Association between thyroid dysfunction and venous thromboembolism in the elderly: a prospective cohort study

Daniel Segna, MD [1] (daniel.segna@insel.ch), Marie Méan, MD [1,2] (marie.meanpascual@insel.ch), Andreas Limacher, PhD, MAS, MSc [3] (andreas.limacher@ctu.unibe.ch), Christine Baumgartner, MD [1] (christine.baumgartner@insel.ch), Manuel R. Blum, MD [1] (manuelraphael.blum@insel.ch), Jürg-Hans Beer, MD [4] (juerg-hans.beer@ksb.ch), Nils Kucher, MD [5] (nils.kucher@insel.ch), Marc Righini, MD [6] (marc.righini@hcuge.ch), Christian M. Matter, MD [7] (christian.matter@usz.ch) , Beat Frauchiger, MD [8] (beat.frauchiger@stgag.ch), Jacques Cornuz, MD, MPH [9] (jacques.cornuz@chuv.ch), Markus Aschwanden, MD [10] (markus.aschwanden@usb.ch), Martin Banyai, MD [11] (martin.banyai@luks.ch), Joseph Osterwalder, MD, MPH [12] (joseph.osterwalder@kssg.ch), Marc Husmann, MD [13] (marc.husmann@usz.ch), Michael Egloff, MD [14] (michael.egloff@hcuge.ch), Daniel Staub, MD [10] (daniel.staub@usb.ch), Bernhard Lämmle, MD [15, 16] (bernhard.laemmle@uni-mainz.de), Anne Angelillo-Scherrer, MD [15, 17] (anne.angelillo-scherrer@insel.ch), Drahomir Aujesky, MD, MSc [1] (drahomir.aujesky@insel.ch), Nicolas Rodondi, MD, MAS [1] (nicolas.rodondi@insel.ch)

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

[2] Service of Internal Medicine, University Hospital of Lausanne, Lausanne, Switzerland.

[3] CTU Bern, Department of Clinical Research, and Institute of Social and Preventive Medicine (ISPM), University of Bern, Switzerland.

[4] Department of Internal Medicine, Cantonal Hospital of Baden, Baden, Switzerland.

[5] Division of Angiology, Bern University Hospital, Bern, Switzerland.

[6] Division of Angiology and Hemostasis, Geneva University Hospital, Geneva, Switzerland.

[7] Cardiology, University Heart Center, University Hospital and University of Zurich, Zurich, Switzerland.

[8] Department of Internal Medicine, Cantonal Hospital of Frauenfeld, Frauenfeld, Switzerland.

[9] Department of Ambulatory Care and Community Medicine, University of Lausanne, Lausanne, Switzerland.

[10] Division of Angiology, Basel University Hospital, Basel, Switzerland.

[11] Division of Angiology, Cantonal Hospital of Lucerne, Lucerne, Switzerland.

[12] Emergency Department, Cantonal Hospital of St. Gallen, St. Gallen, Switzerland.

[13] Division of Angiology, Zurich University Hospital and University of Zurich, Zurich, Switzerland.

[14] Division of Endocrinology, Diabetology, Hypertension and Nutrition, Geneva University Hospitals, Geneva, Switzerland.

[15] University Clinic of Hematology and Central Hematology Laboratory, Bern University Hospital, Bern, Switzerland.

[16] Center for Thrombosis and Hemostasis, University Medical Center, Mainz, Germany

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

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Corresponding author:

Prof. Nicolas Rodondi, MD, MAS,

Department of General Internal Medicine,

Inselspital, University Hospital of Bern,

3010 Bern, Switzerland;

Tel: +41 (0)31 632 41 63; Fax: +41 (0)31 632 88 85;

Email: Nicolas.Rodondi@insel.ch

Essentials:

- Subclinical thyroid dysfunction (SCTD) has been related to hypercoagulability.

- We studied its association with recurrent venous thromboembolism (rVTE).

- Subclinical hyperthyroidism, but not -hypothyroidism, may be associated with lower rVTE risk.

- SCTD is not associated with mortality/differences in thrombophilic biomarkers.

Summary:

Background: Venous thromboembolism (VTE) and subclinical thyroid dysfunction (SCTD) are both common in elderly patients. SCTD has been related to a hypercoagulable state and increased thromboembolic risk. However, prospective data on the relationship between SCTD and VTE are lacking.

Objectives: To investigate the relationship between SCTD and recurrent VTE (rVTE), all-cause mortality, and thrombophilic biomarkers.

Patients: Elderly participants with VTE.

Methods: In a prospective multicenter cohort, thyroid hormones and thrombophilic biomarkers were measured 1 year after acute VTE, as both may be influenced by acute thrombosis. We defined subclinical hypothyroidism (SHypo) as elevated thyroid stimulating hormone levels (TSH=4.50-19.99 mIU/l), and subclinical hyperthyroidism (SHyper) as TSH<0.45, both with normal free thyroxine levels. Outcomes were incidence of rVTE and overall mortality during follow-up starting after the 1-year blood sampling.

