Increasing levels of free thyroxine as a risk factor for a first venous thrombosis: a case-control study

Bregje van Zaane,[1,2] Alessandro Squizzato,[2,3] Roeland Huijgen,[2] Anton P. van Zanten,[4] Eric Fliers,[5] Suzanne C. Cannegieter,[6] Harry R. Büller,[2] Victor E. A. Gerdes,[1,2] and Dees P. M. Brandjes[1]

[1]Department of Internal Medicine, Slotervaart Hospital, Amsterdam, The Netherlands; [2]Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [3]Department of Clinical Medicine, University of Insubria, Varese, Italy; [4]Department of Clinical Biochemistry, Slotervaart Hospital, Amsterdam, The Netherlands; [5]Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; and [6]Department of Clinical Epidemiology, Leiden University Medical Center, University of Leiden, Leiden, The Netherlands

A hypercoagulable state exists in hyperthyroidism, but the association with venous thrombosis (VT) is not fully explored. We aimed to investigate VT risk for different plasma levels of thyroid hormones and thyroid antibodies. We used a case-control study on leg vein thrombosis conducted between September 1999 and August 2006 at the Academic Medical Center, Amsterdam, The Netherlands. Parameters of thyroid function were assessed in 190 cases (mean age, 57 years; range, 19-90 years) and 379 sex-matched controls (mean age, 56 years; range, 18-93 years). Odds ratios (ORs) and 95% confidence intervals (CIs) for VT risk were estimated according to several cutoff levels derived from plasma levels observed in controls. We found the risk of VT to gradually rise with increasing levels of free thyroxine (FT_4). In the absence of traditional acquired risk factors, FT_4 levels above 17 pmol/L yielded a sex- and age-adjusted OR of 2.2 (95% CI, 1.2-4.2) for deep VT, which further increased up to an OR of 13.0 (95% CI, 1.1-154.1) for FT_4 levels above reference range. Our data suggest increasing levels of FT_4 to be a risk factor for VT and may have implications for both the prevention and management of this disease. (*Blood*. 2010; 115(22):4344-4349)

Introduction

Venous thrombosis (VT) is an important cause of morbidity and mortality in developed countries. The estimated incidence rates vary between 1 and 2 per 1000 person-years.[1,2] In the past decades, several risk factors for VT, both genetic and acquired, have been established.[3] Still, in 25% to 50% of first episodes of VT no apparent risk factor can be identified.[4] Identification of additional risk factors associated with VT will improve the understanding and prevention of this disease.

Hyperthyroidism has been associated with a hypercoagulable state and is thus hypothesized to increase the risk of VT.[5] Although there have been several reports on sinus, cerebral, or deep VT (DVT) after thyrotoxicosis, the relation between thyroid function and the risk of VT is not fully explored.[6-10] Although high concentrations of factor VIII and von Willebrand factor contribute to a hypercoagulable state in overt hyperthyroidism, lower von Willebrand factor concentrations found in overt hypothyroidism may, at least in part, protect against VT.[5,11] Regarding these alterations in coagulation factors, similar findings have been described for subclinical thyroid disease.[5] Subclinical thyroid disease has also been linked with arterial vascular disease, and there are good indications that variations in thyroid hormone levels within the physiologic range can modify the function of several organs.[12-15] Therefore, we hypothesized that increasing levels of thyroid hormone may be a risk factor for VT.

In a case-control design, we aimed to clarify the associations between different plasma levels of free thyroxine (FT_4), thyrotropin (TSH), thyroid peroxidase antibodies (antiTPOs) and the presence of VT. Because acute illness such as VT may in itself affect thyroid hormone concentrations by altered protein binding or by inhibition of the conversion of T_4 to triiodothyronine (T_3), T_3 levels were subsequently analyzed to explore whether our findings were influenced by this so-called nonthyroidal illness syndrome (NTIS).

Methods

Study population

Patients with objectively confirmed DVT, calf vein thrombosis, or superficial thrombophlebitis of the lower extremities and control subjects in whom leg vein thrombosis was objectively ruled out were derived from a larger study designed to investigate new risk factors for VT. In this study, all consecutive outpatients suspected of DVT and referred to the Academic Medical Center, Amsterdam, The Netherlands, between September 1999 and August 2006 were recruited (n = 944). Inpatients (n = 58), patients younger than 18 years (n = 3), patients with a previous DVT (n = 119), or patients already receiving vitamin K antagonists or heparin for more than 24 hours (n = 3) were excluded. Among the eligible patients, 7 declined to participate. A total of 754 patients were eligible for the present analysis (Figure 1).

The study was approved by the Academic Medical Center Institutional Review Board, and all patients provided written informed consent in accordance with the Declaration of Helsinki.

