Thyroid



DOI: 10.1089/thy.2017.0480



# Thyroid Dysfunction and Anemia: a Prospective Cohort Study and a Systematic Review

Carmen Floriani[1 §], Martin Feller[1,2 §], Carole E Aubert[1], Khadija M'Rabet-Bensalah[1], Tinh-Hai Collet[3], Wendy PJ den Elzen[4], Douglas C. Bauer[5], Anne Angelillo-Scherrer[6,7], Drahomir Aujesky[1], Nicolas Rodondi[1,2]

**Authors affiliations:**

[1]Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland

[2]Institute of Primary Health Care (BIHAM), University of Bern, Switzerland

[3]Service of Endocrinology, Diabetes and Metabolism, University Hospital of Lausanne, Lausanne, Switzerland

[4]Leiden University Medical Center, Department of Clinical Chemistry and Laboratory Medicine, Leiden, the Netherlands

[5]Departments of Medicine and Epidemiology and Biostatistics, University of California, San Francisco, United States

[6]Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital, University of Bern, Switzerland

[7]Department of Clinical Research, University of Bern, Switzerland

§ contributed equally

**Corresponding author:**

Martin Feller, MD MSc

Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland

Email: Martin.Feller@insel.ch

**ABSTRACT**

**Background:** Even though the association between thyroid dysfunction and anemia is commonly described, we do not know whether it is clinically relevant. We set out to quantify the association of thyroid dysfunction on hemoglobin (Hb) concentration and risk of anemia, and conducted a systematic review (MEDLINE & EMBASE, from inception until May 15$^{th}$, 2017) to interpret our findings in context.

**Methods:** We included participants from the EPIC-Norfolk cohort with available baseline thyroid-stimulating hormone (TSH), free thyroxine (fT4), and Hb. We defined euthyroidism as TSH 0.45-4.49mIU/l (reference category), hypothyroidism as TSH ≥4.50 mIU/l (subclinical (SHypo) with normal fT4 or overt (OHypo) with low fT4), and hyperthyroidism as TSH ≤0.44 mIU/l (subclinical (SHyper) with normal fT4 or overt (OHyper) with elevated fT4). Anemia was defined as Hb <12 g/dl in women and Hb <13 g/dl in men. In the cross-sectional analyses, we used multiple linear regression to compare Hb across TSH categories. In the prospective analysis, we excluded participants with OHypo/OHyper at baseline as we assumed they were treated for overt thyroid disease. We used a covariance model (ANCOVA) to determine change in Hb concentration from baseline to last follow-up, and multivariable Cox regression to analyze anemia risk.

**Results:** In the cross-sectional population (n=12,337), the adjusted Hb was 0.22 g/dl lower (95% confidence interval [95%CI] 0.07 to 0.38) in OHypo than in euthyroids, and 0.08 g/dl lower (95%CI -0.23 to 0.38) in OHyper. In the prospective analysis, 460/7031 participants developed anemia over a median follow-up of 4.7 years. The adjusted mean Hb change over time was -0.04 g/dl in SHypo (95%CI -0.14 to 0.06) and 0.05 g/dl in SHyper (95%CI -0.10 to 0.20). The adjusted hazard ratio for anemia was 0.99 (95%CI 0.67-1.48) in SHypo, and 0.52 (95%CI 0.23-1.16) in SHyper. Our systematic review returned no other prospective studies on this association, but cross-sectional and case-control studies showed comparable results.

**Conclusion:** In this first prospective population-based cohort, subclinical thyroid dysfunction was not associated with a change in Hb concentration during follow-up and

was not an independent risk factor for developing anemia; variations in Hb concentration in patients with overt thyroid dysfunction were not clinically relevant.

**Key Words:** Anemia, Thyroid Dysfuntion, Thyroid Stimulating Hormone, Hemoglobin, Clinical Relevance, Prospective Population-based Cohort

**Funding source:** This project was supported by grants from the Swiss National Science Foundation (SNSF 320030-150025 and 320030-172676 to Prof N Rodondi; P3SMP3-155318, PZ00P3-167826 to Dr T-H Collet).

Introduction

A causal relationship between overt hypothyroidism (OHypo) / overt hyperthyroidism (OHyper) and anemia has been postulated for decades (1-3). Researchers have suggested several pathophysiological mechanisms (4, 5). Hyperthyroidism enhances erythropoiesis, but it also raises the basal metabolic rate. The latter may increase plasma volume (3), counteracting the effect of erythropoiesis and lowering hemoglobin (Hb) concentration, ultimately causing anemia. In hypothyroidism, a drop in basal metabolic rate and a decrease in cellular oxygen consumption may reduce erythropoietin secretion, which would also lower Hb concentration and, ultimately, cause either normocytic, microcytic, or macrocytic anemia, depending on comorbidities (1, 6). Although several studies explored the pathophysiologic link between thyroid dysfunction and erythroid development (5, 7), we do not know whether, or to what extent, thyroid dysfunction alone (in the absence of comorbidities) affects Hb concentration, or if the effect would be clinically relevant in large epidemiological studies.

Limited data exist on the association between thyroid dysfunction and anemia. Case-series from the 1960s and 1970s had no control group (1, 8, 9). More recent cross-sectional studies did not consider potentially relevant confounders, such as renal function or C-reactive protein (10, 11), and included few patients with hypothyroidism (10-13). Only two studies reported on hyperthyroidism and anemia (11, 14), and no prospective study assessed the incidence of anemia in subclinical thyroid dysfunction.

Therefore, we aimed to quantify the effect of thyroid dysfunction on Hb concentration in a large population-based cohort, and assess its association with anemia. We did both a cross-sectional and a prospective analysis, and completed a systematic review.

**Material and Methods**

We report the study in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement (15). We report the results of our systematic review of observational studies according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) statement (16). For our systematic review, one author (CF) searched OVID-MEDLINE and EMBASE (from inception to May 15$^{th}$, 2017) for studies that examined the link between thyroid function and anemia, and checked bibliographies of included articles. We included studies with results on both Hb/anemia and thyroid dysfunction in adult, non-pregnant participants, and excluded those without an euthyroid control group. The appendix contains details of our search strategy. For quality assessment, we rated each included study using the Newcastle-Ottawa Quality Assessment Scale (NOS) for case-control studies or an adapted version for cross-sectional studies.

Setting and study population

We used data from the European Prospective Investigation of Cancer (EPIC) Norfolk cohort, a prospective observational study of 25,639 women and men, aged 40-79 at enrolment (17, 18). Everyone in this age range registered in the general practitioner databases of Norwich, UK, and surroundings, was invited from 1993 to 1997. The Norwich local research ethics committee (United Kingdom) approved the study, and all participants gave written informed consent. We included participants in our cross-sectional analysis if measurements of their baseline thyroid-stimulating hormone (TSH), free thyroxine (fT4), and Hb were available at study entry. We excluded those who self-reported thyroid dysfunction, because we assumed that most would already be in treatment for thyroid dysfunction. For the prospective analysis, we excluded (i) participants with anemia at baseline (as anemia would be the prospective outcome), (ii) those with no available Hb measurments during follow-up, (iii) and those diagnosed with overt thyroid dysfunction at baseline, because we assumed they received thyroid therapy after diagnosis. Descriptive results on thyroid dysfunction and anemia were already reported in this population, in a cross-sectional explanatory analysis without multivariable models to account for confounders through a rapid communication (19). In this study, we report on new multivariable analyses and new prospective analyses that were not available in the previous rapid communication.

Definition of main outcomes, exposures, and potential confounders

The primary outcome of the cross-sectional analysis was Hb concentration. The secondary outcome was anemia, defined as Hb <12 g/dl for women and <13 g/dl for men (binary variable) (20). The main exposure variable was thyroid dysfunction, classified by TSH categories. To make our study easier to compare with others, guided by expert reviews

(21, 22), we defined hypothyroidism as TSH ≥4.50 mIU/l, either subclinical (SHypo), with normal free thyroxine (fT4) or overt (OHypo) with fT4 below the reference range (23). We defined hyperthyroidism as TSH ≤0.44 mIU/l, either subclinical (SHyper) with normal fT4, or overt (OHyper) with fT4 above the reference range (23). The reference category was euthyroidism (TSH 0.45-4.49 mIU/l and fT4 within reference range 9.00-20.00 pmol/l) (23). A secondary exposure variable was fT4. In our prospective analysis, the primary outcome was the difference in Hb concentration between the last available follow-up Hb and baseline Hb (numerical variable, g/dl). The secondary outcome was incident anemia. The main exposure variable was thyroid dysfunction, classified by TSH categories (i.e. SHypo, SHyper, euthyroidism as reference). The secondary exposure variable was fT4 (categorized in quintiles), as in the cross-sectional analysis. We measured the following potential confounders at baseline: age (continuous variable); sex; body mass index (continuous variable); smoking (never, past, present); self-reported history of myocardial infarction, diabetes, stroke (binary variables; yes/no); ferritin (continuous variable); estimated glomerular filtration rate (eGFR, continuous variable); C-reactive Protein (CRP, continuous variable); and mean corpuscular volume (MCV, continuous variable; as a surrogate parameter for B12/folic acid deficiency, since these measurements were unavailable in the EPIC-Norfolk study).

Statistical analyses

In the cross-sectional analysis, we used multiple linear regression to compare Hb across TSH categories (OHypo, SHypo, SHyper, OHyper, euthyroidism as reference category). We also used multiple linear regression to compare Hb concentration across fT4 quintiles (mid quintile as reference). To explore the association between thyroid dysfunction and anemia, we used logistic regression to compare the odds of anemia across TSH categories and fT4 quintiles. For all analyses, we used three models: unadjusted; age- and sex-adjusted; and fully adjusted considering the confounders we already mentioned. We based our inclusion of confounders on a likelihood ratio test ($p<0.1$ as cut-off) that told us which confounders were most likely to confound the association between exposure and outcome in bivariable models.

In the prospective analysis, we analyzed a covariance model (ANCOVA) to determine change in Hb concentration from baseline to last follow-up, taking into account baseline Hb concentrations (24). We presented results as difference in mean change of Hb between SHypo/SHyper and euthyroidism (24, 25). We then used multivariable Cox regression models to compare the incidence of anemia in SHypo/SHyper to euthyroidism and, in a further analysis, the incidence of anemia across fT4 quintiles. Again, we used three models: unadjusted; age- and sex-adjusted; and fully adjusted, similar to the cross-sectional analyses described above.

We used multiple imputation (30 imputations) to complete missing values in potentially relevant confounders in cross-sectional and prospective analyses (for the cross-sectional population: body mass index 24 (0.19%) missing, smoking status 93 (0.75%) missing, ferritin 3,851 (31.2%) missing, eGFR 3,266 (26.5%) missing, and CRP 3,311 (26.8%) missing; for the prospective population: body mass index 11 (0.16%) missing, smoking status 44 (0.63%) missing, ferritin 2068 (29.4%) missing, eGFR 1835 (26.1%) missing, and CRP 1855 (26.4%) missing), and further assessed and ruled out that iron status modifies the association between thyroid dysfunction and Hb.(26) Finally, we conducted sensitivity analyses that used the original, unimputed data. Tests were two-sided, at a 0.05 level of significance. We used STATA, release 14.2 (StataCorp LP, College Station, Texas 77845 USA) for all our analyses.

