The effect of thyroid hormone on haemostasis and thrombosis

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General introduction

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General introduction

In this thesis the relation between thyroid hormones and the coagulation system will be examined. As an introduction, the hypothalamic-pituitary-thyroid axis, the coagulation system and their interactions will be discussed. A short overview of the literature preceding the research reported in this thesis is about the relation between thyroid hormones, the coagulation system, and risk of bleeding and venous thrombosis.

Thyroid hormones

The hypothalamic-pituitary-thyroid axis is the regulatory system of thyroid hormone action. In the hypothalamus, thyroid releasing hormone (TRH) is secreted and transported to the pituitary gland. Here, TRH stimulates the production of thyroid stimulating hormone (TSH), which in its turn is released in the circulation and affects the functioning of the thyroid gland. The thyroid gland is located in het ventral part of the neck and consists of 2 lobes, connected by the isthmus. The thyroid consists of follicles lined by cuboidal epithelioid cells. The thyroid hormones tri-iodothyronine (T3) and tetra-iodothyronine (T4) are synthesized in the gland under influence of TSH and regulated by negative feedback mechanisms. More T4 than T3 is produced and stored, while T3 is the most active form of the hormone. In several peripheral tissues like the liver, muscles and kidney, T4 is converted into the more active T3 (this is done by 5'-monodeiodination). Thyroid hormones bind to nuclear receptors, the thyroid hormone receptors (THR). When T3 or T4 binds to the THR (T3 has a 10-fold greater affinity for the THR), they either activate or repress gene transcription. Thyroid hormone influences the basal metabolic rate, resulting from influence on the carbohydrate, protein and lipid metabolism, the Na-K pump activity and thermogenesis.

Hyperthyroidism is a condition in which the thyroid gland produces and secretes excessive amounts of the free thyroid hormones T3 or T4. Hyperthyroidism is one cause of thyrotoxicosis—the hypermetabolic clinical syndrome which occurs when there are elevated serum levels of T3 or T4. There are several causes of

hyperthyroidism such as Graves' disease, toxic adenoma, and toxic multinodular goitre. Thyroiditis (inflammation of the thyroid gland) may also cause hyperthyroidism, but often progresses to gland dysfunction and, thereby to hormone deficiency and hypothyroidism.
Another cause is oral consumption of excess thyroid hormone tablets (surreptitious use of thyroid hormone), as is the rare event of consumption of ground beef contaminated with thyroid tissue, and consequently thyroid hormone (termed "hamburger hyperthyroidism"). Amiodarone, an anti-arrhythmic drug which is structurally similar to thyroxine, may cause either under- or overactivity of the thyroid. Finally, hypersecretion of thyroid stimulating hormone (TSH), which is almost always caused by a pituitary adenoma, is also a (very rare) cause of hyperthyroidism. Major clinical signs of hyperthyroidism include weight loss (often accompanied by an increased appetite), anxiety, intolerance to heat, hair loss, muscle aches, weakness, fatigue, hyperactivity, irritability, polyuria, polydipsia, delirium, tremor, pretibial myxedema and sweating.

Hypothyroidism is a pathologic condition in which the thyroid gland produces inadequate amounts of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Since iodine is an essential building block to produce T3 and T4, iodine deficiency is the most common cause of hypothyroidism worldwide. In parts of the world where iodine is widely available in food, hypothyroidism is most commonly caused by Hashimoto's thyroiditis; other causes may include an absent thyroid gland or central hypothyroidism due to impaired production of the hypothalamic hormone TRH or the anterior pituitary hormone TSH. Central hypothyroidism may occur following for example injury to these glands from physical trauma, compression by a tumor or vascular insufficiency. Certain medications may cause hypothyroidism, such as lithium-based mood stabilizers, amiodarone and thalidomide. Partial or total removal of the thyroid gland also causes hypothyroidism. Clinical symptoms can be divided in early and late symptoms. Early symptoms are thin, brittle fingernails; dry, itchy skin; weight gain, water retention, myxedema, hair loss, depression, depressed reflexes, hypotonia, muscle cramps, muscle weakness, fatigue, cold intolerance, bradycardia, elevated serum cholesterol. Late symptoms are thinning of the outer

third of the eyebrow, slow speech and hoarse voice, carpal tunnel syndrome and hypotension.

The coagulation system

Haemostasis is the process by which blood loss is prevented. It leads to cessation of blood loss to the extravascular space from a damaged vessel. The endpoint of haemostasis is reached when the damage to a blood vessel wall is covered by a platelets and fibrin-containing plug, thereby stopping bleeding. Thereafter repair of the damaged vessel can start.

Under physiological conditions, the vessel wall prevents platelet adhesion and clot formation. Haemostasis begins almost instantly after an injury has damaged the endothelial lining of the vessel. This damage exposes subendothelium, which contains collagen and von Willebrand factor, to the blood components. This initiates platelets adhesion and aggregation at the site of injury; primary haemostasis. Almost simultaneously with the initiation of primary haemostasis, secondary haemostasis is activated by tissue factor, also released from the vessel wall. Secondary haemostasis is a complex system of coagulation proteins in the blood plasma. Its goal is to generate large amounts of thrombin when needed, ultimately resulting is the generation of insoluble fibrin from soluble fibrinogen to strengthen the platelet plug and allowing adherence and activation of cells involved in vascular repair.

The process of coagulation was originally regarded as a cascade of one factor activating the next. The coagulation system is classically divided in two pathways, extrinsic and the intrinsic pathway, finally leading to fibrin formation through a common pathway (figure 1). The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated which subsequently catalyze the activation of a next zymogen. Rather than a cascade, it is a system in which there are multiple positive and negative feedback loops as well as cross-connections between the pathways.

To achieve fast thrombin generation three phases can be identified:

1. Initiation: Following damage to the blood vessel the extrinsic pathway becomes activated. Tissue factor (TF), which is expressed by endothelial cells, subendothelial tissue and monocytes, is exposed to factor VII (FVII) and forms an activated TF-FVIIa complex. TF-FVIIa in its turn activates Factor X. Together with factor V, calcium and phospholipids, factor X forms the prothrombinase complex. This prothrombinase complex activates prothrombin to thrombin.

2. Amplification: Thrombin formation is amplified by the small amount of thrombin generated by the extrinsic pathway in the initiation phase. Thrombin activates the intrinsic pathway by activation of factor XI, factor VIII and factor V. This leads to the production of activated factor IX and activated factor VIII which together with a phospholipid membrane and calcium form a tenase complex, activating factor X to activated factor X, again leading to formation of the prothrombinase complex which produces large amounts of thrombin from prothrombin. Thrombin converts fibrinogen to fibrin, one of the building block of a haemostatic plug. It also activates factor XIII, which crosslinks the fibrin strands to stabilize the clot. The coagulation system remains in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is inhibited by anticoagulant pathways.

3. Inhibition: Following the amplification phase, several inhibiting mechanisms are activated. The activation of factor X (FX) to form FXa by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI), stopping the initiation phase. Protein C is activated by thrombin. Together with protein S and phospholipids it degrades activated factor V and activated factor VIII, thereby inhibiting coagulation. Antithrombin is also produced, degrading thrombin, activated factor IX, activated factor X, activated factor XI and activated factor XII, this way also inhibiting the propagation of coagulation. Furthermore, plasmin is generated out of plasminogen to cleave fibrin, inhibiting excessive fibrin formation.

The complete coagulation system maintains a balance allowing rapid formation of a clot upon injury that is limited to the site of injury. Any imbalance can induce a bleeding tendency and a protective effect against venous thrombosis or can lead to a low risk of bleeding with a pro-thrombotic tendency, possibly leading to clot formation.

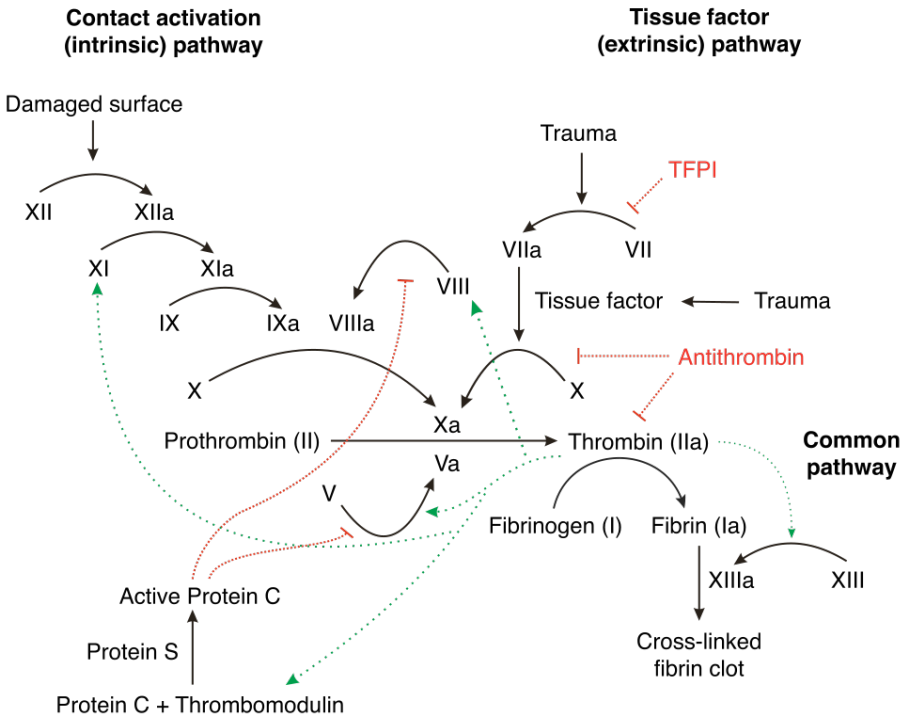

Figure 1: the coagulation system (http://en.wikipedia.org/wiki/File:Coagulation_full.svg).

