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Serum Thyrotropin Elevation and Coronary Artery Calcification in Hemodialysis Patients

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Keywords
Thyroid · Thyrotropin · Calcification · End-stage kidney disease · Dialysis

Abstract
Introduction: Hypothyroidism is highly prevalent in end-stage kidney disease patients, and emerging data show that lower circulating thyroid hormone levels lead to downregulation of vascular calcification inhibitors and coronary artery calcification (CAC) in this population. To date, no studies have examined the association of serum thyrotropin (TSH), the most sensitive and specific single biochemical metric of thyroid function, with CAC risk in hemodialysis patients. ***Methods:*** In secondary analyses of patients from the *Anti-Inflammatory and Anti-Oxidative Nutrition in Hypoalbuminemic Dialysis Patients* trial, we examined serum TSH levels and CAC risk assessed by cardiac computed tomography scans collected within a 90-day period. We evaluated the relationship between serum TSH with CAC Volume (VS) and Agatston score (AS) (defined as >100 mm^3 and >100 Hounsfield Units, respectively) using multivariable logistic regression. ***Results:*** Among 104 patients who met eligibility criteria, higher TSH levels in the highest tertile were associated with moderately elevated CAC VS and AS in case-mix-adjusted analyses (ref: lowest tertile): adjusted ORs (95% CIs) 4.26 (1.18, 15.40) and 5.53 (1.44, 21.30), respectively. TSH levels >3.0 mIU/L (ref: ≤3.0 mIU/L) were also associated with moderately elevated CAC VS and AS. In secondary analyses, point estimates of incrementally lower direct free thyroxine levels trended toward elevated CAC VS and AS, although associations did not achieve statistical significance. ***Conclusions:*** In

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hemodialysis patients, higher serum TSH was associated with elevated CAC VS and AS. Further studies are needed to determine if thyroid hormone supplementation can attenuate CAC burden in this population.

Introduction

Hypothyroidism has a high prevalence in chronic kidney disease (CKD) patients, including those receiving dialysis, but is often under-recognized [1–3]. Large population-based studies have shown that there is an increasing prevalence of thyroid dysfunction with incrementally impaired kidney function [4, 5]. Approximately one-quarter of advanced CKD and end-stage kidney disease (ESKD) patients have underlying hypothyroidism [4–7]. In the general population, overt and subclinical hypothyroidism have each been associated with an increased risk of coronary heart disease (CHD) events and death [8, 9]. Given that both kidney disease and thyroid dysfunction are common in US adults [10, 11], and both are known to adversely affect cardiovascular health [8, 12], there has been growing interest in whether hypothyroidism is a novel, modifiable risk factor for adverse CHD outcomes in ESKD [3].

Emerging data suggest that hypothyroidism is associated with a greater burden of coronary artery calcification (CAC) [3, 13–16], a major pathogenic mechanism for vascular stiffness, left ventricular hypertrophy, and sudden cardiac death in this population [17]. Experimental data suggest that low thyroid function is causally associated with vascular calcification via downregulation of matrix Gla protein and Klotho, which are inhibitors of vascular calcification [15, 16]. These observations have been corroborated by two clinical studies in ESKD patients demonstrating a link between low T3 and thyroxine (T4) levels and heightened risk of CAC [13, 14]. However, given that T3 and T4 levels may be low in the setting of malnutrition, inflammation, and uremia [18–22], it is unclear if these observations represent causal hypothyroidism – CAC relationships, or are instead confounded by non-thyroidal illness (i.e., thyroid functional test alterations associated with underlying ill health in the absence of thyroid pathology [18]).

In contrast, serum thyrotropin (TSH) is considered to be the most sensitive and specific single biochemical metric of thyroid function [23, 24], and a robust indicator of thyroid status less influenced by non-thyroidal illness [18]. However, to date, no studies have detected an association between serum TSH levels and CAC risk in ESKD patients receiving dialysis. To address this knowledge gap, we conducted a study examining the relationship among metrics of thyroid status, serum TSH, and direct free thyroxine (dFT4) levels, as well as anti-thyroid peroxidase (anti-TPO) antibody levels, with elevated CAC scores in a secondary analysis of patients from the prospective *Anti-Inflammatory and Anti-Oxidative Nutrition in Hypoalbuminemic Dialysis Patients* (AIONID) trial [25].

Methods

Source Cohort

We conducted a secondary analysis of the association between thyroid status and CAC among hemodialysis patients from the AIONID trial (Clinicaltrials.gov# NCT00561093) who were recruited across 12 outpatient dialysis units in the Southern California region over the period of June 2008 to June 2010 [25]. The AIONID study was a pilot-feasibility, double-blind randomized controlled trial using a two-by-two factorial design in which patients were randomly assigned to (1) either a nutrition supplement (e.g., Nepro) plus anti-inflammatory antioxidant supplement (e.g., Oxepa) versus placebo, and (2) an anti-inflammatory appetite stimulator (e.g., pentoxifylline) versus placebo. These interventions were administered during routine thrice-weekly hemodialysis treatments over a period of 16 weeks in order to test feasibility and improvements in serum albumin concentrations.