## Results

After we excluded participants with self-reported thyroid disease (n=635), we included 12,337 participants in the cross-sectional analysis. Of these, 976 (7.9%) had anemia, most (11,174, [90.6%]) were euthyroid; 644 (5.2%) had SHypo, 199 (1.6%) OHypo, 270 (2.2%) SHyper, and 50 (0.4%) had OHyper **(Table 1)**. We observed no relevant differences in Hb concentration among TSH categories. In multivariable analyses, Hb was 0.22 g/dl (95%CI 0.07 to 0.38) lower in OHypo than in euthyroidism, and 0.08 g/dl (95%CI -0.23 to 0.38) lower in OHyper **(Table 2)**. The adjusted odds ratio (OR) for anemia was 1.96 (95%CI 1.29-2.98) in OHypo, 2.18 (95%CI 0.98-4.87) in OHyper, but we found no association in SHypo/SHyper (**eTable 2**).

For the prospective analysis, we excluded participants with anemia at baseline (n=976), overt thyroid dysfunction at baseline (n=212), and missing follow-up data on hemoglobin (n=4118). The latter group was similar to the included prospective study population with regard to thyroid (dys-)function, age, sex, and baseline Hb concentration. We followed 7031 participants (**Table 3**) over 55,733 person years (median 4.7 years). Anemia developed during follow-up in 26 of 368 (7.1%) participants with SHypo, 428 of 6496 (6.6%) participants with euthyroidism, and 6 of 167 (4.0%) participants with SHyper. In the ANCOVA analysis, when we compared SHypo/SHyper with euthyroidism, we found no difference in adjusted mean Hb change (SHypo: -0.04 g/dl, 95%CI -0.14 to 0.06; SHyper: 0.05 g/dl, 95%CI -0.10 to 0.20, **Table 4**). When we compared SHypo and SHyper to euthyroidism, the adjusted hazard ratio for anemia was 0.99 for SHypo (95%CI 0.67-1.48) and 0.52 for SHyper (95%CI 0.23-1.16) (**Table 5**).

All results (from cross-sectional and prospective analyses) were similar in our sensitivity analyses, which used the original unimputed data (**eTable 1-4**). Results for fT4 as exposure variable in both the cross-sectional and the prospective analyses, with imputed and original data, yielded no relevant associations (**eTable 1-4**).

Our literature search (see the **Appendix** for detailed search strategy) identified 2721 articles in OVID-MEDLINE and EMBASE. After excluding duplicates, we used title and abstract to screen 2692 articles. We excluded 2661 papers and assessed 31 full-text articles (**eFigure 1**). A manual check of article bibliographies revealed 14 more potentially eligible studies. Of these 45 candidates, 37 articles were excluded after full-text evaluation (**eTable 6**), and eight articles finally met our inclusion criteria (**eTable 5**); four were cross-sectional studies,(10, 12-14) four were case-control studies,(11, 27-29) and none had a prospective design.

Two of the four cross-sectional studies were population-based and of fair methodological quality,  scoring five out of seven points in the Newcastle-Ottawa Quality Assessment Scale (**eTable 7**) (10, 13). Yet, both studies were small, included fewer than 100 patients with SHypo and/or OHypo, excluded participants with hyperthyroidism (both SHyper and OHyper), and did not report significant differences in Hb concentrations across exposure categories. After adjusting for relevant confounders, Bremner et al. found Hb concentration was 13.7 g/dl in participants with SHypo, and 14.2 g/dl (p=0.29) in participants with euthyroidism (13). Den Elzen et al. found Hb concentration was 12.8 g/dl in participants aged >85y with OHypo, 13.1 g/dl in those with SHypo, and 13.0 g/dl in those with euthyroidism (10). In a retrospective analysis of 6534 consecutive female patients referred to a university hospital, Lippi et al. found similar Hb concentrations among participants with hypothyroidism (Hb 13.2 g/dl), euthyroidism (13.3 g/dl), and hyperthyroidism (13.1 g/dl) (14), but used uncommon definitions for thyroid dysfunction (hypothyroidism TSH >2.5 mU/l; euthyroidism TSH 0.2-2.5 mU/l; hyperthyroidism TSH <0.2 mU/l) (14); these could have biased the results towards the null effect (23). Regarding methodological quality, the NOS score was moderate with three out of seven points (**eTable 7**). In their cross-sectional analysis, however, Vitale et al., reported higher prevalence of hypothyroidism in hospitalized patients aged >65 years with anemia (20%) than in control patients without anemia (9.9%; p=0.01) (12). In this study, Hb concentrations increased an average of 1.6 g/dl in nine patients with hypothyroidism and anemia after they were treated with thyroxine (12). It was not clear, however, how the nine out of 21 patients with anemia, hypothyroidism, and no other potential cause of anemia were selected for thyroxine treatment. Overall, the methodological quality was moderate with an NOS score of three out ouf seven points (**eTable 7**). We included four case-control studies in our systematic review (7, 11, 27, 29). Each reported slightly more pronounced differences in Hb concentrations across the categories of thyroid dysfunction. Mean Hb concentrations among euthyroid participants ranged from 12.8 to 14.7 g/dl; corresponding values were 10.8 to 12.7 g/dl in SHypo, and 10.7 to 13.2 g/dl in OHypo. These studies included participants with more severe thyroid dysfunction (e.g., in Jafarzadeh et al., the 50 participants with OHypo had a mean TSH of 136.5mU/l (11)). They were of low methodological quality, scoring two out of 10 points in the adapted NOS (**eTable 8**). These methodological limitations make it hard to determine if the slightly more pronounced differences in Hb concentrations were real (because thyroid dysfunction was more severe) or spurious (a product of bias).

Only two of the eight included studies considered hyperthyroidism (11, 14). Both were negative. In the study by Lippi et al., Hb was 13.1 g/dl among hyperthyroid participants (subclinical and overt) and 13.3 g/dl among euthyroid participants(14); in the study by Jafarzadeh et al., Hb was 13.8 g/dl in OHyper and 14.0 g/dl in euthyroidism (11).

## Discussion

In this first prospective population-based cohort, subclinical thyroid dysfunction was not associated with a change in Hb concentration during follow-up and was not an independent risk factor for developing anemia. OHypo and OHyper were associated with anemia in the cross-sectional analyses. This is congruent with pathophysiologic findings that Thyroid Hormone Receptor alpha (TRα) is expressed by hematopoietic and erythroid progenitors and regulates erythropoiesis (4, 5, 7). However, the differences in mean Hb concentrations between OHypo/OHyper participants and euthyroidism were not clinically relevant. In the cross-sectional and prospective analysis, we found no relevant association between fT4 and anemia. Our systematic review did not identify other prospective cohort studies on the association between thyroid dysfunction and anemia/hemoglobin, and results from the included cross-sectional and case-control studies were comparable to the results of our cohort study.

Our study has limitations. First, we only measured thyroid hormones once, at baseline; we might have misclassified patients who had either transient subclinical thyroid dysfunction, who developed overt thyroid over time, or who reverted to euthyroidism over time. This single measurement may have biased our prospective results towards the null-effect, but the same is true of most large prospective cohorts that actually found associations with other outcomes like coronary heart disease(23) and fractures (30). Second, we had no measurements for vitamin B12 and folic acid. Despite this, the daily intake of participants included the current recommended dose of both (vitamin B12 200-350 µg, folic acid 2-14 mg) (31-33) and we adjusted for MCV (a surrogate parameter for B12/folic acid deficiency). Third, although the EPIC-Norfolk cohort is population-based, we could analyze data from only 12,337 (48.1%) of the 25,639 eligible participants because we had no thyroid function or hemoglobin measurements for some participants. We cannot be sure our study population represents the whole potentially eligible population. Fourth, in our prospective analyses, healthy participants could have been more likely to be available for follow-up examinations than sick participants (healthy cohort effect). If sick participants were both more anemic and more likely to suffer from thyroid dysfunction, then we may have underestimated the association between subclinical thyroid dysfunction and anemia. However, the consistent results between our cross-sectional and the prospective analyses suggest that healthy cohort effect was not substantial. Fifth, we could not exclude participants who developed conditions during follow-up that could affect Hb (e.g. hematological malignancies, chronic diseases), as this information was not available. As hematological malignancies are not associated with thyroid dysfunction, they should not be an important confounder. Chronic diseases could be associated with thyroid dysfunction, at least with subclinical hypothyroidism. In our prospective analyses, we may have attributed incident anemia cases to thyroid dysfunction when, in fact, anemia occurred due to chronic diseases. In this case, however, we would have overestimated the association between thyroid dysfunction and anemia, while we did not find significant large associations. Finally, the observational design of our study precluded causal inference.

Our study also has several strengths. It is the largest population-based study to examine the association between thyroid dysfunction and Hb concentration/anemia, and to consider both hypo- and hyperthyroidism. It is the first prospective cohort study on the topic to have a long follow-up period (almost 5 years). Our results are consistent between our cross-sectional, prospective, and sensitivity analyses, and we could adjust for most potentially relevant confounders. Our findings are strengthened by the systematic review we conducted, since it enabled us to interpret our results in the context of all available literature on the topic.

**Conclusion**

In this first prospective population-based cohort, subclinical thyroid dysfunction was not associated with a change in Hb concentration during follow-up and was not an independent risk factor for developing anemia; variations in Hb concentration in patients with overt thyroid dysfunction were not clinically relevant.

**Acknowledgments**

This project was supported by grants from the Swiss National Science Foundation (SNSF 320030-150025 and 320030-172676 to Prof N Rodondi; P3SMP3-155318, PZ00P3-167826 to Dr T-H Collet). We thank Kali Tal for her editorial assistance.



Thyroid
Thyroid Dysfunction and Anemia: A Prospective Cohort Study and a Systematic Review (DOI: 10.1089/thy.2017.0480)

## References

**1.** Horton L, Coburn RJ, England JM, Himsworth RL 1976 The haematology of hypothyroidism. Q J Med **45**:101-123.

**2.** Fein HG, Rivlin RS 1975 Anemia in thyroid diseases. Med Clin North Am **59**:1133-1145.

**3.** Ford HC, Carter JM 1988 The haematology of hyperthyroidism: abnormalities of erythrocytes, leucocytes, thrombocytes and haemostasis. Postgraduate medical journal **64**:735-742.

**4.** Kendrick TS, Payne CJ, Epis MR, Schneider JR, Leedman PJ, Klinken SP, Ingley E 2008 Erythroid defects in TRalpha-/- mice. Blood **111**:3245-3248.

**5.** Chute JP, Ross JR, McDonnell DP 2010 Minireview: Nuclear receptors, hematopoiesis, and stem cells. Molecular endocrinology **24**:1-10.

**6.** Nightingale S, Vitek PJ, Himsworth RL 1978 The haematology of hyperthyroidism. Q J Med **47**:35-47.

**7.** Erdogan M, Kosenli A, Ganidagli S, Kulaksizoglu M 2012 Characteristics of anemia in subclinical and overt hypothyroid patients. Endocrine journal **59**:213-220.

**8.** Tudhope GR, Wilson GM 1960 Anaemia in hypothyroidism. Incidence, pathogenesis, and response to treatment. Quarterly Journal of Medicine **29**:513-537.

**9.** Rivlin RS, Wagner HN, Jr. 1969 Anemia in hyperthyroidism. Ann Intern Med **70**:507-516.

**10.** den Elzen WP, de Craen AJ, Mooijaart SP, Gussekloo J 2015 Low thyroid function and anemia in old age: the Leiden 85-plus study. Journal of the American Geriatrics Society **63**:407-409.

