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Thyroid antibodies and levothyroxine effects in subclinical hypothyroidism: A pooled analysis of two randomized controlled trials

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Abstract. Lyko C, Blum MR, Abolhassani N, Stuber MJ, Del Giovane C, Feller M, et al. Thyroid antibodies and levothyroxine effects in subclinical hypothyroidism: A pooled analysis of two randomized controlled trials. *J Intern Med.* 2022;**292**:892–903.

Background. Antithyroid antibodies increase the likelihood of developing overt hypothyroidism, but their clinical utility remains unclear. No large randomized controlled trial (RCT) has assessed whether older adults with subclinical hypothyroidism (SHypo) caused by autoimmune thyroid disease derive more benefits from levothyroxine treatment (LT4).

Objective. To determine whether older adults with SHypo and positive antibodies derive more clinical benefits from LT4 than those with negative antibodies.

Methods. We pooled individual participant data from two RCTs, Thyroid Hormone Replacement for Untreated Older Adults with Subclinical Hypothyroidism and IEMO 80+. Participants with persistent SHypo were randomly assigned to receive LT4 or placebo. We compared the effects of LT4 versus placebo in participants with and without anti–thyroid peroxidase (TPO) at baseline. The two primary outcomes were 1-year change in Hypothyroid Symptoms and Tiredness scores on the Thyroid-Related Quality-of-Life Patient-Reported Outcome Questionnaire.

Results. Among 660 participants (54% women) ≥65 years, 188 (28.5%) had positive anti-TPO. LT4 versus placebo on Hypothyroid Symptoms lead to an adjusted between-group difference of −2.07 (95% confidence interval: −6.04 to 1.90) for positive antibodies versus 0.89 (−1.76 to 3.54) for negative antibodies (p for interaction = 0.31). Similarly, there was no treatment effect modification by baseline antibody status for Tiredness scores—adjusted between-group difference 1.75 (−3.60 to 7.09) for positive antibodies versus 1.14 (−1.90 to

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4.19) for negative antibodies (p for interaction = 0.98). Positive anti-TPO were not associated with better quality of life, improvement in handgrip strength, or fewer cardiovascular outcomes with levothyroxine treatment.

Conclusions. Among older adults with SHypo, positive antithyroid antibodies are not associated with more benefits on clinical outcomes with LT4.

Keywords: autoimmune thyroid disease, levothyroxine treatment, subclinical hypothyroidism

Introduction

Subclinical hypothyroidism (SHypo), defined as an elevated thyroid-stimulating hormone (TSH) level with free thyroxine (fT4) in the reference range, affects up to 6%–18% of older adults [1]. Although clinical guidelines differ on which patients should be treated [2–4], SHypo is frequently treated with levothyroxine (LT4) [5]. This contributes to levothyroxine being one of the most prescribed medications in the United States and Europe [2, 3, 5–7]. In older adults, two recent international, double-blind, randomized controlled trials (RCTs)—The Thyroid Hormone Replacement for Untreated Older Adults with Subclinical Hypothyroidism (TRUST) [8] and the IEMO80+ Thyroid Trial [9]—and a meta-analysis [10] found no benefit of levothyroxine on hypothyroid symptoms, tiredness, or quality of life, even among those with high symptom burden [11]. However, specific subgroups might still benefit, such as patients with autoimmune thyroid disease.

Autoimmunity is a common etiology of SHypo [12] characterized clinically by high levels of anti–thyroid peroxidase antibodies (anti-TPO) [2, 13]. Anti-TPO induces cell-mediated cytotoxicity [14, 15] and its presence is associated with the development of thyroid dysfunction [16–19]. Adults with SHypo and positive anti-TPO are more prone to progress to overt hypothyroidism in studies with long-term follow-up of up to 20 years (4.3%/year for positive antibodies vs. 2.6%/year for negative antibodies) [16, 20]. For these patients, the indication for treatment remains controversial. Two guidelines suggest considering treatment in the presence of positive antibodies to prevent progression to overt hypothyroidism, irrespective of TSH levels [2, 4], as do some international experts [21, 22], while another guideline does not mention antibody status as an indication for treatment [3]. Despite controversial recommendations, physicians often use anti-TPO testing to decide whether to initiate treatment [23]. In a survey of American Thyroid Association members and primary care providers, approximately 10%–20% more physicians would initiate therapy in SHypo if antibodies were positive than if antibodies were negative [23]. Little data on the frequency of anti-TPO measurements at the population level exist, but a population-based study of 5552 outpatients found a relatively high proportion of 3%/year of participants with at least one anti-TPO antibody test [24]. Data are very limited on the impact of positive antibodies, as no large RCTs have demonstrated that antibody status influences the effectiveness of levothyroxine on clinical outcomes in adults with SHypo. Previous studies had design limitations such as including only young patients or having small sample sizes ($N = 40$–100) [25–27]. Therefore, in this ancillary analysis of two large RCTs (TRUST and IEMO80+) [8, 9], we aimed to evaluate whether antibody status influences the effectiveness of levothyroxine on clinical outcomes in older adults with SHypo.