Submitted November 15, 2009; accepted March 7, 2010. Prepublished online as *Blood* First Edition paper, March 22, 2010; DOI 10.1182/blood-2009-11-253724.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

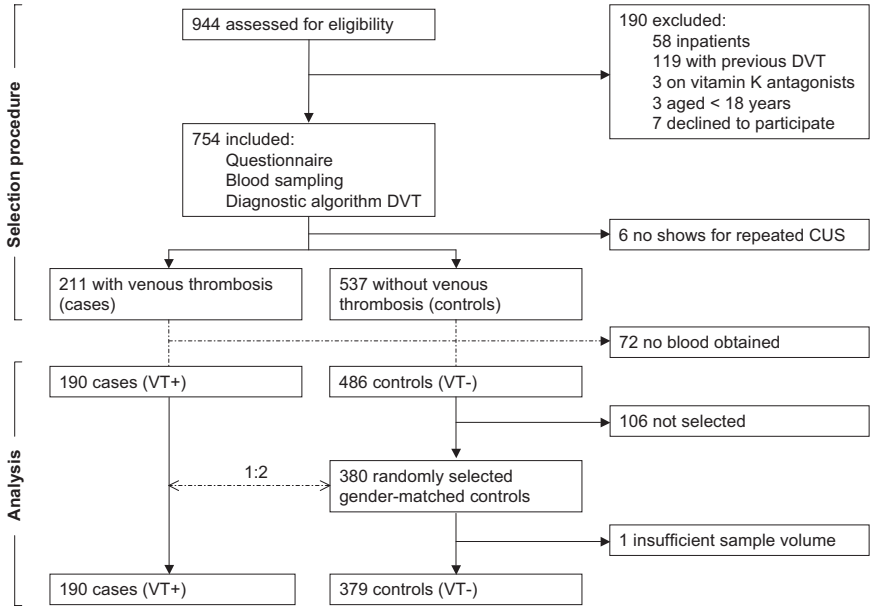

Figure 1. Flow chart of selection procedure and analysis. CUS indicates compression ultrasound; DVT, deep venous thrombosis; and VT, venous thrombosis.

Data collection and diagnosis of VT

At presentation, all patients were asked to complete a detailed questionnaire about family and medical history, medication use, and the presence of predisposing risk factors for VT. Subsequently, venous blood was obtained in a nonfasting state. Blood was collected in 0.109 mol/L trisodium-citrated tubes and immediately centrifuged, and the supernatant was recentrifuged, for 20 minutes at 1600*g* at 4°C to obtain platelet-poor plasma, which was stored at −80°C.

All patients underwent routine workup for diagnosis of DVT according to an algorithmic management strategy combining clinical pretest probability and D-dimer testing (Tinaquant; Roche Diagnostics), followed by compression ultrasound (CUS) scanning of the lower extremities if indicated.[16,17] Failure to fully collapse the lumen of the deep or superficial veins during compression testing was the main criterion for the presence of VT, including DVT, calf vein thrombosis, and superficial thrombophlebitis. DVT was defined as proximal thrombosis of the iliac or superficial femoral vein, or thrombosis of at least the upper third part of the deep calf veins. Thrombosis was considered provoked if at least one of the following criteria was present: use of estrogen- or progesterone-containing agents, malignancy, pregnancy or puerperium, paralysis of the symptomatic leg, recent trauma (within past 60 days), surgery within the past 4 weeks or bedridden for more than 3 days, hospitalization within the previous 6 months, long-distance travel (≥ 6 hours) within the previous 3 months. In the absence of these acquired risk factors, VT was considered unprovoked.

Six patients with a combination of high pretest probability and a positive D-dimer assay, but normal initial ultrasonographic findings, failed to return for repeated CUS scan after 1 week. In total, 211 patients were diagnosed with VT (cases), whereas in 537 patients diagnosis of VT was objectively ruled out with the use of the above-mentioned diagnostic management strategy (controls; Figure 1).

Laboratory assay of thyroid function

Citrated plasma was available for 190 cases and 486 controls. Because we anticipated a control-to-case ratio of 2:1 to suffice, we randomly selected 380 of the 486 controls according to the alphabetical order of their initials. Selection was performed for men and women separately to match for frequency (Figure 1).

Levels of FT_4, T_3, TSH, and antiTPO were assessed in citrated plasma with the use of commercially available assays (ADVIA Centaur immunoassay system; Siemens Healthcare Diagnostics). The intraassay and interassay coefficients of variations (CVs) were below or equal to 4.7% and 4.6% for FT_4, 3.2% and 1.3% for T_3, 9.0% and 4.4% for TSH, and 4.1% and 8.0% for antiTPO, respectively. Because these tests have not been validated by the manufacturer for use with citrated plasma, serum/citrated plasma studies were performed to characterize the correlation. Results in plasma were corrected for dilution with citrate. On the basis of the analysis of at least 21 samples, small systematic differences between serum and citrated plasma were observed, but linear regression analysis showed a significant association between serum and plasma levels of thyroid hormones (regression coefficient $\beta \geq 0.92$; $P < .01$).