Results: Of 561 participants (58% with anticoagulation), 6% had SHypo and 5% SHyper. After 20.8 months of mean follow-up, 9% developed rVTE and 10% died. rVTE incidence rate was 7.2 (95% confidence interval:2.7–19.2) per 100 patient-years in SHypo, 0.0 (0.0-7.6) in SHyper and 5.9 (4.4–

7.8) in euthyroid participants. In multivariate analyses, the sub-hazard ratio [SHR] for rVTE was 0.00 (0.00-0.58) in SHyper and 1.50 (0.52–4.34) in SHypo compared to euthyroids, without increased thrombophilic biomarkers. SHyper (HR 0.80,0.23–2.81) and SHypo (HR 0.99,0.30–3.29) were not associated with mortality.

Conclusion: In elderly patients, SHyper may be associated with lower rVTE risks. SHypo showed a non-statistically significant pattern of an association with rVTE, without increased mortality or differences in thrombophilic biomarkers.

Introduction

Acute venous thromboembolism (VTE), defined as deep vein thrombosis (DVT) or pulmonary embolism (PE), is a major health problem. In 2004, approximately 370,000 disease-related deaths were reported in a study population from six European countries [1] with an all-cause mortality rate of 9.9% in the first month after an index VTE in older adults [2]. VTE is mainly a disease of the elderly [3] with an incidence between 2.8 and 4.1 cases per 1000 person-years in patients older than 65 years [4], leading to important financial and health care issues [5]. VTE may have an increased mortality rate with rising age, as shown among 14,721 hospitalized patients with PE [6] with a relative risk (RR) of 8.48 for death (95% confidence interval [CI] 4.03-17.90) in patients older than 80 years compared to patients below 50 years. Recurrent VTE (rVTE) occurs frequently in up to 4.9 cases per 100 patients-years [7] with an increasing incidence in the elderly [8].

Similar to thromboembolic events, subclinical thyroid dysfunction (SCTD) is a common condition in this population with a higher prevalence upon increasing age [9]. The prevalence of subclinical hypothyroidism (SHypo) reaches up to 10% in the elderly population, while subclinical hyperthyroidism (SHyper) is a less common condition with a prevalence between 0.7 and 3.2% [10], and 4.6% in those ≥ 80 years [11]. Several case-control studies reported an increased thrombophilic risk profile in patients with overt and subclinical hyperthyroidism (SHyper) by enhancing a hypercoagulable and hypofibrinolytic state due to elevated levels of factor VIII (FVIII), factor IX

(FIX), von Willebrand factor (vWF:Ag), fibrinogen, and plasminogen activator inhibitor (PAI-1) [12, 13]. Evidence suggests that hypothyroidism may have a biphasic effect on fibrinolysis, depending on disease severity [14, 15]. For instance, a prospective study on 76 middle-aged women found an increased fibrinolytic activity in severe hypothyroidism, while patients with moderate hypothyroidism (thyroid stimulating hormone [TSH] 10-50 mIU/l) vs. euthyroids may be subject to an increased thrombophilic risk mediated by a decreased fibrinolytic activity with lower D-dimer levels, higher alpha(2)-antiplasmin activities, and higher levels of tissue plasminogen activator (t-PA) and PAI-1. Similarly, patients with SHypo had increased FVII activity [16, 17], high-sensitive C-reactive protein (hs-CRP) [18], PAI-1, fibrinogen, and reduced plasma levels of antithrombin (AT) [17] compared to healthy controls, all potentially contributing to a thrombophilic state. SHypo has been associated with an increased risk of coronary heart disease and mortality [19], whereas the impact of SCTD on the development of VTE remains unclear. A cross-sectional study including 150 patients with or without VTE found a higher prevalence of SHypo in patients with unprovoked VTE compared to euthyroids (Odds' Ratio [OR] 6.8, 95% CI 0.7-64.5) and patients with provoked VTE (OR 5.54, 95% CI 0.6–52.6) [20]. However, due to low case numbers and the cross-sectional study design, prospective cohort studies are needed. Only one recent prospective cohort study found that TSH levels below or above normal range were non-significantly associated with a moderately increased risk for VTE. However, this study was limited by the absence of free thyroxin (fT4) measurements, and could therefore not differentiate between overt and subclinical forms of thyroid dysfunction [21]. As no prospective data on SCTD and rVTE are currently available, we aimed to investigate a possible association between SCTD and rVTE as well as all-cause mortality in a large cohort of elderly patients with VTE. Our second objective was to evaluate whether patients with SCTD and an index VTE showed an increased thrombophilic risk profile compared to euthyroid patients.

Methods

Cohort Sample:

This study was conducted between September 2, 2009 and December 6, 2013 as part of the Swiss Cohort of Elderly Patients with Venous Thromboembolism (SWITCO65+), a prospective multicenter cohort study that assessed long-term medical outcomes in elderly patients with acute VTE from all five university and four high-volume non-university hospitals in Switzerland. As previously published in detail [22], participants aged ≥65 years with an acute, objectively confirmed VTE were prospectively identified in the in- and outpatient services of all participating study sites. Exclusion criteria were catheter-related thrombosis, insufficient German or French-speaking ability, no follow-up possible (i.e., terminal illness), an inability to provide informed consent (i.e., severe dementia), or previous enrollment in the cohort. The study was approved by the Institutional Review Board at each participating center.