In the present study, patients were included provided that they were (1) ≥18 years of age, (2) received thrice-weekly in-center hemodialysis for at least 4 consecutive weeks, (3) had serum albumin levels <4.0 g/dL over a 3-month period, and (4) signed a local institutional review board approved consent form (i.e., eligibility criteria for the parent AIONID trial [25]); (5) underwent a CAC computed tomography (CT) scan during the pretrial phase of the AIONID study; and (6) had available sera collected within 90 days of their CAC measurements. Patients were excluded if they were actively receiving peritoneal dialysis or had a terminal disease (e.g., stage IV cancer). The study was approved by the institutional review boards of the Lundquist Institute (previously known as the Los Angeles Biomedical Research Institute) at Harbor UCLA Medical Center and the University of California Irvine (UCI).

Exposure Ascertainment

The primary exposure of interest was thyroid status as defined by serum TSH level. Serum TSH was measured from thawed serum samples that were collected pre-dialysis at weekday hemodialysis treatments during the baseline period of the AIONID trial (i.e., prior to randomization) and subsequently stored at −80°C. Serum TSH was measured using second-generation chemiluminescent immunoassay tests (Beckman Coulter, Chaska, MN, USA; reference range 0.5–5.0 mIU/L) in the Clinical Pathology Laboratory of the UCI Medical Center. In primary analyses, serum TSH levels were categorized into tertiles of observed baseline values: Tertiles 1, 2, and 3 corresponded to TSH levels of <1.44, 1.44–<2.19, and 2.19–10.0 mIU/L, respectively. In sensitivity analyses, we also examined serum TSH levels (1) dichotomized as ≤3.0 versus >3.0

mIU/L (i.e., threshold for high-normal TSH levels associated with worse survival and patient-reported outcomes in dialysis patients [6, 26–29]), and (2) continuous increments of 1 standard deviation (SD) (+Δ1.9 mIU/L).

In secondary analyses, we examined additional thyroid markers from thawed samples, including (1) serum anti-TPO antibody levels ascertained by the "two-step sandwich" method (Beckman XL, Brea, CA, USA; reference range <35 U/mL) in the UCI Clinical Pathology Laboratory, and (2) serum dFT4 levels ascertained by equilibrium dialysis/tandem mass spectrometry [19, 30–32], which were sent from the UCI Clinical Pathology Laboratory to an outside reference laboratory (ARUP Laboratories, Salt Lake City, UT, USA; reference range: 1.1–2.4 ng/dL).

Outcome Ascertainment

The primary outcome of interest was CAC score measured by E-Speed electron beam scanner (GE-Imatron, South San Francisco, CA) or 64-multidetector CT (LightSpeed VCT, General Electric Medical System, Milwaukee, WI, USA) conducted at Harbor UCLA Medical Center [33–35]. The coronary arteries were imaged with 30–48 continuous 2.5–3-mm slices during mid-diastole using ECG-triggering during a 35-s breath hold. CAC measurements were performed on non-contrast studies and were evaluated at a central reading center by an experienced single-reader blinded to the patient information (MJB). CAC was defined as a plaque of at least three continuous pixels (area 1.02 mm^2) with a density of >130 Houndsfield units (HU).

In primary analyses, we examined CAC measured by Volume Score (VS) (mm^3) given that (1) it represents an actual CAC volume, (2) has shown better reproducibility than other CAC measures such as the Agatston score (AS) (i.e., AS is upwardly weighted by calcium density and may not accurately capture changes in coronary calcium) [36, 37], and (3) has been shown to be more predictive of cardiovascular events than calcium density [38]. VS was calculated by multiplying the number of voxels with calcification by the volume of each voxel for each calcified lesion, and summing individual lesion scores from the four main coronary arteries (left main, left anterior descending, circumflex, and right coronary artery). In secondary analyses, we also examined CAC measured by AS (HU), calculated by multiplying the area of calcium by a factor related to maximum plaque density, and summing lesion scores from the four main coronary arteries. We examined the association between thyroid status and moderately elevated CAC VS and AS (separately), defined as >100 mm^3 and HU, respectively [39–43]. In sensitivity analyses, we also examined varying thresholds of elevated VS and AS as indicators of "severe" and "extensive" CAC using cutoffs of >400 and >1,000 mm^3 or HU, respectively [39–43]. We also estimated associations of thyroid status with CAC VS and AS examined as continuous variables using linear regression.

Socio-Demographic, Comorbidity, Medication, and Laboratory Covariates

Information on socio-demographics, comorbid conditions, medications, and dialysis treatment characteristics (i.e., vascular access type) were collected at study entry by AIONID research coordinators. Dialysis vintage was defined as the time between the date of study entry and the date of hemodialysis initiation. Routine dialysis laboratory measurements were performed by the outpatient dialysis laboratories using automated methods.