Overt and subclinical primary hyperthyroidism were biochemically defined as TSH below the lower limit of the local reference range (0.32-4.32 mU/L) combined with a FT_4 concentration above or within the local reference range (10-24 pmol/L), respectively.[18,19] Primary hypothyroidism, overt and subclinical, was biochemically defined as elevated TSH and a FT_4 concentration below or within the local reference range, respectively.[19,20] AntiTPOs above 80 U/mL were considered elevated.

Statistical analysis

In one plasma sample, thyroid hormones could not be assessed because of insufficient volume. After exclusion of this control subject, FT_4, TSH, and antiTPO levels of 190 cases and 379 controls were used for final analysis (Figure 1). Because levels of T_3 were analyzed only after the initial measurements of FT_4, TSH, and antiTPO had been performed, T_3 levels were available for 186 cases and 375 control subjects; in 8 plasma samples the volume was insufficient for this additional measurement. Subjects taking thyroid medication or with known thyroid dysfunction were not excluded from the analyses.

Categorical variables measured in this study were expressed as number and percentage. FT_4 and T_3 levels were normally distributed in both cases and controls and were presented as means (95% confidence interval [95% CI]). Distributions of TSH and antiTPO levels were skewed and therefore presented as medians (95% CIs). Between-group comparisons were performed with the use of *t* tests or nonparametric tests, depending on the distribution of the data. Odds ratios (ORs) and 95% CIs for the risk of VT at different levels of FT_4, TSH, and antiTPO were calculated with the use of binary logistic regression, taking different percentiles of the values observed in the control subjects as cutoff levels. For each cutoff level below the 50th percentile, we compared subjects below the cutoff to subjects above this level with the use of the latter as reference, and vice versa for cutoff levels above the 50th percentile. Subsequently, the same analyses were performed for levels of T_3, but mostly to support any findings on FT_4 levels. A multivariate model was used to adjust for sex to take the frequency matching on sex into account and for age as a possible confounding factor. In the analysis for TSH, we additionally adjusted for FT_4 to explore the causal relation. Separate analysis was performed for patients with DVT,

Table 1. Patient characteristics

Characteristic	All cases (n = 190)*	DVT only (n = 155)	Controls (n = 379)
Male, n (%)	80 (42)	67 (43)	160 (42)
Median age, y (range)	57 (19-90)	59 (19-90)	56 (18-93)
Unprovoked VT, n (%)	69 (36)	51 (33)	NA
Provoked VT, n (%)	121 (64)	102 (67)	NA
OCP/HRT	41 (22)	31 (20)	40 (11)
Malignancy	24 (13)	22 (14)	24 (6)
Pregnancy/puerperium	0 (0)	0 (0)	3 (1)
Paralysis	16 (8)	15 (10)	30 (8)
Recent trauma	26 (14)	22 (14)	53 (14)
Surgery/bedridden	30 (16)	29 (19)	31 (8)
Hospitalization	35 (18)	33 (21)	48 (13)
Long-distance travel	30 (16)	23 (15)	43 (11)
Use of thyroxine substitution therapy, n (%)	6 (3)	5 (3)	7 (2)
Autoimmune hypothyroidism	1 (1)	1 (1)	2 (1)
Central hypothyroidism	0 (0)	0 (0)	1 (0.3)
Iatrogenic hypothyroidism	3 (2)	3 (2)	0 (0)
Hypothyroidism unknown cause	2 (1)	1 (1)	4 (1)
Use of antithyroidal agents, n (%)	1 (0.5)	0 (0)	0 (0)

DVT indicates deep venous thrombosis; VT, venous thrombosis; NA, not applicable; OCP, oral contraceptive pill; and HRT, hormone replacement therapy.

*All patients with VT (DVT, calf vein thrombosis, or thrombophlebitis).

excluding those with calf vein thrombosis or superficial thrombophlebitis. Chi-square tests, or Fisher exact test in case cells had a count less than 5, were used to compare the 2 study groups with respect to the presence of thyroid abnormalities in comparison with euthyroidism. Statistical analysis was performed with the use of SPSS 15.0 software package (SPSS Inc).

Results

Patient characteristics

Among the 190 cases and 379 controls there were 80 (42%) men in the cases and 160 (42%) in the control subjects. Mean age in the cases (57 years; range, 19-90 years) was similar to that in the control subjects (56 years; range, 18-93 years). Of all patients with VT, 155 (82%) had a DVT, 12 (6%) had an isolated calf vein thrombosis, and 23 (12%) had a superficial thrombophlebitis of the lower extremities. In 69 (36%) VT cases, and in 51 (33%) of the patients with DVT, VT was considered unprovoked.

At inclusion, 5 patients with provoked DVT, 1 with thrombophlebitis, and 7 control subjects were on thyroxine substitution therapy for previously diagnosed hypothyroidism. Only 1 patient, diagnosed with thrombophlebitis, was on thiamazole treatment for known hyperthyroidism. All patients with known hypothyroidism or hyperthyroidism had plasma FT_4 levels within the reference range combined with either TSH levels within reference range or marginally decreased or marginally elevated TSH levels at the time of inclusion.

Baseline characteristics for cases and controls are summarized in Table 1.