From 1003 participants in the SWITCO65+ cohort study, we only considered those who had survived the first 12 months with a blood sample drawn at 12 months after the index VTE, since TSH/fT4 levels may intermittently change due to an acute non-thyroidal illness [23-25], such as VTE. During the first year after baseline, 40 participants withdrew consent, 113 died and 1 was lost to follow-up. TSH and/or fT4 values were not available in another 274 participants 1 year after index VTE for various reasons (no permission by the participant, difficult venipuncture, unanalyzable blood tubes, laboratory error). After categorization into thyroid function groups, 3 participants with overt hyperthyroidism and 11 with overt hypothyroidism were excluded from analyses due to small numbers, leaving 561 participants for our study sample.

Data collection:

At baseline, demographic data, comorbidities and laboratory findings as well as VTE-related treatments of all enrolled patients were assessed according to a standardized data collection form by trained study nurses. Follow-up included two surveillance face-to-face evaluations and one telephone interview during the first year of study participation with subsequent semi-annual contacts, alternating

between face-to-face evaluations (clinic visits or home visits in house-bound patients) and telephone calls as well as periodic reviews of the patient's hospital chart. During each visit/contact, study nurses interviewed patients to obtain information about the date and type of clinical events (recurrent VTE, death). If a clinical event had occurred, this information was complemented by reviewing medical charts and interviewing patients' primary care physicians and family members.

Clinical outcomes

The primary clinical outcome was the recurrence of an objectively confirmed, symptomatic VTE (rVTE), defined as a fatal or new non-fatal PE, or a new DVT [8]. Diagnosis of rVTE during follow-up was established based on the following criteria: for DVT, on the basis of abnormal results on ultrasonography; and for pulmonary embolism, by means of CT or angiography showing new intraluminal defects or on the basis of ventilation-perfusion lung scan showing a high-probability pattern with new perfusion defects. A new proximal DVT, based on abnormal results on ultrasonography, associated with new PE symptom(s) (shortness of breath, chest pain, syncope) was considered as recurrent PE [26].

The secondary clinical outcome was incidence of all-cause mortality during follow-up. Deaths were adjudicated as related, possibly related, or unrelated to PE. Death was judged to be PE-related if confirmed by autopsy, or if death followed a clinically severe PE, either initially or after an objectively confirmed recurrent event. Death in a patient who died suddenly or unexpectedly was classified as possibly PE-related. Unrelated deaths were classified as a result of an obvious cause other than PE [27]. All clinical outcomes were adjudicated by a committee of three blinded clinical experts, and the final decision for diagnosis was based on full consensus.

Laboratory measurements

Blood samples were drawn at baseline (i.e., at the time of VTE diagnosis) and 1 year after index VTE (defining the start of observation time). Samples were immediately centrifuged, frozen, and stored in aliquots at -80°C and secondarily sent for analyses to a core laboratory. All analyses were performed

in study core laboratories and results were not communicated to the patients or their primary care physicians. Specific information about blood collection, storage and laboratory analysis is described elsewhere [28].

Thyroid status was assessed by analyzing serum TSH and fT4 in frozen aliquots in 2014. Thyroid hormone levels were determined by using state-of-the art TSH- and fT4-specific immunoassays named **E**lectro**C**hemi**L**uminescence **I**mmuno**A**ssay (ECLIA, Roche) on a Roche Modular E170 device.

In line with previous large studies [19], euthyroidism was defined as TSH levels between 0.45 and 4.49 mIU/L, SHypo as TSH levels between 4.5 and 19.9 mIU/L, and SHyper as TSH smaller than 0.45 mIU/L , both with fT4 values within normal range (12-22.0 pmol/l).

For the cross-sectional analyses of thrombophilic biomarkers by thyroid status, we included the following laboratory measurements based on previously studied associations with rVTE and/or thyroid dysfunction: D-dimer, measured by an immunoenzymatic assay on a Vidas Biomerieux automated system [29]; fibrinogen, levels of antithrombin, protein C, free protein S, FVIII, FIX, FXI and vWF:Ag on a BCS XP Siemens device [12, 13, 17, 30]; fasting homocysteine level was quantified using an automated high performance liquid chromatography (HPLC) with reverse phase separation and fluorescent detection after the derivation step (with 7-Fluorobenzofurazane-4-sulfonique acid) on a HPLC 1100 from Agilent Technologies.

Participants on oral anticoagulants were excluded from the analyses of Vitamin-K dependent factors, such as FIX and Protein S [31].

Statistical analyses

We compared baseline characteristics and biomarker levels among participant groups with different thyroid function using chi-squared tests and non-parametric Kruskal-Wallis rank tests as appropriate. We present the cumulative incidence of rVTE accounting for non-PE related death as a competing

risk using the method of Coviello and Boggess [32]. For mortality, we present ordinary Kaplan-Meier curves. We calculated incidence rates of a first rVTE and mortality rates for different thyroid function groups and compared survival functions using log-rank tests.

Associations of thyroid function groups and log-transformed TSH values with the time to a first rVTE were examined using competing risk regression according to Fine and Gray [33], accounting for non-PE-related death as a competing event. The method yields sub-hazard ratios (SHR) with corresponding 95% CIs and p values for the failure event of primary interest. We repeated these analyses using cause-specific Cox proportional hazard regression with robust standard errors. For mortality, an ordinary cause-specific Cox-regression with robust standard errors was calculated. In case of no events in a subgroup, we obtained confidence intervals using the profile likelihood method. In order to avoid overfitting in regard to 52 rVTE and 56 death events during observation time, we performed multivariate models with minimal adjustment, adjusting for (1) age and gender, (2) age, gender and rVTE risk factors including: prior VTE [34], active cancer [35], idiopathic VTE [30] and periods of anticoagulation as a time-varying covariate [8], (3) additionally log-transformed fibrinogen, since it has been previously discussed as a mediator between subclinical hyperthyroidism and VTE [36]. There were no missing values in variables of the main analyses (crude and adjusted associations of thyroid function groups and log-transformed TSH values with the time to a first rVTE).

