The Relation Between Thyroid Function and Anemia: A Pooled Analysis of Individual Participant Data

Daisy M. Wopereis,[1,2]* Robert S. Du Puy,[1]* Diana van Heemst,[2] John P. Walsh,[3,4] Alexandra Bremner,[5] Stephan J. L. Bakker,[6] Douglas C. Bauer,[7,8] Anne R. Cappola,[9] Graziano Ceresini,[10] Jean Degryse,[11,12] Robin P. F. Dullaart,[6] Martin Feller,[13,14] Luigi Ferrucci,[15] Carmen Floriani,[13] Oscar H. Franco,[16] Massimo Iacoviello,[17] Georgio Iervasi,[18] Misa Imaizumi,[19] J. Wouter Jukema,[20] Kay-Tee Khaw,[21] Robert N. Luben,[21] Sabrina Molinaro,[22] Matthias Nauck,[23] Kushang V. Patel,[24] Robin P. Peeters,[16,25] Bruce M. Psaty,[26,27] Salman Razvi,[28] Roger K. Schindhelm,[29] Natasja M. van Schoor,[30] David J. Stott,[31] Bert Vaes,[11,12] Mark P. J. Vanderpump,[32] Henry Völzke,[33] Rudi G. J. Westendorp,[34] Nicolas Rodondi,[13,14] Christa M. Cobbaert,[35] Jacobijn Gussekloo,[1,2] and Wendy P. J. den Elzen,[35] for the Thyroid Studies Collaboration

[1]Department of Public Health and Primary Care, Leiden University Medical Center, Leiden 2300RC, Netherlands; [2]Department of Internal Medicine, Gerontology and Geriatrics Section, Leiden University Medical Center, Leiden 2300RC, Netherlands; [3]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Perth 6009, Western Australia; [4]Medical School, The University of Western Australia, Crawley, Perth 6009, Western Australia; [5]School of Population Health, The University of Western Australia, Crawley, Perth 6009, Western Australia; [6]Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen 9700RB, Netherlands; [7]Department of Medicine, University of California San Francisco, San Francisco, California 94143; [8]Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, California 94143; [9]Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104; [10]Department of Clinical and Experimental Medicine, Geriatric Endocrine Unit, University Hospital of Parma, Parma 43126, Italy; [11]Institute of Health and Society, Université catholique de Louvain, Brussels B1200, Belgium; [12]Department of Public Health and Primary Care, Katholieke Universiteit Leuven, Leuven B3000, Belgium; [13]Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, Bern 3010, Switzerland; [14]Institute of Primary Health Care, University of Bern, Bern 3012, Switzerland; [15]National Institute on Aging, Baltimore, Maryland 20892; [16]Department of Epidemiology, Erasmus MC, University Medical Center Rotterdam, Rotterdam 3015GE, Netherlands; [17]University Cardiology Unit, Cardiothoracic Department, University Policlinic Hospital, Bari 70124, Italy; [18]National Council Research Institute of Clinical Physiology/Tuscany Region G. Monasterio Foundation, Pisa 56126, Italy; [19]Department of Clinical Studies, Radiation Effects Research Foundation, Nagasaki 850-0013, Japan; [20]Department of Cardiology, Leiden University Medical Center, Leiden 2300RC, Netherlands; [21]Department of Public Health and Primary Care, University of Cambridge, Cambridge CB1 8RN, England; [22]National Council Research Institute of Clinical Physiology, Pisa 56124, Italy; [23]Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald D-17489, Germany; [24]Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, Washington 98195; [25]Department of Internal Medicine, Rotterdam Thyroid Center, Erasmus MC, Rotterdam 3015GD, Netherlands; [26]Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology, and Health Services, University of Washington, Seattle, Washington 98101; [27]Kaiser Permanente Washington Health Research Institute, Seattle, Washington 98101; [28]Department of Endocrinology, Gateshead Health Foundation NHS Trust, Gateshead, Tyne and Wear NE9 6SX, England; [29]Department of Clinical Chemistry, Haematology and Immunology, Northwest Clinics, Alkmaar 1815JD, Netherlands; [30]Department of Epidemiology and Biostatistics, EMGO Institute for Health and Care Research, Amsterdam 1081BT, Netherlands; [31]Institute of Cardiovascular and Medical Sciences, University of

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*These authors contributed equally to the study.

Abbreviations: CHS, Cardiovascular Health Study; CRP, C-reactive protein; Health ABC, Health, Aging and Body Composition; LASA, Longitudinal Aging Study Amsterdam; PREVEND, Prevention of Renal and Vascular End-Stage Disease.

Glasgow, Glasgow G12 8QQ, United Kingdom; [32]The Physicians' Clinic, London W1G 7AE, England; [33]Institute for Community Medicine, Study of Health in Pomerania/Clinical-Epidemiological Research and German Centre of Cardiovascular Research, University of Greifswald, Greifswald D-17489, Germany; [34]Department of Public Health and Center of Healthy Aging, University of Copenhagen, Copenhagen DK-2200, Denmark; and [35]Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden 2300RC, Netherlands

Context: Anemia and thyroid dysfunction often co-occur, and both increase with age. Human data on relationships between thyroid disease and anemia are scarce.

Objective: To investigate the cross-sectional and longitudinal associations between clinical thyroid status and anemia.

Design: Individual participant data meta-analysis.

Setting: Sixteen cohorts participating in the Thyroid Studies Collaboration (n = 42,162).

Main Outcome Measures: Primary outcome measure was anemia (hemoglobin <130 g/L in men and <120 g/L in women).

Results: Cross-sectionally, participants with abnormal thyroid status had an increased risk of having anemia compared with euthyroid participants [overt hypothyroidism, pooled OR 1.84 (95% CI 1.35 to 2.50), subclinical hypothyroidism 1.21 (1.02 to 1.43), subclinical hyperthyroidism 1.27 (1.03 to 1.57), and overt hyperthyroidism 1.69 (1.00 to 2.87)]. Hemoglobin levels were lower in all groups compared with participants with euthyroidism. In the longitudinal analyses (n = 25,466 from 14 cohorts), the pooled hazard ratio for the risk of development of anemia was 1.38 (95% CI 0.86 to 2.20) for overt hypothyroidism, 1.18 (1.00 to 1.38) for subclinical hypothyroidism, 1.15 (0.94 to 1.42) for subclinical hyperthyroidism, and 1.47 (0.91 to 2.38) for overt hyperthyroidism. Sensitivity analyses excluding thyroid medication or high levels of C-reactive protein yielded similar results. No differences in mean annual change in hemoglobin levels were observed between the thyroid hormone status groups.