FT_4 and risk of VT

Mean FT_4 in all patients with VT was 16.0 pmol/L (95% CI, 15.6-16.4 pmol/L), in patients with DVT it was 16.2 pmol/L (95% CI, 15.8-16.7 pmol/L), and in control subjects it was 15.4 pmol/L (95% CI, 15.1-15.6 pmol/L; Table 2). We found ORs for each cutoff level that clearly increased with the percentiles used (adjusted for the matching factor sex). These ORs were below unity for cutoff levels below the 50th percentile (ie, indicating a protective effect of lower FT_4 levels) but above unity for higher cutoff levels (ie, indicating an increased risk of higher FT_4 levels). Further adjustment for age slightly reduced the crude ORs. Similar results were found when we analyzed only patients with DVT of the leg. In the absence of traditional acquired risk factors for VT, FT_4 above reference range (> 24 pmol/L) yielded an OR of 9.6 (95% CI, 0.8-109.1) for VT and 13.0 (95% CI, 1.1-154.1) for DVT, adjusted for sex and age (Figure 2; Table 3).

Mean T_3 levels were higher in patients with VT (1.94 nmol/L; 95% CI, 1.87-2.00 nmol/L) than in control subjects (1.79 nmol/L; 95% CI, 1.75-1.82 nmol/L), indicating that the association between higher FT_4 and VT as found in the present study is very unlikely to reflect the NTIS because low serum T_3 is the hallmark of NTIS (Table 2). Similar to FT_4, we found the risk of VT to gradually rise with increasing levels of T_3, although the relation was slightly less linear (Figure 2).

TSH and risk of VT

Median TSH in all patients with VT was 1.41 mU/L (95% CI, 1.22-1.51 mU/L), in those with DVT it was 1.37 mU/L (95% CI, 1.17-1.51 mU/L), and in control subjects it was 1.21 mU/L (95% CI, 1.11-1.30 mU/L; Table 2). TSH levels were not associated with the overall risk of VT. The risk of unprovoked DVT was almost

Table 2. Thyroid function

	All cases* (n = 190)*	DVT only (n = 155)	Controls (n = 379)	*P* (2-sided)†	*P* (2-sided)‡
FT_4, pmol/L, mean (95% CI)	16.0 (15.6-16.4)	16.2 (15.8-16.7)	15.4 (15.1-15.6)	<.01	<.01
T_3, nmol/L, mean (95% CI)§	1.94 (1.87-2.00)	1.90 (1.83-1.97)	1.79 (1.75-1.82)	<.01	<.01
TSH, mU/L, median (95% CI)	1.41 (1.22-1.51)	1.37 (1.17-1.51)	1.21 (1.11-1.30)	.03	.09
AntiTPO, U/mL, median (95% CI)	29.0 (27.3-30.8)	28.9 (27.2-30.7)	29.8 (29.0-30.7)	.48	.47
Hyperthyroidism, n (%)	3 (1.6)	3 (1.9)	0 (0)	.04‖	.02‖
Sub hyperthyroidism, n (%)	9 (4.7)	7 (4.5)	18 (4.7)	.96‖	.97‖
Euthyroidism, n (%)	170 (89.5)	138 (89.0)	348 (91.8)	NA	NA
Sub hypothyroidism, n (%)	8 (4.2)	7 (4.5)	12 (3.2)	.50‖	.43‖
Hypothyroidism, n (%)	0 (0.0)	0 (0.0)	1 (0.3)	.999‖	.999‖

DVT indicates deep venous thrombosis; FT_4, free thyroxine; CI, confidence interval; T_3, triiodothyronine; TSH, thyrotropin; antiTPO, thyroid peroxidase antibodies; sub, subclinical; and NA, not applicable.

*All patients with venous thrombosis (deep venous thrombosis, calf vein thrombosis or thrombophlebitis).

†All cases versus controls.

‡Patients with DVT only versus controls.

§Mean T_3 levels involved 186 patients with venous thrombosis, of whom 151 patients were diagnosed with DVT, and 375 control subjects.

‖In comparison with euthyroidism.

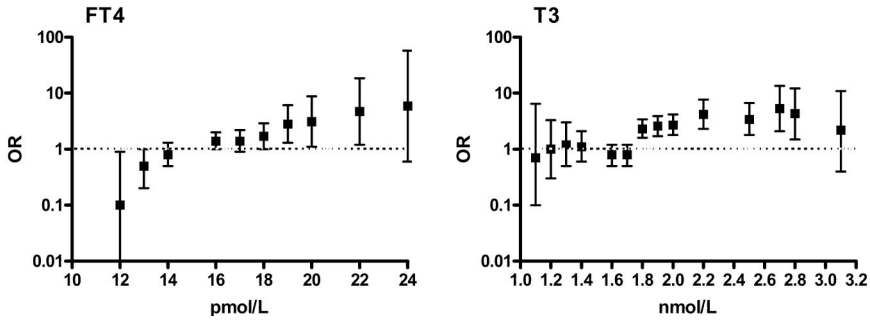

Figure 2. Risk of VT for different levels of FT_4 and T_3 adjusted for sex and age. FT_4 indicates free thyroxine; T_3, triiodothyronine; OR, odds ratio. Reference range for FT_4 was 10-24 pmol/L; reference range for T_3 was 1.2-2.8 nmol/L.