Statistical Analyses

Baseline characteristics between exposure groups were compared using χ^2, analysis of variance, and Kruskal-Wallis tests according to variable type. We examined cross-sectional associations between serum TSH tertiles and CAC score with logistic and linear regression models using four incremental levels of covariate adjustment:

1. Unadjusted model: included serum TSH level as the primary exposure of interest.
2. Case-mix model: adjusted for age, sex, and race (White vs. Non-White).
3. Expanded case-mix-adjusted model: adjusted for covariates in the case-mix model, as well as diabetes.
4. Expanded case-mix + vascular access-adjusted model: adjusted for covariates in the expanded case-mix model, as well as vascular access type.

We a priori defined the case-mix model as our primary model, which forced into the model core socio-demographic measures. The expanded case-mix and expanded case-mix + vascular access-adjusted models were designated as secondary analyses given the high number of parameters relative to the number of cases (i.e., patients with moderately elevated CAC scores). In sensitivity analyses, we also examined expanded case-mix + vascular access + nutritional/inflammatory status-adjusted models, which additionally considered serum albumin and transferrin saturation levels in addition to expanded case-mix + vascular access type covariates; this was based on the observation that serum albumin and transferrin saturation were significantly correlated with serum TSH levels (i.e., Spearman correlation = −0.22 and p value = 0.03 for serum albumin – TSH, and Spearman correlation = −0.21 and p value = 0.04 for transferrin saturation – TSH) and may potentially confound serum TSH-CAC associations. Analogous analyses were conducted in examining the relationship between additional exposure definitions for serum TSH, anti-TPO antibody, and dFT4 levels with CAC. There were no missing values for any of the covariates, except for serum albumin (4.8%) and transferrin saturation (4.8%), which were addressed with multiple imputation. Analyses and figures were generated using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), Stata version 13.1 (Stata Corporation, College Station, TX, USA), and SigmaPlot Version 12.5 (Systat Software, San Jose, CA, USA).

Results

Study Population

Among 104 patients meeting eligibility criteria (Fig. 1), the mean ± SD, median (IQR), and minimum-maximum TSH values were 2.29 ± 1.88, 1.74 (1.22, 2.74), and 0.14–10.0 mIU/L, respectively; the proportion of patients who had TSH levels in the high-normal (>3.0 mIU/L) and hypothyroid (>5.0 mIU/L) ranges were 18.3% and 9.6%, respectively.

Baseline characteristics of the cohort stratified by TSH tertile are shown in Table 1. Compared to patients in the lowest TSH tertile, those in the highest tertile tended to

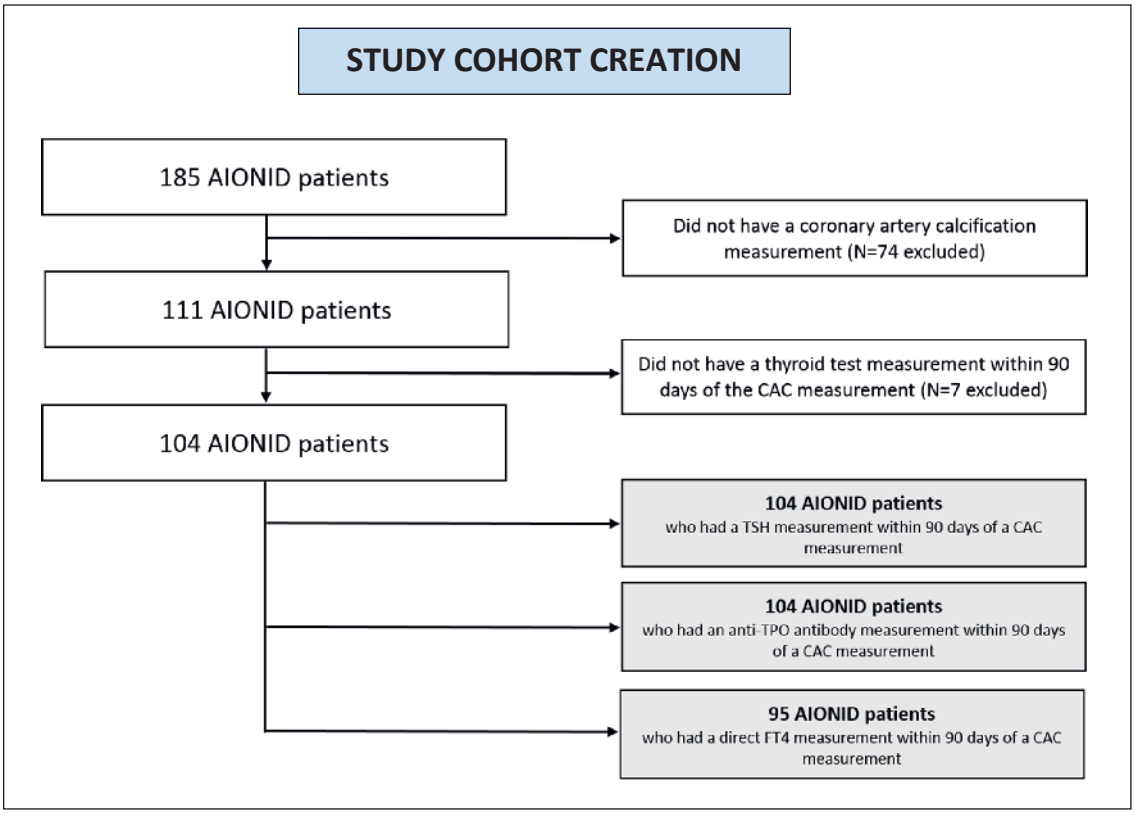

Fig. 1. Study cohort creation. AIONID, *Anti-Inflammatory and Anti-Oxidative Nutrition in Hypoalbuminemic Dialysis Patients*; CAC, coronary artery calcification; anti-TPO antibody, anti-thyroid peroxidase antibody; FT4, free thyroxine.