Venous thrombosis

With an incidence of 1-2 per 1000 person-years, venous thrombosis is the third most common cardiovascular disease in Western society (after acute coronary syndrome and stroke). Clinical manifestations of venous thrombosis are deep venous thrombosis and pulmonary embolism. Deep venous thrombosis is a condition where a thrombus develops in the deep veins of the calf or in more proximal veins, such as the popliteal, femoral or iliac veins. This leads to obstruction of venous drainage of blood. Characteristic symptoms of deep venous thrombosis are a red, swollen, painful leg. Pulmonary emboli develop from deep venous thrombi. A venous thrombus formed anywhere in the body can dislodge from its location and move

more proximally. The emboli are caught in the first web of smaller vessels they encounter: the lungs. This leads to various symptoms: dyspnea, tachypnea, pleuric pain, cough and wheezing, although the clinical presentation is highly variable. The 30 days case fatality rate for deep venous thrombosis and pulmonary embolism combined is 11% and the 1-year case-fatality rate is 23% [1,2]. In more than 6 percent of all deaths massive pulmonary emboli are found by autopsy. Much research has been done on risk factors for venous thrombosis. These risk factors can be divided in genetic and acquired risk factors. Genetic risk factors include antithrombin deficiency, factor V Leiden mutation, prothrombin 20120A mutation, ABO blood group, protein C deficiency and protein S deficiency. Acquired risk factors include surgery, plaster cast, cancer, long haul travel, pregnancy, and oral contraceptive use [3-6].

The recurrence risk of a venous thrombotic event is much higher than the risk of a primary event. The cumulative incidence of recurrent venous thrombosis has been described to be 4-11% within the first year and 12-30% in the five years after the first event. Incidence rates of recurrence vary between 25 and 46 per 1000 person-years [7-10]. While many risk factors for first venous thrombosis are known, this is not the case for recurrent events. Furthermore, the risk profile for recurrent venous thrombosis is different from that of first venous thrombosis. This is the case for for example thrombophilia, an increased tendency of the blood to clot. Thrombophilia includes deficiencies of the natural anticoagulants antithrombin, protein C, and protein S, as well as carriership of factor V Leiden, prothrombin G20210A, and high levels of factors VIII, IX, or XI, homocysteine, and fibrinogen. While thrombophila is associated with a 2-10 fold increased risk of a first event, it does not predict risk of recurrence [7,9]. Age, also an important risk factor for a first venous thrombosis, does not influence risk of recurrence [11,12].

Thyroid hormones and the coagulation system

The first time a relation between thyroid disease and venous thrombosis was described was in 1913 by Kaliebe [13]. He described a patient with Graves disease and cerebral venous thrombosis and proposed a relation between thyroid hormone

and venous thrombosis. Other case reports followed, again describing subjects with hyperthyroidism and cerebral venous thrombosis, suggesting an effect of thyroid hormone excess on the coagulation system [14-18]. Subsequent studies focused on alterations in levels of coagulation factors in patients with thyroid disease and mostly confirmed that hyperthyroidism was associated with prothrombotic changes.

Squizzato et al. reviewed the available literature [19] until 2007 on thyroid hormones and their effect on the coagulation system. An important conclusion of their study was that there were no high quality papers on this subject. After pooling the available studies of low and medium quality they arrived at the following conclusions: In subjects with elevated levels of thyroxine, high levels of von Willebrand factor and fibrinogen were observed. In subjects with decreased levels of thyroxine, an increased bleeding time was seen together with a prolonged activated partial thromboplastin time and prothrombin time and decreased levels of factor VIII, von Willebrand factor and fibrinogen. Apart from studies into the effect of thyroid hormones on the coagulation system, only one small study had been performed on the relation between thyroxine and the risk of venous thrombosis before the start of this thesis in 2007. This study concerned 50 patients with provoked, 50 patients with unprovoked and 50 controls with no venous thrombosis [20]. No increased prevalence of hyperthyroidism was found in patients with venous thrombosis compared with controls.

The objective of this thesis is to clarify the relation between thyroid hormones, the coagulation factors and their effect on the risk of haemorrhage, first venous thrombosis and recurrent venous thrombosis. The effect of thyroid hormones and thyroid stimulating hormones in the thyroid axis on the coagulation system is not clear in all details. Importantly, it is unknown which coagulation factors are influenced by either TSH of FT4 and which are not. Also, claims have been made on a possible auto-immune effect of anti-thyroid peroxidase antibodies on the coagulation system, and so there could be an effect on the coagulation system of these antibodies. Because large studies concerning the effect of thyroid function on the risk of venous thrombosis and haemorrhage are missing, these risks in patients with aberrant thyroid function remain unknown. Since risk factors for a first venous thrombosis

differ from those of a recurrent venous event, it is also of interest to assess the effect of thyroid function on recurrent venous thrombosis.

Outline of this thesis

In chapter 2, the effect of levels of free thyroxine (FT4) and thyroid stimulating hormone on individual coagulation factors is evaluated in a group of patients treated for thyroid cancer. The effect of levels of free thyroxine on the risk for major bleeding in a population using vitamin K antagonists is described in Chapter 3. In the TROL study, a large Norwegian prospective cohort study, the relation between levels of free thyroxine and risk of venous thrombosis was studied, which is described in Chapter 4. Chapter 5, 6 and 7 describe data from the MEGA study, a population based case-control study in Leiden on the aetiology of venous thrombosis. In Chapter 5, the relation between levels of free thyroxine and risk of thrombosis is studied in more detail and in several subgroups. Furthermore, the relation between free thyroxine and coagulation factors is assessed within controls. Because contradictory findings have been reported on the effect of low levels of free thyroxine on the risk of venous thrombosis, this was assessed in the MEGA study and discussed in chapter 6. In chapter 7 the effect of levels of free thyroxine on the risk of recurrent venous thrombosis is reported, as estimated from the MEGA follow up study. Chapter 8 presents a review of the literature incorporating the findings of this thesis.

The effect of changes in thyroxine and thyroid stimulating hormone levels on the coagulation system

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Introduction

Thyroid dysfunction is known to affect the coagulation system [19,21-23]. In patients with overt hypothyroidism decreased levels of factor VIII (FVIII), fibrinogen and von Willebrand factor (VWF) have been observed [24,25]. In hyperthyroidism the opposite has consistently been described, i.e. elevated levels of VWF and fibrinogen. Free thyroxine (FT4) and thyrotropin (TSH) are inversely related: when FT4 rises, TSH drops and vice versa. Although it is generally assumed that levels of FT4 are related to altered coagulation parameters, it remains unknown whether this effect on coagulation is partially mediated by thyrotropin (TSH). Recently more evidence is provided showing that TSH can have a direct effect itself in peripheral tissues such as bone, adipose tissue and muscle, mediated via the TSH receptor [26-28]. The question whether TSH, independently of FT4, also affects the coagulation system remains therefore to be answered. We had the opportunity to study the separate effects of these hormones in two series of patients successfully treated for well-differentiated thyroid carcinoma. We set out to examine and disentangle the effect of changes in TSH and serum FT4 levels on FVIII, VWF and fibrinogen. Additionally, we explored the influence of FT4 and TSH levels on other coagulation factors.

Methods

The analyses were based on data from patients treated for well-differentiated thyroid carcinoma. Patients were derived from two studies initiated to assess the influence of thyroid hormone on:

1. Metabolism and gene expression in relation to heart rate and blood pressure [29].
2. Bone metabolism [30].

Eligibility criteria for these studies have been described previously [29,30]. In short: patients > 18 years and cured from well-differentiated thyroid carcinoma in need for diagnostic thyroglobulin (TG) stimulation were eligible. Prior to diagnostic protocol, patients from the Department of Endocrinology of the Leiden University Medical

Center (LUMC) were asked to participate in the study. For both studies written informed consent was obtained and the Medical Ethics Committee of the LUMC approved both protocols.

The rationale behind the design is that serum TG determined after maximal TSH stimulation is used as tumour marker in these patients. Cure is defined as undetectable TG levels and negative total-body scintigraphy. To assess maximally stimulated TG, high levels of TSH are induced by withdrawal of levothyroxine, or by administration of recombinant human TSH (rhTSH) while continuing levothyroxine treatment [31]. Characteristics of patients assessed with either thyroxin-withdrawal or rhTSH stimulation can assumed to be similar. According to method of TG stimulation two groups were studied:

1. Eleven patients undergoing thyroxine-withdrawal were included. Blood was sampled at two points. The first blood sampling was performed after participants had stopped their levothyroxine treatment for 4 weeks. Participants restarted their levothyroxine treatment and eight weeks following the first blood sampling, the second blood sample was drawn.
2. Seventeen patients received rhTSH (Thyrogen) and blood samples were again drawn at two time points. The first sample of blood was drawn when participants were at their regular substitution level. During two subsequent days 0.9 mg rhTSH was administered intramuscularly, and after 4 days the second sample was drawn.