3-fold increased for TSH below 0.02 mU/L (OR, 2.9; 95% CI, 0.7-12.0) but returned to unity after adjustment for FT_4 (OR, 0.9; 95% CI, 0.2-5.2; data not shown).

AntiTPO and risk of VT

Median antiTPO in all patients with VT was 29.0 U/mL (95% CI, 27.3-30.8 U/mL), in those with DVT it was 28.9 U/mL (95% CI, 27.2-30.7 U/mL), and in control subjects it was 29.8 U/mL (95% CI, 29.0-30.7 U/mL; Table 2). No association between different levels of antiTPO and VT was observed (data not shown).

Thyroid function at the time of thrombotic event

Three patients with DVT had thyroid hormone concentrations consistent with primary hyperthyroidism (1.6% of all patients with VT; 1.9% of patients with DVT only), whereas this was not observed in any of the control subjects. Statistical analysis confirmed that hyperthyroidism and (deep) VT co-occurred more frequently than expected by chance ($P = .04$, all patients with VT; $P = .02$, patients with DVT only, 2-sided Fisher exact test). No such association was found for subclinical hyperthyroidism or for overt or subclinical primary hypothyroidism (Table 2).

All 3 patients with biochemical hyperthyroidism appeared to have clinically manifest hyperthyroidism during follow-up that was diagnosed 2, 54, and 65 months after presentation for DVT, respectively. For a description of these patients, see supplemental Document 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Discussion

Using laboratory assessment of thyroid function in 190 patients with confirmed VT of the lower extremities and 379 control subjects, we were able to show a clear gradual relation between plasma FT_4 levels and the risk of VT. Notably, the thrombotic risk was substantially increased for FT_4 levels well within the physiologic range. Moreover, FT_4 levels were particularly associated with the risk of unprovoked DVT, indicating FT_4 as a potential novel risk factor. Similar to FT_4, the risk of VT also increased with higher levels of T_3, but the relation was slightly less linear. No clear association was found for TSH or antiTPO.

The present analysis is to our knowledge the first to study the effect of increasing levels of thyroid hormones on the risk of VT. Over the past decades we have witnessed a multiplicity of genetic or acquired risk factors for VT to be identified. The estimated magnitude of each varies widely. To illustrate, thrombosis risk after surgery is 6- to 15-fold increased; hospitalization is associated with an 8- to 11-fold increased risk, whereas in pregnancy the VT risk is 1- to 5-fold increased, in puerperium 14- to 60-fold, in users of oral contraceptives 1.4- to 5-fold, and in heterozygotes of factor V Leiden 3- to 8-fold increased.[21-23] Generally, newly identified genetic or acquired risk factors tend to confer less thrombotic risk than the well-established ones. Although confirmation is required, the here reported risk of increasing FT_4 levels, varying from 1.7- to 13-fold, appears to be of a similar magnitude.

Table 3. Risk of venous thrombosis for different FT_4 levels (adjusted for sex and age)

			VT (n = 190)‡		DVT (n = 155)		Unprovoked VT (n = 69)‡		Unprovoked DVT (n = 51)	
FT_4*	Percentile†	Controls (n = 379)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
Less than 10 pmol/L	LoRef	2	0	NA	0	NA	0	NA	0	NA
Less than 11 pmol/L	1	3	0	NA	0	NA	0	NA	0	NA
Less than 12 pmol/L	5	16	1	0.1 (0.0-0.9)	1	0.2 (0.0-1.2)	1	0.4 (0.0-2.7)	1	0.5 (0.1-4.0)
Less than 13 pmol/L	10	36	9	0.5 (0.2-1.0)	6	0.4 (0.2-1.0)	5	0.8 (0.3-2.2)	2	0.4 (0.1-1.9)
Less than 14 pmol/L	30	85	36	0.8 (0.5-1.3)	28	0.8 (0.5-1.3)	14	1.0 (0.5-1.8)	8	0.8 (0.3-1.7)
Greater than 16 pmol/L	60	121	76	1.4 (1.0-2.0)	69	1.7 (1.1-2.4)	25	1.2 (0.7-2.0)	23	1.6 (0.9-3.0)
Greater than 17 pmol/L	80	68	46	1.4 (0.9-2.2)	41	1.6 (1.0-2.5)	18	1.5 (0.8-2.7)	18	2.2 (1.2-4.2)
Greater than 18 pmol/L	90	35	28	1.7 (1.0-2.9)	27	2.0 (1.1-3.4)	13	2.0 (1.0-4.1)	13	2.8 (1.4-6.0)
Greater than 19 pmol/L	95	12	16	2.8 (1.3-6.1)	15	3.1 (1.4-6.9)	9	3.9 (1.6-9.9)	9	5.4 (2.1-13.8)
Greater than 20 pmol/L	97.5	6	9	3.1 (1.1-8.8)	9	3.7 (1.3-10.6)	5	4.2 (1.2-14.5)	5	5.8 (1.7-20.3)
Greater than 22 pmol/L	99	3	7	4.7 (1.2-18.6)	7	5.7 (1.5-22.5)	5	8.9 (2.0-38.5)	5	12.5 (2.8-55.4)
Greater than 24 pmol/L	UpRef	1	3	5.9 (0.6-57.3)	3	6.9 (0.7-67.7)	2	9.6 (0.8-109.1)	2	13.0 (1.1-154.1)