Materials and methods

Design

This study is a combined analysis of two coordinated RCTs, the TRUST [8] and IEMO80+ [9] Thyroid Trials, whose designs have been previously described [9, 28]. Participants from both trials were pooled for the purpose of this analysis as the RCTs were led with similar designs and included a prespecified planned combined analysis of all participants [9]. The trials were conducted in accordance with the principles of the Declaration of Helsinki [29] and Good Clinical Practice guidelines [30]. They were approved by the ethics committees and regulatory authorities in all four countries (Switzerland, the Netherlands, Ireland, and the UK) involved in the trials. All participants provided written informed consent (trials registrations: ClinicalTrials.gov NCT01660126 [TRUST and IEMO], Netherlands Trial Register NTR3851 [IEMO]).

Study population

As previously described, [8] TRUST included community-dwelling individuals aged ≥65 years with persistent SHypo, defined by TSH levels between 4.6 and 19.9 mIU/L on two measurements at least 3 months apart and fT4 levels within the assay reference range. IEMO80+ had identical inclusion criteria for individuals aged ≥80 years [9]. Exclusion criteria were published previously [8, 9]. In the present study, we only included participants whose anti-TPO status was measured at baseline. Of the 842 participants who underwent randomization, 182 had no anti-TPO data at baseline (no consent for blood draw or for technical reasons, such as too limited blood samples), resulting in 660 participants with available anti-TPO data (Fig. 1).

Randomization and blinding

Randomization was done for each trial in an independent data center through a computer-based program [8, 9]. The treatment started with levothyroxine 50 μg daily (25 μg in individuals <50 kg body weight or with coronary heart disease) or matching placebo. Levothyroxine dose was titrated every 6–8 weeks to target TSH levels ≥0.4 and <4.6 mIU/L. In the placebo group, a mock titration was performed. During the trial, neither the participants, investigators, nor the treating physicians were aware of the treatment allocation and of the results of TSH measurements.

Laboratory assays

Nonfasting venous blood samples were collected from each participant at baseline and at follow-up with the same analyzer method. Chemiluminescent immunoassays for TSH and fT4 were performed in local labs. Antibody status was measured after the end of the RCT in stored baseline serum samples collected before randomization at the Department of Clinical Chemistry and Laboratory Medicine laboratory of the Leiden University Medical Center with an immuno-assay from Roche (Cobas 8000 E602), which uses electrochemiluminescence with a competition principle. Anti-TPO was used in the analysis, as anti-TPO is the most sensitive marker for thyroid autoimmunity [13–19]. Repeated quality control testing was done under the supervision of a European Specialist in Laboratory Medicine. The measuring range of anti-TPO is between 5 and 600 kU/L (defined by the lower detection limit and the maximum of the master curve). Measurements exceeding the measuring range were set to the high or low limit, respectively. Positivity was defined as >34 kU/L, which corresponds to the 95th percentile of the assay reference range measured in healthy subjects [31]. This threshold is consistent with reference ranges used in other immune assays and studies [32–34].

Study outcomes

The two primary outcomes were the changes from baseline to 12 months in the widely established ThyPRO Hypothyroid Symptoms score (four items) and Tiredness score (seven items), [35] each item ranging from 0 to 100, with higher scores corresponding with more symptoms. The minimal clinically important difference (MCID) is 9 points [36].

The secondary outcomes were the variation from baseline to 12 months in health-related quality of life (EuroQoL [EQ] Group 5-Dimension [5D] 3 levels [3L] Self-Report Questionnaire [EQ-5D-3L] [37] ranging from −0.59 to 1 on the descriptive index and from 0 to 100 on the visual-analog scale, with higher scores indicating better quality of life), handgrip strength (maximum static force that a hand can apply by squeezing a dynamometer), and fatal or nonfatal cardiovascular events (acute myocardial infarction, stroke, amputations for peripheral vascular disease, revascularization for atherosclerotic vascular disease including for acute coronary syndrome, heart failure hospitalizations). Executive cognitive function (the letter-digit coding test, which calculates the speed for matching nine letters with nine digits in 90 seconds with higher scores indicating better executive function) was assessed only at the end of the study.

Statistical methods

Baseline data are expressed as the number of participants (percentage) for categorical characteristics or as mean ± standard deviation (SD) or median (interquartile range [IQR]) for continuous characteristics.