**11.** Jafarzadeh A, Poorgholami M, Izadi N, Nemati M, Rezayati M 2010 Immunological and hematological changes in patients with hyperthyroidism or hypothyroidism.

Clin Invest Med **33**:E271-279.

**12.** Vitale G, Fatti LM, Prolo S, Girola A, Caraglia M, Marra M, Abbruzzese A, Gerli G, Mari D 2010 Screening for hypothyroidism in older hospitalized patients with anemia: a new insight into an old disease. Journal of the American Geriatrics Society **58**:1825-1827.

**13.** Bremner AP, Feddema P, Joske DJ, Leedman PJ, O'Leary PC, Olynyk JK, Walsh JP 2012 Significant association between thyroid hormones and erythrocyte indices in euthyroid subjects. Clinical endocrinology **76**:304-311.

**14.** Lippi G, Montagnana M, Salvagno GL, Guidi GC 2008 Should women with abnormal serum thyroid stimulating hormone undergo screening for anemia? Arch Pathol Lab Med **132**:321-322.

**15.** Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, Poole C, Schlesselman JJ, Egger M, Initiative S 2014 Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Int J Surg **12**:1500-1524.

**16.** Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB 2000 Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA **283**:2008-2012.

**17.** Hayat SA, Luben R, Keevil VL, Moore S, Dalzell N, Bhaniani A, Khawaja AP, Foster P, Brayne C, Wareham NJ, Khaw KT 2014 Cohort profile: A prospective cohort study of objective physical and cognitive capability and visual health in an ageing population of men and women in Norfolk (EPIC-Norfolk 3). Int J Epidemiol **43**:1063-1072.

**18.** Day N, Oakes S, Luben R, Khaw KT, Bingham S, Welch A, Wareham N 1999 EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. British journal of cancer **80 Suppl 1**:95-103.

**19.** M'Rabet-Bensalah K, Aubert CE, Coslovsky M, Collet TH, Baumgartner C, den Elzen

WP, Luben R, Angelillo-Scherrer A, Aujesky D, Khaw KT, Rodondi N 2016 Thyroid dysfunction and anaemia in a large population-based study. Clinical endocrinology **84**:627-631.

**20.** Organization WH 2011 Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. VMINS/Vitamin and Mineral Nutrition Information System (WHO/NMH/NHD/MNM/111)

**21.** Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, Franklyn JA, Hershman JM, Burman KD, Denke MA, Gorman C, Cooper RS, Weissman NJ 2004 Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA **291**:228-238.

**22.** Helfand M 2004 Screening for subclinical thyroid dysfunction in nonpregnant adults: a summary of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med **140**:128-141.

**23.** 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 C 2010 Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA **304**:1365-1374.

**24.** Vickers AJ, Altman DG 2001 Statistics notes: Analysing controlled trials with baseline and follow up measurements. BMJ **323**:1123-1124.

**25.** Vickers AJ 2001 The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. BMC Med Res Methodol **1**:6.

**26.** Beard JL, Borel MJ, Derr J 1990 Impaired thermoregulation and thyroid function in iron-deficiency anemia. The American journal of clinical nutrition **52**:813-819.

**27.** Srikrishna R, Girishbabu RJ, Ramesh ST 2015 Study of association of anemia in sub-clinical and overt hypothyroid patients. Research Journal of Pharmaceutical, Biological and Chemical Sciences **6**:368-373.

**28.** Mehmet E, Aybike K, Ganidagli S, Mustafa K 2012 Characteristics of anemia in subclinical and overt hypothyroid patients. Endocrine journal **59**:213-220.

**29.** Bashir H, Bhat MH, Farooq R, Majid S, Shoib S, Hamid R, Mattoo AA, Rashid T, Bhat AA, Wani HA, Masood A 2012 Comparison of hematological parameters in untreated and treated subclinical hypothyroidism and primary hypothyroidism patients. Med J Islam Repub Iran **26**:172-178.

**30.** Blum MR, Bauer DC, Collet TH, Fink HA, Cappola AR, da Costa BR, Wirth CD, Peeters RP, Asvold 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, Juni P, Aujesky D, Rodondi N, Thyroid Studies C 2015 Subclinical thyroid dysfunction and fracture risk: a meta-analysis. JAMA **313**:2055-2065.

**31.** Park JY, Nicolas G, Freisling H, Biessy C, Scalbert A, Romieu I, Chajes V, Chuang SC, Ericson U, Wallstrom P, Ros MM, Peeters PH, Mattiello A, Palli D, Maria Huerta J, Amiano P, Halkjaer J, Dahm CC, Trichopoulou A, Orfanos P, Teucher B, Feller S, Skeie G, Engeset D, Boutron-Ruault MC, Clavel-Chapelon F, Crowe F, Khaw KT, Vineis P, Slimani N 2012 Comparison of standardised dietary folate intake across ten countries participating in the European Prospective Investigation into Cancer and Nutrition. Br J Nutr **108**:552-569.

**32.** Olsen A, Halkjaer J, van Gils CH, Buijsse B, Verhagen H, Jenab M, Boutron-Ruault MC, Ericson U, Ocke MC, Peeters PH, Touvier M, Niravong M, Waaseth M, Skeie G, Khaw KT, Travis R, Ferrari P, Sanchez MJ, Agudo A, Overvad K, Linseisen J, Weikert C, Sacerdote C, Evangelista A, Zylis D, Tsiotas K, Manjer J, van Guelpen B, Riboli E, Slimani N, Bingham S 2009 Dietary intake of the water-soluble vitamins B1, B2, B6, B12 and C in 10 countries in the European Prospective Investigation into Cancer and Nutrition. Eur J Clin Nutr **63 Suppl 4**:S122-149.

**33.** de Bree A, van Dusseldorp M, Brouwer IA, van het Hof KH, Steegers-Theunissen RP 1997 Folate intake in Europe: recommended, actual and desired intake. Eur J Clin Nutr **51**:643-660.

Thyroid
Thyroid Dysfunction and Anemia: A Prospective Cohort Study and a Systematic Review (DOI: 10.1089/thy.2017.0480)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

**Table 1: Baseline characteristics of the cross-sectional study population (N=12,337)**

| | OHypo | SHypo | Euthyroidism | SHyper | OHyper | p-value° |
|---|---|---|---|---|---|---|
| Participants | 199 (1.6) | 644 (5.2) | 11,174 (90.6) | 270 (2.2) | 50 (0.4) | |
| **Demographics** | | | | | | |
| Age [y] | 60.4 (9.2) | 60.7 (9.0) | 58.6 (9.5) | 59.1 (10.0) | 62.3 (9.9) | <0.001 |
| Female | 149 (74.9) | 444 (68.9) | 5,785 (51.8) | 151 (55.9) | 28 (56.0) | <0.001 |
| **Laboratory parameters** | | | | | | |
| TSH [mIU/l] | 25.66 (31.5) | 7.78 (16.6) | 1.84 (0.8) | 0.27 (0.1) | 0.16 (0.2) | <0.001 |
| Free T4 [pmol/l] | 7.3 (1.6) | 11.2 (1.5) | 12.4 (1.8) | 14.0 (2.2) | 35.5 (19.6) | <0.001 |
| Anemia* | 29 (14.6) | 55 (8.5) | 866 (7.8) | 18 (6.7) | 8 (16.0) | 0.001 |
| Hemoglobin [g/dl] | 13.4 (1.2) | 13.6 (1.4) | 13.9 (1.3) | 13.8 (1.3) | 13.7 (1.6) | <0.001 |
| MCV [fl] | 89.4 (4.5) | 88.9 (4.5) | 89.3 (4.4) | 89.2 (4.5) | 88.5 (4.3) | 0.196 |
| Ferritin [ng/ml] | 78.5 (71.9) | 79.2 (63.6) | 89.6 (75.2) | 84.2 (77.2) | 95.6 (101.4) | 0.019 |
| CRP [mg/l] | 3.6 (6.5) | 4.2 (12.0) | 3.0 (5.6) | 3.9 (11.2) | 3.8 (6.1) | <0.001 |
| eGFR [ml/min] | 70.9 (20.0) | 72.5 (19.5) | 77.7 (24.9) | 82.9 (35.7) | 74.3 (25.9) | <0.001 |
| **BMI categories** | | | | | | |
| <25.0 kg/m$^2$ | 66 (33.2) | 232 (36.0) | 4,432 (39.7) | 122 (45.2) | 20 (40.0) | overall 0.046 |
| 25.0-29.9 kg/m$^2$ | 90 (45.2) | 292 | 5,099 (45.6) | 112 | 23 (46.0) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | (45.3) | | (41.5) | | |
| ≥30.0 kg/m$^2$ | 42 (21.1) | 118 (18.3) | 1,623 (14.5) | 35 (13.0) | 7 (14.0) | |
| **Smoking status** | | | | | | |
| Never | 88 (44.2) | 342 (53.1) | 5,055 (45.2) | 107 (39.6) | 25 (50.0) | overall <0.001 |
| Past | 89 (44.7) | 253 (39.3) | 4,693 (42.0) | 122 (45.2) | 20 (40.0) | |
| Present | 19 (9.6) | 41 (6.4) | 1,348 (12.1) | 38 (14.1) | 4 (8.0) | |
| **Medical History** | | | | | | |
| Myocardial Infarction | 4 (2.0) | 18 (2.8) | 342 (3.1) | 3 (1.1) | 0 | 0.216 |
| Stroke | 0 | 16 (2.5) | 134 (1.2) | 5 (2.2) | 2 (4.0) | 0.004 |
| Diabetes mellitus | 2 (1.0) | 13 (2.0) | 241 (2.2) | 4 (1.5) | 3 (6.0) | 0.247 |

**Table 2: Multiple linear regression: hemoglobin concentrations [g/dl] compared across thyroid dysfunction categories in the cross-sectional study population (N=12,337)**

| | OHypo | SHypo | Euthyroidism | SHyper | OHyper |
|---|---|---|---|---|---|
| Participants (%) | 199 (1.6) | 644 (5.2) | 11,174 (90.6) | 270 (2.2) | 50 (0.4) |
| Crude analysis | -0.52 (-0.71 to -0.33) | -0.29 (-0.40 to -0.18) | Ref. | -0.10 (-0.26 to 0.06) | -0.16 (-0.53 to 0.21) |
| Age- and sex-adjusted analysis | -0.19 (-0.35 to -0.03) | -0.05 (-0.14 to 0.04) | Ref. | -0.04 (-0.18 to 0.10) | -0.11 (-0.43 to 0.20) |
| Multivariable° analysis with imputed§ data | -0.22 (-0.38 to -0.07) | -0.03 (-0.11 to 0.06) | Ref. | -0.02 (-0.15 to 0.11) | -0.08 (-0.38 to 0.23) |
| Multivariable° analysis with original+ data | -0.28 (-0.45 to -0.11) | -0.04 (-0.14 to 0.05) | Ref. | -0.01 (-0.17 to 0.16) | -0.42 (-0.82 to -0.03) |

Thyroid
Thyroid Dysfunction and Anemia: A Prospective Cohort Study and a Systematic Review (DOI: 10.1089/thy.2017.0480)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