Biochemical analysis

Levels of fT4 and TSH were measured with an ELISA with a Modular Analytics E-170 system; intra-assay CV 1.6-2.2% and 1.3-5.0% respectively (Roche Diagnostics, Almere, The Netherlands). Coagulation levels were measured by activity assays for factor II, (STA Factor II), factor VII (STA Factor VII), factor VIII (STA deficient VIII), factor IX (STA factor IX) antithrombin (STA Antithrombin III) and protein C (chromogenic method, STA protein C chromogen) and by antigen assays for von Willebrand Factor (STA Liatest® VWF) and protein S (Liatest® Protein S and Liatest® Free Protein S), all produced by Diagnostica Stago, Asnières, France.

Furthermore, fibrinogen (method according to Claus, STA Fibrinogen, Diagnostica Stago, Asnières, France), thrombin-antithrombin complex antigen (Enzygnost TAT micro; Dade Behring, Marburg, Germany) and prothrombin fragment 1+2 antigen (Enzygnost F1+2 (monoclonal); Dade Behring, Marburg, Germany) were measured. All tests were performed in duplicates.

Statistical analysis

Median values and their ranges were determined for all measurements. Mean differences of the pre- and post-treatment levels for each coagulation factor within each patient were calculated with their 95% confidence intervals (CI95). All statistical analyses were performed using SPSS 16.0 for OS X (SPSS Inc, Chicago, IL).

Results

The thyroxine withdrawal group study group consisted of 7 women and 4 men, median age 44 years (range: 29-56). Ten participants were treated for papillary carcinoma, 1 for a follicular carcinoma. The median time from surgery and radioactive iodine ablation therapy was 16 months (range: 8-64). At measurement 1 (during levothyroxine withdrawal), levels of TSH were high with a median of 133.4 mU/l while median levels of fT4 were extremely low: 1.5 pmol/L. At measurement 2 (upon return of the normal suppleted situation), median TSH levels had decreased to 0.7 mU/l with median fT4 levels of 24.2 pmol/l. For FVIII, VWF and fibrinogen a clear difference was observed between measurement 1 and measurement 2 (FVIII (+39.1 U/dl CI95 6.8 to 71.4), FIX (+30.8 U/dl CI95 20.8 to 40.9), VWF (+32.0 U/dl CI95 13.1 to 50.8) and fibrinogen (+0.6 g/l CI95 0.2 to 1.0)). In addition as shown in Table 1, factor II (FII), antithrombin (AT), and total protein S increased to a lesser extend at measurement 2, while levels of factor VII (FVII) and protein had decreased. No clear changes were observed for prothrombin fragment 1+2 (F1+2) and thrombin-antithrombin complexes (TAT) (Table 1).

The recombinant TSH group consisted of 1 male and 16 female patients. Median age of these patients was 49 (range 25-86) years. Fifteen participants had a papillary carcinoma and 2 patients a follicular carcinoma. The median time from surgery and

radioactive iodine ablation therapy was 37 months (range 13-303). A strong increase in median TSH levels was seen between measurements, i.e. from 0.1 mU/L in the normal situation to 23.9 mU/L after rTSH suppletion. Median levels of fT4 did not differ materially between the two measurements: 21.3 pmol/L versus 22.1 pmol/L. In this group, no clear differences in levels of FVIII, FIX, VWF, fibrinogen or the other coagulation factors were observed between the two measurements (Table 2).

Discussion

In patients in whom stable fT4 levels accompanied increasing levels of TSH, no clear effect on coagulation parameters was observed. In patients who changed from a state of high TSH and low fT4 to low TSH and slightly elevated fT4 levels, a clear rise in FVIII, FIX, VWF and fibrinogen was demonstrated. These results suggest that changes in coagulation factors related to thyroid function are mainly mediated by fT4 and not by TSH.

The findings of an increase of FVIII, fibrinogen, FIX and VWF with rising levels of fT4 is consistent with previous literature [19,32-34]. Furthermore we showed an effect on nearly all other measured coagulation factors with changing levels of fT4. There are two possible mechanisms for the effect of thyroid dysfunction on coagulation: first excess or deficiency of thyroid hormone and secondly, auto immune diseases associated with thyroid disease can have an effect on coagulation [35]. In this study we focused on the first option. Excess or deficiency of thyroid hormone can lead to either a disequilibrium in the production and clearance of coagulation factors or a heightened of lowered direct genetic transcription of coagulation factors [36]. Up to this point, no definitive answer is at hand explaining the exact mechanism.

Due to the setting of this study within patient care, there were differences between the two groups: Firstly, the mean TSH that we measured was higher in the withdrawal (141.7 mU/l) than in the recombinant TSH group (23.2 mU/l). However, it is known that shortly after rhTSH administration levels of TSH rise to values above 100 mU/L [37] and decrease gradually towards the values like those we measured a few days later. This means that both groups are likely to have been exposed to

similar peak levels of TSH. Secondly, there were only 4 days of rhTSH stimulation in the recombinant TSH group. It may be argued that this period is too short to demonstrate an effect on the coagulation system. However, in several studies using a direct trigger for activation of the coagulation system changes in coagulation parameters were observed within 5 to 8 hours [38-40]. Also, direct in-vivo stimulation of hepatocytes with T3 showed a clear response in fibrinogen and coagulation factor II and X within 12 hours. This effect was even more pronounced after 24 hours [36]. In studies on venous thrombosis after orthopaedic surgery, within 4 to 5 days after surgery, fatal pulmonary emboli occurred [41], reflecting a clear activation of the coagulation system within this time frame. Given these observations, if any effect is present, we would expect that 4 days of high TSH exposure would be more than sufficient to result in a demonstrable difference between pre- and post-testing.

Despite these limitations we observed clear changes in levels of coagulation parameters only with rising levels of FT4, and not with changing TSH levels. Recently it was found that higher levels of FT4 resulted in an increased risk of venous thrombosis [42]. In our study, the changes in coagulation parameters we found did not point unequivocal in a pro- or anticoagulant direction, but the large increases in FVIII and FIX accompanying support a prothrombotic effect of hyperthyroidism.

In conclusion, the results from our study suggest that a rise in thyroxine level is associated with increasing FVIII, FIX, VWF and fibrinogen levels, and that this shift is not primarily a result of high TSH levels. An overall shift in other coagulation factors, mostly towards a pro-thrombotic tendency was also observed.

Table 1: Effect on coagulation parameters of increasing levels of thyroxine and decreasing levels of TSH (thyroxine withdrawal group)

	Measurement 1 Median (range)	Measurement 2 Median (range)	Mean difference	CI 95
Thyroxine (pmol/L)	1.5 (0.0-2.4)	24.2 (17.4-31.2)	23.4	20.4 to 26.3
Ref: 10-24 pmol/L				
TSH (mU/L)	133.4 (99.1-191.9)	0.7 (0.0-3.0)	-141.7	-164.7 to -118.6
Ref: 0.3-4.7 mU/L				
F1+2 (pmol/l)	125 (100-284)	158 (91-1888)	7.7	-47.0 to 62.5
TAT (ug/l)	2.3 (1.9-18.2)	2.7 (2.1-72.5)	-1.0	-6.5 to 4.6
AT (U/dl)	104 (82-141)	116 (90-152)	13.4	5.0 to 22.0
Protein C (U/dl)	132 (99-200)	113 (91-163)	-13.6	-22.8 to -4.3
Total Protein S (U/dl)	107 (88-143)	106 (93-160)	7.0	-0.1 to 14.2
FII (U/dl)	114 (83-137)	122 (92-150)	8.5	2.1 to 14.9
FVII (U/dl)	136 (106-213)	105 (88-157)	-29.9	-46.4 to -13.4
FVIII (U/dl)	95 (55-155)	133 (84-219)	39.1	6.8 to 71.4
FIX (U/dl)	96 (70-134)	141 (101-171)	30.8	20.8 to 40.9
Fibrinogen (g/l)	3.1 (2.0-3.9)	3.7 (2.5-4.6)	0.6	0.2 to 1.0
VWF (U/dl)	79 (51-120)	102 (67-186)	32.0	13.1 to 50.8

TSH: Thyroid stimulating hormone; AT: Antithrombin; F1+2: prothrombin fragment 1 and 2; FII: factor II; FVII: factor VII; FVIII: factor VIII; FIX: factor IX; TAT: thrombin-antithrombin complex; VWF: von Willbrand factor; CI 95; 95% confidence interval.

Table 2: effect on coagulation parameters of increasing levels of TSH with stable levels of fT4. (Recombinant human TSH group)

	Measurement 1 Median (range)	Measurement 2 Median (range)	Mean difference	CI 95
Thyroxine (pmol/L) Ref: 10-24 pmol/L	21.3 (15.2-27.0)	22.1 (17.8-27.2)	1.0	0.1 to 1.9
TSH (mU/L) Ref: 0.3-4.7 mU/L	0.1 (0.0-3.0)	23.9 (5.5-46.7)	23.2	17.4 to 29.1
F1+2 (pmol/l)	258 (124-392)	204 (108-343)	-30.5	-69.0 to 7.9
TAT (ug/l)	3.9 (2.3-21.9)	3.4 (2.0-23.8)	1.3	-3.2 to 5.8
AT (U/dl)	126 (107-145)	118 (101-140)	-1.7	-6.4 to 3.0
Protein C (U/dl)	125 (90-150)	122 (80-140)	-3.9	-8.0 to 0.2
Total Protein S (U/dl)	126 (94-159)	124 (88-169)	-2.1	-5.6 to 1.4
FII (U/dl)	113 (95-130)	111 (99-126)	-3.0	-7.1 to 1.1
FVII (U/dl)	118 (90-172)	112 (95-153)	-8.8	-15.3 to -2.3
FVIII (U/dl)	123 (71-324)	138 (59-258)	-10.3	-54.1 to 33.4
FIX (U/dl)	160 (114-234)	160 (97-214)	-12.7	-25.7 to 0.3
Fibrinogen (g/l)	3.7 (2.4-4.6)	3.8 (2.3-5.6)	0.1	-0.2 to 0.4
VWF (U/dl)	105 (61-316)	150 (44-293)	7.3	-16.8 to 31.4

TSH: Thyroid stimulating hormone; AT: Antithrombin; F1+2: prothrombin fragment 1 and 2; FII: factor II; FVII: factor VII; FVIII: factor VIII; FIX: factor IX; TAT: thrombin-antithrombin complex; VWF: von Willbrand factor; CI 95; 95% confidence interval.