In line with the protocol and first reports [9], the primary analysis considered all participants in the intention-to-treat population who had the 1-year follow-up visit within the protocol-specified window. In this study, only participants with an anti-TPO status at baseline were included. Between-group comparisons on the difference between 1-year follow-up and baseline values were performed using linear mixed-effects regression

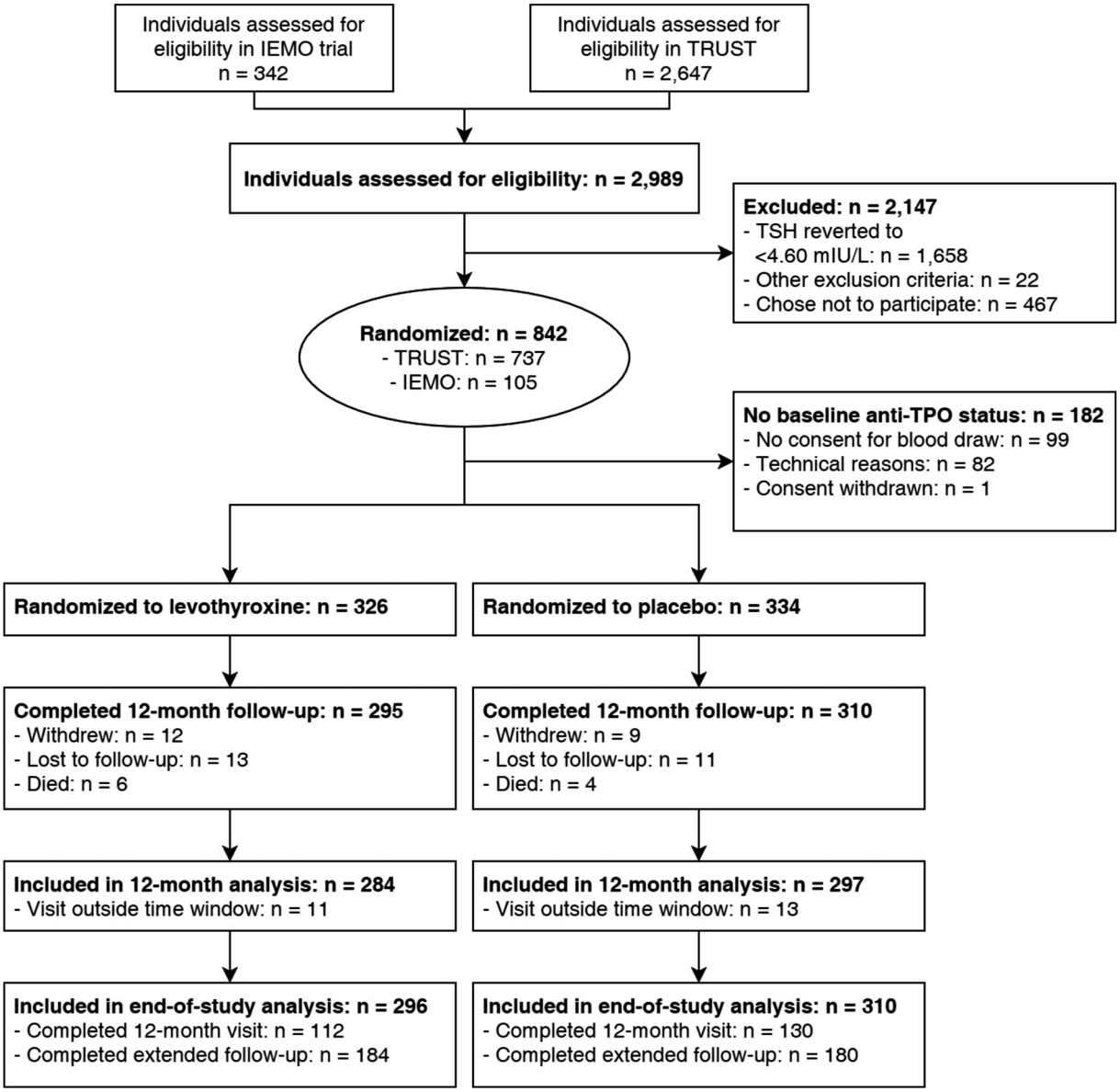

Fig. 1 *Study flow chart. Abbreviations: anti-TPO, anti–thyroid peroxidas; IEMO, Institute for Evidence-Based Medicine in Old Age; TRUST, Thyroid Hormone Replacement for Untreated Older Adults with Subclinical Hypothyroidism Trial; TSH, thyroid-stimulating hormone. Visit time window: The 12-month window was defined as between 334 and 397 days after randomization. N = 310 had 12-month follow-up (n = 297 within the visit window). Of these, n = 180 had extended follow-up and 130 did not. The end-of-study analysis takes the latest available visit (extended, n = 180; or 12-months n = 130) with a mean follow-up duration of 20 months. For the primary analysis at 12 months, participants with visits outside the time window were excluded, as previously done [8, 9].*

models with an interaction term between the treatment group and baseline anti-TPO status, with adjustment for stratification variables (sex, country, and randomization dose) and the respective baseline values (given that randomization was not stratified by baseline antibody status, as measured after the trial end), and study as a random effect. Basing the effect size for the interaction (i.e., the difference between the differences between the treatment groups) on the SD of the difference