**Table 3: Baseline characteristics of the prospective study population (N=7,031)***

| | SHypo | Euthyroidism | SHyper | p-value° |
|---|---|---|---|---|
| Participants | 368 (5.2) | 6,496 (92.4) | 167 (2.4) | |
| **Demographics** | | | | |
| Age [y] | 60.1 (8.8) | 58.2 (9.0) | 57.5 (10.0) | <0.001 |
| Female | 248 (67.4) | 3,302 (50.8) | 95 (56.9) | <0.001 |
| **Laboratory parameters** | | | | |
| TSH [mIU/l] | 7.1 (4.5) | 1.8 (0.9) | 0.27 (0.13) | <0.001 |
| Free T4 [pmol/l] | 11.2 (1.6) | 12.4 (1.7) | 13.9 (2.2) | <0.001 |
| Hemoglobin [g/dl] | 13.8 (1.1) | 14.0 (1.2) | 14.0 (1.3) | <0.001 |
| MCV [fl] | 89.0 (4.1) | 89.5 (3.8) | 89.6 (3.7) | 0.057 |
| Ferritin [ng/ml] | 77.1 (59.4) | 89.9 (74.8) | 89.5 (84.7) | 0.021 |
| CRP [mg/l] | 3.6 (11.8) | 2.6 (4.7) | 3.9 (13.4) | 0.001 |
| eGFR [ml/min] | 72.2 (18.4) | 77.7 (24.0) | 82.1 (36.9) | <0.001 |
| **BMI categories** | | | | |
| <25.0 kg/m$^2$ | 143 (39.0) | 2,715 (41.9) | 80 (47.9) | overall 0.288 |
| 25.0-29.9 kg/m$^2$ | 163 (44.4) | 2,926 (45.1) | 66 (39.5) | |
| ≥30.0 kg/m$^2$ | 61 (16.6) | 845 (13.0) | 21 (12.6) | |
| **Smoking status** | | | | |
| Never | 205 (56.3) | 3,114 (48.2) | 70 (41.9) | overall 0.008 |
| Past | 136 (37.4) | 2,681 (41.5) | 75 (44.9) | |
| Present | 23 (6.3) | 661 (10.2) | 22 (13.2) | |
| **Medical History** | | | | |
| Myocardial Infarction | 9 (2.5) | 155 (2.4) | 1 (0.6) | 0.319 |
| Stroke | 9 (2.5) | 58 (0.9) | 1 (0.6) | 0.011 |
| Diabetes mellitus | 7 (1.9) | 112 (1.7) | 3 (1.8) | 0.966 |

**Table 4: Changes in hemoglobin concentration [g/dl] compared across thyroid dysfunction categories in the prospective cohort (N=7,031)***

| | SHypo | Euthyroidism | SHyper |
|---|---|---|---|
| Participants (%) | 368 (5.2%) | 6496 (92.4%) | 167 (2.4%) |
| Baseline hemoglobin, mean (SD) | 13.8 (1.1) | 14.0 (1.2) | 14.0 (1.3) |
| Follow-up hemoglobin, mean (SD) | 13.8 (1.2) | 14.1 (1.3) | 14.1 (1.1) |
| Difference in adjusted° mean change of hemoglobin (95%CI), imputed[§] dataset | -0.04 (-0.14 to 0.06) | Ref. | 0.05 (-0.10 to 0.20) |

**Table 5: Hazard ratio for anemia: subclinical thyroid dysfunction compared to euthyroidism in the prospective study population (N=7,031)***

| | SHypo | Euthyroidism | SHyper |
|---|---|---|---|
| Anemia N (%) | 26 (7.1) | 428 (6.6) | 6 (3.6) |
| **HR for anemia (95%CI)** | | | |
| Crude analysis | 0.94 (0.63-1.39) | Ref. | 0.52 (0.23-1.16) |
| Age- and sex-adjusted analysis | 1.00 (0.67-1.49) | Ref. | 0.51 (0.23-1.13) |
| Multivariable° analysis with imputed[§] data | 0.99 (0.67-1.48) | Ref. | 0.52 (0.23-1.16) |
| Multivariable° analysis with original data[+] | 0.86 (0.53-1.40) | Ref. | 0.69 (0.29-1.68) |

**Table 1:** Results are presented as: number (percentage) for categorical variables, mean (standard deviation) for continuous variables

°p-values derived from one-way analysis of variance models in case of continuous variables and from $\chi^2$ tests in case of categorical variables

*Definition of anemia: Hb<12g/dl for women, Hb<13g/dl for men

Missing data: BMI (24 [0.19%]), smoking status (93 [0.75%]), ferritin (3,851 [31.2%]), eGFR (3,266 [26.5%]), and CRP (3,311 [26.8%])

Abbreviations: N, number; OHypo, overt hypothyroidism; SHypo, subclinical hypothyroidism; SHyper, subclinical hyperthyroidism; OHyper, overt hyperthyroidism; y, years; TSH, thyroid stimulating hormone; T4, thyroxine; MCV, mean corpuscular volume; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; BMI, body mass index

**Table 2:** In brackets are 95% confidence intervals, if not otherwise stated

°Adjustments: age, sex, BMI, smoking status, myocardial infarction, diabetes mellitus, stroke, ferritin, eGFR, CRP, MCV

§Imputation for BMI (24 [0.19%] missing), smoking status (93 [0.75%] missing), ferritin (3,851 [31.2%] missing), eGFR (3,266 [26.5%] missing), and CRP (3,311 [26.8%] missing). All other variables were complete

+N = 8,275

Abbreviations: N, number; OHypo, overt hypothyroidism; SHypo, subclinical hypothyroidism; SHyper, subclinical hyperthyroidism; OHyper, overt hyperthyroidism; Ref., reference; BMI, body mass index; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; MCV, mean corpuscular volume

**Table 3:** Results are presented as: number (percentage) for categorical variables, mean (standard deviation) for continuous variables

*We included only participants without anemia or overt thyroid dysfunction at baseline, and with available hemoglobin assessment during follow-up

°p-values derived from one-way analysis of variance models in case of continuous variables and from $\chi^2$ tests in case of categorical variables

Missing data: BMI (11 [0.16%]), smoking status (44 [0.63%]), ferritin (2,068 [29.4%]), eGFR (1,835 [26.1%]), and CRP (1,855 [26.4%])

Abbreviations: N, number; SHypo, subclinical hypothyroidism; SHyper, subclinical hyperthyroidism; y, years; TSH, thyroid stimulating hormone; T4, thyroxine; MCV, mean corpuscular volume; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; BMI, body mass index

**Table 4:**

*We included only participants without anemia or overt thyroid dysfunction at baseline, and with available hemoglobin assessment during follow-up

°Adjustments: baseline hemoglobin, length of follow-up in years, age, sex, BMI, smoking status, myocardial infarction, diabetes mellitus, stroke, ferritin, eGFR, CRP, MCV

§Imputation for BMI (11 [0.16%] missing), smoking status (44 [0.63%] missing), ferritin (3,851 [31.2%] missing), eGFR (3,266 [26.5%] missing), and CRP (3,311 [26.8%] missing). All other variables were complete

Abbreviations: N, number; SHypo, subclinical hypothyroidism; SHyper, subclinical hyperthyroidism; SD, standard deviation; CI, confidence interval; Ref., reference; BMI, body mass index; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; MCV, mean corpuscular volume

**Table 5:** In brackets are 95% confidence intervals, if not otherwise stated

*We included only participants without anemia or overt thyroid dysfunction at baseline, and with available hemoglobin assessment during follow-up

°Adjustments: age, sex, BMI, diabetes mellitus, ferritin, MCV

§Imputation for BMI (11 [0.16%] missing), smoking status (44 [0.63%] missing), ferritin (3,851 [31.2%] missing), eGFR (3,266 [26.5%] missing), and CRP (3,311 [26.8%] missing). All other variables were complete

+N = 4,955

Abbreviations: N, number; SHypo, subclinical hypothyroidism; SHyper, subclinical hyperthyroidism; HR, hazard ratio; CI, confidence interval; Ref., reference; BMI, body mass index; MCV, mean corpuscular volume; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein

Thyroid
Thyroid Dysfunction and Anemia: A Prospective Cohort Study and a Systematic Review (DOI: 10.1089/thy.2017.0480)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

## Appendix

### Literature Search

### Data base for literature search

In Ovid: Medline and Old Medline 15.05.2017

http://gateway.ovid.com/autologin.html

In EMBASE 15.05.2017

link: http://www.embase.com/home

### Inclusion criteria:

- Patients with thyroid dysfunction: subclinical and/or overt hypothyroidism/ hyperthyroidism
- Exposure: at least two categories of thyroid (dys-)function, including a euthyroid category
- Outcome: hemoglobin concentrations and/or proportion of patients with/without anemia reported for each of the exposure categories

### Exclusion criteria:

- Studies exclusively on patients younger than 18 years or on pregnant women
- Studies exclusively on patients with anemia due to a specific cause (e.g. iron-deficiency) and co-occurrence of thyroid dysfunction (because in these studies, the specific effect of thyroid dysfunction on anemia/hemoglobin cannot be disentangled from e.g. iron-deficiency)
- Case reports/Case series/Reviews (due to lack of comparison group)

### Search strategy MEDLINE/OLD MEDLINE (n= 393)

Exp thyroid disease/, exp hypothyroidism/, exp hyperthyroidism/, exp thyroid hormones/, exp thyrotropin/, (subclinical adj2 (hyperthyr$ or hypothyr$ or dysthyr$ or thyr$)).ti,ab./, exp anemia/, (anem$ or anaem$).ti,ab./, exp cohort studies/, cohort$.tw/, controlled clinical trial.pt./, exp epidemiologic methods/

**Search strategy in Embase (n=2,328)**

thyroid disease/exp, hypothyroidism/exp, hyperthyroidism/exp, thyroid hormones/exp, thyrotropin/exp, (subclinical NEAR/2 (hyperthyr* or hypothyr* or dysthyr* or thyr*)):ab,ti, anemia/exp, anem*:ab,ti or anaem*:ab,ti, cohort analysis/exp, cohort*:ab,ti, controlled clinical trial/exp, epidemiology/exp

**References**

**1.** Lippi G, Montagnana M, Salvagno GL, Guidi GC 2008 Should women with abnormal serum thyroid stimulating hormone undergo screening for anemia? Arch Pathol Lab Med **132**:321-322.

**2.** Vitale G, Fatti LM, Prolo S, Girola A, Caraglia M, Marra M, Abbruzzese A, Gerli G, Mari D 2010 Screening for hypothyroidism in older hospitalized patients with anemia: a new insight into an old disease. Journal of the American Geriatrics Society **58**:1825-1827.

**3.** Bremner AP, Feddema P, Joske DJ, Leedman PJ, O'Leary PC, Olynyk JK, Walsh JP 2012 Significant association between thyroid hormones and erythrocyte indices in euthyroid subjects. Clinical endocrinology **76**:304-311.

**4.** den Elzen WP, de Craen AJ, Mooijaart SP, Gussekloo J 2015 Low thyroid function and anemia in old age: the Leiden 85-plus study. Journal of the American Geriatrics Society **63**:407-409.

**5.** Jafarzadeh A, Poorgholami M, Izadi N, Nemati M, Rezayati M 2010 Immunological and hematological changes in patients with hyperthyroidism or hypothyroidism. Clin Invest Med **33**:E271-279.

**6.** Mehmet E, Aybike K, Ganidagli S, Mustafa K 2012 Characteristics of anemia in subclinical and overt hypothyroid patients. Endocrine journal **59**:213-220.