FT_4 indicates free thyroxine; VT indicates venous thrombosis; DVT, deep venous thrombosis; OR, odds ratio; CI, confidence interval; LoRef, lower limit of reference range; NA, not applicable; and UpRef, upper limit of reference range.

*Reference range is 10-24 pmol/L.

†Analysis was performed for the 1st, 2.5th, 5th, 10th, 20th, 30th, 40th, 50th, 60th, 70th, 80th, 90th, 95th, 97.5th, and 99th percentiles, as well as for FT_4 levels below or above reference range. Because of laboratory reportage of FT_4 results in round figures, the 1st percentile corresponded with the 2.5th percentile, the 10th percentile with the 20th percentile, the 50th percentile with the 60th percentile, and the 70th percentile with the 80th percentile. For each cutoff level below the 50th percentile, we compared subjects below the cutoff to subjects above this level, thus setting the latter as reference; vice versa for each cutoff level above the 50th percentile.

‡DVT, calf vein thrombosis, or thrombophlebitis.

It is probable that the risk of VT associated with FT_4 levels reflects thyroid hormone-induced alterations in factor VIII synthesis and secretion, high concentrations of which are an independent risk factor for VT, and possibly other procoagulant yet unknown changes.[24] Indeed, our findings corroborate clinical data suggesting both high concentrations of factor VIII in thyroid hormone excess and low factor VIII concentrations in overt hypothyroidism.[5,11] However, the reduced risk of VT for lower FT_4 levels, yet still within the physiologic range, is in contrast with some studies, including one of our own, that suggest the hypercoagulable state to extend into subclinical hypothyroidism.[25-27] In a previous pilot study, we found an increased frequency of subclinical hypothyroidism in patients with unprovoked DVT (OR, 6.8; 95% CI, 0.7-64.5). The different outcome is best explained by both the limited sample size and the fact that, in this pilot study, thyroid function was assessed only several months after the thrombotic event.[27] Although in the present study we did not observe any association between biochemical diagnosis of subclinical thyroid disease and thrombotic risk, it is now clearly shown that FT_4 levels and risk of VT constitute a continuum.

Putative mechanisms by which thyroid hormone may influence coagulation proteins are ill defined, yet it is most likely that it does so by thyroid hormone receptor–mediated regulation of gene transcription at the hepatic or endothelial cell level, or both.[28,29] Other hypotheses include indirect effects mediated through β-adrenergic receptor function.[30,31]

VT is a multicausal disease in which a combination of more than one genetic or acquired risk factor is needed to pass the thrombosis threshold.[3] Thus, the clinical utility of our findings may be of particular relevance for subjects with additional risk factors for VT, eg, women on oral contraceptive agents or patients undergoing surgery. If confirmed, screening for thyroid function in high-risk patients may improve our ability to predict and prevent this disease. For patients with VT, knowledge of thyroid function could be of importance in decisions about the duration of anticoagulant treatment in those 25% to 50% in whom no additional risk factor is identified. In addition, screening for thyroid function in patients presenting with a new VT could help us to early detect thyroid disease, especially because clinical symptoms and signs of (subclinical) hyperthyroidism might easily go unnoticed during the early years of the disease. For patients with overt hyperthyroidism, physicians should be aware that these patients might be at increased risk of VT.

Several potential limitations should be addressed. First, the present analysis was limited to a population suspected of DVT. As mentioned, acute or chronic disease is known to affect thyroid function, and multiple alterations in thyroid hormone levels have been observed in patients with this NTIS.[32] The most common and earliest change is inhibition of T_4-to-T_3 conversion, with a resulting decrease in the circulating T_3 level. In general, levels of FT_4 are usually less affected by NTIS than T_3, but values may be higher than normal in mild or moderate forms of the syndrome.[32,33] T_4 is present in the circulation either free or bound to thyroxine-binding globulin, thyroxine-binding prealbumin, or albumin. As such, changes in protein binding probably decrease the total amount of T_4 (free and bound), whereas levels of the unbound hormone may increase. If, in the present study, decreased protein binding was more present in the patients with VT than in control subjects, this would have resulted in higher FT_4 levels in cases compared with controls and, thus, to an overestimation of our effect measures. However, to discriminate between NTIS (low T_3) or a direct association between high FT_4 (and T_3) and the risk of VT, we subsequently performed additional assessment of T_3 levels. Although a certain degree of NTIS was present in some patients with VT, or in some control subjects (eg, those with erysipelas), the higher T_3 levels in cases compared with controls, as well as the clear association between T_3 and VT, make it highly unlikely that our findings are solely a reflection of NTIS. Nonetheless, prospective epidemiologic studies are needed to further confirm our findings.