between the treatment groups in both anti-TPO groups and averaging the patient numbers within both groups, a difference in the difference of 6.9 points on the scale could be detected significantly with 80% power at a two-sided alpha of 0.05. The analysis was repeated for the per-protocol population, including all participants who pursued the trial regimen (i.e., on treatment at the time of the analysis being conducted and who had their 12-month visit at 12 months ± 31 days after randomization) [8]. In addition, the analysis was repeated for outcomes at the final visit, which was either the 12-month visit or the last visit after more than 12 months of follow-up, further adjusting for time to visit. We conducted subgroup analyses for sex, age (<80 vs. ≥80 years), and baseline TSH categories (<7.0 mIU/L vs. ≥7.0 mIU/L) [38]. We further adjusted for pre-existing medical conditions (hypertension, ischemic heart disease, atrial fibrillation, and diabetes mellitus) in sensitivity analyses. Results were expressed as adjusted between-group differences of the various outcome scores from baseline to follow-up with 95% confidence interval (CI).

Two-sided statistical significance was considered for p-values <0.05. Analyses were performed using Stata version 15 (StataCorp, College Station, TX) and R version 3.6.0 (R Foundation) for Windows.

Patient and public involvement

Patients and the public were involved in the design through the Leyden Academy on Vitality and Ageing in the Netherlands (www.leydenacademy.nl) with a mission to enhance the quality of life of older adults.

Results

Trial population

The characteristics at baseline were similar between participants who had available antibody status and those who did not (Table S1). A total of 296 participants in the levothyroxine group and 310 in the placebo group completed the study with a mean follow-up of 20 months. The mean age was 75.9 ± 7.1 years. A total of 188 participants (28.5%) had positive anti-TPO and 472 participants had negative anti-TPO (Table 1). The proportion of positive anti-TPO was not significantly different between the treatment group and the placebo group (31.9% and 25.1%, respectively, $p = 0.06$, Table S2). In participants with positive anti-TPO, the median level was 156.0 kU/L (IQR 97.6–288.3) in the levothyroxine group and 183.8 kU/L (IQR 88.0–325.2) in the placebo group. Some between-group differences in baseline characteristics were present (Table 1), given that randomization was not stratified by baseline antibody status. The Hypothyroid symptoms score was nonsignificantly higher in the positive anti-TPO group (18.9 ± 18.6 vs. 17.5 ± 19.8, $p = 0.40$), and the Tiredness symptoms score was significantly higher with positive anti-TPO (28.1 ± 22.8 vs. 23.4 ± 19.1, $p = 0.01$).

TSH patterns

At baseline, TSH levels were comparable between the treatment and placebo groups within each anti-TPO group (Table 1), with higher TSH in positive anti-TPO participants (mean TSH 7.0 ± 2.6 mUL/L) compared to negative anti-TPO (6.2 ± 1.7 mU/L, $p < 0.001$). The TSH levels were reduced to a mean 3.5 ± 1.9 mIU/L after 6–8 weeks of treatment and to 3.8 ± 2.1 after 12 months. In the placebo group, they did not significantly change during follow-up (Figure S1).

Primary outcomes

In positive anti-TPO participants, the mean adjusted between-group difference of Hypothyroid symptoms score between the levothyroxine and placebo group was −2.07 (95% CI −6.04 to 1.90) (Table 2, Fig. 2); a positive difference would favor for placebo, while a negative difference would favor treatment with a MCID of 9 points. In negative anti-TPO participants, the mean adjusted between-group difference of Hypothyroid symptoms between levothyroxine and placebo group was 0.89 (95% CI −1.76 to 3.54). There was no evidence for effect modification by anti-TPO status (p for interaction = 0.31).

In positive anti-TPO participants, the mean adjusted between-group difference of Tiredness score between the levothyroxine and placebo group was 1.75 (95% CI −3.60 to 7.09). In negative anti-TPO participants, the adjusted between-group difference was 1.14 (95% CI −1.90 to 4.19). Overall, the changes between treatment groups did not differ between positive and negative anti-TPO participants (p for interaction = 0.98).

These results were similar in the per protocol (Table S3) as well as in the end of study analysis (Fig. 2, Table S4). The sensitivity analyses with adjustment for baseline imbalances (pre-existing medical