**7.** Bashir H, Bhat MH, Farooq R, Majid S, Shoib S, Hamid R, Mattoo AA, Rashid T, Bhat AA, Wani HA, Masood A 2012 Comparison of hematological parameters in untreated and treated subclinical hypothyroidism and primary hypothyroidism patients. Med J Islam Repub Iran **26**:172-178.

**8.** Srikrishna R, Girishbabu RJ, Ramesh ST 2015 Study of association of anemia in sub-clinical and overt hypothyroid patients. Research Journal of Pharmaceutical, Biological and Chemical Sciences **6**:368-373.

**9.** Christ-Crain M, Meier C, Huber P, Zulewski H, Staub JJ, Muller B 2003 Effect of restoration of euthyroidism on peripheral blood cells and erythropoietin in women with subclinical hypothyroidism. Hormones (Athens) **2**:237-242.

**10.** Cinemre H, Bilir C, Gokosmanoglu F, Bahcebasi T 2009 Hematologic effects of levothyroxine in iron-deficient subclinical hypothyroid patients: A randomized, double-blind, controlled study. Journal of Clinical Endocrinology and Metabolism **94**:151-156.

**11.** Ravanbod M, Asadipooya K, Kalantarhormozi M, Nabipour I, Omrani GR 2013 Treatment of iron-deficiency anemia in patients with subclinical hypothyroidism. American Journal of Medicine **126**:420-424.

**12.** Choi CW, Lee J, Kyong HP, In KC, Seok JK, Jae HS, Byung SK, Sang WS, Yeul HK, Kim JS 2005 Incidence of anemia in older Koreans: Community-based cohort study. Archives of Gerontology and Geriatrics **41**:303-309.

**13.** Gianoukakis AG, Leigh MJ, Richards P, Christenson PD, Hakimian A, Fu P, Niihara Y, Smith TJ 2009 Characterization of the anaemia associated with Graves' disease. Clinical endocrinology **70**:781-787.

**14.** Joosten E, Lioen P 2015 Iron deficiency anemia and anemia of chronic disease in geriatric hospitalized patients: How frequent are comorbidities as an additional explanation for the anemia? Geriatrics and Gerontology International **15**:931-935.

**15.** Bahemuka M, Hodkinson HM 1975 Screening for hypothyroidism in elderly inpatients. Br Med J **2**:601-603.

**16.** Carmel R, Spencer CA 1982 Clinical and subclinical thyroid disorders associated with pernicious anemia. Observations on abnormal thyroid-stimulating hormone levels and on a possible association of blood group O with hyperthyroidism. Arch Intern Med **142**:1465-1469.

**17.** Volzke H, Wallaschofski H, Wolff B, Berger K, John U, Dorr M 2006 Thyroid function and serum ferritin levels: the study of health in Pomerania. Thyroid **16**:681-686.

**18.** Omar S, Hadj Taeib S, Kanoun F, Hammami MB, Kamoun S, Ben Romdhane N, Feki

M, Slimane H, Kaabachi N 2010 [Erythrocyte abnormalities in thyroid dysfunction]. Tunis Med **88**:783-788.

**19.** Schindhelm RK, Ten Boekel E, Heima NE, Van Schoor NM, Simsek S 2013 Thyroid hormones and erythrocyte indices in a cohort of euthyroid older subjects. European Journal of Internal Medicine **24**:241-244.

**20.** Petrosyan I, Blaison G, Andrès E, Federici L 2012 Anaemia in the elderly: An aetiologic profile of a prospective cohort of 95 hospitalised patients. European Journal of Internal Medicine **23**:524-528.

**21.** Lopez-Berastegui O, Gomez-Antunez M, San Julian-Romero M, Lavilla-Olleros C, Santos-Martinez J, Aldezabal-Polo N, Pinilla-Llorente B, Muiño-Miguez A, Millan-Nuñez-Cortes J 2013 Anemia in older patients: An etiologic profile. European Journal of Internal Medicine **24**:e160.

**22.** Velarde-Mayol C, de la Hoz-García B, del Cañizo-Fernández-Roldán C, Hernández-López AM, Loza-Candia I, Cardona-Hernández A 2015 Pernicious anemia and autoimmune thyroid diseases in elderly people. Revista Espanola de Geriatria y Gerontologia **50**:126-128.

**23.** M'Rabet-Bensalah K, Aubert CE, Coslovsky M, Collet TH, Baumgartner C, Den Elzen WPJ, Luben R, Angelillo-Scherrer A, Aujesky D, Khaw KT, Rodondi N 2016 Thyroid dysfunction and anaemia in a large population-based study. Clinical endocrinology **84**:627-631.

**24.** Ottesen M, Feldt-Rasmussen U, Andersen J, Hippe E, Schouboe A 1995 Thyroid function and autoimmunity in pernicious anemia before and during cyanocobalamin treatment. J Endocrinol Invest **18**:91-97.

**25.** Kosenli A, Erdogan M, Ganidagli S, Kulaksizoglu M, Solmaz S, Kosenli O, Unsal C, Canataroglu A 2010 Anemia frequency and etiology in primary hypothyroidism. Endocrine Abstracts **20**:P140.

**26.** Price EA, Mehra R, Holmes TH, Schrier SL 2011 Anemia in older persons: Etiology and evaluation. Blood Cells, Molecules, and Diseases **46**:159-165.

**27.** Dorgalaleh A, Mahmoodi M, Varmaghani B, Kiani Node F, Saeeidi Kia O, Alizadeh S, Tabibian S, Bamedi T, Momeni M, Abbasian S, Kashani Khatib Z 2013 Effect of thyroid dysfunctions on blood cell count and red blood cell indice. Iran J Ped Hematol Oncol **3**:73-77.

**28.** Aktas G, Sit M, Dikbas O, Tekce BK, Savli H, Tekce H, Alcelik A 2014 Could red cell distribution width be a marker in Hashimoto's Thyroiditis? Experimental and Clinical Endocrinology and Diabetes **122**:572-574.

**29.** Tudhope GR, Wilson GM 1960 Anaemia in hypothyroidism. Incidence, pathogenesis, and response to treatment. Quarterly Journal of Medicine **29**:513-537.

**30.** Rivlin RS, Wagner HN, Jr. 1969 Anemia in hyperthyroidism. Ann Intern Med **70**:507-516.

**31.** Horton L, Coburn RJ, England JM, Himsworth RL 1976 The haematology of hypothyroidism. Q J Med **45**:101-123.

**32.** Das KC, Mukherjee M, Sarkar TK, Dash RJ, Rastogi GK 1975 Erythropoiesis and erythropoietin in hypo- and hyperthyroidism. The Journal of clinical endocrinology and metabolism **40**:211-220.

**33.** Nightingale S, Vitek PJ, Himsworth RL 1978 The haematology of hyperthyroidism. Q J Med **47**:35-47.

**34.** Sims EG 1983 Hypothyroidism causing macrocytic anemia unresponsive to B12 and folate. J Natl Med Assoc **75**:429-431.

**35.** Bertola G, Bocchia M, Ribotto P, Orlandini B, Torchio G 1998 [Hematological changes in hyperthyroidism. A case report]. Recenti Prog Med **89**:180-182.

**36.** Dharmarajan TS, Thadisina S 2011 Anemia in the old: Possible indicator of serious underlying disease,warrants evaluation. S. Thadisina MD, RO Russell MD, TS

Dharmarajan MD, AGSF Montefiore Medical Center (North), Bronx, NY. Journal of the American Geriatrics Society **59**:S120.

**37.** Fein HG, Rivlin RS 1975 Anemia in thyroid diseases. Med Clin North Am **59**:1133-1145.

**38.** Ford HC, Carter JM 1988 The haematology of hyperthyroidism: abnormalities of erythrocytes, leucocytes, thrombocytes and haemostasis. Postgraduate medical journal **64**:735-742.

**39.** Antonijevic N, Nesovic M, Trbojevic B, Milosevic R 1999 [Anemia in hypothyroidism]. Med Pregl **52**:136-140.

**40.** Levy C, Siguret V 2007 Anaemia in the elderly: multiple etiological categories. Immuno-Analyse et Biologie Specialisee **22**:215-221.

**41.** Kaferle J, Strzoda CE 2009 Evaluation of macrocytosis. Am Fam Physician **79**:203-208.

**42.** Stein SA, Wartofsky L 2010 Screening for thyroid disease: Defining high-risk populations. Expert Review of Endocrinology and Metabolism **5**:663-671.

**43.** Hennessey JV, Espaillat R 2015 Reversible morbidity markers in subclinical hypothyroidism. Postgrad Med **127**:78-91.

**44.** Erdogan M, Kosenli A, Ganidagli S, Kulaksizoglu M 2012 Characteristics of anemia in subclinical and overt hypothyroid patients. Endocrine journal **59**:213-220.

Thyroid
Thyroid Dysfunction and Anemia: A Prospective Cohort Study and a Systematic Review (DOI: 10.1089/thy.2017.0480)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

**eTable 1**: **Multiple linear regression: hemoglobin concentrations [g/dl] compared across fT4 quintiles in the cross-sectional study population (N=12,337)**

| | 1st fT4 quintile | 2nd fT4 quintile | 3rd fT4 quintile | 4th fT4 quintile | 5th fT4 quintile |
|---|---|---|---|---|---|
| Crude analysis | -0.30 (-0.38 to -0.23) | -0.11 (-0.19 to -0.04) | Ref. | 0.09 (0.02 to 0.17) | 0.15 (0.07 to 0.22) |
| Age- and sex-adjusted analysis | -0.16 (-0.22 to -0.10) | -0.08 (-0.14 to -0.02) | Ref. | 0.05 (-0.02 to 0.11) | 0.02 (-0.04 to 0.08) |
| Multivariable° analysis with imputed§ data | -0.16 (-0.22 to -0.10) | -0.08 (-0.14 to -0.02) | Ref. | 0.05 (-0.01 to 0.11) | 0.01 (-0.06 to 0.07) |
| Multivariable° analysis with original data+ | -0.13 (-0.20 to -0.06) | -0.09 (-0.16 to -0.02) | Ref. | 0.05 (-0.02 to 0.11) | 0.03 (-0.04 to 0.10) |

**eTable 2: Odds ratio for anemia in the cross-sectional study population (N=12,337)**

| | OHypo | SHypo | Euthyroidism | SHyper | OHyper |
|---|---|---|---|---|---|
| Anemia N (%) | 29 (14.97) | 55 (8.54) | 866 (7.75) | 18 (6.67) | 8 (16.00) |
| **OR for anemia (95%CI)** | | | | | |
| Crude analysis | 2.03 (1.36-3.02) | 1.11 (0.84-1.48) | Ref. | 0.85 (0.52-1.38) | 2.27 (1.06-4.84) |
| Age- and sex-adjusted analysis | 1.75 (1.17-2.62) | 1.00 (0.75-1.33) | Ref. | 0.83 (0.51-1.34) | 2.28 (1.06-4.90) |
| Multivariable° with imputed§ data | 1.96 (1.29-2.98) | 0.93 (0.69-1.25) | Ref. | 0.74 (0.44-1.23) | 2.18 (0.98-4.87) |
| Multivariable with original data+ | 2.00 (1.17-3.44) | 0.88 (0.57-1.34) | Ref. | 0.46 (0.17-1.21) | 2.13 (0.62-7.31) |

