 7.1)	1.9 (0.5 – 7.5)	2.2 (0.5 – 8.8)
T4 Decrease	2.9 (1.0 – 8.4)	3.2 (1.0 – 10.0)	3.0 (0.9 – 9.3)	2.6 (0.8 – 8.0)
Persistently low T4	2.6 (1.0 – 7.2)	3.6 (1.2 – 10.6)	3.7 (1.3 – 10.8)	3.8 (1.3 – 11.2)

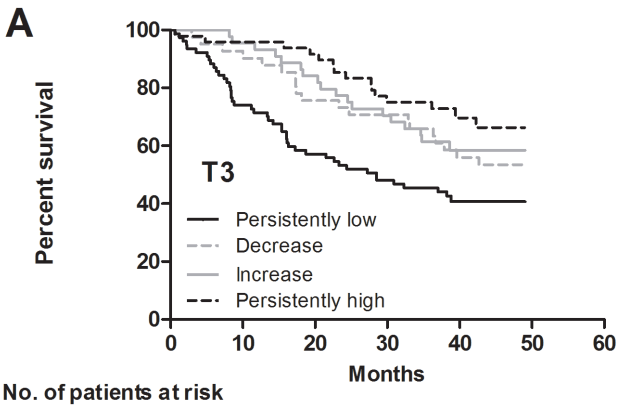
1. The group with persistently elevated levels served as the reference category in each analysis.
2. Model 1: Adjusted for age, sex, Davies Comorbidity score, protein-energy wasting (SGA>1), smoking history, log (vintage on dialysis), and serum albumin levels.
3. Model 2: Additionally adjusted for log-IL-6 levels.
4. Model 3: Variables included in Model 2 plus T3 changes (as a continuous variable).

Discussion

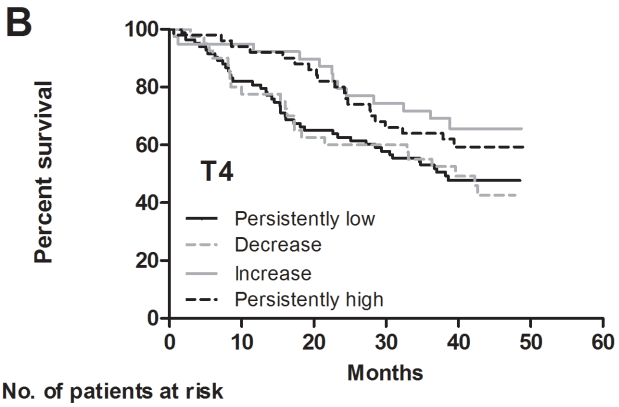
In this study, we show that both basal levels and trimestral variation of T3 and T4 are associated with increased mortality, particularly due to cardiovascular causes. Adjusting in the causal pathway suggests that the mechanisms associating low T4 levels with an elevated mortality rate may be, at least in part, independent of T3 levels.

Our observation linking low basal T3 levels with increased all-cause mortality is in agreement with some,[5;14;16;17] but not all preceding literature.[15] While earlier studies[5;14;17] only studied the association between T3 and fT3 and all-cause mortality, our study also shows that mortality prediction is mainly attributable to CV causes of death. The lack of mortality prediction observed by Fernandez-Reyes et al.[15] could relate to the selection of patients having survived at least 12 months, the low number of events encountered and the strict inclusion criteria which excluded, among others, patients with abnormal fT3 and fT4 levels. In this sense, our analysis shows that patients with both baseline and longitudinal low T3 levels have a relatively shorter time to death, possibly suggesting a stronger impact of thyroid hormones on short-term outcome. A recent study from Ozen et al.[5] showed that adjustment for CRP and s-albumin abrogated the association between fT3 and all-cause mortality, and the authors concluded that poor nutritional status confounded the investigated association. The acute-phase reactant nature of s-albumin

Figure 1. Kaplan–Meier curves for T3 and T4 trimestral variation. Kaplan–Meier survival curves for all-cause mortality according to T3 (A) and T4 (B) trimestral variation in 210 prevalent hemodialysis patients.