Table 1. *Baseline characteristics by anti-TPO status and treatment group*

	Positive anti-TPO, $n = 188$		Negative anti-TPO, $n = 472$	
	Levothyroxine, $n = 104$	Placebo, $n = 84$	Levothyroxine, $n = 222$	Placebo, $n = 250$
Demographics				
Age, mean (SD) (years)	74.6 (6.3)	75.0 (7.2)	76.1 (6.8)	76.6 (7.4)
Women, *n* (%)	71 (68.3)	65 (77.4)	105 (47.3)	119 (47.6)
Caucasians (self identified), *n* (%)[a]	103 (99.0)	80 (95.2)	219 (98.6)	247 (98.8)
Pre-existing medical conditions, *n* (%)				
Hypertension	52 (50.0)	34 (41.0)	113 (50.9)	125 (50.2)
Ischemic heart disease[b]	13 (12.5)	4 (4.8)	36 (16.2)	43 (17.2)
Atrial fibrillation	8 (7.8)	9 (10.7)	38 (17.1)	35 (14.0)
Diabetes mellitus	17 (16.3)	9 (10.7)	39 (17.6)	38 (15.2)
Clinical data				
Number of medications, median (IQR)	4 (2–6)	4 (1–6)	4 (2–6)	4 (2–6)
MMSE score, median (IQR)[c]	29.0 (27.0–30.0)	29.0 (28.0–30.0)	29.0 (27.0–29.0)	29.0 (27.0–30.0)
Thyrotropin level (mIU/L)				
Mean (SD)	7.1 (2.6)	7.0 (2.5)	6.3 (1.8)	6.2 (1.5)
Median (IQR)	6.0 (5.5–7.6)	6.4 (5.1–7.6)	5.7 (5.1–6.8)	5.7 (5.1–6.8)
Free thyroxine, mean (SD) (pmol/L)	12.8 (1.8)	13.0 (1.8)	13.7 (2.0)	13.5 (2.0)
Anti-TPO antibody level, median (IQR) (kU/L)	156.0 (97.6–288.3)	183.8 (88.0–325.2)	13.1 (10.5–16.5)	12.7 (10.1–15.9)
ThyPRO Hypothyroid Symptoms and Tiredness scores				
Hypothyroid Symptoms score, mean (SD)[d]	18.4 (20.4)	19.4 (19.1)	18.1 (19.2)	16.9 (18.2)
Tiredness score, mean (SD)[d]	28.0 (23.3)	28.2 (22.4)	23.9 (20.4)	22.8 (17.9)
Secondary outcomes				
EuroQoL-5D-3L score, mean (SD)[e]	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)
Handgrip strength, mean (SD) (kg)[f]	26.7 (9.9)	25.2 (8.7)	27.9 (10.4)	27.7 (11.5)
Letter-digit coding test, mean (SD)[g]	25.6 (7.0)	26.7 (9.1)	24.2 (7.1)	24.3 (7.3)

Abbreviations: anti-TPO, anti–thyroid peroxidase; IQR, interquartile range; MMSE, mini-mental score examination; SD, standard deviation; ThyPRO, Thyroid-Related Quality of Life Patient-Reported Outcome; 3L, three levels; 5D, five dimensions.
[a]Race was reported by the patient.
[b]Ischemic heart disease was a history of previous myocardial infarction or angina pectoris.
[c]MMSE score ranges from 0 to 30, with a higher score corresponding to better cognitive function.
[d]The hypothyroid symptoms and the tiredness score range from 0 to 100, with a higher score indicating more symptoms. The minimal clinically important difference is 9 points.
[e]The EuroQoL-5D-3L score is a standardized instrument for measuring generic health status. The 5D score is going from –0.59 to 1, with a higher score indicating a better quality of life.
[f]Handgrip strength is a measure of the maximum static force that a hand can squeeze using a dynamometer.
[g]Letter-digit coding test measures executive function by calculating the speed for matching nine letters with nine digits in 90 seconds (higher score indicating better executive function).

conditions) yielded similar results (Table S5). There were also no differences according to anti-TPO status and subgroups analyses for TSH (<7 mIU/L vs. ≥7.0 mIU/L), age (<80 vs. ≥80 years), or sex (Figures S2 and S3).

Secondary outcomes

The effect of levothyroxine versus placebo on the EQ-5D-3L score was similar for those with positive and negative antibodies, with a mean adjusted between-group difference of −0.004 (95%

Table 2. *Twelve-months change in primary and secondary outcomes by treatment group and baseline anti-TPO status*