**eTable 3: Odds ratio for anemia in the cross-sectional study population, using fT4 quintiles as exposure (N=12,337)**

| | 1st fT4 quintile | 2nd fT4 quintile | 3rd fT4 quintile | 4th fT4 quintile | 5th fT4 quintile |
|---|---|---|---|---|---|
| Anemia N (%) | 249 (9.58) | 205 (8.60) | 187 (7.39) | 173 (6.90) | 162 (7.00) |
| **OR for anemia (95%CI)** | | | | | |
| Crude analysis | 1.33 (1.09-1.62) | 1.18 (0.96-1.45) | Ref. | 0.93 (0.75-1.15) | 0.94 (0.76-1.17) |
| Age- and sex-adjusted analysis | 1.24 (1.01-1.51) | 1.16 (0.94-1.43) | Ref. | 0.95 (0.77-1.18) | 1.02 (0.81-1.26) |
| Multivariable° with imputed§ data | 1.25 (1.02-1.54) | 1.18 (0.95-1.46) | Ref. | 0.96 (0.77-1.19) | 1.03 (0.82-1.29) |
| Multivariable with original data+ | 1.34 (0.99-1.80) | 1.42 (1.05-1.90) | Ref. | 1.09 (0.79-1.49) | 1.11 (0.80-1.53) |

**eTable 4: Hazard ratio for anemia in the prospective study population, using fT4 quintiles as exposure (N=7,031)***

| | **1st fT4 quintile** | **2nd fT4 quintile** | **3rd fT4 quintile** | **4th fT4 quintile** | **5th fT4 quintile** |
|---|---|---|---|---|---|
| Anemia N (%) | 96 (6.79) | 85 (5.96) | 111 (7.57) | 72 (4.99) | 96 (7.49) |
| **HR for anemia (95%CI)** | | | | | |
| Crude analysis | 0.87 (0.66-1.15) | 0.74 (0.56-0.99) | Ref. | 0.68 (0.50-0.91) | 0.99 (0.75-1.30) |
| Age- and sex-adjusted analysis | 0.86 (0.65-1.13) | 0.75 (0.56-0.99) | Ref. | 0.68 (0.50-0.91) | 1.02 (0.77-1.34) |
| Multivariable° with imputed§ data | 0.85 (0.64-1.11) | 0.75 (0.56-1.00) | Ref. | 0.68 (0.50-0.91) | 1.05 (0.80-1.38) |
| Multivariable° with original data+ | 0.80 (0.59-1.10) | 0.56 (0.40-0.79) | Ref. | 0.57 (0.40-0.82) | 1.09 (0.80-1.49) |

**Table 5**: **Description of included studies about thyroid function and anemia**

| Author, year | Study Design | Study population, n | Definition of thyroid dysfunction | Outcome | Results | Quality assessment/Comments |
|---|---|---|---|---|---|---|
| Lippi G, 2008(1) | Cross-sectional* | 6,534 consecutive female referrals from GPs to a university hospital | Hypothyroidism (TSH >2.5mU/l, n=1988) Euthyroidism (TSH 0.20-2.5mU/l, n=4426) Hyperthyroidism (TSH <0.20mU/l, n=120) | Anemia defined as Hb<12g/dl | Hypothyroidism: Hb 132g/l (p=0.03), prevalence of anemia 15.5% (p=0.64) Euthyroidism (ref. group): Hb 133g/l, prevalence of anemia 13.8% Hyperthyroidism: Hb 131g/l (p=0.04), prevalence of anemia 15.8% (p=0.58) | - Only females<br>- Selected study population (consecutive referrals)<br>- Uncommon definition of hypothyroidism (usually TSH >4.5 mU/l) and hyperthyroidism (usually TSH <0.5mU/l)<br>- No adjustment for potential confounders |

| | | | | | | |
|---|---|---|---|---|---|---|
| Vitale G, 2010(2) | Cross-sectional* | 316 hospitalized patients >65 years | Hypothyroidism according to TSH (no clear definition reported) | Anemia (female Hb <12g/dl, male Hb <13g/dl) | 155 (49%) patients with anemia. Prevalence of hypothyroidism in anemic patients: 20% in non-anemic patients: 9.9% p=0.01 | - Selected study population (hospitalized patients)<br>- Definition of hypothyroidism is not reported<br>- No adjustment for potential confounders<br>- 9/31 hypothyroid patients were selected for thyroxine treatment and Hb improved 1.6g/dl on average<br>- It is reported that 21 patients with hypothyroidism and anemia had no other reason for anemia. It remains unclear |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | why/how only 9 out of 21 were selected for thyroxine treatment. |
| Bremner AP, 2012(3) | Cross-sectional, population-based cohort | 1,098 outpatients (patients with hyperthyroidism were excluded) | SHypo (TSH 0.4-4.0mU/l, fT4 9-23pmol/l, n=87) Euthyroidism defined as TSH and fT4 both in the ref. range, n=1011 | Hb and other erythrocyte parameters | SHypo: Hb 136.9g/l Euthyroidism: Hb 141.8g/l p=0.29 | - Small likelihood of selection bias<br>- Small number of participants with SHypo<br>- Relevant confounders considered in analysis |
| Den Elzen WPJ, 2015(4) | Cross-sectional, population-based cohort | 526 outpatients >85 years | OHypo (TSH >4.5mU/L, fT4 <13 pmol/l, n=40) SHypo (TSH >4.5mU/L, fT4 13-23pmol/l, n=35) Euthyroidis | Anemia (female Hb<12g/dl, male Hb<13 g/dl) | OHypo: Hb 12.8g/dl (p=0.37), prevalence of anemia 35% (OR 1.76, 95% CI 0.89-3.51) SHypo: Hb 13.1g/dl (p=0.68), prevalence | - Small likelihood of selection bias<br>- Small number of participants with OHypo and SHypo<br>- Not reported that patients with hyperthyroidism were |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | m (TSH 0.5-4.5mU/l, n=451) | | of anemia 26% (OR 1.16, 95% CI 0.52-2.57) Euthyroidism (ref. group): Hb 13.0g/dl, prevalence of anemia 25% | probably excluded<br>- Analyses only adjusted for sex, other potential confounders not mentioned |
| Jafarzadeh A, 2010(5) | Case-control | Patients admitted to a hospital: 50 women with OHypo 50 women with OHyper 50 sex-and age-matched euthyroid controls | OHypo (TSH >3.5mIU/l, fT3 <1.6nmol/l, fT4 <60nmol/l) Euthyroidism defined as TSH and fT4/3 in ref. range OHyper (TSH <0.35mIU/l, fT3 >3.6nmol/l, fT4 >160nmol/l) | Hb and other erythrocyte parameters | OHypo: Hb 13.2g/dl (p=0.01) Euthyroidism: Hb 14.0g/dl (ref.) OHyper: Hb 13.8g/dl (p=not significant) | - Unclear how participants with thyroid dysfunction were selected and if they were representative of all potential patients with thyroid dysfunction<br>- Unclear how controls were selected (except for age- and sex-matching)<br>- Uncommon |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | TSH ref. range (0.35 – 3.6 mU/l)<br>- No adjustment for potential confounders (except for age- and sex-matching)<br>- Patients suffered from severe OHypo and OHyper (mean TSH 136.5mU/l and 0.06mU/l, respectively) |
| Erdogan M, 2012(6) | Case-control | Participants were selected from 4,800 patients who presented for the first time at the endocrinology department of a | OHypo (TSH >4.2mU/l, fT4 <0.70ng/dl) SHypo (TSH >4.2mU/l, fT4 in ref. range) Euthyroid (TSH 0.27-4.2mU/l, fT4 0.70 | Anemia (female Hb<12g/dl, male Hb< 13g/dl) | OHypo: Hb 11.9g/dl, prevalence of anemia 43% SHypo: Hb 12.4g/dl, prevalence of anemia 39% Euthyroidism: Hb 12.8g/dl, | - Although the derivation of the study population is well reported, it is astonishing that the numbers come down to round figures (100, 100, 200), leaving the |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | university hospital: 100 OHypo 100 SHypo 200 healthy, euthyroid controls | 1.48ng/dl) | | prevalence of anemia 26% Compared to euthyroidism, all results are statistically significant | potential for selection bias<br>- No community controls (persons attending outpatient clinic)<br>- No adjustment for potential confounders<br>- Patients suffered from severe OHypo and SHypo (mean TSH 43.1mU/l and 13.9mU/l, respectively) |
| Bashir H, 2012(7) | Case-control | Participants were referred to the endocrinology department of a university hospital: 100 | OHypo (TSH >4.3mU/l, fT4 below ref. range) SHypo (TSH >4.3mU/l, normal fT4) | Hb and other erythrocyte parameters | Untreated OHypo: Hb 10.7g/dl Treated OHypo: Hb 12.6g/dl Untreated SHypo: Hb 10.8g/dl Treated SHypo: Hb | - Unclear how the study population was derived<br>- No community controls (persons attending outpatient clinic)<br>- No adjustment |

Thyroid
Thyroid Dysfunction and Anemia: A Prospective Cohort Study and a Systematic Review (DOI: 10.1089/thy.2017.0480)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

| | | | | | | |
|---|---|---|---|---|---|---|
| | | untreated OHypo<br>100 treated OHypo<br>110 untreated SHypo<br>110 treated SHypo<br>180 age- and sex-matched euthyroid controls | | | 12.0g/dl<br>Euthyroidism: Hb 14.7g/dl | for potential confounders |
| Srikrishna R, 2015(8) | Case-control | Participants were randomly selected from patients attending an outpatient university hospital:<br>108 OHypo<br>123 SHypo<br>209 age- and sex-matched | OHypo (TSH above and fT4 below ref. range)<br>SHypo (TSH above and fT4 within ref. range)<br>Euthyroids (TSH and fT4 in ref. range) | Anemia (female Hb<12g/dl, male Hb< 13g/dl) | OHypo: Hb 11.3g/dl (p<0.001), prevalence of anemia 49.3% (p<0.001)<br>SHypo: Hb 12.7g/dl (p<0.001), prevalence of anemia 46.8% (p=0.018)<br>Euthyroidism (ref. | - Unclear how cases were "randomly" selected<br>- No community controls (persons attending outpatient clinic)<br>- No ref. ranges of thyroid hormones<br>- No adjustment for potential confounders |

| | | | |
|---|---|---|---|
| healthy, euthyroid controls | | group)): Hb 13.3g/dl, prevalence of anemia 28% | - Patients suffered from severe OHypo and SHypo (mean TSH 57.0mU/l and 11.4mU/l, respectively) |