As described above, we started observation time 12 months after index VTE, but conducted sensitivity analyses with: 1) observation time starting at the index VTE; 2) excluding patients with rVTE within 4 weeks before the 1-year blood samples; 3) observation time lasting for 12 months after TSH/fT4 analyses. In order to investigate associations between thrombophilic biomarkers and thyroid status, we compared mean serum levels using analysis of variance (ANOVA) and analysis of covariance (ANCOVA). For this analysis, levels of D-dimer, fibrinogen, protein S, homocysteine, and FVIII were log-transformed due to their skewed, log-normal distribution (Supplementary Table 2). Levels of protein C could not be normalized due to its U-shaped and censored distribution and were thus evaluated non-parametrically using a Kruskal-Wallis rank test. In ANCOVA, minimal adjustment was done for age and gender, full adjustment for age, gender and factors known to be

associated with thrombophilic states, namely smoking status [37], active cancer [38] and diabetes mellitus [39]. There were only a few missing values in thrombophilic biomarkers. We therefore followed a complete case analysis when adjusting for fibrinogen and evaluating thrombophilic biomarkers. All analyses were done using Stata 14 (Stata Corporation, College Station, Texas).

This study is an ancillary study of the SWITCO65+ cohort study, which was powered in order to detect a $\geq$2-fold risk increase of VTE recurrence in patients with compared to patients without a risk factor if the prevalence of the risk factor is at least 10% [22].

Results

Participants

Baseline characteristics are presented in Table 1. Overall, 35 participants (6%) had SHypo, 500 (89%) were euthyroid and 26 (5%) had SHyper. The median TSH level for the entire study population was 1.9 mIU/l (interquartile range 1.2-2.7) with 2 patients showing a TSH$\geq$10 mIU/l. At 12 months after the index VTE, 326 patients (58%) were still receiving anticoagulant treatment, in most instances vitamin K antagonists (56%). The distribution or quality of anticoagulant therapy did not significantly differ at the time of thyroid hormone measurements (Table 1).

Participants with SHyper were more likely to be female and immobilized than SHypo and euthyroid participants. Non-analyzed patients were generally older (76 years, interquartile range [IQR]: 71.0–83.0 vs. 74 years, IQR 69.0-80.0, p<0.01), suffered more frequently from cardiovascular diseases (3% vs. 1%, p=0.02), active cancer (27% vs 11%, p<0.01), as well as immobilization (26% vs. 19%, p<0.01), and comprised more women (55% vs. 41%, p<0.01) than analyzed participants (Supplementary Table 1).

Association between Thyroid Status and rVTE

After a mean follow-up of 20.8 months (standard deviation ± 9.1) starting one year after index VTE, 52 (9.2%) participants had experienced a rVTE, thereof 87% were off anticoagulation. rVTE incidence rates were 7.20 per 100 patient-years (95% CI 2.70–19.19) among participants with SHypo vs. 5.85 per 100 patient-years (95% CI 4.41–7.77) in euthyroid controls, whereas no event occurred among participants with SHyper (Table 2).

In multivariate competing risk analyses, SHyper was significantly associated with lower rVTE (Table 2). When adjusting for age and gender, participants with SHyper had a SHR of 0.00 (95% CI 0.00-0.63, p=0.01), whereas those with SHypo had a sub-hazard ratio [SHR] of 1.23 for rVTE (95% CI 0.42-3.67, p=0.70), compared to euthyroid controls. After further adjustment for known rVTE risk factors, the association between SHyper and rVTE remained significant (Table 2). TSH as a continuous variable was not associated with rVTE (adjusted SHR per log-unit increase: 1.18, 95% CI 0.87–1.60, p=0.28, Table 3). Additional adjustment for fibrinogen, a possible mediator between thyroid function and VTE, yielded fairly the same results. In a sensitivity analysis observing recurrence rate from the time of the index VTE, the SHR was 0.20 (95% CI 0.03–1.56, p=0.13) for SHyper and 1.15 (95% CI 0.45–2.93, p=0.77) for SHypo, as compared to euthyroid controls. Analyses according to TSH quartiles showed no difference in rVTE incidence (p=0.98). Considering the higher rVTE incidence rate in SHypo vs. euthyroidism during the first year (Figure 1, 13.27 vs. 6.56 per 100 patient-years, respectively), we performed a secondary analysis with an observation time limited to the first 12 months after thyroid hormone measurements and found a HR of 2.50 (95% CI 0.89-7.00, p=0.08) for the association between SHypo and rVTE, as compared to euthyroid participants. Overall, we obtained similar results using Cox regression for the cause specific hazards.

