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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

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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 15th, 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

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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 15th, 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

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(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**).

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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

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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).

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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

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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)	

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		(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)

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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)

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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)

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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 χ^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 χ^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

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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

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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

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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)

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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)

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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)

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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 - Selected study population (consecutive referrals) - Uncommon definition of hypothyroidism (usually TSH >4.5 mU/l) and hyperthyroidism (usually TSH <0.5mU/l) - No adjustment for potential confounders

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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) - Definition of hypothyroidism is not reported - No adjustment for potential confounders - 9/31 hypothyroid patients were selected for thyroxine treatment and Hb improved 1.6g/dl on average - It is reported that 21 patients with hypothyroidism and anemia had no other reason for anemia. It remains unclear

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						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 - Small number of participants with SHypo - 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 - Small number of participants with OHypo and SHypo - Not reported that patients with hyperthyroidism were

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			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 - 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 - Unclear how controls were selected (except for age- and sex-matching) - Uncommon

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						TSH ref. range (0.35 – 3.6 mU/l) - No adjustment for potential confounders (except for age- and sex-matching) - 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 departmen t 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

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		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 - No community controls (persons attending outpatient clinic) - No adjustment for potential confounders - 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 - No community controls (persons attending outpatient clinic) - No adjustment

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		untreated OHypo 100 treated OHypo 110 untreated SHypo 110 treated SHypo 180 age- and sex-matched euthyroid controls			12.0g/dl 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: 108 OHypo 123 SHypo 209 age- and sex-matched	OHypo (TSH above and fT4 below ref. range) SHypo (TSH above and fT4 within ref. range) 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) SHypo: Hb 12.7g/dl (p<0.001), prevalence of anemia 46.8% (p=0.018) Euthyroidism (ref.	- Unclear how cases were "randomly" selected - No community controls (persons attending outpatient clinic) - No ref. ranges of thyroid hormones - 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)

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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

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						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

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						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

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				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

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					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 <0.10mUl/l Hypothyroidism TSH >5.0mUl/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

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					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-Berasteguio, 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-

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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

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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 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) 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

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		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

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				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
Dharmaraj an MD,	Case Report	-	No clear definition	Anemia (female	-	Excluded because lack

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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

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

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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 descri ption	No adjustm ent for	Secure record (1	Yes (1 point)	No design ation	2 of m

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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.

Thyroid
Thyroid Dysfunction and Anemia: A Prospective Cohort Study and a Systematic Review (DOI: 10.1089/thy.2017.0480)
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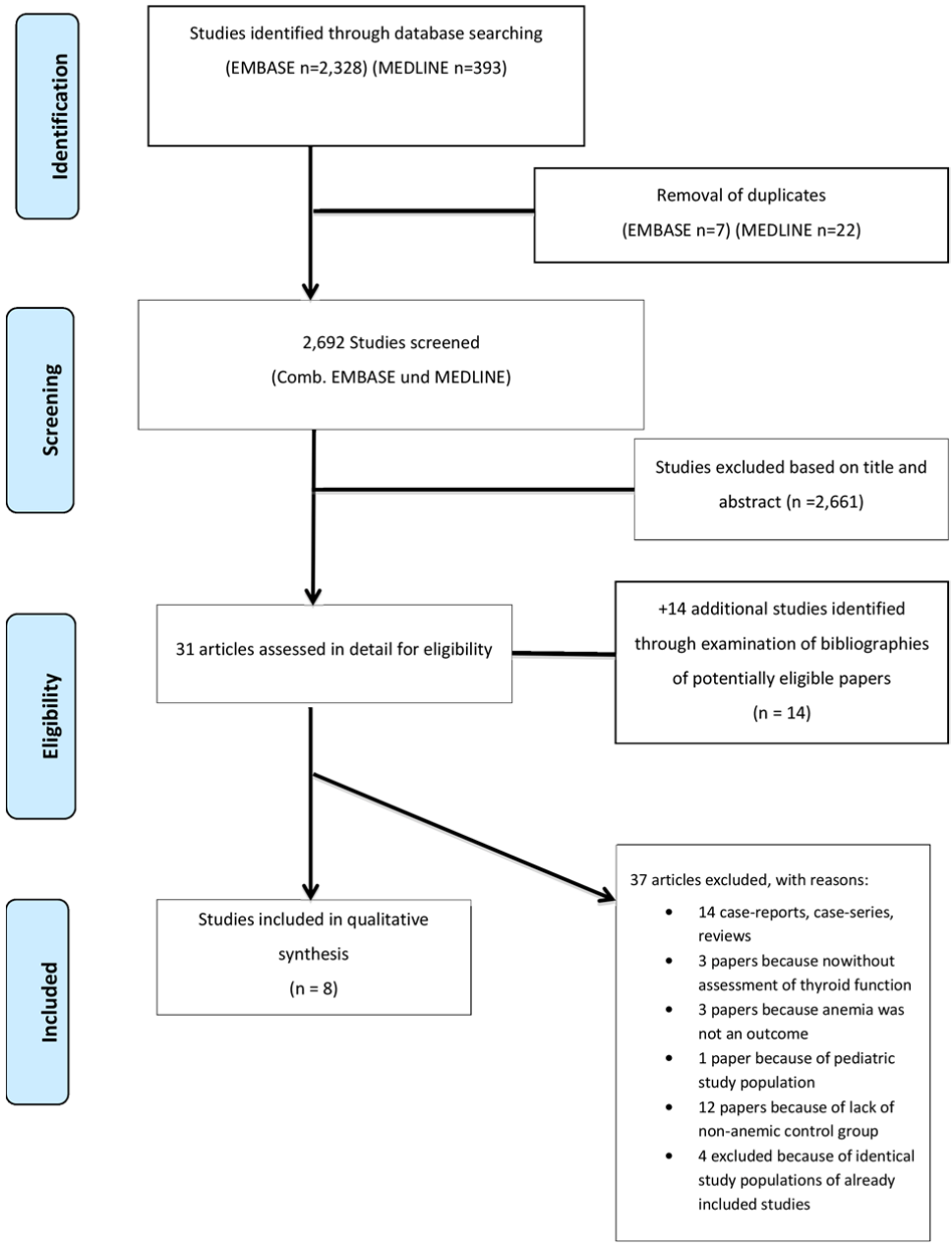
eFigure 1: Flow diagram of the systematic review



eFigure 1: Flow diagram of the systematic review

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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

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

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*

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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.

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°App, Appendix

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

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