**eTable 6**: **Description of excluded studies about thyroid function and anemia**

| **Author, year** | **Study Design** | **Study population, n** | **Definition of thyroid dysfunction** | **Outcome** | **Results** | **Comments** |
|---|---|---|---|---|---|---|
| Christ-Crain M, 2003(9) | RCT | 66 women | TSH reference range 0.3-4mU/l | Not clearly defined | LT4 treatment in SCH results in increased erythropoietin levels | Excluded because no euthyroid control group |
| Cinemre H, 2009(10) | RCT | 51 Consecutive outpatients | SHypo: Elevated TSH in the setting of normal total or free fT4 and fT3 | Clinical satisfactory Increase in Hb | Hb increased by 0.4 g/dl in the iron treatment group [95% confidence interval (CI) 0.2-0.7], by 1.9g/dl in the iron/levothyroxine treatment group [95% CI 1.5-2.3] | RCT in patients with SHypo and iron-deficiency anemia examining the effect of iron substitution with and without Levothyroxine. Excluded because the main cause of anemia is iron deficiency |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | and the contribution of SHypo to the development of anemia cannot be discerned |
| Mohammadreza R, 2013(11) | RCT | 60 | SHypo defined as TSH 4.5-10mU/l and normal fT3 and fT4 Iron deficiency Anemia | Change from BL in Hb, ferritin and TSH | A combination of iron salt and levothyroxine is better than each alone | RCT in patients with SHypo and iron-deficiency anemia examining the effect of iron substitution with and without Levothyroxine. Excluded because the main cause of anemia is iron deficiency and the contribution of SHypo to |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | the development of anemia cannot be discerned |
| Choi CW, 2005(12) | Prospective study | 332 outpatients | No assessment of thyroid dysfunction at BL | Incidence of Anemia (Hb<12g/dl, Hb< 13g/dl) | 24 (7.2%) incident anemia cases during 3y of FUP, 1 subject with hypothyroidism | TSH only assessed in incident anemia cases (no TSH assessment in non-anemic group) |
| Gianoukakis AG, 2009(13) | Prospective Study | 98 Consecutive outpatients | OHyper due to Grave's disease | Anemia (Hb reference range females 11.9-14.9g/dl, men 13.9-16.9g/dl) | 31 participants had anemia, mean Hb 11.3g/dl | Limited to patients with Graves' disease (no control group) |
| Joosten E, 2015(14) | Prospective study | 191 Consecutive hospitalized older patients | - | Iron deficiency anemia, Anemia of | Of 56 patients with iron deficiency anemia 3 had thyroid | Only patient with iron-deficiency and anemia of chronic disease |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | chronic disease | dysfunction, of 135 patients with anemia of chronic disease had thyroid dysfunction | |
| Bahemuka M, 1975(15) | Cross-sectional | 2000 >60 y inpatients | Hypothyroidism according to TSH (no clear definition reported) | - | 46 (2.3%) were hypothyroid 7 had pernicious anemia, no other results on anemia reported | Anemia due to autoimmune disorder (pernicious anemia). Excluded due to lacking information on hematological parameters/ anemia in euthyroid comparison group |
| Carmel R, 1982(16) | Cross-sectional | 162 with pernicious anemia | TSH according 1982 | Not clearly defined | High prevalence of pernicious anemia in | Excluded because only on patients with pernicious |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | OHyper (8.6%) and OHypo (11.7%) | anemia |
| Völzke H, 2006(17) | Cross-sectional | 4111 outpatients | TSH and fT4 and/or fT3 tertiles | Ferritin serum levels | No association between thyroid function and serum ferritin levels | Excluded because no assessment of anemia |
| Omar S, 2010(18) (French article) | Cross-sectional | Hyperthyroidism 235 Hypothyroidism 177 | Hyperthyroidism TSH <0.10mUI/l Hypothyroidism TSH >5.0mUI/l | Anemia (female Hb <12g/dl, male Hb <13g/dl) | Prevalence of anemia 40.9% in hyperthyroidism, prevalence of anemia 57.1% in hypothyroidism | Excluded because no euthyroid control group |
| Schindhelm RK, 2012(19) | Cross-sectional | 762 | TSH, only euthyroid | Erythrocyte indices | Free T4, but not TSH is associated with erythrocyte indices (positive association | Excluded because only euthyroid subjects |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | with Hb, erythrocyte count and hematocrit) | |
| Petrosyan I, 2012(20) | Cross-sectional | 95 patients >/= 65 y with anemia | - | Cause of anemia | 2 of 360 participants (2.1%) had hypothyroidism | Excluded because no non-anemic control group and no assessment of thyroid function |
| Lopez-Berastegui O, 2013(21) | Cross-Sectional | 581 65 y or alder | TSH >10mIU/l | Anemia (female Hb <12g/dl, male Hb <13g/dl) | - | Relevant information on thyroid function and anemia lacking |
| Velarde-Mayol C, 2015(22) (Spanish article) | Cross sectional | 5,082 Consecutive outpatients | Autoimmune thyroid disease without further definition | Pernicious anemia | Prevalence of Thyroid autoimmune disease 8.2%, prevalence of pernicious anemia 3.3% | Only on autoimmune thyroid diseases |
| M'Rhabet-Bensalah | Cross-section | - | - | - | - | Same study as M'Rhabet- |

| | | | | | | |
|---|---|---|---|---|---|---|
| K, 2015(23) | al (conference abstract) | | | | | Bensalah K, Clinical Endocrinology 2016 |
| M'Rhabet-Bensalah K, 2015(23) | Cross-sectional | - | - | - | - | Same study as M'Rhabet-Bensalah K, Clinical Endocrinology 2016 |
| M'Rhabet-Bensalah K, 2016(23) | Cross-sectional | 8971 population-based participants | Thyroid dysfunction According TSH | Anemia (female Hb <12g/dl, male Hb <13g/dl) | The prevalence of anemia was higher in OHyper (14.6%) but not increased in SHypo/SHyper | Same study population as present study |
| Ottesen M, 1995(24) | Case Control | 35 patients with pernicious anemia 22 healthy controls | Cobalamin Treatment | Thyroid function and autoimmunity | No effect of cobalamin treatment on thyroid function | Cases were patients with decreased plasma cobalamin, and anemia was not the outcome |
| Kosenli A, | Case | - | - | - | - | Same study |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2009(25) | Control Study (conference abstract) | | | | | as Erdogan M, Endocrine Journal 2012 |
| Price EA, 2011(26) | Case-control | 190 older outpatients (>65y) referrals to hematology<br>Non anemic controls (n=not specified) | According to TSH | Anemia (Hb<12g/dl, Hb< 13g/dl), Cause of anemia | No subject was found to have anemia related to thyroid dysfunction | No information about thyroid function of the control group -> excluded (none of 190 anemic patients had thyroid dysfunction) |
| Dorgalaleh A, 2013(27) | Case Control | 102 patients with OHypo, 84 with OHyper, 118 healthy controls | OHypo (TSH >5.5mU/l )<br>OHyper (TSH <0.3mU/l) | Blood cell count/ red blood cell indices | Statistically relevant difference in RBC, MCH, MCHC, RDW and HCT | Pediatric population |
| Aktas G, 2014(28) | Case Control | 102 patients | Hashimoto Thyroiditis | RDW | Patients with | Anemia was an exclusion |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | with HT 63 healthy controls | | | elevated RWD should be further evaluated for HT | criterion |
| Thudhope GR, 1960(29) | Case series | 166 | Hypothyroidism according to standard in 1960 | Prevalence of anemia (female Hb <12g/dl, male Hb <13 g/dl) | 10% iron deficiency anemia 7.8% pernicious anemia | Excluded because lack of control group |
| Rivlin RS, 1969(30) | Case series | 9 | According standard in 1969 | Iron utilization | There might be an impairment in the iron utilization of hyperthyroid patients developing anemia | Excluded because lack of control group |
| Horton L, 1975(31) | Case series | 202 | OHypo according to standard in 1975 | Anemia (female Hb <12g/dl, male Hb <13 g/dl) | Anemia was present in 39 of 172 women and 14 of 30 men | Excluded because lack of control group |
| Das KC, 1975(32) | Case series | 44 | Not clearly defined | Not clearly | Stimulation of | Excluded because no |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | defined | erythropoiesis by thyroid hormones seems to be erythropoietin mediated | euthyroid control group |
| Nightingale S, 1977(33) | Case series | 239 | OHyper according to standard in 1977 | Anemia (female Hb <12g/dl, male Hb <13 g/dl) | Anemia was present in 37 of 207 women and 9 of 39 men | Excluded because lack of control group |
| Sims EG, 1983(34) | Case Report | - | Not specified | Not specified | - | Excluded because lack of control group |
| Bertola G, 1998(35) (Italian article) | Case Report | - | OHyper in Basedow TSH <0.3mU/l, fT4 5ng/l | Pancytopenia | Normalization of hematological parameters after treatment of hyperthyroidism | Excluded because lack of control group |
| Dharmarajan MD, | Case Report | - | No clear definition | Anemia (female | - | Excluded because lack |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2004(36) | | | | Hb <12g/dl, male Hb <13g/dl) | | of control group |
| Fein HG, 1975(37) | Review | - | Not clearly defined | - | - | Excluded because it's a review |
| Ford HC, 1988(38) | Review | - | OHyper Not clear defined | Ery-/Ery-indices | - | Excluded because it's a review |
| Antonijevic N, 1999(39) (Croatian article) | Review | - | - | - | - | Excluded because it's a review |
| Levy C, 2007(40) (French article) | Review | - | - | - | - | Excluded because it's a review |
| Kaferle J, 2009(41) | Review | - | - | Macrocytosis | Non-alcoholic liver disease and hypothyroidism account for a substantial proportion of macrocytosi | Excluded because it's a review |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | s | |
| Stein SA, 2010(42) | Review | - | - | - | - | Excluded because it's a review |
| Hennessey JV(43), 2015 | Review | - | SHypo | Iron-deficiency anemia | Anemia is significantly more common in OHypo/SHypo | Excluded because it's a review |

**Table 7: Newcastle-Ottawa Quality Assessment Scale Cross-Sectional Studies**

| Study | Selection | | | Comparability | Outcome | | Score |
|---|---|---|---|---|---|---|---|
| | Representativeness of the sample | Non-responders | Ascertainment of exposure | Confounding Factors are controlled | Assessment of outcome | Statistical Test | |
| Lippi G, 2008(1) | Selected Group | No description | Validated measurement tool (1 point) | No | Record linkage (1 point) | Clearly described (1 point) | 3 of max. 7 |
| Vitale G, 2010(2) | Selected Group | No description | Validated measurement tool (1 point) | No | Record linkage (1 point) | Clearly described (1 point) | 3 of max. 7 |
| Bremner AP, 2012(3) | Truly representative of the average in the target population (1 point) | No description | Validated measurement tool (1 point) | Yes (1 point) | Record linkage (1 point) | Clearly described (1 point) | 5 of max. 7 |
| Den Elzen WPJ, 2015(4) | Truly representative of the average in the target population (1 point) | No description | Validated measurement tool (1 point) | Yes (1 point) | Record linkage (1 point) | Clearly described (1 point) | 5 of max. 7 |

**eTable 8: Newcastle-Ottawa Quality Assessment Scale Case-Control Studies**

| Study | Selection | | | | Comparability | Exposure | | | Score |
|---|---|---|---|---|---|---|---|---|---|
| | Case definition | Representativeness of the case | Selection of controls | Definition of controls | Comparability of the cases and controls | Ascertainment of exposure | Same method of ascertainment for cases and controls | Non-Response Rate | |
| Jafarzadeh A, 2010(5) | Record linkage | Potential for selection bias | No description | No description | No adjustment for potential confounding | Secure record (1 point) | Yes (1 point) | No designation | 2 of max. 10 |
| Erdogan M, 2012(44) | Record linkage | Potential for selection bias | Hospital controls | No description | No adjustment for potential confounding | Secure record (1 point) | Yes (1 point) | No designation | 2 of max. 10 |
| Bashir H, 2012( | Record linkag | Potential for selection | Hospital contro | No description | No adjustment for | Secure record (1 | Yes (1 point) | No designation | 2 of m |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 7) | e | bias | ls | | potential confounding | point) | | | 60 ax. 10 |
| Srikrishna R, 2015(8) | Record linkage | Potential for selection bias | Hospital controls | No description | No adjustment for potential confounding | Secure record (1 point) | Yes (1 point) | No designation | 2 of max. 10 |