Table 1. Baseline characteristics of cohort according to thyroid status defined by serum TSH levels categorized as tertiles

	TSH category				*p* value
	Overall	Tertile 1	Tertile 2	Tertile 3	
Patients, *n*	104	35	34	35	N/A
TSH, mIU/L, min-max	0.14–10.02	0.14–1.43	1.44–2.12	2.19–10.02	N/A
Age, years, mean±SD	58±13	60±13	57±13	57±12	0.60
Female, %	52	57	47	51	0.70
Race, %					
White	63	69	50	71	0.13
Non-White	37	31	50	29	
Hispanic, %	55	63	41	60	0.15
Vascular access, %					
AVF/AVG	80	86	76	77	0.56
Catheter	20	14	24	23	
Diabetes, %	68	69	68	69	>0.99
Anti-TPO antibody level, median (IQR)	1.0 (1.0, 2.0)	1.0 (1.0, 4.0)	1.0 (1.0, 1.0)	1.0 (1.0, 4.0)	0.17
Direct free T4 level, median (IQR)	1.6 (1.4, 2.0)	1.7 (1.5, 2.0)	1.6 (1.3, 1.8)	1.7 (1.5, 2.2)	0.17

TSH, thyrotropin; AVF, arteriovenous fistula; AVG, arteriovenous grant; Anti-TPO level, anti-thyroid peroxidase antibody level; T4, thyroxine.

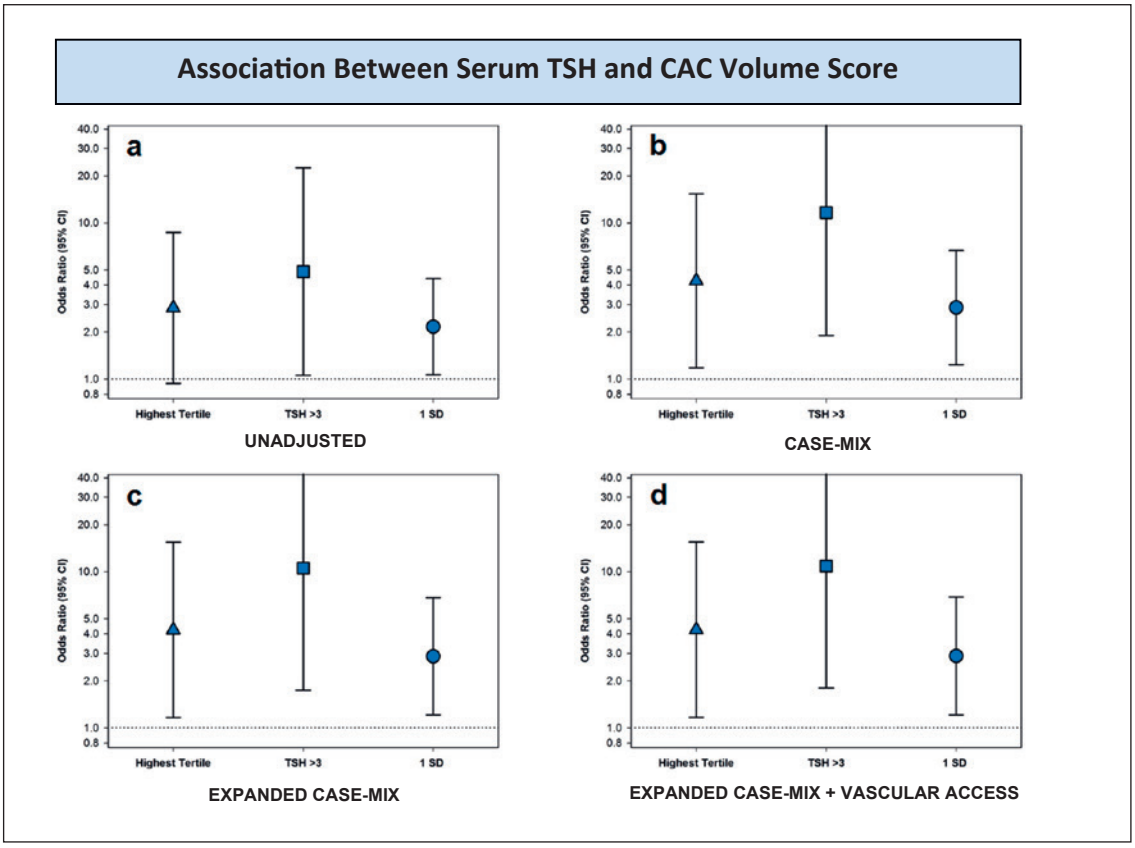

Fig. 2. Association between serum TSH category and total VS using logistic regression in unadjusted (**a**), case-mix (**b**), expanded case-mix (**c**), and expanded case-mix + vascular access (**d**) models.

be younger; were more likely to be male; and were less likely to have an arteriovenous fistula or graft but more likely to have a tunneled dialysis catheter, although differences were not statistically significant. Baseline characteristics of the cohort stratified by TSH levels ≤3.0 versus >3.0 mIU/L are shown in online supplementary Table S1 (see www.karger.com/doi/10.1159/000525037 for all online suppl. material).