Conclusion: Higher odds of having anemia were observed in participants with both hypothyroid function and hyperthyroid function. In addition, reduced thyroid function at baseline showed a trend of increased risk of developing anemia during follow-up. It remains to be assessed in a randomized controlled trial whether treatment is effective in reducing anemia. (***J Clin Endocrinol Metab*** **103: 3658–3667, 2018)**

Thyroid diseases and anemia are common disorders, and their prevalence increases with age (1–4). Hypothyroidism and anemia can each cause nonspecific symptoms of ill health like fatigue, and both lead to decreased quality of life. The combination of anemia and abnormal thyroid function may therefore be accompanied by serious morbidity and further effects on quality of life.

The co-occurrence of anemia and hypothyroidism is not only a challenging diagnostic problem in allocating symptoms to one of the diseases, but may also point to a causal relationship between thyroid disease and anemia (5). Indeed, relationships between thyroid disease and anemia have already been documented in experimental animal studies in the distant past (5). For instance, hypophysectomized mammals were found to have decreased red blood cell counts that corrected after administration of thyroid hormones (6, 7). Additionally, mice deficient in the thyroid hormone receptor TRα have been found to have decreased hematocrit values (8).

However, human data regarding relationships between thyroid disease and hematologic anomalies are scarce. Researchers investigating potential altered erythropoiesis as a result of thyroid dysfunction found red cell abnormalities and a reduced proliferative potential of hematopoietic progenitor cells in both patients with hypothyroidism and hyperthyroidism, but the total number of studied participants was low (9, 10).

In addition, a higher prevalence of anemia was identified in older male patients with subclinical hypothyroidism (11) and in patients with clinical hypothyroidism (12), but incidence estimates were not available due to the cross-sectional study design. Additionally, a rise of thyroid hormone levels or a decrease in levels of TSH within the reference ranges was associated with higher erythropoietic activity (13), but the low number of studied participants precluded stratification by hyperthyroid subgroups. In one population-based cohort, both hypothyroidism and hyperthyroidism were associated

with decreased hemoglobin in cross-sectional analyses but not in longitudinal analyses (14).

Clinical experimental evidence on the causal relation between low thyroid function and anemia is currently limited to a number of small case series in which treatment of hypothyroidism with levothyroxine resulted in a considerable increase in hemoglobin and resolution of anemia (12, 15, 16). Alternatively, and in line with the observational data, in a cohort of patients with hyperthyroidism, a high prevalence of anemia was found, which returned to normal following antithyroid therapy (17).

Despite the myriad of smaller studies hinting at a potential relationship between thyroid dysfunction and anemia, methodologically sound pooled estimates drawn from large and representative populations are missing. In the current study, we sought to determine the association between thyroid hormone status and anemia in cross-sectional and longitudinal analyses by performing an individual participant data meta-analysis on data from 16 independent observational cohort studies participating in the Thyroid Studies Collaboration.

Methods

Study population

We performed an individual participant data meta-analysis of cohorts participating in the Thyroid Studies Collaboration. The cohorts are summarized in Table 1 and described elsewhere in detail (2, 18–21). For the current project, we included the 16 cohorts in which thyroid function tests and hemoglobin were measured at baseline.

Table 1. Baseline Characteristics of Individuals in Included Studies (N = 42,162)

Study	Study Population	Total Number of Participants: Baseline/ Follow-up	Age, Median (Range), y	Women (%)	Antithyroid or Thyroid Medication at Baseline (%)	Anemia at Baseline (%)	Anemia During Follow-up (%)	Duration of Follow-up, Median (IQR), y	Total Person-Years
Total		42,162/25,466	14–103	22,308 (52.9)	1067 (2.5)	4274 (10.1)	2423 (5.7)	5.7 (3–9.5)	162,583
Bari study	Outpatients with heart failure followed up by cardiology department in Bari, Italy	337/206	66 (21–92)	78 (20.5)	23 (6.8)	69 (20.5)	30 (8.9)	1.4 (0.7–1.9)	273
BELFRAIL	Subjects aged ≥80 y in three well-circumscribed areas of Belgium	524/331	84 (80–100)	331 (63.2)	52 (9.9)	106 (20.2)	52 (9.9)	1.6 (1.4–1.8)	521
Busselton Health study	Adults living in Busselton, Western Australia	2074/1245	51 (17–90)	1030 (49.7)	27 (1.3)	76 (3.7)	54 (2.6)	14.0 (14.0–14.0)	17,164
Cardiovascular Health study	Community-dwelling adults with Medicare eligibility in four US communities	3106/2314	71 (64–100)	1864 (60.0)	0	259 (8.3)	321 (10.3)	3.0 (3.0–3.0)	12,552
EPIC-Norfolk study	Adults aged 45–79 y living in Norfolk, England	13,286/7657	59 (40–78)	7276 (54.8)	NA	1090 (8.2)	499 (3.8)	4.3 (3.4–12.3)	57,604
Health ABC study	Community-dwelling adults aged 70–79 y with Medicare eligibility in two US communities	2531/1236	74 (70–81)	1305 (51.6)	253 (10.0)	384 (15.2)	195 (7.7)	7.5 (7.5–7.5)	8543
InCHIANTI study	Community dwelling from two small towns in Tuscany, Italy; Invecchiare in Chianti, "Aging in the Chianti Area" (InCHIANTI) study	1200/944	72 (21–103)	675 (56.3)	30 (2.5)	120 (10.0)	177 (14.8)	9.0 (6.0–9.2)	6958
LASA (20)	Random sample of older men and women (aged 55–85 y) in Amsterdam, Zwolle, and Oss, Netherlands	766/329	68 (55–85)	393 (51.3)	14 (1.8)	43 (5.6)	28 (3.7)	3.0 (3.0–3.1)	974
Leiden 85-plus Study	All adults aged 85 y living in Leiden, Netherlands	555/397	85 (NA)	368 (66.3)	20 (3.6)	158 (28.5)	98 (17.7)	3.0 (0.5–5.0)	1324
Nagasaki Adult Health study	Atomic bomb survivors in Nagasaki, Japan	965/753	57 (38–92)	578 (59.9)	11 (1.1)	179 (18.5)	196 (20.3)	11.9 (7.4–12.0)	7196
Pisa cohort	Patients admitted to cardiology department in Pisa, Italy	2259/NA	68 (14–96)	785 (34.7)	NA	490 (21.7)	NA	NA	NA
PREVEND study	Inhabitants, aged 28–75 y, of the city of Groningen, Netherlands	934/779	60 (35–82)	397 (42.5)	NA	106 (11.3)	82 (8.8)	5.7 (5.7–5.7)	8247
PROSPER study	Older community-dwelling adults at high cardiovascular risk in Netherlands, Ireland, and Scotland	5769/5138	75 (69–83)	2983 (51.7)	256 (4.4)	402 (7.0)	203 (3.5)	0.25 (0.25–0.25)	1261
Rotterdam Study	All inhabitants of the suburb Ommoord in Rotterdam, Netherlands, aged ≥55 y	1835/1322	69 (55–93)	1135 (61.9)	45 (2.5)	109 (5.9)	214 (11.7)	11.1 (6.6–17.4)	14,066
SHIP (21)	Adults living in Western Pomerania, Germany	4214/2882	50 (20–81)	2139 (50.8)	263 (6.2)	589 (14.0)	274 (6.5)	10.0 (5.0–11.0)	25,900
Whickham Survey	Adults living in and near Newcastle-upon-Tyne, England	1807/NA	46 (18–93)	971 (53.7)	73 (4.0)	94 (5.2)	NA	NA	NA