Major haemorrhage during vitamin K antagonist treatment: the influence of thyroid hormone levels

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Abstract

Background

Annually, approximately 1-3% of patients treated with vitamin K antagonists (VKA) suffer from major haemorrhage. Since high levels of free thyroxine (FT4) are associated with increased thrombosis risk, the aim was to assess whether low levels of FT4 contribute to major haemorrhage in patients under VKA treatment.

Methods

The FACTORS study is a case control study in patients using VKA treatment including 110 cases with major haemorrhage. Controls were 220 matched participants treated with VKA without major haemorrhage. Odds ratios (OR) and 95% confidence intervals (CI95) for the association of FT4 levels and major haemorrhage were calculated for different FT4 cutoffs by conditional logistic regression.

Results

In patients with a FT4 level below 13 pmol/l the risk of major haemorrhage was 5-fold increased (OR 5.1, CI95 0.9 to 28.6) compared with patients with a FT4 above 13 pmol/l. At a cut-off of 14 pmol/l the risk was 3-fold increased (OR 2.9, CI95 1.0 to 8.5). High levels of FT4 did not affect bleeding risk. No clear effect of TSH and thyroid peroxidase antibodies was seen on the risk of major haemorrhage.

Conclusions

These results indicate that FT4 levels below 14 pmol/l play a role in the aetiology of major haemorrhage in VKA users.

Introduction

Vitamin K antagonist treatment (VKA) is used for several indications where anti-coagulation is needed, such as the treatment and prevention of venous thrombosis or the prevention of cerebrovascular accidents in patients with atrial fibrillation. Haemorrhage is an important complication of anticoagulant treatment. Annually, 7-10 % of patients treated with VKA suffer from haemorrhage, and 1-3% from major haemorrhage [43,44]. Several risk factors for a bleeding tendency have been identified, such as co-morbidities, older age and use of co-medication [43,44].

Recently the relation between thyroid hormone and the coagulation system has gained interest as a focus of research [19,21,22,35,45]. High levels of free thyroxine (FT4) are associated with high levels of factor VIII (FVIII), von Willebrand factor (vWF), fibrinogen and factor IX (FIX) [19,46,47] and are a risk factor for venous thrombosis [42,48,49]. On the other side of the spectrum, low levels of FVIII, vWF and fibrinogen have been described in hypothyroidism resulting in a protective effect on the risk for venous thrombosis [19]. It has also been reported that low levels of FT4 may lead to acquired von Willebrand syndrome [45]. Low levels of FVIII and vWF are a risk factor for major haemorrhage [50]. Since FVIII and vWF are influenced by FT4, low FT4 levels may also influence bleeding risk in patients under VKA treatment.

FT4 can exert its effect on the coagulation system also in yet another way. It is known that FT4 has an effect on the pharmacodynamics of VKA, with different levels of FT4 resulting in different international normalized ratio (INR) values [51,52]. Importantly, VKA dose is continuously adapted by the anticoagulation clinics to ensure therapeutic INR range. This prevents a clinical effect of FT4 on INR levels in this study.

The aim of the present study was to assess whether the level of FT4 plays a role in the aetiology of major bleeding complications in patients under VKA treatment.

Materials and Methods

Patients and data collection

The study design of the FACTORS (FACTors in ORal anticoagulant Safety) case-control study has been described in a previous paper [53]. In short, in the registries of two Anticoagulation Clinics (Leiden and Amsterdam, The Netherlands) all patients with bleeding complications between 1999 and 2001 were identified and these complications were classified as minor or major. Patients with major haemorrhage under VKA treatment were included as cases. Major haemorrhage was defined as haemorrhage leading to death or hospitalization; a haemoglobin decrease >1.25 mmol/l; intracranial, intramuscular, joint or intraocular haemorrhage. In total, 110 cases were included in the study. Two control subjects (i.e. patients under VKA treatment without haemorrhage) per case were selected from the same registries and matched for age (10 year age strata), sex, indication of VKA therapy, anticoagulation clinic, type of VKA and whether individuals were still on active treatment at time of data collection. If, for example, a case on VKA treatment for atrial fibrillation was included, two controls on VKA treatment for atrial fibrillation were included. A total of 220 controls was included. The study protocol was approved by both the ethics committees of the Leiden University Medical Center and the Amsterdam Medical Center.

Patients and controls were visited at home by a trained research nurse, with a median of 14 months after the bleeding event. Patients completed a questionnaire and citrated blood was drawn from the antecubital veins, kept at 4°C and centrifuged for 20 minutes at 2250*g within 2 hours from collection and stored at -80°C. Information on medication use was collected. Ten cases and 15 controls were treated with amiodarone, 0 cases and 9 controls received levothyroxine treatment and 2 controls were on thyreostatics. Bearing in mind the 1:2 ratio in which the cases and controls were recruited, amiodarone use was evenly distributed in cases and controls while levothyroxine treatment was only present in controls.

Laboratory measurements

Levels of FT4, thyroid stimulating hormone (TSH) and thyroid peroxidise antibodies (antiTPO) were measured in the available citrated plasma samples (103 cases and 213 controls; TSH in 103 cases and 208 controls) using commercially available assays (ADVIA Centaur® immunoassay system, Siemens Healthcare Diagnostics, Marburg, Germany). As these tests have not been validated by the manufacturer for use with citrated plasma, studies comparing TSH and FT4 in plasma and serum have been performed [42]. Only small systematic differences were detected and linear regression analysis showed a strong association between levels of FT4, TSH and AntiTPO measured in serum and plasma (regression coefficients: $\beta \geq 0.92$). The laboratories reference range in plasma was 10 to 24 pmol/l for FT4 and 0.32-4.32 mU/l for TSH.

Statistical analysis

FT4 results were returned by the routine laboratory in round numbers. Actual reported values of FT4 were used as cut-off points (FT4 <13, <14, <15, <16, >21, >22, >23, >24 pmol/l). Categorizing FT4 based on percentiles was not possible due to the rounded number used to report FT4. TSH and AntiTPO results were returned with 2 and 1 decimals respectively, and cut-off values for the 2.5^{th}, 5^{th}, 10^{th}, 20^{th}, 80^{th}, 90^{th}, 95^{th} and 97.5^{th} percentiles were calculated in the control population. To study the effect on bleeding risk of high levels of FT4, TSH or anti-TPO, we contrasted individuals with levels above the cut-off to those with levels below the cut-off, and vice versa for the analysis of the effect of low levels. These analyses were repeated for the various cut-off levels. Odds ratios (OR) and 95% confidence intervals (CI95) were calculated with conditional logistic regression to take the matched design into account. As density sampling for the controls was performed, the OR is identical to the rate ratio [54]. Analyses were stratified for men and women. In our analysis we tried to disentangle pathways through which FT4 might influence bleeding risk. An effect of FT4 via VKA metabolism was considered unlikely, as changing INR levels are continuously adapted to and kept in tight range. To assess whether FT4 levels exert an effect on bleeding risk mediated by VWF and FVIII levels, we ran two logistic models: one including VWF and FVIII and a model without these two variables. If the effect of FT4 is mediated through VWF and FVIII, the adjusted model

is likely to show an attenuated effect (i.e. an odds ratio towards 1.0), compared to the unadjusted model. The INR used was the last known INR before the event for cases and the last known INR before blood sampling in the controls. All statistical analyses were performed in R version 2.12.1 [55] (packages: foreign_0.8-41 [56] and survival_2.36-5 [57]). (R Development Core Team (2005). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria)

Results

Patient characteristics

The study population consisted of 110 cases and 220 controls (Table 1). Median age of the cases was 66.5 years (p2.5 to p97.5: 32.6 to 84.4) and 70.9 years (p2.5 to p97.5: 38.3 to 85.1) in the controls. Of both cases and controls 60% were men. Forty four percent of the cases and 38% of the controls used phenprocoumon, the others used acenocoumarol.

Effect of FT4 on bleeding risk

Patients with a FT4 level below 13 pmol/l had a 5-fold increased risk (OR 5.1 CI95 0.9 to 28.6) for major bleeding compared with patients with FT4 levels above or equal to 13 pmol/l (Table 2). At a cut-off of 14 pmol/l the risk was 3-fold increased (OR 2.9, CI95 1.0 to 8.5). No clear attenuation of the effect was seen adjusting for INR, VWF or FVIII, meaning that it is unlikely that the effect of FT4 on bleeding risk is mediated through these factors. No clearly increased or decreased risk could be shown for high levels of FT4, i.e., an OR of 0.6 (CI95 0.2 to 2.3) was found for a cut-off of 23 pmol/l and an OR of 1.2 (CI95 0.3 to 5.0) for a cut-off of 24 pmol/l. Stratified analysis by sex revealed odds ratios in women ranging from 5.1 (CI95 0.9 to 28.6) at a FT4 level of 13 pmol/l to an OR of 0.8 (CI95 0.2 to 4.4) at 24 pmol/l. In men, the odds ratio ranged from 2.9 (CI95 0.5 to 17.6) at 13 pmol/l to 0.3 (CI95 0.0 to 3.5) at 23 pmol/l (Table 3).