Serum TSH Levels and Elevated CAC VSs

Among patients who had concurrent serum TSH and CAC measurements, the mean ± SD, median (IQR), and minimum-maximum VSs were 860 ± 1,160, 448 (34, 1,157), and 0–5,606 mm^3, respectively. There were a total of 71 patients in the overall cohort who had a moderately elevated VS defined as >100 mm^3. When examined across serum TSH tertiles, moderately elevated VSs were observed in 22, 20, and 29 patients in the lowest, middle, and highest TSH tertiles, respectively.

In unadjusted logistic regression analyses, there was a trend toward a significant association between the highest TSH tertile (i.e., as an indicator of lower thyroid function) and moderately elevated VS (ref: lowest TSH tertile): OR (95% CI) 2.86 (0.94, 8.71), $p = 0.07$ (Fig. 2; online suppl. Table S2). Following adjustment for case-mix covariates, this association became stronger and statistically significant: adjusted OR (aOR) (95% CI): 4.26 (1.18–15.40), $p = 0.03$. The association between the highest TSH tertile and moderately elevated VS persisted with incremental adjustment for expanded case-mix and expanded case-mix + vascular access covariates: aORs (95% CI) 4.24 (1.16, 15.50), $p = 0.03$ and 4.25 (1.17, 15.50), $p = 0.03$, respectively. Adjustment covariates found to be important in the relationship between the main exposure, serum TSH tertiles, and the primary outcome, CAC VS, are presented in online supplementary Table S3 (all covariates with p values <0.05 in the expanded case mix + vascular access model).

In sensitivity analyses examining varying serum TSH cutoffs (Fig. 2; online suppl. Table S2), we also found that TSH levels >3.0 mIU/L were significantly associated with moderately elevated VSs across all adjustment levels (ref: ≤3.0 mIU/L): aOR (95% CI) 10.90 (1.80, 65.50), $p = 0.009$ in expanded case-mix + vascular access-adjusted analyses. When examined as continuous increments, each 1-SD higher TSH level (i.e., +Δ1 SD higher TSH = +Δ1.9 mIU/L) was significantly associated with moderately elevated VS across all adjustment levels: aOR (95% CI) 2.89 (1.21, 6.90), $p = 0.02$ in expanded case-mix + vascular access-adjusted analyses.

Upon examination of higher VS thresholds as an indicator of greater CAC severity (severe and extensive CAC defined as >400 and >1,000 mm^3, respectively), point estimates for the highest TSH tertile demonstrated a trend toward elevated VSs (online suppl. Table S2). Notably, in expanded case-mix + vascular access-adjusted analyses, TSH levels >3.0 mIU/L were significantly associated with severe CAC VSs: aOR (95% CI) 3.52 (1.08, 11.50). Similarly, each 1-SD higher TSH level was associated with severe and extensive CAC VSs: aORs (95% CIs) 1.64 (1.00, 2.65) and 1.60 (1.02, 2.51), respectively, in expanded case-mix + vascular access-adjusted analyses.

When VS was examined as a continuous variable, we also found a significant association between higher TSH levels with higher CAC VSs (online suppl. Table S4). Incrementally higher TSH levels (+Δ1 mIU/L and +Δ1 SD higher TSH level) were each associated with increasingly higher CAC VSs in analyses adjusted for expanded case-mix + vascular access covariates: $\beta = +126.7$, $p = 0.03$ and $\beta = +240.7$, $p = 0.03$, respectively.

To account for nutritional/inflammatory status as a potential confounder of the thyroid status – CAC relationship, we conducted sensitivity analyses that adjusted for serum albumin and transferrin saturation levels in addition to expanded case-mix covariates. We observed robust associations between the highest TSH tertile and moderately elevated VS in the expanded case-mix + vascular access + nutritional/inflammatory status-adjusted model: aOR (95% CI) 4.36 (1.12, 17.01), $p = 0.03$ (online suppl. Table 5). Additionally, serum TSH >3.0 mIU/L and higher serum TSH levels examined as continuous increments (i.e., +Δ1 SD higher TSH) showed robust associations with moderately elevated VS in expanded case-mix + vascular access + nutritional/inflammatory status-adjusted analyses.

Serum TSH Levels and Elevated CAC AS

Among patients who had concurrent serum TSH and CAC measurements, the mean ± SD, median (IQR), and minimum-maximum CAC ASs were 1,075 ± 1,468, 502 (42, 1,451), and 0–7,130 HU, respectively. There were a total of 72 patients in the overall cohort who had a moderately elevated AS defined as >100 HU. When examined across serum TSH tertiles, moderately elevated ASs were observed in 22, 20, and 30 patients in the lowest, middle, and highest TSH tertiles, respectively.