No. of patients at risk					
Persist. high	48	47	46	37	22
Increase	44	43	38	32	17
Decrease	41	38	32	30	23
Persist. low	77	58	45	39	21



No. of patients at risk					
Persist. high	50	48	44	34	23
Increase	39	38	36	30	17
Decrease	40	32	26	25	16
Persist. low	83	69	55	49	28

and its strong associations with the inflammatory status however, make these results difficult to contextualize.[24;25] In our own patient population, adjustment for inflammation (IL-6) and malnutrition (SGA) did not affect this association. Furthermore, Zoccali et al.[14] reported in their study that, since adjustment for fT3 abrogated the association between inflammation and patient outcome, fT3 may be an intermediate pathological of systemic inflammation. In our data, as well as in the study conducted by Ozen et al.[5], adjustment for inflammation did not affect the link between T3 and mortality. Also, associations between trimestral thyroid hormone changes and variation of inflammatory markers were somewhat weak and inconsistent in our study. One limitation that makes our studies not fully comparable is, that while Zoccali et al.[14] and Ozen et al.[5] measured free fractions, we report on total T4 and T3 levels only. Although adjustment for s-albumin (one of the main transporters of T3 in plasma) did not modify the observed associations, we should acknowledge that fT3 is not tantamount of T3. Several studies have, nevertheless, suggested anti-atherosclerotic effects of fT3 on the vascular bed via its effect on mitochondrial oxidative systems, induction of vasodilatatory molecules, inhibition of angiotensin II receptor expression and down-stream signal transduction, mechanisms all of which do not necessarily involve the inflammatory cascade.[26-28]

In support of the association between T3 and the prediction of cardiovascular death in our study, a previous report[11] in patients with moderate to severe kidney disease reported that T3 levels inversely associated with flow mediated vasodilatation, and that this observation was dependent on multivariate adjustment for asymmetric dimethylarginine levels. At a cardiac level, low T3 levels in CKD patients are associated with reduced left ventricular function, increased left ventricular mass[13] and elevated intima-media thickness[12]. Our study also adds novel evidence on the suggested impact of trimestral T3 variation on all-cause and CV mortality in ESRD. Patients with persistently low T3 levels exhibited the highest hazards of dying irrespective of other concomitant risk factors. Interestingly, also an increased variation in T3 during the observation period (e.g. both increases and decreases in concentration) resulted in elevated hazards albeit statistically not significant, leading us to hypothesize that also an increased T3 fluctuation may link to an adverse outcome. This finding accords with a previous study in critically ill patients,[29] in which a fast decline in T3 and T4 (without a concomitant rise in TSH levels) was observed prior to death. Our longitudinal analysis, by including two observations 3 months apart in the same individual, represents a step forward from previous evidence by virtue of observing the subject specific temporal order of events. Therefore, it is interesting to pinpoint that fluctuation of thyroid hormones over time exists in ESRD patients, and that this fluctuation links to a worse outcome. What factors drive this, and whether uremic thyroid fluctuation is higher than in other diseases remains, for now, unknown.

Another novelty in our analysis is the association between baseline and trimestral variation of T4 levels and (cardiovascular) mortality. Although this is the first study to report so in a dialysis population, findings are in line with previous evidence in non-renal patients with non-thyroidal illness.[29;30] An interesting aspect in our analysis is that the strength of the association between low T4 levels and mortality was not fully affected by further adjustment for T3 levels. This may suggest

that the effect of T4 on outcome is, at least in part, not dependent on its metabolite and that both may participate in the increased CV risk. According to recent literature however, adjusting in the causal pathway may not be so straightforward as initially thought of,[31] and caution is needed in the interpretation of this finding which warrants confirmation in further studies. Nevertheless, our previous report in incident dialysis patients also observed, by means of receiving operator characteristics (ROC) analysis a significant, albeit weak association between T4 levels and all-cause mortality.[16] The recent study by Takamura et al.[32] in euthyroid patients demonstrated that carotid intima-media thickness was inversely and independently associated with T4 and fT4, suggesting an increased cardiovascular risk in subjects with low T4 even within the normal reference range. Differential effects of T4 and T3 on immune cells have also been reported: T4 stimulated while T3 inhibited peripheral lymphocyte proliferation.[33]

In the interpretation of our results, some additional limitations must be addressed: Causes of death were extracted by death records and not confirmed by autopsies, which could result in some degree of misclassification. As unknown causes of death were denoted as non-CV, this could translate, in any case, into an underestimation of the observed effect towards the null hypothesis, and the true hazards may possibly be bigger. The inclusion of prevalent dialysis patients may infer into a survival bias, although preceding literature contemplates also prevalent patients in their designs. Finally, ours is an observational study and confounding by unmeasured factors cannot be ruled out.

To conclude, patients with reduced T3 and/or T4 levels bear an increased mortality risk, especially due to cardiovascular causes. This was true when considering both baseline measurements and trimestral variation patterns. Our longitudinal design adds important observational evidence -although non-decisive- that the link may underlie a causal effect.

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