Abbreviations: EPIC, European Prospective Investigation of Cancer; Health ABC, Health, Aging and Body Composition; IQR, interquartile range (25th–75th percentiles); NA, not available; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; SHIP, Study of Health in Pomerania.

Anemia

Anemia was defined according to the World Health Organization criteria (hemoglobin concentration <130 g/L in men and <120 g/L in women) (22). In 14 cohorts, a follow-up measurement of hemoglobin was available.

Thyroid function

TSH and free T4 concentrations were measured at baseline in all cohorts. Cohort-specific cutoff values were applied for free T4 concentrations (Supplemental Table 1). Participants with a TSH level of 0.45 to 4.5 mIU/L were categorized as euthyroid. Overt hypothyroidism was defined as a TSH level >4.5 mIU/L in combination with reduced free T4 concentration. Subclinical hypothyroidism was defined as a TSH level >4.5 mIU/L in combination with a normal free T4 concentration. A TSH level <0.45 mIU/L with normal free T4 levels was defined as subclinical hyperthyroidism. Overt hyperthyroidism was defined as a TSH level <0.45 mIU/L with an elevated free T4 concentration (2).

Statistical analyses

We performed a two-stage individual participant data meta-analysis to allow for consistent definitions and analyses across the cohorts, increased analytical flexibility, and decreased complexity of the analyses (18, 23–26). In the first step, the cross-sectional and longitudinal associations between thyroid hormone status and anemia in each study cohort were estimated separately from supplied original study datasets with data on the participant level. In the second step, all effect estimates found in step one were pooled using random-effects models (DerSimonian and Laird) with inverse variance weighting.

For the cross-sectional association between thyroid hormone status and anemia at baseline, logistic regression models were constructed. Prospectively, we investigated the risk of developing anemia during follow-up using Cox regression models; participants with pre-existing anemia were excluded. The analyses were based on the thyroid function category at baseline. If a new case of anemia was identified, it was assumed that the anemia had developed halfway through the follow-up period.

Thyroid status was included as a categorical variable (overt hypothyroidism, subclinical hypothyroidism, subclinical hyperthyroidism, and overt hyperthyroidism), with euthyroidism as the reference group. All models were adjusted for age and sex. A *P* value for trend was obtained for both overt and subclinical hypothyroid and hyperthyroid categories. Subgroup analyses, including calculations of a *P* value for interaction, were performed separately for sex, age groups, and ethnicity.

In sensitivity analyses, we excluded all participants who used antithyroid medication or thyroid hormone replacement therapy at baseline or during follow-up. We also compared mean hemoglobin levels at baseline between thyroid status groups and differences in mean annual change in hemoglobin levels during follow-up between thyroid status groups using linear regression models. Additionally, we excluded all participants with a high level of C-reactive protein [(CRP); >20 mg/L] as a proxy for chronic inflammatory disease.

Data analyses were performed using IBM SPSS Statistics Version 23 and Review Manager 5.3 from the Cochrane Collaboration.

Results

For this study, individual participant data of 56,297 participants from 16 different cohorts participating in the Thyroid Studies Collaboration were available. At baseline, thyroid function (TSH and free T4) and hemoglobin measurements were available from 42,162 participants, of whom 459 (1.1%) had overt hypothyroidism, 2930 (6.9%) had subclinical hypothyroidism, 36,081 (85.6%) were euthyroid, 2386 (5.7%) had subclinical hyperthyroidism, and 306 (0.7%) had overt hyperthyroidism.

Baseline characteristics of the cohorts are presented in Table 1. The overall median age of each cohort ranged from 46 to 85 years, and the overall percentage of women was 51.0%. More detailed information about the study participants is presented in Supplemental Tables 2 and 3. The participants excluded because their thyroid function or hemoglobin measurement were not available had a median age ranging from 45 to 84 years; the percentage of women was 51.5%.

Cross-sectional analyses

At baseline, 4274 (10.1%) participants had anemia: 15.9% in the overt hypothyroid group, 11.6% in the subclinical hypothyroid group, 9.7% in the euthyroid group, 13.6% in the subclinical hyperthyroid group, and 11.1% in the overt hyperthyroid group. Participants with subclinical or overt hypothyroidism and subclinical or overt hyperthyroidism had increased odds of having anemia compared with participants with euthyroidism (Table 2; Fig. 1). The pooled OR for the overt hypothyroid group was 1.84 (95% CI 1.35 to 2.50), 1.21 (1.02 to 1.43) for the group with subclinical hypothyroidism, 1.27 (1.03 to 1.57) for those with subclinical hyperthyroidism, and 1.69 (1.00 to 2.87) for those in the overt hyperthyroid group. We observed statistically significant trends from euthyroidism to hypothyroidism (*i.e.*, from subclinical hypothyroidism to overt hypothyroidism; $P = 0.01$) and from euthyroidism to hyperthyroidism (*i.e.*, from subclinical hyperthyroidism to overt hyperthyroidism; $P = 0.04$). When the analyses were stratified by sex, we observed no statistically significant differences (all *P* values for interaction >0.05) between men and women (Table 2). Also, no statistically noteworthy differences were observed among different age categories or among white, black, or Asian participants.