Across all adjustment levels, we observed a significant association between the highest TSH tertile and moderately elevated AS (ref: lowest TSH tertile): aOR (95% CI) 5.49 (1.41, 21.50), $p = 0.01$ in expanded case-mix + vascular access-adjusted analyses (Fig. 3; online suppl. Table S5). Adjustment covariates found to be important in the relationship between serum TSH tertiles and CAC VS are presented in online supplementary Table S3 (all covariates with p values <0.05 in the expanded case mix + vascular access model).

Using this AS threshold, we also found that TSH levels >3.0 mIU/L (ref: ≤3.0 mIU/L) were significantly associated with moderately elevated ASs across all adjustment levels: aORs (95% CIs) 9.79 (1.63, 59.00), $p = 0.01$, in expanded case-mix + vascular access-adjusted analyses. When examined as continuous increments, each 1-SD higher TSH level was significantly associated with moderately elevated AS across all adjustment levels: aOR (95% CI) 3.05 (1.23, 7.57), $p = 0.01$ in expanded case-mix + vascular access-adjusted analyses.

In sensitivity analyses that examined higher AS thresholds as an indicator of greater CAC severity (severe and extensive CAC defined as >400 and >1,000 HU, respectively), point estimates for the highest TSH tertile demonstrated a trend toward elevated ASs (online suppl. Table S5). In expanded case-mix + vascular access-adjusted analyses, TSH levels >3.0 mIU/L trended toward extensive CAC AS but did not reach statistical significance: aOR (95% CI) 2.82 (0.89, 8.90), $p = 0.08$. However, each 1-SD higher TSH level was significantly associated with extensive CAC AS in expanded case-mix + vascular access-adjusted analyses: aOR (95% CI) 1.55 (0.99, 2.41), $p = 0.05$.

When AS was examined as a continuous variable, we also found a significant association between higher TSH levels with higher CAC ASs (online suppl. Table S6). Incrementally higher TSH levels (+Δ1 mIU/L and +Δ1 SD higher TSH level) were each associated with increasingly higher CAC ASs in analyses adjusted for expanded case-mix + vascular access covariates: $\beta = +158.1$, $p = 0.03$.

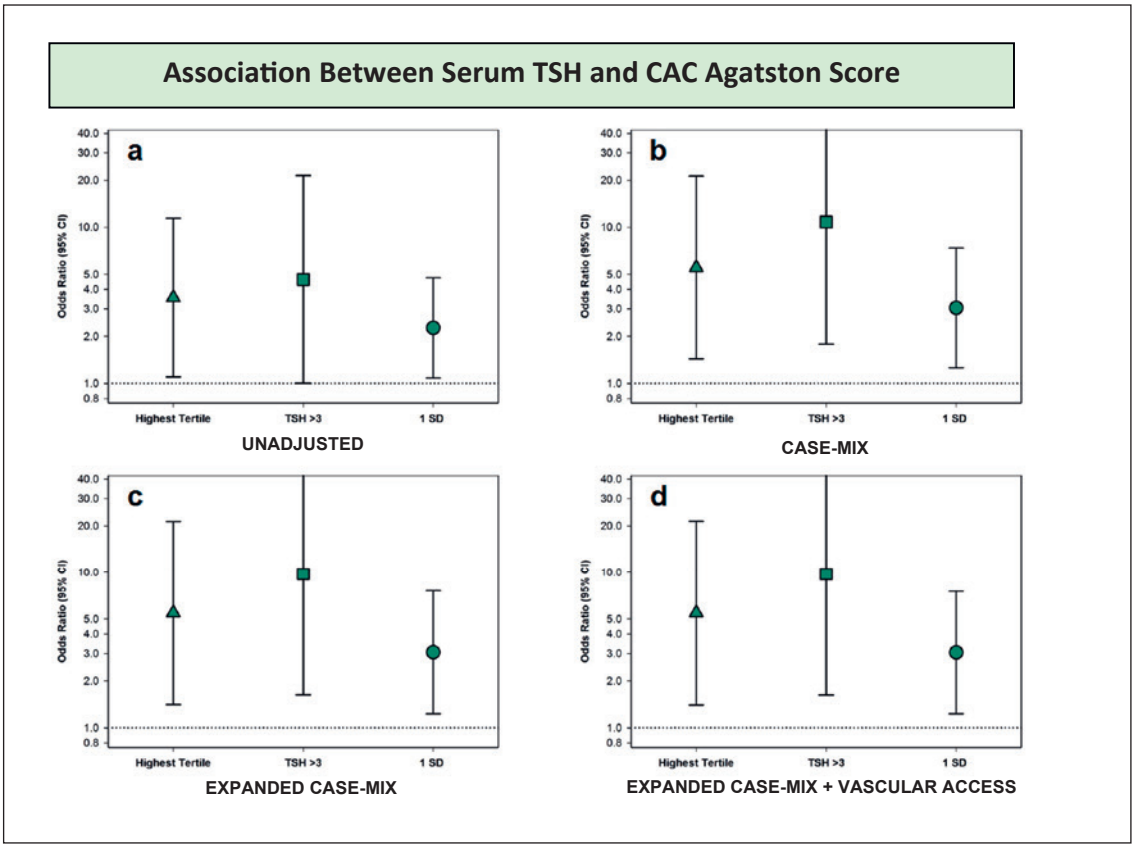

Fig. 3. Association between serum TSH category and total AS using logistic regression in unadjusted (**a**), case-mix (**b**), expanded case-mix (**c**), and expanded case-mix + vascular access (**d**) models.