Measure	Mean value (SD)		Treatment effect (levothyroxine vs. placebo)		
	Baseline	12-months follow-up	Mean within-group change (95% CI)	Adjusted between-group difference (95% CI)[a]	*P* for interaction
ThyPRO Hypothyroid Symptoms score[b]					
Positive anti-TPO					
Levothyroxine ($n = 86$)	17.4 (19.3)	14.2 (15.2)	−3.12 (−6.4 to 0.2)	−2.07	0.31
Placebo ($n = 74$)	19.8 (19.8)	17.2 (16.9)	−2.53 (−6.3 to 1.2)	(−6.04 to 1.90)	
Negative anti-TPO					
Levothyroxine ($n = 198$)	17.3 (18.4)	17.9 (17.8)	0.6 (−1.6 to 2.8)	0.89 (−1.76 to 3.54)	
Placebo ($n = 223$)	16.6 (18.0)	16.7 (18.1)	0.1 (−1.9 to 2.1)		
ThyPRO Tiredness score[b]					
Positive anti-TPO					
Levothyroxine ($n = 86$)	26.8 (22.4)	28.7 (20.4)	2.0 (−2.4 to 6.3)	1.75	0.98
Placebo ($n = 74$)	28.3 (23.1)	28.4 (20.8)	0.1 (−4.8 to 4.9)	(−3.60 to 7.09)	
Negative anti-TPO					
Levothyroxine ($n = 198$)	23.1 (19.6)	28.2 (20.6)	5.1 (2.4–7.8)	1.14 (−1.90 to 4.19)	
Placebo ($n = 223$)	22.1 (17.1)	26.5 (18.1)	4.3 (2.2–6.5)		
EuroQoL-5D-3L[c]					
Positive anti-TPO					
Levothyroxine ($n = 86$)	0.839 (0.209)	0.828 (0.214)	−0.011 (−0.050 to 0.028)	−0.004	0.56
Placebo ($n = 74$)	0.833 (0.192)	0.822 (0.225)	−0.011 (−0.054 to 0.032)	(−0.059 to 0.051)	
Negative anti-TPO					
Levothyroxine ($n = 198$)	0.847 (0.163)	0.814 (0.223)	−0.033 (−0.058 to −0.009)	−0.025 (−0.057 to 0.008)	
Placebo ($n = 222$)	0.855 (0.175)	0.843 (0.205)	−0.012 (−0.036 to 0.012)		
Handgrip strength (kg)[d]					
Positive anti-TPO					
Levothyroxine ($n = 84$)	27.4 (10.3)	25.6 (9.9)	−1.8 (−3.0 to −0.6)	−0.35	0.59
Placebo ($n = 73$)	25.8 (8.7)	24.5 (8.3)	−1.3 (−2.4 to −0.2)	(−1.90 to 1.19)	
Negative anti-TPO					
Levothyroxine ($n = 186$)	28.5 (10.6)	27.2 (11.0)	−1.2 (−2.0 to −0.5)	0.28 (−0.79 to 1.35)	
Placebo ($n = 203$)	28.4 (11.5)	27.0 (11.3)	−1.4 (−2.2 to −0.6)		

Note: There are fewer patients with available measured outcomes because of missing data; for details, see Fig. 1.
Abbreviations: anti-TPO, anti–thyroid peroxidase; CI, confidence interval; SD, standard deviation; ThyPRO, Thyroid-Related Quality of Life Patient-Reported Outcome; 3L, three levels; 5D, five dimensions.
[a]Adjusted for stratification variables (country, sex, and starting dose of levothyroxine). Differences should be read as follows: for ThyPRO Hypothyroid Symptoms and Tiredness Score, negative values indicate benefit of levothyroxine treatment, whereas for EQ-5D and handgrip strength, positive values indicate benefit of levothyroxine.
[b]ThyPRO: the Hypothyroid Symptoms and the Tiredness score range from 0 to 100, with a higher score indicating more symptoms. The minimal significant clinical change is from 9 points. Total ThyPRO scores per domain with missing items were scaled to maintain the maximum possible score for analyses. The score was considered to be missing if >50% of the score items were missing.
[c]The EuroQoL-5D-3L score is a standardized instrument for measuring generic health status. The 5D score is going from −0.59 to 1, with a higher score indicating a better quality of life.
[d]Handgrip strength is a measure of the maximum static force that a hand can squeeze using a dynamometer.