Longitudinal analyses

In the longitudinal analyses, 25,466 participants from 14 cohorts were included, with a median follow-up time of 5.7 years (interquartile range 3.0 to 9.5). A total of 2423 participants developed anemia during follow-up (14.9 per 1000 person-years): 12.2% in the overt hypothyroid group, 12.0% in the subclinical hypothyroid group, 9.2% in the euthyroid group, 10.7% in the subclinical hyperthyroid group, and 8.7% in the overt hyperthyroid group (Table 3; Fig. 2). The pooled hazard ratios for the risk of developing anemia were 1.38 (95% CI

Table 2. The Risk of Having Anemia at Baseline According to Thyroid Hormone Status (N = 42,162 From 16 Cohorts)

	Overt Hypothyroidism	Subclinical Hypothyroidism	Euthyroidism	Subclinical Hyperthyroidism	Overt Hyperthyroidism	N Overt Hypothyroidism/ Subclinical Hypothyroidism/ Subclinical Hyperthyroidism/ Overt Hyperthyroidism
All[a]	1.84 (1.35–2.50)	1.21 (1.02–1.43)	1 (ref)	1.27 (1.03–1.57)	1.69 (1.00–2.87)	459/2930/36,081/2386/306
Sex						
Male	2.45 (1.45–4.12)	1.27 (1.03–1.57)	1 (ref)	1.19 (0.95–1.49)	1.59 (0.80–3.14)	122/1029/17,546/1055/102
Female	1.79 (1.30–2.47)	1.23 (0.99–1.52)	1 (ref)	1.42 (1.11–1.81)	1.78 (0.99–3.21)	337/1901/18,535/1331/204
Age, y						
<50[b]	2.25 (1.10–4.60)	1.15 (0.77–1.74)	1 (ref)	1.27 (0.73–2.21)	3.53 (0.26–48.39)	48/452/6763/599/27
50–65	5.53 (0.93–33.03)	1.44 (0.94–2.21)	1 (ref)	1.88 (1.09–3.24)	4.71 (1.25–17.78)	132/677/9719/670/63
65–80	2.02 (1.02–3.99)	1.40 (1.10–1.78)	1 (ref)	1.21 (0.85–1.73)	1.49 (0.89–2.51)	215/1711/16,814/949/186
>80	1.91 (1.01–3.62)	1.03 (0.68–1.54)	1 (ref)	1.49 (0.99–2.23)	2.66 (0.35–20.26)	65/234/2646/168/27
Ethnicity						
White[c]	1.97 (1.37–2.82)	1.29 (1.11–1.51)	1 (ref)	1.30 (1.04–1.63)	1.56 (1.03–2.34)	431/2687/34,154/2339/303
Black[d]	0.96 (0.20–4.58)	1.51 (0.74–3.05)	1 (ref)	0.77 (0.31–1.89)	—	12/98/1013/35/2
Asian[e]	2.01 (0.63–6.39)	0.87 (0.54–1.39)	1 (ref)	0.82 (0.10–6.83)	—	13/143/828/8/1
Other[f]	—	—	1 (ref)	—	—	0/0/22/1/0

Data are pooled hazard ratio (95% CI) unless otherwise noted. Results were obtained by logistic regression analysis, adjusted for age (if applicable) and sex.

[a]*P* for trend: overt hyperthyroidism to euthyroidism, *P* = 0.01; euthyroidism to overt hyperthyroidism, *P* = 0.04.

[b]Reference group is <50 y.

[c]Reference group is white.

[d]Only data from CHS, Health ABC, and PREVEND.

[e]Only data from LASA, Nagasaki, PREVEND, and Rotterdam.

[f]Only data from LASA, PREVEND, and Rotterdam.

0.86 to 2.20) for the overt hypothyroid group, 1.18 (1.00 to 1.38) for the group with subclinical hypothyroidism, 1.15 (0.94 to 1.42) for the group with subclinical hyperthyroidism, and 1.47 (0.91 to 2.38) in the overt hyperthyroid group. We observed a statistically significant trend from euthyroidism to hypothyroidism (*P* = 0.02). No statistically significant trend was observed for euthyroidism to hyperthyroidism (*P* = 0.20). When the participants were stratified by sex, age, or ethnicity, these findings remained unchanged. Associations were more pronounced in those studies with a median follow-up ≥5 years (Supplemental Table 4).

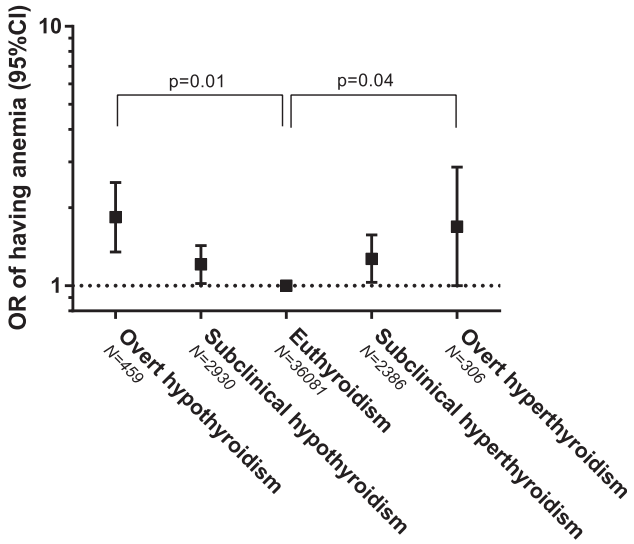

Figure 1. The pooled ORs of the risk of having anemia at baseline with the 95% CI and *P* value for trend. Logistic regression models corrected for age and sex; reference group is euthyroidism.