At the 97.5th percentile, no association between TSH and bleeding risk was shown (OR 1.1, CI95 0.3 to 4.6) (Table 4). At lower levels of TSH, an increased risk for major haemorrhage was observed, gradually rising to an OR of 3.6 (CI95 1.0 to 13.3)

at the 2.5th percentile. Analysis of AntiTPO showed no effect of these antibodies on the risk for major haemorrhage under VKA treatment (Table 4).

Discussion

In this case-control study we assessed the association between FT4 and the occurrence of major haemorrhage in patients under VKA treatment. We found a 5-fold increased risk of major haemorrhage in patients with low levels of FT4 (<13 pmol/l) relative to patients with higher FT4 levels. Notably, this effect was found within the normal range of FT4 levels. In this study, pathway via INR, neither a pathway via VWF and FVIII could be found as explanation for the association between FT4 levels and bleeding risk.

Unexpectedly, an association with bleeding risk was observed for lower TSH levels. This, however, is not in accordance with the increased risk at lower levels of FT4 bearing in mind the negative feedback connecting TSH and FT4. An explanation for the increased risk with both low TSH and low FT4 levels could be the presence of non-thyroidal illness (NTI) (i.e. sick euthyroid syndrome). As we only found one case in our population with both a FT4 and TSH below the 5th percentile, indicating possible NTI, NTI as explanation for the found effect is unlikely. FT4 is associated with change in coagulation factors whereas TSH is not [46,47]. Therefore a direct effect of TSH seems to be biologically less plausible. Alternatively, because levels of TSH are related to many cardiovascular parameters and diseases (blood pressure, coronary heart disease, glomerular filtration rate and serum lipid concentrations) [58-63], it is possible that low TSH is a marker for other factors associated with haemorrhage. TSH also tends to increase over time while FT4 levels remain stable [64], potentially making that measured TSH levels do not accurately reflect TSH levels at time of the event.

There are several limitations to this case-control study. By design, patients were included after the occurrence of a major haemorrhage and blood was drawn after the event. Therefore measured FT4 levels in our study might not reflect FT4 levels at time of the event. It has however been shown that FT4 levels are very stable over

time [64,65], making it likely that FT4 levels after the event are a good reflection of FT4 levels at time of the event. Blood was sampled after the acute phase of disease, making it more likely for the coagulation parameters to reflect the levels before the event. Because of the study size, presented confidence intervals were relatively wide and subanalyses were not possible. Lastly, the storage time of the blood samples may theoretically have caused changes in the parameters measurable in the samples. If this were the case, these changes would have resulted in random misclassification, also resulting in an underestimation of the true effect. Altogether, none of the above mentioned limitations can have explained the increased odds ratios found in our study.

Since this is the first study to show an increased risk for major bleeding with lower levels of FT4, it is too premature to conclude on possible clinical implications. To speculate, a more strict regulation of thyroid function in patients on VKAs could be indicated, aiming at FT4 levels > 15 pmol/l; also, in patients with spontaneous bleeding on VKA, thyroid status could be checked and, if necessary, corrected to prevent more bleeding episodes. At any rate, more research is needed to confirm our findings in larger studies and to study clinical implications.

In conclusion, our findings indicate that VKA users with lower levels of FT4 have an increased risk for major bleeding.

Table 1: Patient characteristics.

	Cases (n=110)	Controls (n=220)
Male, n (%)	66 (60%)	131 (60%)
Age at baseline, median (p2.5 to p97.5)	66.5 (32.6 to 84.4)	70.9 (38.3 to 85.1)
Phenprocoumon (%)	48 (44%)	82 (38%)
Indication		
Atrial fibrillation	27	62
Cardioversion	1	11
Venous thrombosis	3	6
Mechanical heart valve	18	38
Recurrent venous thrombosis	8	14
Peripheral atherosclerosis	16	29
Ischemic heart disease	19	33
Prophylactic	7	14
Stroke	5	5
Other	6	8
Type of haemorrhage (n)		
GE bleeding	50	
Epistaxis	8	
Muscle bleeding	27	
Intra-cranial bleeding	5	
Retinal bleeding	9	
Haematuria	9	
Other bleeding	2	
Thyroid function		
FT4, pmol/l (p2.5 to 97.5)	17.7 (12.2 to 23.2)	17.7 (13.3 to 24.1)
TSH, mU/l, (p2.5 to p97.5)	1.09 (0.03 to 7.17)	1.17 (0.15 to 6.46)
ATPO, U/dl, (p2.5 to p97.5)	16.6 (16.6 to 1444.4)	16.6 (16.6 to 1444.4)
Subclinical hypothyroidism	6	10
Hypothyroidism	0	0
Subclinical hyperthyroidism	10	8
Hyperthyroidism	1	2
Coagulation		
FVIII, IU/dl, (p2.5 to p97.5)	118.5 (75.9 to 190.1)	115.0 (79.4 to 172.3)
vWF ag, IU/dl, (p2.5 to p97.5)	160.0 (66.4 to 323.4)	148.0 (79.0 to 250.7)

N indicates number; p, percentile; FT4, thyroxine; TSH, thyroid stimulating hormone; ATPO, thyroid peroxidase antibodies; FVIII, factor VIII and GE, gastroeophageal.

Table 2: risk for major bleeding in VKA users with different levels of FT4.

FT4	Cases	Controls	OR[1] (CI95)	OR[2] (CI95)	OR[3] (CI95)	OR[4] (CI95)
<13.3	4/99	2/211	5.1 (0.9 to 28.6)	3.5 (0.4 to 31.2)	5.3 (0.9 to 30.8)	3.8 (0.3 to 40.7)
<14.4	9/94	8/205	2.9 (1.0 to 8.5)	5.1 (0.7 to 35.6)	2.5 (0.8 to 7.8)	7.1 (0.9 to 56.1)
<15.6	14/89	29/184	1.0 (0.5 to 2.1)	2.8 (0.9 to 8.8)	1.1 (0.5 to 2.2)	3.6 (1.0 to 12.8)
<16.7	20/83	58/155	0.6 (0.3 to 1.1)	0.9 (0.3 to 2.3)	0.7 (0.4 to 1.3)	1.3 (0.5 to 3.8)
>21.1	14/89	29/184	1.2 (0.5 to 2.4)	0.6 (0.2 to 1.9)	1.0 (0.5 to 2.1)	0.6 (0.2 to 1.7)
>22.2	6/97	20/193	0.6 (0.2 to 1.7)	0.4 (0.1 to 2.0)	0.6 (0.2 to 1.7)	0.4 (0.1 to 2.0)
>23.3	3/100	11/202	0.6 (0.2 to 2.3)	0.3 (0.0 to 2.8)	0.5 (0.1 to 2.1)	0.2 (0.0 to 2.4)
>24.4	3/100	6/207	1.2 (0.3 to 5.0)	0.7 (0.1 to 6.7)	1.1 (0.3 to 4.7)	0.7 (0.1 to 7.5)

Reference group is the group above (for the lower cut-offs) and below (for the higher cut-offs) the cutoff value. CFT4 indicates citrated free thyroxine; FT4, free thyroxine; OR, odds ratio and CI95, 95% confidence interval. OR[1]: crude odds ratio. OR[2]: odds ratio adjusted for last measured INR. OR[3]: odds ratio adjusted for factor VIII and vWF. OR[4]: odds ratio adjusted for last measured INR, factor VIII and vWF.

Table 3: risk for major bleeding in VKA users with different levels of FT4, in men and women.

FT4	Cases[male]	Controls[male]	OR[male] (CI95)	Cases[female]	Controls[female]	OR[female] (CI95)
<13.3	0/61	0/126	na	4/38	2/85	5.1 (0.9 to 28.6)
<14.4	3/58	2/124	2.9 (0.5 to 17.6)	6/36	6/81	2.9 (0.8 to 11.0)
<15.6	7/54	16/110	0.9 (0.4 to 2.4)	7/35	13/74	1.2 (0.4 to 3.3)
<16.7	10/51	29/97	0.6 (0.3 to 1.5)	10/32	29/58	0.6 (0.3 to 1.5)
>21.1	7/54	20/106	0.7 (0.3 to 1.9)	7/35	9/78	2.6 (0.7 to 9.0)
>22.2	3/58	13/113	0.5 (0.1 to 1.7)	3/39	7/80	1.0 (0.2 to 4.7)
>23.3	1/60	6/120	0.3 (0.0 to 3.5)	2/40	5/82	0.8 (0.2 to 4.4)
>24.4	1/60	1/125	3.5 (0.2 to 55.8)	2/40	5/82	0.8 (0.2 to 4.4)

Reference group is the group above (for the lower cut-offs) and below (for the higher cut-offs) the cutoff value. CFT4 indicates citrated free thyroxine; FT4, free thyroxine; OR, odds ratio; CI95, 95% confidence interval and na, not applicable.

Table 4: risk for major bleeding in VKA users with different levels of TSH and AntiTPO.