Second, the design of our study that included blood sampling in the acute phase of VT did not allow us to investigate the relationship between thyroid hormone and factor VIII, because factor VIII levels measured during the acute phase are unlikely to be representative for levels before the event: increased consumption of factor VIII has been reported during coagulation activation as well as up-regulation of factor VIII synthesis during the acute phase response.[34] Therefore, in the present study we cannot confirm thyroid hormone–induced alterations in factor VIII concentrations.

Third, not all patients suspected of DVT of the lower extremities underwent CUS scanning to exclude VT. Nevertheless, because the diagnostic management strategy combining clinical pretest probability and D-dimer testing has repeatedly proven to be equally safe in refuting the diagnosis of VT, mismatching seems unlikely.[17,35,36]

In conclusion, our data suggest that increasing levels of FT_4 are a risk factor for VT, whereas lower FT_4 levels are protective of VT. Future studies are needed to further broaden our knowledge on this issue and to evaluate whether implementation of this novel risk factor in daily practice can improve our ability to prevent and manage VT in terms of reducing its risks. In particular, epidemiologic studies in which thyroid hormone levels are measured before or after the thrombotic event may provide definitive answers on the influence of NTIS. Furthermore, it would be interesting to investigate whether patients with VT with higher FT_4 levels also have an increased risk of recurrent thrombosis, or, vice versa, whether subjects who have been treated for hyperthyroidism have a lower risk. In addition, further insights in the clinical relevance of our findings could be obtained by assessing the relation between thyroid hormone levels and VT in high-risk patients, such as those undergoing surgery.

Acknowledgments

We thank the "vasculisten," a group of medical students at Academic Medical Center, University of Amsterdam, who had an essential role in the execution of this study, especially the recruitment and data collection. We thank Huib Bout and Jan van Veen, Department of Clinical Biochemistry, Slotervaart Hospital (Amsterdam, The Netherlands) for their laboratory efforts.

Authorship

Contribution: B.v.Z. had full access to all of the data in the study and takes responsibility for the integrity of the data, the accuracy of the data analysis, and the decision to submit for publication. B.v.Z. helped to obtain data, perform statistical analysis, and write the report; A.S. helped to design the study and participated in writing the report; R.H. helped to design and coordinate the study, recruit participants, obtain data, and write the report; A.P.v.Z. performed laboratory analysis of thyroid function and participated in writing the report; E.F. participated in writing the report; S.C.C. helped to

perform statistical analysis and write the report; H.R.B. helped to design and coordinate the study and write the report; V.E.A.G. helped to design the study, perform statistical analysis, and write the report; and D.P.M.B. participated in designing the study and writing the report.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: B. van Zaane, Department of Internal Medicine, Slotervaart Hospital, Louwesweg 6, 1066 EC, Amsterdam, The Netherlands; e-mail: b.vanzaane@amc.uva.nl.