Additional analyses

Cross-sectionally, hemoglobin levels (as a continuous variable) were lower (mean difference between −0.06 and −0.19 g/dL) in all groups compared with participants with euthyroidism (Supplemental Table 5). Prospectively, no differences in mean annual change in hemoglobin levels were observed among the thyroid hormone status groups (Supplemental Table 6). Similar results were observed when analyses were stratified on sex. In addition, sensitivity analyses excluding participants who used thyroid hormone medication or with high levels of CRP yielded higher ORs in line with the unrestricted results but with wider CIs (Supplemental Tables 7 and 8).

For all main analyses, I^2 statistics remained <40% (Supplemental Tables 9 and 10), and, in combination with size and direction of effects, statistical heterogeneity was deemed low to negligible (27).

Discussion

In this individual participant data meta-analysis, we observed a cross-sectional relation between thyroid

Table 3. The Risk of Developing Anemia During Follow-up According to Thyroid Hormone Status at Baseline (N = 25,466 From 14 Cohorts)

	Overt Hypothyroidism	Subclinical Hypothyroidism	Euthyroidism	Subclinical Hyperthyroidism	Overt Hyperthyroidism	N Overt Hypothyroidism/ Subclinical Hypothyroidism/ Subclinical Hyperthyroidism/ Overt Hyperthyroidism
All[a]	1.38 (0.86–2.20)	1.18 (1.00–1.38)	1 (ref)	1.15 (0.94–1.42)	1.47 (0.91–2.38)	270/1678/21,965/1358/195
Sex						
Male	2.14 (0.79–5.79)	1.05 (0.83–1.34)	1 (ref)	1.41 (0.92–2.18)	0.83 (0.26–2.61)	62/577/10,450/584/63
Female	1.19 (0.75–1.88)	1.37 (1.05–1.80)	1 (ref)	1.22 (0.95–1.57)	2.27 (1.30–3.93)	207/1096/11,487/773/131
Age, y						
<50[b]	17.81 (4.06–78.24)	1.48 (0.78–2.83)	1 (ref)	1.09 (0.68–1.73)	—	16/106/3857/343/21
50–65	1.58 (0.14–18.00)	1.14 (0.82–1.58)	1 (ref)	1.02 (0.70–1.50)	2.97 (0.56–15.64)	85/384/5872/414/32
65–80	1.39 (0.81–2.37)[c]	1.20 (0.96–1.51)	1 (ref)	1.14 (0.83–1.57)	1.51 (0.85–2.69)	130/1069/10,737/521/124
>80	1.48 (0.55–4.03)[c]	1.30 (0.80–2.10)	1 (ref)	1.57 (0.98–2.50)	3.59 (0.49–26.06)	39/119/1499/80/18
Ethnicity						
White[d]	1.38 (0.75–2.54)	1.21 (1.00–1.45)	1 (ref)	1.16 (0.93–1.44)	1.52 (0.93–2.50)	257/1527/20,849/1336/193
Black[e]	2.80 (0.38–20.76)	1.30 (0.59–2.83)	1 (ref)	1.57 (0.57–4.36)	—	5/41/415/16/1
Asian[f]	1.68 (0.53–5.29)	1.03 (0.70–1.51)	1 (ref)	—	—	6/109/654/6/1
Other[g]	—	—	1 (ref)	—	—	0/0/12/0/0

Data are pooled hazard ratio (95% CI) unless otherwise noted. Results were obtained by Cox regression analysis, adjusted for age (if applicable) and sex.

[a] *P* for trend: overt hyperthyroidism to euthyroidism, $P = 0.02$; euthyroidism to overt hyperthyroidism, $P = 0.20$.

[b] Reference group is <50 y.

[c] *P* value for interaction ($P < 0.05$).

[d] Reference group is white.

[e] Only data from CHS, Health ABC, and PREVEND.

[f] Only data from LASA, Nagasaki, PREVEND, and Rotterdam.

[g] Only data from LASA, PREVEND, and Rotterdam.

function and anemia; higher odds of anemia were observed in participants with both overt and subclinical hypothyroidism as well as overt and subclinical hyperthyroidism. In addition, reduced thyroid function at baseline showed a trend of increased risk of developing anemia during follow-up. The longitudinal association between overt and subclinical hyperthyroidism and the risk of developing anemia did not reach statistical significance. Prospectively, no differences in mean annual change in hemoglobin levels were observed among the thyroid hormone status groups.

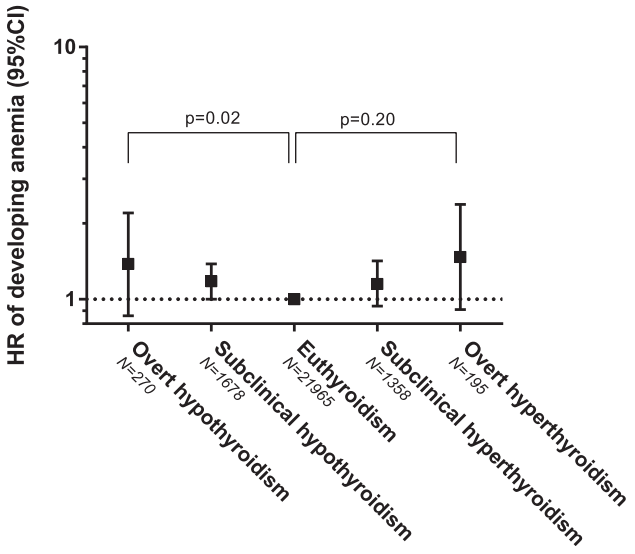

Figure 2. The pooled hazard ratios of developing anemia during follow-up in the thyroid function groups with the 95% CI and *P* value for trend. Cox regression models corrected for age and sex; reference group is euthyroidism.

The findings in the current individual participant data meta-analysis build on findings from earlier studies in which thyroid dysfunction was associated with abnormal red blood cell indices (11–13). In this study, thyroid dysfunction, whether overt or subclinical hypothyroidism and hypothyroidism, was associated with slightly lower hemoglobin levels. Given the small difference in hemoglobin levels among thyroid function groups, the contribution of thyroid dysfunction on low hemoglobin levels or anemia may be small. It remains to be assessed in a randomized controlled trial whether treatment of (subclinical) hypothyroidism is effective in reducing anemia to further decide whether the findings are thought to be clinically relevant and whether these should influence practice and policies. Christ-Crain *et al.* (28) showed that erythropoietin levels increased after thyroxin treatment in patients with subclinical hypothyroidism. In addition, a number of studies have also shown a beneficial effect of thyroid hormone treatment in patients with hypothyroidism on erythropoietin levels (12, 15, 16).