Association between Thyroid Status and Thrombophilic Biomarkers

The vWF antigen was the sole thrombophilic biomarker which significantly differed between thyroid groups in crude ANOVA (p<0.01), as well as in age-and sex-adjusted ANCOVA (p=0.03), but this association was weaker after fully adjusted ANCOVA (p=0.05, Table 4). The highest mean plasma

concentration was found in SHyper (243%, 95% CI 186-301), followed by subclinically hypothyroid (213%, 95% CI 191-235) and euthyroid (198%, 95% CI 192-205) participants, but differences were only statistically significant between those with SHyper and euthyroidism (p=0.02; SHypo vs euthyroids: p=0.32). Likewise, the FVIII/vWF:Ag ratio varied significantly between SHypo, SHyper and euthyroidism, but after adjusting for smoking, diabetes mellitus and active cancer, it was only significantly different for SHyper compared to euthyroids (p=0.04; SHypo vs euthyroid: p=0.18). Median and interquartile ranges of thrombophilic biomarker levels by thyroid status are presented in Supplementary Table 2.

Mortality by Thyroid Status

During a mean observation time of 20.8 months, 56 (10.0%) participants died. Mortality did not differ by thyroid status (Figure 2). The highest mortality was observed in participants with SHyper (6.2 per 100 patient-years, 95% CI 2.0-19.2) followed by euthyroids (5.8 per 100 patient-years, 95% CI 4.4-7.7) and participants with SHypo (5.1 per 100 patient-years, 95% CI 1.6–15.8). Thyroid status and TSH as continuous variable were not associated with mortality in multivariate models (Tables 2, 3).

Discussion:

In this prospective multicenter cohort study of elderly VTE patients, SHyper was associated with a lower rVTE risk. On the other hand, SHypo was not significantly associated with an increased rVTE risk, although there was a pattern thereof, particularly during the first year of follow-up. Moreover, SCTD was neither associated with mortality nor increased levels of several thrombophilic biomarkers, except for vWF:Ag which was borderline significantly increased in SHyper.

Data on SCTD and VTE are limited. A recently published prospective cohort study on 11,900 subjects aged 25-89 years [21] found a non-significantly increased risk for rVTE among both patients with elevated and decreased TSH values compared to healthy controls (HRs: 1.55, 95% CI 0.87-2.77 and 2.16, 95% CI 0.69-6.76, respectively). Results of this previous study were limited by the absence of fT4 measurements, so that the authors could not discriminate between overt and subclinical forms of thyroid dysfunction, and confidence intervals were rather wide. In contrast to these findings, we found

a potentially lower rVTE risk in SHyper vs. euthyroids. Squizzato et al. [20] investigated the association between DVT and SHypo/SHyper in a pilot case-control study of 150 patients, comparing groups with unprovoked DVT, provoked DVT and healthy controls. The prevalence of SHypo was higher (OR 5.54; 95% CI: 0.6-52.6) in patients with unprovoked DVT (14.0%), as compared to healthy subjects (2%) and those with provoked DVT (1%), but results were limited by the cross-sectional design, large confidence intervals. Furthermore, we could not corroborate the hypothesis of a hypercoagulable or hypofibrinolytic state in those patients in our analysis of thrombophilic biomarkers. In comparison, the present prospective cohort included also PE and was 3 to 4-fold greater in sample size.

Previous findings on thrombophilic biomarkers in SCTD were mostly obtained from case-control studies with low sample sizes (i.e. 65 to 156 patients) [17, 36] and thus may be subject to substantial selection bias. In our large prospective study, we found no increase in most thrombophilic biomarkers among participants with SCTD compared to euthyroid controls. For example, we did not detect an increased concentration of fibrinogen in contrast to previous studies [40]. Moreover, D-dimer levels resulted to be fairly the same across all thyroid function groups (p=0.98). These findings do not support the concept of a hypercoagulable state, and an increased or decreased fibrinolysis in participants with SCTD. We generally observed increased vWF:Ag concentrations, whereas SHyper, but not SHypo participants had significantly higher vWF:Ag levels than euthyroid controls. Similarly, the association between vWF:Ag levels and SHyper has previously been shown in a meta-analysis of observational studies among patients with SHyper and overt hyperthyroidism (standardized mean difference, i.e. the difference between the group means divided by the pooled standard deviation: 2.9, 95% CI 1.4-3.3) compared to euthyroids [12]. In line with our findings, a case-control study found higher mean concentrations of vWF in SHyper than in controls (111.8 vs. 94.2 ng/ml) but this difference was not statistically significant [36]. The pathogenic role of vWF as a thrombophilic risk factor has been studied in knock-out mice models [41]. However, there are conflicting data regarding the role of vWF in the development of VTE in humans, independently from increased FVIII concentrations [42, 43]. In our analyses, FVIII concentrations were within normal range and an

increased FVIII/vWF ratio as an indicator for higher levels of unbound FVIII [44] could not be demonstrated.

Our study has several strengths. First, to our knowledge, this is the first prospective cohort study investigating the relationship between SCTD and rVTE. Second, rVTE was adjudicated by a blinded expert committee using clearly defined objective criteria, reducing the risk of detection bias. Finally, both in- and outpatients with index VTE were included in a nationwide multicenter setting, leading to an increased generalizability.