Percentile	TSH (mU/l)	Cases	Controls	OR (CI95)
<2.5	0.12	6/97	5/203	3.6 (1.0 to 13.3)
<5	0.29	10/93	10/198	2.5 (1.0 to 6.3)
<10	0.45	17/86	20/188	2.2 (1.0 to 4.8)
<20	0.72	27/76	41/167	1.8 (1.0 to 3.3)
>80	2.14	21/82	41/167	1.0 (0.6 to 1.9)
>90	2.74	14/89	22/188	1.5 (0.7 to 3.3)
>95	4.47	6/97	10/198	1.2 (0.4 to 3.2)
>97.5	6.60	3/100	5/203	1.1 (0.3 to 4.6)
Percentile	**AntiTPO (U/dl)**	**Cases**	**Controls**	**OR (CI95)**
>80	22.4	19/84	42/171	0.9 (0.5 to 1.6)
>90	155.1	12/91	21/192	1.3 (0.6 to 2.7)
>95	585.2	7/96	10/203	1.5 (0.5 to 4.3)
>97.5	1444.4	5/98	9/204	1.3 (0.4 to 4.1)

Reference group is the group above (for the lower cut-offs) and below (for the higher cut-offs) the cutoff value. TSH indicates thyroid stimulating hormone; AntiTPO, thyroid peroxidase antibodies; OR, odds ratio and CI95, 95% confidence interval.

Increased levels of free thyroxine and risk of venous thrombosis in a large population-based prospective study

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Abstract

Background

Recent studies have shown that high levels of free thyroxine (FT4), even without leading to hyperthyroidism, are associated with a procoagulant state.

Objectives

The aim of our study was to determine whether high levels of thyroid hormones are associated with an increased risk of venous thrombosis.

Patients/Methods

From a prospective nested case-cohort design within the Norwegian HUNT2 cohort (1995 – 1997; 66 140 subjects) all patients with venous thrombosis during follow-up (n=515) and 1476 randomly selected age and sex-stratified controls were included. Relative and absolute risks for venous thrombosis were calculated for different cut-off levels of thyroid hormones based on percentiles in the controls. and different times between blood sampling and event,

Results

In subjects with a FT4 level above the 98th percentile (17.3 pmol/l) the odds ratio (OR) was 2.5 (CI95 1.3 to 5.0) compared with subjects with levels below this percentile. For venous thrombosis within one year from blood sampling, this relative risk was more pronounced with an OR of 4.8 (CI95 1.7 to 14.0). Within half a year the association was even stronger with an OR of 9.9 (CI95 2.9 to 34.0, adjusted for age, sex and BMI). For TSH the relation was inverse and less pronounced. The absolute risk within 6 months in the population for FT4 levels above the 98th percentile was 6.1 per 1000 person-years (CI95 1.7 to 15.7).

Conclusions

Levels of free thyroxine at the upper end of the normal range are a strong risk factor for venous thrombosis. The risk increased with higher levels of thyroxine and shorter time between blood sampling and event. Further studies into the effect of clinical hyperthyroidism are warranted.

Introduction

Thyroid hormone is a main regulator of metabolism. Thyroid hormones influence the coagulation system, mainly through factor VIII (FVIII) and von Willebrand factor (VWF) [19,22,46,47]. These coagulation factor levels rise with increasing levels of free thyroxine (FT4) and vice versa. In overt hypothyroidism, this latter effect is reversible with hormone substitution [33,34]. Recent findings indicate that the effect on the coagulation system is mainly mediated via FT4. Changing thyroid stimulating hormone (TSH) levels with stable free thyroxine (FT4) levels did not result in changes in coagulation factors in two recent studies [46,47,66], while FT4 acts not only on FVIII and VWF but also on factor IX (FIX) and fibrinogen levels [19,46,47,67]. Whether or not these alterations in coagulation factors translate into a higher risk of venous thrombosis has not been studied extensively.

Although there have been several case reports on the relation between (cerebral) venous thrombosis and hyperthyroidism, an association between levels of thyroid hormone within the normal range and the risk of venous thrombosis has only recently been described in a case control study [42]. In this study a decreased risk of venous thrombosis was described for low levels of FT4 and an overall 6-fold increased risk for FT4 levels above 24 pmol/l compared with levels below this cut-off value was found. Due to its design, only relative risks could be calculated in this case-control study. Furthermore, blood was drawn at the time of thrombosis, and the FT4 levels could potentially have been influenced by the event itself. To avoid these problems, we prospectively studied the relationship between levels of FT4, thyroid stimulating hormone (thyrotropin, TSH) and the risk of venous thrombosis in a case-control study nested within a large population-based cohort (the HUNT2 cohort), where blood was drawn at baseline, before the thrombotic event occurred.

Our aim was to calculate absolute and relative risks for the effect of FT4 and TSH on the occurrence of venous thrombosis, with particular attention to the effect of different cut-off levels and to time between blood sampling and occurrence of

thrombosis. To exclude direct immune-mediated effects, we also measured anti-thyroid peroxidase antibodies (AntiTPO).

Methods

Between August 1995 and June 1997 all inhabitants of the Nord-Trøndelag county in Norway aged 20 years or older (n=94 140) were invited to participate in the second Nord-Trøndelag Health Study (HUNT2) [68]. In total, 71% (n=66 140) of all eligible individuals participated in the study. All participants gave their informed consent at enrollment in the HUNT2 study. They underwent a physical examination and received a questionnaire on several demographic measures, risk factors for cardiovascular disease, lifestyle, quality of life, medication use, and co-morbidity such as thyroid disease. In addition, serum blood samples were collected (65 291 samples, 98.7%) at start of follow-up. The inhabitants of the Nord- Trøndelag County mainly go to Levanger hospital and Namsos hospital and to the St. Olav university hospital in the neighboring county for clinical care, as other hospitals are far removed. In these centers, diagnostic ICD 9 and ICD 10 codes for venous thrombosis were collected up until 1 January 2002 which were subsequently verified with hospital records. Events were confirmed when they fulfilled the following criteria: DVT was defined by an intraluminal filling defect on ascending contrast venography in the leg or arm, a non-compressible venous segment in the popliteal, femoral or axillar veins on duplex ultrasound, or a positive computed tomography (CT) scan. Probable DVT was defined as no venous filling on ascending contrast venography or no venous flow in femoral, femoral or axillar veins on duplex ultrasound. PE was defined according to the PIOPED criteria[69,70] as a high-probability ventilation/ perfusion (V/Q) scan, i.e. ≥ 2 segmental perfusion defects (V/Q mismatch), a perfusion scan with ≥ 2 segmental perfusion defects associated with normal chest X-ray (X/Q mismatch), or a positive CT scan or by autopsy. Probable PE was defined as intermediate probability V/Q scan (1 moderate or large V/Q mismatch). Both definite and probable events were included in the analyses. Of all cases, only those who participated in the HUNT2 study were included. Patients with previous thrombosis, thrombosis before enrollment in the HUNT2 cohort or retinal-vein thrombosis were excluded [68]. In total, we identified 515 venous thrombotic events

occurring between baseline and 2002. A random sample of 1476 age and sex-stratified controls was drawn from the original HUNT2 cohort [68].

Laboratory measurements

In both the cases and the control subjects FT4, TSH and AntiTPO were measured in the baseline sample. Serum concentrations of FT4, TSH and AntiTPO were analysed at Levanger hospital. FT4, TSH and antiTPO were analysed by chemiluminescent microparticle immunoassays (CMIA) on an Architect ci8200 (Abbott Laboratories, Abbott Park, IL, USA), using reagent kits from Architect iSystem, USA (free T4 and antiTPO) or Ireland (TSH). The laboratory's reference ranges are 9.0 to 19.0 pmol/l for FT4, 0.20 to 4.50 mU/l for TSH and <5.61 for AntiTPO. In 446 cases sufficient blood was available to measure FT4, TSH and AntiTPO levels. In the controls 1231 FT4, 1228 TSH and 1230 AntiTPO levels could be measured within the available samples.

Statistical analysis

Descriptives are given as median (2.5^{th} percentile to 97.5^{th} percentile). As cut-off points for FT4, TSH and AntiTPO levels we used the 2^{nd}, 5^{th}, 10^{th}, 90^{th}, 95^{th} and 98^{th} percentiles in the control group. For the lower percentiles we compared the numbers of case and control subjects below the cut-off percentile with the numbers above this cut-off percentile. For the higher percentiles we did the opposite. We used logistic regression to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for the association between FT4, TSH and antiTPO and thrombosis risk, adjusting for age and sex to take the frequency matching into account. We additionally adjusted for body mass index (BMI) as a continuous variable. Patients with a DVT and PE were analyzed separately. We also repeated the analysis excluding all subjects who indicated some form of thyroid disease, i.e., history of hyperthyroidism or hypothyroidism, goiter, thyroid disease, use of levothyroxine, use of anti-thyroid drugs, thyroid surgery or treatment with radioactive iodine. Since FT4 levels are not constant over time, samples that were taken a long time before the event may not reflect the actual FT4 level at the time of thrombosis. Therefore we performed subanalyses for cases in whom the time between blood sampling and venous thrombosis was less than 1 year or less than 0.5 year. In addition, we stratified the

analyses for provoked (immobilisation, travel >8h, paresis, oral contraceptives, pregnancy, trauma, surgery and active or non-active cancer) and unprovoked thrombosis.

As our case-control design was nested within a cohort (a case-cohort design), we could use numbers of person-years that were available from the original HUNT2 cohort (data on person-years were available for 65 215 subjects) as a basis for the calculation of absolute risks. For each 10 year age-category we calculated the 80th and the 90th percentile of FT4 distribution in the controls. For each age-specific incidence rate we took the number of cases that occurred for this age group above this cut-off in the numerator, and 10% or 20% of the person years in the denominator. As reference incidence we did the opposite, and took all cases that occurred below this cut-off in the numerator and 80 or 90% of the person-years in the denominator. We repeated this analysis using only 1 and 0.5 years of follow-up by taking the total number of participants times 1 and times 0.5 for the person-years calculation. For the calculation of the confidence intervals the Poisson distribution was used. Statistical analyses were performed using SPSS 17.0 (SPSS Inc, Chicago, IL).