There are numerous types of anemia that can be classified according to whether the anemia is primarily

the result of blood loss, deficits in the production of healthy erythrocytes, or by reduced erythrocyte survival. Currently, it is unclear what mechanisms exactly allow thyroid function and erythropoiesis to be linked pathophysiologically and how both ends of the thyroid disease spectrum might lead to an anemic state. However, for subclinical and overt hyperthyroidism, several pathways have been proposed. Hyperthyroidism might be associated with anemia via reduced erythrocyte survival due to altered iron metabolism and utilization, enhanced oxidative stress, and increased hemolysis (29, 30). Thyroid hormones stimulate energy metabolism, resulting in an enhanced requirement of oxygen delivery to the tissues speeding up destructive processes.

For subclinical and overt hypothyroidism, there is accumulating evidence that indicates low thyroid function may be causally related to anemia via deficits in the production of healthy erythrocytes, although the underlying mechanisms by which thyroid hormones and TSH may lead to anemia are not fully understood (31). T3, T4, and TSH may play a direct role in erythropoiesis (32). For instance, both T3 and T4 are involved in the regulation of hematopoiesis by influencing erythroid precursor proliferative capacity (33). In addition, a direct β2-adrenergic receptor–mediated stimulation of red cell precursors by T4 has been shown (34). T4 has also been found to stimulate the initiation and completion of hemoglobin protein chains *in vitro* and to enhance red blood cell formation (5). Thyroid hormones were also shown to promote erythropoiesis by increasing the production of erythropoietin by the kidneys (35). Furthermore, there is evidence that thyroid hormones affect iron transport and utilization. TSH could affect hematopoiesis by binding to a functional TSH receptor, which can be found in erythrocytes and some extrathyroidal tissues (10). Another explanation for the co-occurrence of low thyroid function and anemia is that there are common causes for abnormal thyroid status and anemia. Chronic (inflammatory) diseases, malnutrition, and malabsorption may all result in reduced thyroid status as an adaptive response to energetic deficits. In addition, malnutrition and malabsorption may cause deficiencies of micronutrients that are crucial for erythropoiesis, like iron, vitamin B12, and folate, as well as iodine deficiency, which is crucial for normal thyroid function. Interestingly, iron deficiency, which is the most common cause of anemia, was also found to decrease the activity of thyroid peroxidase, an iron-containing enzyme involved in the synthesis of thyroid hormones (36).

Strengths of the current individual participant data meta-analysis are the inclusion of individual participant data of large cohort studies from across the globe. The availability of individual participant data allowed us to choose clinically relevant categories of thyroid function and anemia, standardize these definitions, and perform several standardized subgroup analyses.

An individual participant data meta-analysis of well-designed observational studies can be considered an important tool in assessing causality. When studying causality, the nine considerations of Hill in 1965 (37) can be used as a checklist. In our study, many of these considerations are met. Although the individual study cohorts and individual subgroups may have been small, we had sufficient power to study the associations in this pooled analysis because of the increased combined sample size. Because multiple studies were included, we could also study consistency in the results of the different cohorts (*e.g.*, effect estimates all pointing in the same direction); the low level of heterogeneity also aids in considering a causal relation. In addition, the availability of the individual participant data allowed us to define identical subgroups for each study in a biological gradient, from overt hypothyroidism to overt hyperthyroidism. The availability of prospective observational data are also in compliance with the fourth consideration of temporality; in 14 studies, a baseline measurement of the determinant (thyroid function) and (baseline and) follow-up measurements of the outcome of interest (hemoglobin) were available. Therefore, our pooled analysis of observational studies satisfies multiple criteria of Hill. However, it remains to be assessed in a well-designed, randomized controlled trial with a considerable number of participants with (subclinical) hypothyroidism if treatment is effective in reducing anemia. Further analysis of the data from two well-designed, randomized controlled trials for subclinical hypothyroidism in older persons [TRUST and IEMO Thyroid Trial (38, 39)] could be a first attempt at uncovering the clinical relevance of thyroid influences on hemoglobin levels.

Some limitations of this study have to be acknowledged as well. First, a limitation of this pooled analysis is that TSH and free T4 were only measured once at baseline. Because subclinical hypothyroidism has been shown to normalize in one-third of cases (40), in guidelines, it is often recommended that measurements of these parameters are repeated. Unfortunately, repeated TSH and free T4 measurements were not available in many cohorts. Erroneously classifying patients with euthyroidism based on one measurement may have led to an underestimation of the associations found. Second, the statistical power was more limited in the longitudinal models than in the baseline, cross-sectional analysis. The association between overt and subclinical hyperthyroidism and the risk of developing anemia did not reach statistical significance, but the results of the longitudinal

analyses followed a similar pattern. Third, we did not apply age-adjusted reference ranges as per current consensus and usual practice. However, evidence in favor of age-specific TSH reference ranges is starting to accumulate (41); so, too, is evidence to the contrary (42–44). This is an important topic of future research. Fourth, we performed sensitivity analyses excluding participants with high CRP levels as a proxy for chronic diseases that might predispose to anemia, but this only excluded diseases associated with inflammation. Particularly in the group of participants with subclinical hypothyroidism, the possibility of the presence of nonthyroidal illness cannot be fully excluded. As a result, possible residual errors caused by residual bias and confounding may have deflated the results. Unfortunately, information on additional potential confounding factors, like thyroid medication dose titrations, other diseases relating to anemia (cancer, chronic kidney disease, leukemia, gastric ulcers, arthritis, or chronic obstructive pulmonary disease), menopausal state, nonthyroidal illness, concomitant medications, and iron or vitamin supplements, was not available for most cohorts.

In conclusion, we observed higher odds of anemia in both participants with hypothyroid and hyperthyroid function. In addition, reduced thyroid function at baseline showed a trend of increased risk of developing anemia during follow-up. It remains to be assessed in a randomized controlled trial whether treatment of (subclinical) hypothyroidism is effective in reducing anemia.

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Correspondence and Reprint Requests: Wendy P. J. den Elzen, PhD, Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Post Zone E2-P, PO Box 9600, Leiden 2300RC, Netherlands. E-mail: w.p.j.den_elzen@lumc.nl.