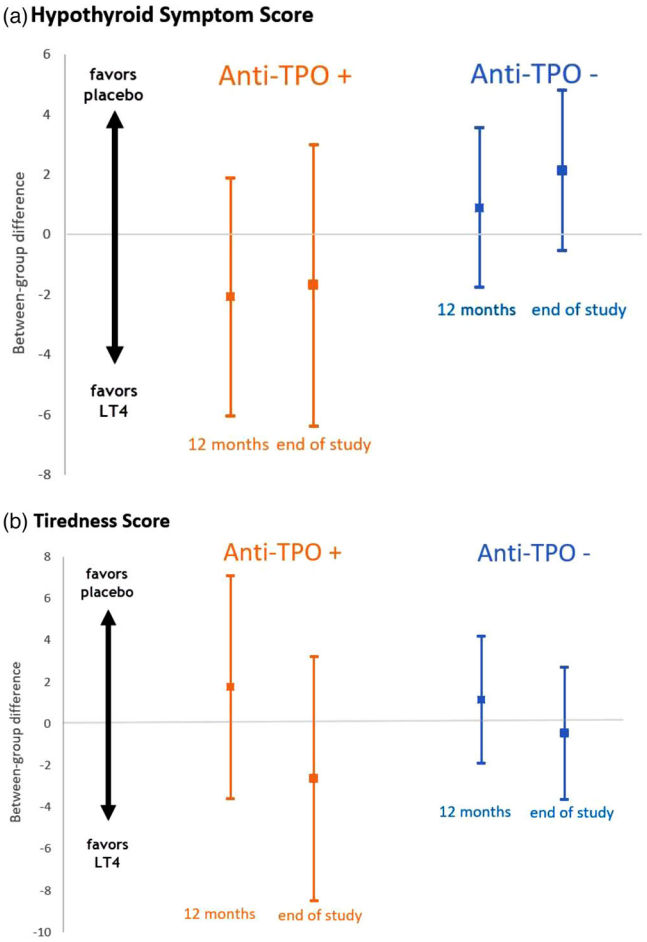

Fig. 2 *Between-group differences in outcome measures at 12 months and at the end of study, stratified by baseline anti–thyroid peroxidase (TPO) status. The figure shows treatment effect estimates (levothyroxine treatment [LT4] vs. placebo) with 95% confidence intervals for the ThyPRO (Thyroid-Related Quality of Life Patient-Reported Outcome) Hypothyroid Symptoms score (a) and Tiredness Score (b), stratified by baseline anti-TPO status (positive vs. negative). For exact numbers, refer to Table 2 and Table S4. The 12-month window was defined as between 334 and 397 days after randomization and the end-of-study analysis takes the latest available visit with a mean follow-up duration of 20 months. The left panel (orange) corresponds to the subgroup with positive anti-TPO and the right panel (blue) corresponds to negative anti-TPO. Positive difference would favor for no treatment, while negative difference with a minimum of 9 points would favor to treatment.*

CI −0.059 to 0.051) in positive anti-TPO, and −0.025 (95% CI −0.057 to 0.008) in negative anti-TPO participants. There was no evidence for effect modification by anti-TPO status (p for interaction = 0.56). Similarly, for handgrip strength, the adjusted between-groups difference was −0.35 (95% CI −1.90 to 1.19) in positive anti-TPO participants and 0.28 (95% CI −0.79 to 1.35) in negative anti-TPO participants. There were no statistically different results between positive and negative antibody status (p for interaction = 0.59). In the population with available baseline anti-TPO status, the overall risk of fatal and nonfatal cardiovascular events did not differ among those treated with levothyroxine and placebo (hazard ratio 0.91, 95% CI 0.43–1.95), and there was no evidence that these relationships differed among those with positive anti-TPO (hazard ratio 1.19, 95% CI 0.32–4.44) and those with negative anti-TPO (hazard ratio 0.75, 95% CI 0.28–1.96) (p for interaction = 0.58). The per-protocol analysis (Table S3) and further adjustment for pre-existing medical conditions (Table S5) yielded similar results. In the analysis at the end of the study, letter-digit coding to assess cognitive function was, in addition, measured and we found no statistical difference according to antibody status (Table S4).

Discussion

In this large study assessing whether antibody status influences the effectiveness of levothyroxine in older adults with SHypo, the effect on clinical outcomes did not differ between participants with positive or negative anti-TPO. Although the Hypothyroid symptoms score decreased more in the positive anti-TPO group, the decrease was not statistically significant, and importantly, the CI did not include the MCID of 9 points [36]. Tiredness scores increased in both groups with no

significant difference between positive and negative anti-TPO participants. For secondary outcomes including health-related quality of life, handgrip strength, cognitive function tests, and cardiovascular events, the treatment effect did not differ according to the anti-TPO status.

Comparison with other evidence

These results are consistent with the few previous smaller studies, although they analyzed clinical outcomes different from ours. A 6-months RCT of levothyroxine replacement in 40 women with SHypo did not show any relationship between positive antimicrosomal antibodies (a previous-generation measurement of anti-TPO) and changes in cholesterol levels or weight [27]. These results were limited by the small number of participants (N = 40–100). Similarly, in a post hoc subgroup analysis of a randomized crossover trial comparing levothyroxine substitution versus placebo in 100 participants (mean age 53.8 years), cardiovascular risk factors and endothelial function after treatment did not show significant differences between positive and negative anti-TPO participants [25]. This trial was limited by a small sample size and short duration (12 weeks), and it was a post hoc subgroup analysis. Conversely, an RCT of levothyroxine replacement in 60 young participants (mean age 34 years) with SHypo and positive anti-TPO demonstrated a significant improvement in depressive symptoms (Beck Depression Inventory decreased from 16.8 ± 13.3 to 12.4 ± 10.0, p = 0.04) [26]. However, the clinical relevance is unclear and there was no comparison with patients with negative anti-TPO. In contrast, our study analyzed more participants over a longer period and is the first to have included an older population.