Results

In the HUNT2 cohort a total of 66 140 subjects participated. 515 cases of confirmed venous thrombosis were found in this cohort. In the cases, 44.3% were men and in the controls 45.8%. The median age of the cases (70.0 years (p2.5-p97.5: 29.9 to 87.0)) at the time of inclusion was similar to the median age at the time of inclusion in the controls (70.0 years (p2.5-p97.5: 31.0 to 88.0)). The thrombotic events occurred at a median of 33 months (p2.5 to p97.5: 1 to 68 ;range: 2 days to 76 months) after baseline. The median follow up in the controls was 66 months (p2.5 to p97.5: 55 to 77). In the cases 63.3% had a deep venous thrombosis, 30.1% had a pulmonary embolism and 6.6% had both a deep venous thrombosis and a pulmonary embolism (Table 1). No differences in basic characteristics were observed between these 515 cases and the 446 patients in whom blood was available for thyroid hormone measurements (Table 1).

The 446 cases had a median FT4 level of 13.5 pmol/L; (p2.5-p97.5: 10.7 to 17.9), a median TSH level of 1.40 mU/L (p2.5-p97.5: 0.37 to 3.53) and a median AntiTPO level of 0.8 U/ml (p2.5-p97.5: 0.1 to 433.8). The 1231 controls had a median FT4 level of 13.4 pmol/L (p2.5-p97.5: 10.8 to 17.2), a median TSH level of 1.43 mU/L (p2.5-p97.5: 0.43 to 3.48) and a median AntiTPO level of 0.8 U/ml (p2.5-p97.5: 0.2 to 360.7). In total, 28 (6.3%) cases and 66 (5.4%) controls reported some form of thyroid disease (Table 1).

As shown in Table 2 we found a higher risk for venous thrombosis (OR 2.0, 95%CI 1.1 to 3.7) for participants with high levels of FT4 (>98th percentile (17.3 pmol/l)) compared with subjects with levels of FT4 below this percentile. For participants below the 5th percentile the odds ratio was 1.1 (CI95 0.7 to 1.9). When we repeated this analysis after restricting to cases in whom the thrombotic event happened within one year from the blood draw, an odds ratio of 0.6 (CI95: 0.2 to 2.6) was observed for participants with FT4 below the 5th percentile versus participants above the 5th percentile. In this group the odds ratios increased in a dose response relation from 0.6 towards 3.8 (CI95: 1.4 to 10.3) for participants with FT4 above the 98th percentile versus participants below the 98th percentile. When we further restricted the time between blood sampling and thrombotic event to half a year, the dose-response relation became even more apparent, with ORs rising from 0.8 (CI95: 0.1 to 6.0) for the 5th percentile cut-off to 7.7 (CI95: 2.5 to 23.9) for the 98th percentile cut-off. After additional adjustment for BMI, we observed an OR of 9.9 (CI95: 2.9 to 34.0) for this highest cut-off. Adjustment for TSH had no effect on the risk estimates (Table 2)

We also performed this analysis for levels of TSH (Table 3). No relation with risk of thrombosis was observed when all cases were taken into account. When the time period between blood sampling and event was restricted to one year or half a year, lower levels of TSH were associated with a somewhat increased risk of venous thrombosis. For participants with a TSH below the 2nd percentile an OR of 3.4 (CI95: 0.7 to 15.4) was found for a time frame of half a year between blood sampling and thrombosis. Adjustment for FT4 led to attenuation of all associations (Table 3).

Neither for high nor for low levels of AntiTPO did we find an effect on risk of venous thrombosis (Table 4). Also when calculating the OR for cases and controls within and out of the reference range, no effect of AntiTPO was seen.

In a subsequent analysis we looked into the effect of FT4 on the different manifestations of venous thrombosis. For patients with a deep venous thrombosis we found an OR of 2.3 (CI95: 1.1 to 5.1), while for patients with a pulmonary embolism (with or without proven DVT) the OR was 2.5 (CI95: 1.0 to 6.5) both at the 98th percentile. The odds ratios for FT4 on the risk of venous thrombosis after exclusion of all patients with a history of thyroid disease did not substantially differ from the main findings (data in appendix 1). Also, the odds ratios for patients with provoking factors did not differ from patients with unprovoked thrombosis (Table 5).

In all unexposed groups (with FT4 levels below each cut-off) we found incidence rates of approximately 1 per 1000 person years. Incidence rates in the exposed group ranged from 1.6 per 1000 person years (CI95 1.2 to 2.1) when all cases were considered using the p90 as cut-off, to an incidence of 6.1 per 1000 person years (CI95 1.7 to 15.7) using the p98 as cut-off for subjects with time between blood sampling and event of less than 0.5 year. (Table 6).

Discussion

In this prospective population based case-cohort study of 66 140 individuals we examined the relation between levels of FT4, TSH and AntiTPO and the risk of venous thrombosis in 446 patients with thrombosis. We found a clearly higher risk for increasing levels of FT4, with an up to a 2.5 fold increased risk for the highest FT4 levels (above p98). We also observed higher odds ratios with shorter time from blood sampling (up to a 10-fold increased risk for the 98th percentile as cut-off for events that occurred within 6 months from blood sampling). The association of TSH with thrombosis risk was much less pronounced. Since we neither observed a dose-response relation for AntiTPO levels nor an effect of decreasing time between the blood draw and the thrombotic event, we suspect that a direct relation between AntiTPO and risk of venous thrombosis is unlikely.

Other earlier studies have looked into associations between thyroid disease and concentrations of coagulation factors, and generally described increased levels of coagulation factors for hyperthyroidism and lower levels for hypothyroidism [19,24,32-34,45-47]. A recent study found in 11 patients treated for thyroid carcinoma that a general rise of coagulation factors occurred towards a pro-thrombotic tendency when FT4 levels in these patients went from almost zero to a level slightly above the normal range. The rise in FVIII and VWF was most pronounced, but there were also obvious effects on FIX and fibrinogen levels [46]. In these patients, a similar increase in TSH alone (with stable FT4 levels) had no effect on these coagulation factors. This phenomenon, i.e. a direct effect of FT4 on coagulation levels where TSH appears to have no such action, was recently confirmed in a similar study [47]. Also, in our analysis, adjustment for FT4 led to reduction of the effect sizes for TSH, which did not occur the other way around. This suggests that the association between TSH and thrombosis risk is explained by FT4. It therefore appears that, in contrast to FT4, TSH has no direct effect on the coagulation system and that the association is not explained through thyroid disease. It is not known what mechanism explains the association between thyroid hormone and the coagulation system. A direct effect of FT4 on gene transcription in hepatocytes and endothelial cells leading to a higher production of coagulation factors is likely [19,36,69]. Another possible mechanism for the higher risk for thrombosis are thyroid associated auto-immune processes [70], but the lack of association between AntiTPO and risk of thrombosis in the present study does not support this hypothesis for this auto-immune antibody. More research needs to be done to establish the pathophysiology of this relationship.

Few clinical, controlled studies have been carried out on this topic. Recently, a case-control study was performed on the relation between levels of FT4 and the risk of venous thrombosis. In this study, where an overall 6-fold increased risk for FT4 levels above 24 pmol/l compared to levels below this cut-off was found, blood was sampled at the time of thrombosis. This had the advantage that the thyroid hormone levels were measured more or less at the same time that the thrombosis had developed, but it could not be excluded that some of the observed effects were due

to acute phase effects or non-thyroidal illness [42]. Another study by Danescu et al. showed no relation between hyperthyroidism and venous thrombosis. Although this is a study with a large number of subjects, there were several major limitations. No data were available on FT4 levels and no information on time between venous thrombosis and diagnosis of hyperthyroidism was given [71]. The design of this study combined the strengths of both a case-control and a follow-up design: the former has the advantage that an exposure does not need to be measured in the total population or cohort, but that a random selection suffices. So, we only needed to measure FT4, TSH and Anti-TPO in about 2000 subjects. However, an important drawback of a case-control approach is that it is generally not possible to calculate absolute risks. Yet, as our case-control design was nested within a cohort (a case-cohort design), we could still use data from the original cohort for the calculation of absolute risks. A second disadvantage of a case-control study is that the exposure is often measured after the event. This can lead to a dilution of the association, especially when, as in the case of thyroid hormone, any abnormalities may have been treated by the time the blood is sampled. It is also impossible to exclude that the event is the cause of the "exposure". These drawbacks were also prevented in our case-cohort design, as the blood was sampled before the event.