Similarly, each 1-SD higher TSH level was associated with a ~300 mm^3 increase in CAC AS: $\beta = +300.3$, $p = 0.03$.

In sensitivity analyses accounting for nutritional/inflammatory status as a potential confounder, we observed robust associations between the highest TSH tertile and moderately elevated AS in the expanded case-mix + vascular access + nutritional/inflammatory status-adjusted model: aOR (95% CI) 6.16 (1.42, 26.60), $p = 0.02$ (online suppl. Table 5). Additionally, serum TSH levels >3.0 mIU/L and higher serum TSH levels examined as continuous increments (i.e., +Δ1 SD higher TSH) showed persistent associations with moderately elevated AS in expanded case-mix + vascular access + nutritional/inflammatory status-adjusted analyses.

Serum Anti-TPO Antibody and Direct Free Thyroxine Levels and CAC Scores

In secondary analyses, we examined additional thyroid markers, including serum anti-TPO antibody and dFT4 levels. Examination of varying exposure definitions of serum anti-TPO antibody levels did not show associations with moderately elevated CAC VS (online suppl. Table S7) nor AS (online suppl. Table S8).

In analyses of serum dFT4 levels, point estimates suggested a trend toward an association between incrementally higher dFT4 levels (i.e., +Δ1 SD higher FT4 = +Δ0.7 ng/dL) as an indicator of higher thyroid function and lower likelihood of moderately elevated CAC VS (online suppl. Table S7) and AS (online suppl. Table S8), although estimates did not achieve statistical significance.

Discussion

In a well-defined cohort of prevalent hemodialysis patients who underwent assessment of various thyroid markers and CAC measurements using coronary CT scanning, we found that lower levels of thyroid function, as assessed by an increase in serum TSH, were associated with elevated CAC VS and AS indicating arterial calcifi-

cation burden. In primary analyses, higher serum TSH levels (i.e., as an indicator of lower thyroid function) using various definitions (e.g., highest TSH tertile, TSH levels >3.0 mIU/L, and continuous increments of TSH) were associated with moderately elevated VS and AS independent of socio-demographics, comorbidity, and dialysis-related factors. In sensitivity analyses examining higher VS and AS thresholds as an indicator of greater CAC severity, we also found that incrementally higher levels of serum TSH (+Δ1 SD) were associated with severely elevated CAC VS (>400 mm^3), as well as extensively elevated VS and AS (>1,000 mm^3 and >1,000 HU, respectively).

An increasing body of evidence has uncovered a relationship between thyroid status and vascular calcification [3, 13–16]. First, experimental models show that the expression of vascular calcification inhibitors, namely matrix Gla protein and Klotho, are dependent on the presence of thyroid hormones. In a study of rat aortic smooth muscle cells, physiological concentrations of thyroid hormone (i.e., tri-iodothyroxinine [T3]) were shown to directly facilitate gene expression of matrix Gla protein in smooth muscle cells via thyroid hormone nuclear receptors, leading to the prevention of vascular calcification in vivo [16]. In contrast, hypothyroid-induced reductions in matrix Gla protein mRNA levels led to an increase in calcium content in aortic smooth muscle tissues. In another study of murine adipocytes, thyroid hormone (i.e., T3) was also shown to significantly upregulate the expression levels of the membrane form of the Klotho gene [15]. With respect to clinical data, it was reported over five decades ago that adults with cretinism due to congenital hypothyroidism were observed to have extensive arterial calcification [44]. These observations have been corroborated by two recent clinical studies in dialysis patients showing that lower levels of circulating thyroid hormone are associated with CAC. In a study of 66 peritoneal dialysis patients who underwent assessment of thyroid markers and CAC AS measurement using cardiac CT, there was an inverse association between thyroid hormone levels (i.e., free T3) and CAC [13]. In a study of 97 ESKD patients, free T3 and FT4 levels were inversely associated with CAC ASs, and free T3 levels were positively associated with circulating desphospho-uncarboxylated matrix Gla protein and soluble Klotho concentrations [14].

To our knowledge, ours is the first study to observe an association between elevated serum TSH levels, a more sensitive metric of thyroid status before serum FT4 levels are reduced, with elevated CAC VS and Agaston Scores. Given that T3 is largely derived from the peripheral deiodination of T4-to-T4 [21, 45], a process highly sensitive to inflammation, malnutrition, and non-thyroidal illness [18, 22], lower T3 levels may be more indicative of underlying ill health as opposed to low thyroid function in the dialysis population. In addition, as the vast majority (>99.9%) of T4 is protein-bound [19], routinely used free T4 assays, which are dependent on hormone-protein binding, may result in spurious results in the presence of uremic toxins, non-thyroidal illness, or certain medications (e.g., furosemide, heparin) commonly used in CKD/ESKD patients that may interfere with hormone-protein binding [19, 20]. In contrast, serum TSH is considered the single most sensitive and specific biochemical metric of thyroid function in the general population given its negative logarithmic association with T4 levels (i.e., small changes in T4 lead to exponential changes in TSH) [23, 24].