**eTable 1:** In brackets are 95% confidence intervals

°Adjustments**:** age, sex, BMI, smoking status, myocardial infarction, diabetes mellitus, stroke, ferritin, eGFR, CRP, MCV

§Imputation for BMI (24 [0.19%] missing), smoking status (93 [0.75%] missing), ferritin (3,851 [31.2%] missing), eGFR (3,266 [26.5%] missing), and CRP (3,311 [26.8%] missing), complete data for age- and sex-adjusted analysis

+N = 8,275

Abbreviations: fT4, free thyroxine; N, number; Ref., reference; BMI, body mass index; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; MCV, mean corpuscular volume

**eTable 2:** In brackets are 95% confidence intervals, if not stated otherwise

°Adjustments: age, sex, BMI, smoking status, myocardial infarction, ferritin, eGFR, CRP, MCV

§ Imputation for BMI (24 [0.19%] missing), smoking status (93 [0.75%] missing), ferritin (3,851 [31.2%] missing), eGFR (3,266 [26.5%] missing), and CRP (3,311 [26.8%] missing), complete data for age- and sex-adjusted analysis

+N = 8,275

Abbreviations**:** N, number; OHypo, overt hypothyroidism; SHypo, subclinical hypothyroidism; SHyper, subclinical hyperthyroidism; OHyper, overt hyperthyroidism; OR, odds ratio; CI, confidence interval; Ref., reference; BMI, body mass index; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; MCV, mean corpuscular volume

**eTable 3:** In brackets are 95% confidence intervals, if not stated otherwise

°Adjustments: age, sex, BMI, smoking status, myocardial infarction, ferritin, eGFR, CRP, MCV

§ Imputation for BMI (24 [0.19%] missing), smoking status (93 [0.75%] missing), ferritin (3,851 [31.2%] missing), eGFR (3,266 [26.5%] missing), and CRP (3,311 [26.8%] missing), complete data for age- and sex-adjusted analysis

+N = 8,275

Abbreviations: fT4, free thyroxine; N, number; OR, odds ratio; CI, confidence interval; Ref., reference; BMI, body mass index; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; MCV, mean corpuscular volume

**eTable 4:** In brackets are 95% confidence intervals, if not stated otherwise

*We included only participants without anemia or overt thyroid dysfunction at baseline, and with available hemoglobin assessment during follow-up

°Adjustments: age, sex, BMI, diabetes mellitus, ferritin, MCV

§Imputation for BMI (11 [0.16%] missing), smoking status (44 [0.63%] missing), ferritin (2,068 [29.4%] missing), eGFR (1,835 [26.1%] missing), and CRP (1,855 [26.4%] missing), complete data for age- and sex-adjusted analysis

+N = 4,955

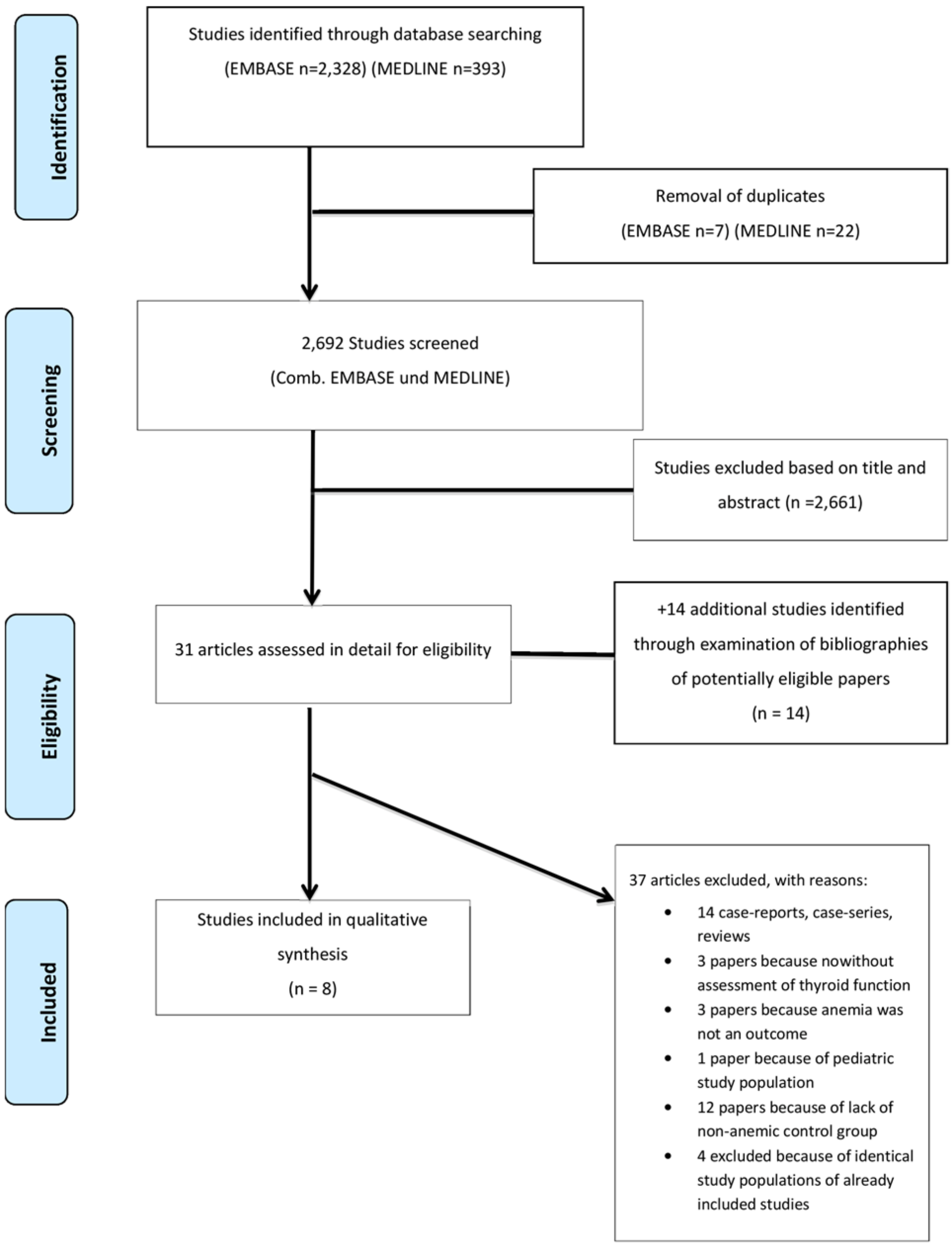
Abbreviations: fT4, free thyroxine; N, number; HR, Hazard ratio; Ref., reference; BMI, body mass index; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; MCV, mean corpuscular volume

**eTable 5:** Abbreviations: CI, confidence interval; fT3, free triiodothyronine; fT4, free thyroxine; Hb, hemoglobin; LT4, levothyroxine; n, number; ng, nanogramm; OHyper, overt hyperthyroidism; OHypo, overt hypothyroidism; OR, odds ratio; pmol, picomol; SHyper, subclinical hyperthyroidism; SHypo, subclinical hypothyroidism; TSH, thyroid stimulating hormone; y, year.

*cross-sectional in terms of assessment of exposure (thyroid hormones) and outcome (hemoglobin/anemia) at the same time.

**eTable 6:** Abbreviations: CI, confidence Interval; Ery, erythrocyte; Ery-Indices, erythrocyte-indices; fT3, free triiodothyronine; fT4, free thyroxin; Hb, hemoglobin; HT, Hashimoto thyroiditis; n, number; ng, nanogramm; OHyper, overt hyperthyroidism; OHypo, overt hypothyroidism; SHyper, subclinical hyperthyroidism; SHypo, subclinical hypothyroidism; TSH, Thyroid Stimulating Hormone; RCT, randomized controlled trial; RDW, red blood cell distribution width; y, year.

**eFigure 1: Flow diagram of the systematic review**




eFigure 1: Flow diagram of the systematic review

**MOOSE Checklist for Meta-analyses of Observational Studies**

**General comment on this checklist:** We would like to highlight that we primarily report results from a cohort study, and only additionally performed a systematic review (without meta-analysis) of the literature to interpret the results of our cohort study in the light of the whole body of evidence on the topic. Therefore, as our manuscript reports results from a cohort study and a systematic review without meta-analysis, it is not exactly structured and as detailed as if it only reported results from a systematic review including a meta-analysis.

| Item No | Recommendation | Reported on Page No |
|---|---|---|
| Reporting of background should include | | |
| 1 | Problem definition | 3 |
| 2 | Hypothesis statement | 3 |
| 3 | Description of study outcome(s) | 3 |
| 4 | Type of exposure or intervention used | na |
| 5 | Type of study designs used | 3 |
| 6 | Study population | 3 |
| Reporting of search strategy should include | | |
| 7 | Qualifications of searchers (eg, librarians and investigators) | 3 |
| 8 | Search strategy, including time period included in the synthesis and key words | 3 |
| 9 | Effort to include all available studies, including contact with authors | na |
| 10 | Databases and registries searched | 3 |
| 11 | Search software used, name and version, including special features used (eg, explosion) | na |
| 12 | Use of hand searching (eg, reference lists of obtained articles) | 3 |
| 13 | List of citations located and those excluded, including justification | App° page 8 |

| | | |
|---|---|---|
| 14 | Method of addressing articles published in languages other than English | na |
| 15 | Method of handling abstracts and unpublished studies | na |
| 16 | Description of any contact with authors | na |
| Reporting of methods should include | | |
| 17 | Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested | 3 |
| 18 | Rationale for the selection and coding of data (eg, sound clinical principles or convenience) | na* |
| 19 | Documentation of how data were classified and coded (eg, multiple raters, blinding and interrater reliability) | na* |
| 20 | Assessment of confounding (eg, comparability of cases and controls in studies where appropriate) | App° pages 13 and 14 |
| 21 | Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results | App° pages 13 and 14 |
| 22 | Assessment of heterogeneity | na* |
| 23 | Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated | na* |
| 24 | Provision of appropriate tables and graphics | na* |
| Reporting of results should include | | |
| 25 | Graphic summarizing individual study estimates and overall estimate | na* |
| 26 | Table giving descriptive information for each study included | App° page 5 |
| 27 | Results of sensitivity testing (eg, subgroup analysis) | na* |

| | | |
|---|---|---|
| 28 | Indication of statistical uncertainty of findings | na* |
| Reporting of discussion should include | | |
| 29 | Quantitative assessment of bias (eg, publication bias) | na* |
| 30 | Justification for exclusion (eg, exclusion of non-English language citations) | na |
| 31 | Assessment of quality of included studies | 6-7 |
| Reporting of conclusions should include | | |
| 32 | Consideration of alternative explanations for observed results | We only drew conclusions about our cohort study, not about the systematic review |
| 33 | Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review) | We only drew conclusions about our cohort study, not about the systematic review |
| 34 | Guidelines for future research | We only drew conclusions about our cohort |

| | | |
|---|---|---|
| | | study, not about the systematic review |
| 35 | Disclosure of funding source | 2 |

*Checklist from*: Stroup DF, Berlin JA, Morton SC, et al, for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. Meta-analysis of Observational Studies in Epidemiology. A Proposal for Reporting. *JAMA*. 2000;283(15):2008-2012. doi: 10.1001/jama.283.15.2008.

Transcribed from the original paper within the NEUROSURGERY® Editorial Office, Atlanta, GA, United Sates. August 2012.

°App, Appendix

* as we performed no meta-analysis, we did not