On the other hand, there were several limitations. First, there were relatively few participants and rVTE events in the SHypo and SHyper group, leading to rather wide confidence intervals. The Kaplan-Meier curves and multivariate analyses indicated that patients with SHypo had an increased risk of rVTE, but confidence intervals of sub-hazard ratios were rather wide and associations not statistically significant. Although we could identify a significant association between SHyper and a lower rVTE risk in our cohort, caution should be taken before definitive conclusion, because patient numbers were low for SHyper and a possible chance-finding cannot be excluded. However, prevalence of SHyper was higher in the present cohort (5%) compared to 0.7- 3.2% described in literature [10]. Second, we planned to investigate a dose-dependent effect of TSH on rVTE risk in stratified analyses, including assessment of the impact of higher TSH concentrations, but these analyses could not be performed owing to no rVTE event in this subgroup of only 2 participants with TSH between 10-19.9 mIU/l. Consistently, stratified analyses for provoked/unprovoked index VTE and its subtypes could not be performed due to few vs. no rVTE events in SHypo vs. SHyper, respectively. Third, any acute non-thyroidal illness [23-25], such as VTE and related radiologic procedures involving iodinated contrast media [45], may temporarily influence thyroid function. Therefore, we measured thyroid hormones 1 year after the index VTE and obtained comparable results from a sensitivity analysis excluding participants with rVTE within one month before blood sampling. Since our observation time started 12 months after the index VTE, we could not assess the association between SCTD and short-term risk of rVTE.

Fourth, the SWITCO65+ study was not specifically designed for thyroid disease. Thus, history of thyroid disease or change in thyroid status were not assessed. SHypo has an annual rate of 3-4.3% for spontaneous progression to overt thyroid dysfunction [46], whereas 15 to 62% revert to euthyroidism over follow-up periods of one to six years [47]. SHyper similarly progresses to overt disease in 1-2% of affected individuals per year [48]. Finally, as thyroid hormone measurements were retrospectively obtained from the SWITCO65+ biobank, information on thyroid medication use was not available and we could not exclude those patients, which may have decreased the association between SHypo and rVTE. As laboratory results were neither communicated to participants nor to treating physicians due to unclear evidence for benefit of treatment, new prescription of thyroid medication was likely low during follow-up; based on a survey at the two largest study sites (Bern, Lausanne), only 3 patients with SHypo received thyroid hormone replacement therapy until the end of follow-up. According to previous prospective cohorts on SCTD, the incidence of new thyroid drug users ranged between 0% to 3.3% in studies with mean follow-up durations of 2 to 5 years [19]. Despite a higher incidence rate of rVTE among SHypo vs. euthyroidism in the first 1 year after TSH measurement (Figure 1), we did not find an initially significant association with rVTE, which may have been washed out by newly prescribed thyroid replacement therapy during follow-up.

Conclusions

In this prospective multicenter cohort study, SHyper was associated with a decreased recurrence risk in elderly VTE patients. SHypo showed a non-statistically significant pattern of an association with rVTE, especially in the first year of follow-up. SCTD was neither associated with mortality nor increased levels of several thrombophilic biomarkers, except for vWF:Ag which was borderline significantly increased in SHyper.

Future research projects should verify our results, particularly in younger populations (requiring larger study samples or longer observation times), and additionally assess a possible dose-dependent effect of TSH on coagulation parameters and clinical outcomes, which would need larger cohort studies with

a higher prevalence of SHypo. Finally, to definitively prove a causal relationship between thyroid replacement therapy and rVTE, randomized controlled trials would be needed.

Addendum

D. Segna, M. Méan, A. Limacher and N. Rodondi were responsible for study design and statistical analyses; D. Segna, M. Méan and N. Rodondi wrote the manuscript; D. Aujesky, N. Rodondi, B. Lämmle, M. Righini, H.-J. Beer, B. Frauchiger, J. Osterwalder, N. Kucher and A. Angelillo-Scherrer collected data and obtained funding from the Swiss National Science Foundation; B. Lämmle, M. Righini, J. Cornuz, H.-J. Beer, B. Frauchiger, J. Osterwalder, N. Kucher, A. Angelillo-Scherrer, M.R. Blum, C. Baumgartner, C.M. Matter, M. Banyai, M. Egloff, D. Staub, M. Aschwanden and M. Husmann critically reviewed the manuscript.