To address these limitations, we utilized serum TSH levels as a more reliable indicator of lower thyroid function and observed robust associations with elevated CAC VS and AS across multiple secondary and sensitivity analyses. Furthermore, we also conducted novel dFT4 measurements, which more accurately discern FT4 levels by physically separating free from protein-bound hormone using equilibrium dialysis, followed by FT4 quantification using tandem mass spectrometry [19, 31], and has shown more potent associations with TSH in populations with altered hormone-protein binding compared with routinely used FT4 assays [30, 32]. We found that point estimates of incrementally lower dFT4 levels as an indicator of lower thyroid function also trended toward elevated CAC VS and AS, although associations did not achieve statistical significance. These findings are directly germane to ESKD and dialysis patients among whom hypothyroidism is a highly prevalent yet modifiable endocrine complication, and by extension provide insights potentially relevant to other high cardiovascular risk non-CKD populations in whom the higher burden of CAC has also been linked with CHD events, stroke, heart failure, and death [46–49].

Notably, in the present study, we also conducted anti-TPO antibody assessments but did not detect an association with elevated CAC scores. In a prior study of 1,149 women from the Netherlands Rotterdam cohort, those with subclinical hypothyroidism and concomitantly elevated anti-TPO antibody levels had a heightened risk of both aortic atherosclerosis and myocardial infarction [50]. In the general population, it has been hypothesized that elevated anti-TPO antibody levels leading to autoimmune thyroiditis and chronic inflammation contribute to

a heightened risk of atherosclerotic disease [50–52]. Given that nontraditional risk factors (e.g., mineral bone disease, oxidative stress, and uremic toxins) may play a more dominant role in the pathogenesis of fibrocalcific coronary lesions in ESKD [53], this may explain the lack of an observed association between anti-TPO antibody levels and elevated CAC in the present study although further confirmatory studies are needed.

The strengths of our study include its well-characterized cohort of hemodialysis patients who underwent thyroid status evaluation with "gold-standard" metrics; rigorous assessment of both CAC VS and AS using cardiac CT as a noninvasive method to measure coronary calcification with low intra- and interobserver variability; and comprehensive availability of detailed patient-level data on socio-demographics, comorbidities, and laboratory data collected in the ambulatory setting. However, several limitations of our study bear mention. First, given that the serum samples in which thyroid tests were conducted and CAC measurements were concurrently collected within a 90-day period, we cannot confirm a longitudinal relationship nor causal association between thyroid status and coronary calcification from the present study. Measurements of CAC at a single time point may not reflect the ideal time for risk prediction. Second, the limited sample size of our cohort may have precluded the ability to detect significant associations between some of the thyroid markers (anti-TPO antibody and dFT4) and elevated CAC scores. Third, as our study was a secondary analysis of participants from a clinical trial, it is possible that patients who agreed to participate in the AIONID study may have been healthier than the broader US hemodialysis population. Fourth, due to data limitations, we did not have the opportunity to examine the relationship between thyroid status and CAC with other markers of subclinical atherosclerosis (i.e., carotid-intima media thickness) [54]. While this present study focused on CAC as a stronger indicator of cardiovascular risk than other subclinical atherosclerosis markers in both the CKD and non-CKD populations [55, 56], given the multiple ill effects of thyroid dysfunction on cardiovascular health observed in the non-CKD population (i.e., impaired cardiac contractility, endothelial function, cardiac conduction) [8], future studies examining the impact of thyroid status on other cardiovascular endpoints in CKD patients are warranted. Lastly, given that our recruitment was restricted to 12 outpatient dialysis units in Southern California, our findings may not be generalizable to other geographic regions in which patients' case-mix characteristics and dialysis practice patterns may differ.

In conclusion, we observed that lower levels of thyroid function ascertained by elevated serum TSH levels were significantly associated with a greater burden of CAC in hemodialysis patients. Given the high prevalence of thyroid dysfunction and cardiovascular disease in ESKD patients, further studies are needed to confirm findings and determine whether thyroid hormone replacement ameliorates CAC and its downstream consequences in this population.

Statement of Ethics

Study participants have given their written informed consent. The study was approved by the Institutional Review Boards and Committee on Human Studies at the University of California Irvine.

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Author Contributions

Research idea and study design: C.M.R.; data acquisition: C.M.R. and K.K.Z.; data analysis/interpretation: C.M.R. and A.S.Y.; statistical analysis: A.S.Y.; supervision or mentorship: C.M.R. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Data Availability Statement

The data that support the findings of this study are not publicly available due to containing information that could compromise the privacy of research participants. Further inquiries can be directed to the corresponding author.

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