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References

1. Cooper DS, Biondi B. Subclinical thyroid disease. *Lancet*. 2012; 379(9821):1142–1154.
2. Rodondi N, den Elzen WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, Asvold BO, Iervasi G, Imaizumi M, Collet TH, Bremner A, Maisonneuve P, Sgarbi JA, Khaw KT, Vanderpump MP, Newman AB, Cornuz J, Franklyn JA, Westendorp RG, Vittinghoff E, Gussekloo J; Thyroid Studies Collaboration. Subclinical

hypothyroidism and the risk of coronary heart disease and mortality. *JAMA*. 2010;**304**(12):1365–1374.
3. Beghé C, Wilson A, Ershler WB. Prevalence and outcomes of anemia in geriatrics: a systematic review of the literature. *Am J Med*. 2004;**116**(7, Suppl 7A):3S–10S.
4. Gaskell H, Derry S, Andrew Moore R, McQuay HJ. Prevalence of anaemia in older persons: systematic review. *BMC Geriatr*. 2008;**8**(1):1.
5. Fein HG, Rivlin RS. Anemia in thyroid diseases. *Med Clin North Am*. 1975;**59**(5):1133–1145.
6. Evans ES, Rosenberg LL, Simpson ME. Erythropoietic response to calorigenic hormones. *Endocrinology*. 1961;**68**(3):517–532.
7. Horsley V. The Brown lectures on pathology. *BMJ*. 1885;**1**(1261):419–423.
8. Kendrick TS, Payne CJ, Epis MR, Schneider JR, Leedman PJ, Klinken SP, Ingley E. Erythroid defects in TRalpha-/- mice. *Blood*. 2008;**111**(6):3245–3248.
9. Das KC, Mukherjee M, Sarkar TK, Dash RJ, Rastogi GK. Erythropoiesis and erythropoietin in hypo- and hyperthyroidism. *J Clin Endocrinol Metab*. 1975;**40**(2):211–220.
10. Kawa MP, Grymula K, Paczkowska E, Baskiewicz-Masiuk M, Dabkowska E, Koziolek M, Tarnowski M, Kłos P, Dziedziejko V, Kucia M, Syrenicz A, Machalinski B. Clinical relevance of thyroid dysfunction in human haematopoiesis: biochemical and molecular studies. *Eur J Endocrinol*. 2010;**162**(2):295–305.
11. den Elzen WP, de Craen AJ, Mooijaart SP, Gussekloo J. Low thyroid function and anemia in old age: the Leiden 85-plus study. *J Am Geriatr Soc*. 2015;**63**(2):407–409.
12. Horton L, Coburn RJ, England JM, Himsworth RL. The haematology of hypothyroidism. *Q J Med*. 1976;**45**(177):101–123.
13. Bremner AP, Feddema P, Joske DJ, Leedman PJ, O'Leary PC, Olynyk JK, Walsh JP. Significant association between thyroid hormones and erythrocyte indices in euthyroid subjects. *Clin Endocrinol (Oxf)*. 2012;**76**(2):304–311.
14. Floriani C, Feller M, Aubert CE, M'Rabet-Bensalah K, Collet TH, den Elzen WPJ, Bauer DC, Angelillo-Scherrer A, Aujesky D, Rodondi N. Thyroid dysfunction and anemia: a prospective cohort study and a systematic review. *Thyroid*. 2018;**28**(5):575–582.
15. Tudhope GR, Wilson GM. Anaemia in hypothyroidism. Incidence, pathogenesis, and response to treatment. *Q J Med*. 1960;**29**:513–537.
16. Vitale G, Fatti LM, Prolo S, Girola A, Caraglia M, Marra M, Abbruzzese A, Gerli G, Mari D. Screening for hypothyroidism in older hospitalized patients with anemia: a new insight into an old disease. *J Am Geriatr Soc*. 2010;**58**(9):1825–1827.
17. Gianoukakis AG, Leigh MJ, Richards P, Christenson PD, Hakimian A, Fu P, Niihara Y, Smith TJ. Characterization of the anaemia associated with Graves' disease. *Clin Endocrinol (Oxf)*. 2009;**70**(5):781–787.
18. Blum MR, Bauer DC, Collet TH, Fink HA, Cappola AR, da Costa BR, Wirth CD, Peeters RP, Åsvold BO, den Elzen WP, Luben RN, Imaizumi M, Bremner AP, Gogakos A, Eastell R, Kearney PM, Strotmeyer ES, Wallace ER, Hoff M, Ceresini G, Rivadeneira F, Uitterlinden AG, Stott DJ, Westendorp RG, Khaw KT, Langhammer A, Ferrucci L, Gussekloo J, Williams GR, Walsh JP, Jüni P, Aujesky D, Rodondi N; Thyroid Studies Collaboration. Subclinical thyroid dysfunction and fracture risk: a meta-analysis. *JAMA*. 2015;**313**(20):2055–2065.
19. Meuwese CL, van Diepen M, Cappola AR, Sarnak MJ, Shlipak MG, Bauer DC, Fried LP, Iacoviello M, Vaes B, Degryse J, Khaw KT, Luben RN, Åsvold BO, Bjøro T, Vatten LJ, de Craen AJM, Trompet S, Iervasi G, Molinaro S, Ceresini G, Ferrucci L, Dullaart RPF, Bakker SJL, Jukema JW, Kearney PM, Stott DJ, Peeters RP, Franco OH, Völzke H, Walsh JP, Bremner A, Sgarbi JA, Maciel RMB, Imaizumi M, Ohishi W, Dekker FW, Rodondi N, Gussekloo J, den Elzen WPJ; Thyroid Studies Collaboration. Low thyroid function is not associated with an accelerated deterioration in renal function [published online ahead of print 18 April 2018]. *Nephrol Dial Transplant*. doi: 10.1093/ndt/gfy071.
20. Huisman M, Poppelaars J, van der Horst M, Beekman AT, Brug J, van Tilburg TG, Deeg DJ. Cohort profile: the Longitudinal Aging Study Amsterdam. *Int J Epidemiol*. 2011;**40**(4):868–876.
21. Ittermann T, Haring R, Sauer S, Wallaschofski H, Dörr M, Nauck M, Völzke H. Decreased serum TSH levels are not associated with mortality in the adult northeast German population. *Eur J Endocrinol*. 2010;**162**(3):579–585.
22. Nutritional anaemias. Report of a WHO scientific group. *World Health Organ Tech Rep Ser*. 1968;**405**:5–37.
23. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;**7**(3):177–188.
24. Fowkes FG, Murray GD, Butcher I, Heald CL, Lee RJ, Chambless LE, Folsom AR, Hirsch AT, Dramaix M, deBacker G, Wautrecht JC, Kornitzer M, Newman AB, Cushman M, Sutton-Tyrrell K, Fowkes FG, Lee AJ, Price JF, d'Agostino RB, Murabito JM, Norman PE, Jamrozik K, Curb JD, Masaki KH, Rodríguez BL, Dekker JM, Bouter LM, Heine RJ, Nijpels G, Stehouwer CD, Ferrucci L, McDermott MM, Stoffers HE, Hooi JD, Knottnerus JA, Ogren M, Hedblad B, Witteman JC, Breteler MM, Hunink MG, Hofman A, Criqui MH, Langer RD, Fronek A, Hiatt WR, Hamman R, Resnick HE, Guralnik J, McDermott MM; Ankle Brachial Index Collaboration. Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta-analysis. *JAMA*. 2008;**300**(2):197–208.
25. Simmonds MC, Higgins JP, Stewart LA, Tierney JF, Clarke MJ, Thompson SG. Meta-analysis of individual patient data from randomized trials: a review of methods used in practice. *Clin Trials*. 2005;**2**(3):209–217.
26. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: rationale, conduct, and reporting. *BMJ*. 2010;**340**:c221.
27. Cochrane Handbook for Systematic Reviews of Interventions. Available at: www.handbook.cochrane.org. Accessed 31 May 2018.
28. Christ-Crain M, Meier C, Huber P, Zulewski H, Staub JJ, Müller B. Effect of restoration of euthyroidism on peripheral blood cells and erythropoietin in women with subclinical hypothyroidism. *Hormones (Athens)*. 2003;**2**(4):237–242.
29. Yücel R, Ozdemir S, Darıyerli N, Toplan S, Akyolcu MC, Yiğit G. Erythrocyte osmotic fragility and lipid peroxidation in experimental hyperthyroidism. *Endocrine*. 2009;**36**(3):498–502.
30. Asl SZ, Brojeni NK, Ghasemi A, Faraji F, Hedayati M, Azizi F. Alterations in osmotic fragility of the red blood cells in hypo- and hyperthyroid patients. *J Endocrinol Invest*. 2009;**32**(1):28–32.
31. Maggio M, De Vita F, Fisichella A, Lauretani F, Ticinesi A, Ceresini G, Cappola A, Ferrucci L, Ceda GP. The role of the multiple hormonal dysregulation in the onset of "anemia of aging": focus on testosterone, IGF-1, and thyroid hormones. *Int J Endocrinol*. 2015;**2015**:292574.
32. Perrin MC, Blanchet JP, Mouchiroud G. Modulation of human and mouse erythropoiesis by thyroid hormone and retinoic acid: evidence for specific effects at different steps of the erythroid pathway. *Hematol Cell Ther*. 1997;**39**(1):19–26.
33. Golde DW, Bersch N, Chopra IJ, Cline MJ. Thyroid hormones stimulate erythropoiesis in vitro. *Br J Haematol*. 1977;**37**(2):173–177.
34. Sullivan PS, McDonald TP. Thyroxine suppresses thrombocytopoiesis and stimulates erythropoiesis in mice. *Proc Soc Exp Biol Med*. 1992;**201**(3):271–277.
35. Fandrey J, Pagel H, Frede S, Wolff M, Jelkmann W. Thyroid hormones enhance hypoxia-induced erythropoietin production in vitro. *Exp Hematol*. 1994;**22**(3):272–277.
36. Khatiwada S, Gelal B, Baral N, Lamsal M. Association between iron status and thyroid function in Nepalese children. *Thyroid Res*. 2016;**9**(1):2.