Disclosure of Conflict of Interests

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Tables

Table 1: Patient Characteristics

	All	Subclinical hypothyroidism TSH 4.50-19.99 mIU/l, normal fT4	Euthyroidism TSH 0.45-4.49 mIU/l, normal fT4	Subclinical hyperthyroidism TSH<0.45 mIU/l, normal fT4	p
Total N	**561 (100%)**	**35 (6%)**	**500 (89%)**	**26 (5%)**	
Demographics					
Gender (female)	229 (41%)	14 (40%)	196 (39%)	19 (73%)	**0.003**
Age, years	74 (69.0; 80.0)	75.0 (69.0; 81.0)	74.0 (68.0; 79.0)	79.0 (73.3; 84.3)	0.059
Clinical characteristics					
Obesity (BMI ≥30 kg/m^2)	152 (27%)	11 (31%)	133 (27%)	8 (31%)	0.751
Diabetes mellitus	93 (17%)	8 (23%)	80 (16%)	5 (19%)	0.535
Arterial hypertension	364 (65%)	25 (71%)	319 (64%)	20 (77%)	0.277
History of cardiovascular and cerebrovascular disease [a]	6 (1%)	0 (0%)	5 (1%)	1 (4%)	0.317
Current smoking	39 (7%)	1 (3%)	37 (7%)	1 (4%)	0.482
Active cancer [b]	63 (11%)	2 (6%)	57 (11%)	4 (15%)	0.465
Immobilization [b]	104 (19%)	5 (14%)	88 (18%)	11 (42%)	**0.005**
Oestrogen replacement therapy [b]	16 (3%)	2 (6%)	13 (3%)	1 (4%)	0.537
Prior VTE	166 (30%)	18 (51%)	139 (28%)	9 (35%)	0.011
Postthrombotic syndrome *	201 (36%)	14 (40%)	173 (35%)	14 (54%)	0.119
Any acute rheumatic disease [b]	22 (4%)	1 (3%)	19 (4%)	2 (8%)	0.575
Inflammatory bowel disease	20 (4%)	0 (0%)	20 (4%)	0 (0%)	0.282
History of major surgery [b]	87 (16%)	4 (11%)	75 (15%)	8 (31%)	0.076
Anticoagulation at the time of index VTE					
Initial anticoagulation	558 (99%)	35 (100%)	498 (100%)	25 (96%)	0.057
Initial anticoagulation < 3 months	48 (9%)	2 (6%)	40 (8%)	6 (23%)	**0.023**
Parenteral anticoagulation	544 (97%)	35 (100%)	484 (97%)	25 (96%)	0.548
Oral anticoagulation	522 (93%)	34 (97%)	467 (93%)	21 (81%)	**0.029**
Concomitant antiplatelet therapy	177 (32%)	11 (31%)	158 (32%)	8 (31%)	0.996
Percent time in therapeutic range (between index VTE and time of thyroid hormone measurements)	65.3 (47.2; 80.9)	55.9 (37.9; 74.5)	66.8 (47.7; 81.2)	55.2 (38.7; 65.7)	**0.048**
Duration of initial anticoagulation (months)	11.1 (5.9; 29.7)	23.2 (6.4; 30.5)	10.1 (5.9; 29.6)	6.7 (3.2; 29.8)	0.214
Anticoagulation at the time of thyroid hormone measurements *					
Any anticoagulation	326 (58%)	23 (66%)	289 (58%)	14 (54%)	0.593
Parenteral anticoagulation	20 (4%)	0 (0%)	19 (4%)	1 (4%)	0.502
Oral anticoagulation	314 (56%)	23 (66%)	277 (55%)	15 (54%)	0.481
Percent time in therapeutic range (between thyroid hormone measurement and 1 year thereafter)	71.1 (53.7; 88.1)	69.2 (47.2; 83.4)	72.5 (55.1; 89.1)	50.9 (26.7; 88.3)	0.100

Variables recorded at the time of index venous thromboembolic event (VTE), if not otherwise indicated.

Values given as total number (N, %) or median (interquartile range). BMI= Body Mass Index; *p*= p-value

There were missing values in fibrinogen (7%) and hsCRP (7%).

[a] defined as patient-reported myocardial infarction/stroke during the last 3 months

[b] within 3 months before index VTE.

[*] Variables recorded at the time of thyroid hormone measurement 1 year after index VTE

Table 2:

Association of Thyroid Status with Recurrent Venous Thromboembolisms (VTE) and All-Cause Mortality

	Subclinical hypothyroidism TSH 4.50 - 19.99 mIU/l, normal fT4	p	Euthyroidism TSH 0.45 - 4.49 mIU/l, normal fT4	p	Subclinical hyperthyroidism TSH < 0.45 mIU/l, normal fT4	Overall p
Total N	**35 (6%)**		**500 (89%)**		**26 (5%)**	
Recurrent VTE						
Number of events/person-years	4/55.5		48/820.1		0/48.4	
Incidence rate per 100 person-years (95% CI)	7.20 (2.70 - 19.19)		5.85 (4.41 - 7.77)		0.00 (0.00 - 7.63)*	
Crude SHR (95% CI)	1.24 (0.43 - 3.57)	0.70	reference	0.02	0.00 (0.00 – 0.71)[a]	0.06
Age-and sex-adjusted SHR (95% CI)	1.23 (0.42 - 3.67)	0.70	reference	0.01	0.00 (0.00 – 0.63)[a]	0.04
Multivariate-adjusted SHR (95% CI)[b]	1.50 (0.52 - 4.34)	0.46	reference	0.01	0.00 (0.00 – 0.58)[a]	0.02
Fibrinogen-adjusted SHR (95% CI)[c]	1.49 (0.51 - 4.33)	0.46	reference	0.01	0.00 (0.00 – 0.57)[a]	0.02
All-cause mortality						
Number of events/person-years	3/59.0		50/862.8		3/48.4	
Incidence rate per 100 person-years (95% CI)	5.08 (1.64 - 15.76)		5.80 (4.39 - 7.65)		6.20 (2.00 - 19.23)	
Crude HR (95% CI)	0.87 (0.27 - 2.84)	0.82	reference	0.91	1.08 (0.32 - 3.63)	0.96
Age-and sex-adjusted HR (95% CI)	0.77 (0.23 - 2.60)	0.67	reference	0.98	0.98 (0.29 - 3.35)	0.90
Multivariate-adjusted HR (95% CI)[b]	0.99 (0.30 - 3.29)	0.98	reference	0.73	0.80 (0.23 - 2.81)	0.93
Fibrinogen-adjusted HR (95% CI)[c]	0.88 (0.27 - 2.86)	0.83	reference	0.90	0.92 (0.26 - 3.31)	0.91

Sub-hazard and hazard ratios (SHR and HR) and incidence rates given with 95% confidence interval (95% CI). The overall p-value is from a likelihood ratio test. *p* = p-value. VTE: venous thromboembolism defined as any event of deep vein thrombosis and/or pulmonary embolism

[a] Confidence intervals from profile-likelihood method and p-value from likelihood-ratio test in case of zero events.