References

1. Naess IA, Christiansen SC, Romundstad P, et al. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost.* 2007;5(4):692-699.
2. Silverstein MD, Heit JA, Mohr DN, et al. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. *Arch Intern Med.* 1998;158(6):585-593.
3. Rosendaal FR. Venous thrombosis: a multicausal disease. *Lancet.* 1999;353(9159):1167-1173.
4. White RH. The epidemiology of venous thromboembolism. *Circulation.* 2003;107(23 suppl 1):I4-I8.
5. Squizzato A, Romualdi E, Buller HR, Gerdes VE. Clinical review: thyroid dysfunction and effects on coagulation and fibrinolysis: a systematic review. *J Clin Endocrinol Metab.* 2007;92(7):2415-2420.
6. Dai A, Wasay M, Dubey N, Giglio P, Bakshi R. Superior sagittal sinus thrombosis secondary to hyperthyroidism. *J Stroke Cerebrovasc Dis.* 2000;9(2):89-90.
7. Lodha A, Haran M, Frankel R, Shani J. Thyrotoxicosis causing arterial and venous thrombosis. *Am J Med Sci.* 2009;338(5):428.
8. Siegert CE, Smelt AH, de Bruin TW. Superior sagittal sinus thrombosis and thyrotoxicosis. Possible association in two cases. *Stroke.* 1995; 26(3):496-497.
9. Squizzato A, Vitale J, Gerdes VE, et al. Recurrent deep venous thrombosis during optimal anticoagulation and overt hyperthyroidism: a case report. *Blood Coagul Fibrinolysis.* 2007;18(8):801-803.
10. Verberne HJ, Fliers E, Prummel MF, et al. Thyrotoxicosis as a predisposing factor for cerebral venous thrombosis. *Thyroid.* 2000;10(7):607-610.
11. Manfredi E, van Zaane B, Gerdes VE, Brandjes DP, Squizzato A. Hypothyroidism and acquired von Willebrand's syndrome: a systematic review. *Haemophilia.* 2008;14(3):423-433.
12. Kim BJ, Kim TY, Koh JM, et al. Relationship between serum free T4 (FT4) levels and metabolic syndrome (MS) and its components in healthy euthyroid subjects. *Clin Endocrinol (Oxf).* 2009; 70(1):152-160.
13. Ochs N, Auer R, Bauer DC, et al. Meta-analysis: subclinical thyroid dysfunction and the risk for coronary heart disease and mortality. *Ann Intern Med.* 2008;148(11):832-845.
14. Roos A, Bakker SJ, Links TP, Gans RO, Wolffenbuttel BH. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. *J Clin Endocrinol Metab.* 2007;92(2):491-496.
15. Takamura N, Akilzhanova A, Hayashida N, et al. Thyroid function is associated with carotid intima-media thickness in euthyroid subjects. *Atherosclerosis.* 2009;204(2):e77-e81.
16. Lensing AW, Prandoni P, Brandjes D, et al. Detection of deep-vein thrombosis by real-time B-mode ultrasonography. *N Engl J Med.* 1989;320(6):342-345.
17. Wells PS, Anderson DR, Rodger M, et al. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. *N Engl J Med.* 2003; 349(13):1227-1235.
18. Cooper DS. Hyperthyroidism. *Lancet.* 2003; 362(9382):459-468.
19. Surks MI, Ortiz E, Daniels GH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. *JAMA.* 2004;291(2):228-238.
20. Roberts CG, Ladenson PW. Hypothyroidism. *Lancet.* 2004;363(9411):793-803.
21. Heit JA, Silverstein MD, Mohr DN, et al. Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case-control study. *Arch Intern Med.* 2000;160(6):809-815.
22. Pomp ER, Lenselink AM, Rosendaal FR, Doggen CJ. Pregnancy, the postpartum period and prothrombotic defects: risk of venous thrombosis in the MEGA study. *J Thromb Haemost.* 2008;6(4): 632-637.
23. Rosendaal FR. Venous thrombosis: the role of genes, environment, and behavior. *Hematology Am Soc Hematol Educ Program.* 2005;1-12.
24. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. *Lancet.* 1995;345(8943):152-155.
25. Canturk Z, Cetinarslan B, Tarkun I, et al. Hemostatic system as a risk factor for cardiovascular disease in women with subclinical hypothyroidism. *Thyroid.* 2003;13(10):971-977.
26. Chadarevian R, Bruckert E, Ankri A, et al. Relationship between thyroid hormones and plasma D-dimer levels. *Thromb Haemost.* 1998;79(1):99-103.
27. Squizzato A, Romualdi E, Piantanida E, et al. Subclinical hypothyroidism and deep venous thrombosis. A pilot cross-sectional study. *Thromb Haemost.* 2007;97(5):803-806.
28. Baumgartner-Parzer SM, Wagner L, Reining G, et al. Increase by tri-iodothyronine of endothelin-1, fibronectin and von Willebrand factor in cultured endothelial cells. *J Endocrinol.* 1997;154(2): 231-239.
29. Shih CH, Chen SL, Yen CC, et al. Thyroid hormone receptor-dependent transcriptional regulation of fibrinogen and coagulation proteins. *Endocrinology.* 2004;145(6):2804-2814.
30. Burggraaf J, Lalezari S, Emeis JJ, et al. Endothelial function in patients with hyperthyroidism before and after treatment with propranolol and thiamazol. *Thyroid.* 2001;11(2):153-160.
31. Liu L, Wang X, Lin Z, Wu H. Elevated plasma levels of VWF:Ag in hyperthyroidism are mediated through beta-adrenergic receptors. *Endocr Res.* 1993;19(2-3):123-133.
32. Docter R, Krenning EP, de Jong M, Hennemann G. The sick euthyroid syndrome: changes in thyroid hormone serum parameters and hormone metabolism. *Clin Endocrinol (Oxf).* 1993;39(5): 499-518.
33. Fliers E, Guldenaar SE, Wiersinga WM, Swaab DF. Decreased hypothalamic thyrotropin-releasing hormone gene expression in patients with nonthyroidal illness. *J Clin Endocrinol Metab.* 1997;82(12):4032-4036.
34. Begbie M, Notley C, Tinlin S, Sawyer L, Lillicrap D. The factor VIII acute phase response requires the participation of NFkappaB and C/EBP. *Thromb Haemost.* 2000;84(2):216-222.
35. Kraaijenhagen RA, Piovella F, Bernardi E, et al. Simplification of the diagnostic management of suspected deep vein thrombosis. *Arch Intern Med.* 2002;162(8):907-911.
36. Schutgens RE, Ackermark P, Haas FJ, et al. Combination of a normal D-dimer concentration and a non-high pretest clinical probability score is a safe strategy to exclude deep venous thrombosis. *Circulation.* 2003;107(4):593-597.