Clinical implications

In clinical practice, the utility of measuring antithyroid antibodies remains controversial, as reflected by different recommendations in international guidelines, with two guidelines suggesting treatment in the presence of positive antibodies to prevent progression to overt hypothyroidism [2, 4] and another guideline not mentioning antibody status as an indication for treatment [3]. Our analysis showed no clear evidence for levothyroxine treatment in older adults with SHypo and positive anti-TPO on a large range of patient-relevant outcomes, underlining that anti-TPO in SHypo should not guide treatment decisions in older adults. While we could not assess the long-term impact on progression to overt hypothyroidism due to limited follow-up duration, the rate of progression has been described as relatively low (4.3%/year for positive antibodies vs. 2.6%/year for negative antibodies) based on studies with 10–20 years of follow-up [16, 20], and it may not be of substantial clinical relevance in an ageing population. Some studies have also shown an increase in TSH in older adults, which has not been associated with clinical outcomes in observational studies [39–42]. Our study now adds interventional data that treatment of Shypo is not associated with better outcomes—also in those with elevated anti-TPO—and underscores the interpretation that treatment threshold in older adults could be safely raised [43]. It might be argued that therapy might also start later when overt hypothyroidism (or important symptoms) developed by following TSH values. Furthermore, we found no evidence that the risk of fatal and nonfatal cardiovascular events differed by anti-TPO status; this is in line with evidence from the National Health and Nutrition Examination Survey (NHANES), which found no correlation of anti-TPO with cardiovascular disease risk in SHypo, and it further adds against using anti-TPO to guide treatment decisions [44].

Strengths and limitations of this study

Among the strengths, our study is the largest RCT analyzing the effect of levothyroxine in SHypo for older persons according to antibody status. Although SHypo is often found along with positive anti-TPO, very few studies have investigated this aspect and they have been mostly small, often emphasizing on young participants and with a short follow-up. Another strength is the use of the same laboratory assay for all antibody measurements, which minimizes the measurement bias. The internal validity is strengthened by the use of standardized scores in two fully blinded RCTs to assess nonspecific symptoms such as hypothyroid symptoms and tiredness, which have been shown to be sensitive to change [45]. In addition, we analyzed a wide range of patient-centered outcomes of clinical relevance in an elderly population.

This study has limitations. First, the participants were all ≥65 years old and mostly Caucasian, and it is unknown whether these results apply to other populations. Second, the number of participants with positive antibody status was relatively small (N = 180) albeit far larger than in all previous trials (N = 40–100) [25–27], and our study had adequate

power to detect a clinically meaningful difference in the treatment effect. Third, the randomization was not stratified by antibody status and some baseline imbalances were found, but adjustment for these imbalances yielded similar results. Fourth, there was no information on over-the-counter medications (e.g., multivitamin use such as biotin), which could interact with thyroid function measurements. Fifth, the number of fatal and nonfatal events during the follow-up period was relatively low, limiting power to detect a significant difference by antibody status. Finally, there were relatively few participants with elevated TSH ≥10 mU/L or high symptom burden precluding a meaningful subgroup analysis on these. Participants with SHypo are expected to have low scores in the ThyPRO scores [36] but we found some participants with higher symptom burden and even in these, we did not find a larger benefit of levothyroxine in a secondary analysis [11]. However, in positive anti-TPO patients with elevated TSH ≥10 mU/L or high symptom burden, we cannot exclude a potential clinically relevant treatment effect.

Conclusion

In this pooled analysis of data from two randomized trials in older participants with SHypo, positive antithyroid antibodies were not associated with more benefits on clinical outcomes with levothyroxine treatment. The role of measuring anti-TPO for treatment decisions in SHypo needs further study.

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Conflict of interest

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and have confirmed that they have no conflict of interest. The lead author (N. R.) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as

originally planned (and if relevant, registered) have been explained.

Author contributions

C. L. and M. B. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. N. R., T. Q., P. K., J. G., R. W., and S. M. conceived and designed the study. All authors contributed to data acquisition, analysis, and interpretation. M. B., N. A., and C. D. performed the statistical analyses. C. L. and M. B. drafted the manuscript. All authors revised the manuscript for important intellectual content. N. R. obtained funding. N. R., T. Q., P. K., J. G., R. W., and S. M. were responsible for administrative, technical, and material support. N. R., T. Q., P. K., J. G., R. W., and S. M. supervised the study. N. R. is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Data availability statement

Data for this study will be made available to others in the scientific community upon request after publication. Data will be made available for scientific purposes for researchers whose proposed use of the data has been approved by a publication committee. For data access, please contact the corresponding author.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary Figure 1: TSH levels over time according to anti-TPO status.
Supplementary Figure 2: ThyPRO Hypothyroid Symptoms score by anti- TPO and subgroups.
Supplementary Figure 3: ThyPRO Tiredness Score by anti-TPO and subgroups.
Supplementary Table 1: Baseline characteristics in TRUST and IEMO80+ participants with and without measurement of anti-TPO antibodies.
Supplementary Table 2: Baseline characteristics by treatment group in all TRUST and IEMO80+ participants.
Supplementary Table 3: Outcomes after 12 months of treatment in per-protocol population.
Supplementary Table 4: Outcomes at the end of the study by baseline anti-TPO status.
Supplementary Table 5: 12-months change in outcomes by treatment group and baseline anti-TPO status after adjustment for stratification variables, pre-existing medical condition and TSH at baseline. ■