37. Hill AB. The environment and disease: Association or causation? *Proc R Soc Med*. 1965;**58**:295–300.
38. Stott DJ, Rodondi N, Kearney PM, Ford I, Westendorp RGJ, Mooijaart SP, Sattar N, Aubert CE, Aujesky D, Bauer DC, Baumgartner C, Blum MR, Browne JP, Byrne S, Collet TH, Dekkers OM, den Elzen WPJ, Du Puy RS, Ellis G, Feller M, Floriani C, Hendry K, Hurley C, Jukema JW, Kean S, Kelly M, Krebs D, Langhorne P, McCarthy G, McCarthy V, McConnachie A, McDade M, Messow M, O'Flynn A, O'Riordan D, Poortvliet RKE, Quinn TJ, Russell A, Sinnott C, Smit JWA, Van Dorland HA, Walsh KA, Walsh EK, Watt T, Wilson R, Gussekloo J; TRUST Study Group. Thyroid hormone therapy for older adults with subclinical hypothyroidism. *N Engl J Med*. 2017;**376**(26):2534–2544.
39. IEMO 80-plus Thyroid Trial: Nederlands Trial Register. Available at: www.trialregister.nl/trialreg/admin/rctview.asp?TC=3851. Accessed 30 July 2018.
40. Díez JJ, Iglesias P, Burman KD. Spontaneous normalization of thyrotropin concentrations in patients with subclinical hypothyroidism. *J Clin Endocrinol Metab*. 2005;**90**(7):4124–4127.
41. Surks MI, Boucai L. Age- and race-based serum thyrotropin reference limits. *J Clin Endocrinol Metab*. 2010;**95**(2):496–502.
42. Fatourechi V. Upper limit of normal serum thyroid-stimulating hormone: a moving and now an aging target? *J Clin Endocrinol Metab*. 2007;**92**(12):4560–4562.
43. Kahapola-Arachchige KM, Hadlow N, Wardrop R, Lim EM, Walsh JP. Age-specific TSH reference ranges have minimal impact on the diagnosis of thyroid dysfunction. *Clin Endocrinol (Oxf)*. 2012;**77**(5):773–779.
44. Laurberg P, Andersen S, Carlé A, Karmisholt J, Knudsen N, Pedersen IB. The TSH upper reference limit: where are we at? *Nat Rev Endocrinol*. 2011;**7**(4):232–239.