[b] adjusted for age, sex, prior VTE, active cancer, idiopathic VTE, and periods of anticoagulation as a time-varying covariate.

[c] adjusted for fibrinogen as continuous log-transformed variable, age, sex, prior VTE, active cancer, idiopathic VTE, and periods of anticoagulation as a time-varying covariate.

* Exact confidence interval based on a Poisson distribution

Table 3:

Association of Log-transformed TSH with Recurrent Venous Thromboembolisms (VTE) and All-Cause Mortality

	n/N	SHR or HR (95% CI)	*p*
Recurrent VTE			
Crude (95% CI)	52/561	1.10 (0.84-1.45)	0.48
Age-and sex-adjusted (95% CI)	52/561	1.13 (0.85-1.51)	0.39
Multivariate-adjusted (95% CI)[a]	52/561	1.18 (0.87-1.60)	0.28
Fibrinogen adjusted (95% CI)[b]	52/558	1.19 (0.87-1.61)	0.28
All-cause-mortality			
Crude (95% CI)	56/561	0.83 (0.64-1.08)	0.17
Age-and sex-adjusted (95% CI)	56/561	0.84 (0.64-1.11)	0.22
Multivariate-adjusted (95% CI)[a]	56/561	0.88 (0.66-1.18)	0.41
Fibrinogen-adjusted (95% CI)[b]	56/558	0.85 (0.64-1.14)	0.28

N: number of patients, n: number of events, HR: hazard ratio, SHR: sub-hazard ratio, CI: confidence interval, *p*= p-value.

SHR and HR are expressed per one log-unit increase. VTE: venous thromboembolism defined as any event of deep vein thrombosis and/or pulmonary embolism

[a] adjusted for age, sex, prior VTE, active cancer, idiopathic VTE, and periods of anticoagulation as a time-varying covariate.

[b] adjusted for fibrinogen as continuous log-transformed variable, age, sex, prior VTE, active cancer, idiopathic VTE, and periods of anticoagulation as a time-varying covariate.

Table 4:

Association between Thrombophilic Biomarkers and Thyroid Status 1 year after index VTE

	Subclinical hypothyroidism TSH 4.5-19.99 mIU/l, normal fT4		Euthyroidism TSH 0.45-4.49 mIU/l, normal fT4		Subclinical hyperthyroidism TSH<0.45 mIU/l, normal fT4		*p* 1st adjustment	*p* 2nd adjustment
vWF:Ag (%)	213	(191-235)	198	(192-205)	243	(186-301)	0.03	0.05 [a]
D-dimer [§]	6.60 (6.31-6.89)		6.54 (6.47-6.61)		6.71 (6.46-6.96)		0.97	0.98
Fibrinogen [§]	1.26 (1.19-1.34)		1.21 (1.19-1.23)		1.24 (1.14-1.34)		0.55	0.52
Protein S [§] [#]	4.59 (4.46-4.72)		4.48 (4.45-4.52)		4.57 (4.52-4.63)		0.13	0.17
Antithrombin (%)	101	(95-106)	101 (99-102)		104	(98-110)	0.85	0.89
Homocysteine [§]	2.73 (2.61-2.85)		2.64 (2.60-2.68)		2.61 (2.43-2.78)		0.59	0.59
Factor VIII [§]	5.00 (4.90-5.10)		5.00 (4.97-5.03)		4.91 (4.78-5.04)		0.19	0.15
Factor IX (%) [#]	128 (123-133)		117 (115-119)		115 (101-130)		0.26	0.28
Factor XI (%)	110	(105-116)	108	(106-109)	115	(105-124)	0.32	0.38
Factor VIII/ vWF:Ag ratio	0.77	(0.69-0.85)	0.84 (0.81-0.86)		0.71	(0.58-0.84)	0.04	0.05 [b]

There were missing values in fibrinogen (7%) and hsCRP (7%). Missingness for other variables was below 1%.

Values given as means and 95% confidence interval. *p*= p-value, vWF:Ag: von Willebrand factor antigen; VTE: venous thromboembolism defined as any event of deep vein thrombosis and/or pulmonary embolism. § log-transformed for ANOVA/ANCOVA analysis due to skewed log-normal distribution; # Participants on oral anticoagulants were excluded from these analyses.

1st adjustment: adjusting for age and gender, 2nd adjustment: further adjusting for smoking status, active cancer, diabetes mellitus

[a] Pairwise p-values from ANCOVA analyses after 2nd adjustment for vWF:Ag:

subclinical hyperthyroidism vs. euthyroidism, p=0.02, subclinical hypothyroidism vs. euthyroidism, p=0.32

[b] Pairwise p-values from ANCOVA analyses after 2nd adjustment for FVIII/ vWF:Ag ratio:

subclinical hyperthyroidism vs. euthyroidism, p=0.04, subclinical hypothyroidism vs. euthyroidism, p=0.18