There are potential limitations to the study. Some measurements were missing, i.e. in 69 patients (13%) and 245 (17%) controls. However, as the only reason why they were missing was a technical one (not sufficient blood available or a test failure), we assumed these measurements to be missing completely at random. This was also confirmed by the fact that the general characteristics did not differ between the complete group and the included group. Secondly, the time between blood sampling and measurement of the thyroid parameters was approximately 12 years, which may have made the measurements less reliable. However, such misclassification of FT4 levels will at most have led to an underestimation of the effect measures, as such misclassification will have been random (i.e. independent of whether or not a subject develops venous thrombosis later on). This could therefore not explain our findings of a clearly increased risk. Furthermore, blood was sampled at only one moment in time and since FT4 levels are not constant over time, they might not reflect the actual FT4 level at the time of thrombosis. Such misclassification would again have

been random and led to an underestimation of the ORs. We countered this limitation by analyses restricted to individuals with blood sampling shortly before the thrombosis, and indeed found higher ORs than in the overall analysis. An alternative explanation for the higher ORs closer to the event could have been that non-thyroidal illness due to co-morbidity was present, which co-morbidity in fact led to the thrombosis, rather than the thyroid dysfunction. However, we performed a stratified analysis in which we found similar ORs for provoked (i.e. where co-morbidity was present) and unprovoked events. We therefore feel that this explanation is unlikely. Furthermore, in this study, no citrated blood was sampled at baseline so we had no opportunity to link the clinical data to levels of coagulation factors, to further study the underlying mechanism. Lastly, as this study was performed in a general population where overt hyperthyroidism is rare (prevalence 0.5% [68]), we found too few patients with biochemical hyperthyroidism at the time of blood sampling to reliably conclude on the effect of clinical thyroid disease on the risk of thrombosis. However, the clear graded association makes it likely that risks are even higher for those with overt hyperthyroidism. Our study could form the basis of studies in such populations.

Our findings may have clinical implications. From an endocrinological point of view, one should be alert on signs of venous thrombosis in patients with hyperthyroidism. From a haematological point of view, especially patients with unprovoked thrombosis could be screened for hyperthyroidism.

In the previous study on this subject [42,67], the increased risk for venous thrombosis was also found well within reference values for FT4. Interestingly, other studies on the association of thyroid function with other clinical states (such as glomerular filtration rate, coronary heart disease, serum lipid concentrations and blood pressure) have also shown an association of thyroid function within the reference range [58-63]. These findings suggest that thyroid function should perhaps not be roughly categorised into hypo-, hyper- and euthyroidism, but be used as a continuous variable in etiological or predictive studies into disease states related to thyroid function.

In conclusion, in this large, prospective study we showed that high levels of free thyroxine are a strong risk factor for venous thrombosis.

Table 1: Baseline characteristics.

	Cases (n=446)	Controls (n=1228)
male, n (%)	208 (46.6)	575 (46.8)
BMI, median (p2.5 to p97.5)	27.2 (19.7 to 38.6)	26.5 (19.8 to 36.7)
Age at baseline, median (p2.5 to p97.5)	68.5 (29.0 to 87.0)	69.0 (30.0 to 87.3)
DVT, n (%)	282 (63.2)	
PE, n (%)	164 (36.8)	
History of thyroid disease,n(%)	28 (6.3)	66 (5.4)
Radioactive Iodine treatment, n (%)	7 (1.6)	12 (1.0)
Thyroid surgery, n (%)	9 (2.0)	18 (1.5)
Use anti-thyroid drugs, n (%)	1 (0.2)	1 (0.1)
Use levothyroxine, n (%)	16 (3.6)	44 (3.6)
Thyroid disease reported, n (%)	7 (1.6)	10 (0.8)
Goiter, n (%)	11 (2.5)	20 (1.6)
Hyperthyroidism reported, n (%)	15 (3.4)	17 (1.4)
Hypothyroidism reported, n (%)	18 (4.0)	42 (3.4)

n indicates number of subjects; BMI, body mass index; p, percentile; DVT, deep venous thrombosis and PE, pulmonary embolism.

Table 2: Numbers of subjects and odds ratios for several percentiles of FT4 as well as for different time intervals.

All cases							TTE <1 year				TTE <0.5 year			
P	FT4	Controls*	Cases*	OR (CI95)	OR# (CI95)	OR^ (CI95)	Cases*	OR (CI95)	OR# (CI95)	OR^ (CI95)	Cases*	OR	OR# (CI95)	OR^ (CI95)
2	10.7	21/1210	10/436	1.3 (0.7 to 2.8)	1.3 (0.6 to 2.8)	1.3 (0.6 to 3.0)	0/71	na	na	na	0/30	na	na	na
5	11.1	54/1177	22/424	1.1 (0.7 to 1.9)	1.1 (0.6 to 1.8)	1.1 (0.6 to 1.8)	2/69	0.6 (0.2 to 2.6)	0.6 (0.2 to 2.7)	0.7 (0.2 to 3.0)	1/29	0.8 (0.1 to 5.6)	0.8 (0.1 to 6.0)	0.8 (0.1 to 6.5)
10	11.7	122/1109	59/387	1.4 (1.0 to 1.9)	1.3 (0.9 to 1.8)	1.4 (1.0 to 1.9)	6/65	0.8 (0.4 to 2.0)	0.8 (0.4 to 2.0)	1.0 (0.4 to 2.3)	3/27	1.0 (0.3 to 3.4)	1.0 (0.3 to 3.4)	1.1 (0.3 to 3.8)
90	15.5	120/1111	55/391	1.3 (0.9 to 1.8)	1.5 (1.0 to 2.1)	1.4 (1.0 to 2.1)	14/57	2.3 (1.2 to 4.2)	2.7 (1.4 to 5.2)	2.5 (1.3 to 4.9)	6/24	2.3 (0.9 to 5.8)	2.8 (1.1 to 7.2)	2.7 (1.0 to 7.1)
95	16.3	58/1173	30/416	1.5 (0.9 to 2.3)	1.7 (1.1 to 2.8)	1.7 (1.0 to 2.7)	9/62	2.9 (1.4 to 6.2)	3.6 (1.7 to 7.9)	3.2 (1.4 to 7.2)	5/25	4.0 (1.5 to 10.9)	5.0 (1.8 to 14.3)	4.9 (1.7 to 14.2)
98	17.3	24/1207	17/429	2.0 (1.1 to 3.7)	2.5 (1.3 to 5.0)	2.4 (1.2 to 4.9)	5/66	3.8 (1.4 to 10.3)	4.8 (1.7 to 14.0)	4.1 (1.4 to 12.2)	4/26	7.7 (2.5 to 23.9)	9.9 (2.9 to 34.0)	9.7 (2.7 to 34.4)

P indicates percentile; FT4, free thyroxine(pmol/l); TSH, thyrotropin or thyroid stimulating hormone; OR, odds ratio; CI95, 95% confidence interval; TTE <1 year, cases with thrombosis less than one year from blood draw, TTE <0.5 year, cases with thrombosis less than half a year from blood draw, na, not applicable.
*: For the lower percentiles the numbers of case and control subjects below the cut-off percentile are compared to numbers above this cut-off percentile. For the higher percentiles the opposite applies.
OR: odds ratio adjusted for age and sex
OR#: odds ratios adjusted for age, sex and BMI.
OR^: odds ratios adjusted for age, sex, BMI and TSH

Table 3: Odds ratios for percentiles of TSH and different time intervals. Adjusted for age and sex.

All cases						TTE <1 year			TTE <0.5 year		
P	TSH	Controls*	Cases*	OR (CI95)	OR# (CI95)	Cases*	OR (CI95)	OR# (CI95)	Cases*	OR	OR# (CI95)
2	0.37	24/1204	11/435	1.3 (0.6 to 2.6)	1.2 (0.6 to 2.5)	4/67	2.8 (0.9 to 8.4)	1.8 (0.5 to 5.7)	2/28	3.4 (0.7 to 15.4)	2.2 (0.4 to 11.7)
5	0.54	58/1170	29/417	1.4(0.9 to 2.2)	1.4 (0.9 to 2.2)	7/64	2.1 (0.9 to 4.9)	1.6 (0.7 to 3.9)	2/28	1.4 (0.3 to 6.0)	1.0 (0.2 to 4.7)
10	0.71	118/1110	50/396	1.2 (0.8 to 1.7)	1.2 (0.8 to 1.7)	11/60	1.7 (0.9 to 3.4)	1.4 (0.7 to 2.9)	5/25	1.9 (0.7 to 5.0)	1.6 (0.6 to 4.4)
90	2.70	122/1106	38/408	0.9 (0.6 to 1.3)	0.9 (0.6 to 1.3)	5/66	0.6 (0.3 to 1.6)	0.7 (0.3 to 1.9)	3/27	0.9 (0.3 to 3.1)	1.1 (0.3 to 3.7)
95	3.15	61/1167	20/426	0.9 (0.5 to 1.5)	0.9 (0.6 to 1.6)	1/70	0.3 (0.0 to 1.9)	0.3 (0.0 to 2.3)	1/29	0.6 (0.1 to 4.6)	0.7 (0.1 to 5.7)
98	3.63	24/1204	8/438	0.9 (0.4 to 2.1)	1.0 (0.4 to 2.2)	0/71	na	na	0/30	na	na

P indicates percentile; TSH, thyrotropin or thyroid stimulating hormone **(mU/l)**; FT4, free thyroxine; OR, odds ratio; CI95, 95% confidence interval; TTE <1 year, cases with thrombosis less than one year from blood draw; TTE <0.5 year, cases with thrombosis less than a half year from blood draw and na, not applicable.
*: For the lower percentiles the numbers of case and control subjects below the cut-off percentile are compared to numbers above this cut-off percentile. For the higher percentiles the opposite applies.
OR: odds ratio adjusted for age and sex
OR#: odds ratios adjusted for age, sex, BMI and FT4

Table 4: Odds ratios for percentiles of AntiTPO and different time intervals (adjusted for age and sex).

			All cases		TTE <1 year		TTE <0.5 year	
P	**AntiTPO**	**Controls***	**Cases***	**OR (CI95)**	**Cases***	**OR (CI95)**	**Cases***	**OR (CI95)**
2	0.14	20/1210	10/436	1.4 (0.6 to 3.0)	1/70	1.0 (0.1 to 7.4)	1/29	2.0 (0.2 to 16.8)
5	0.22	61/1169	27/419	1.2 (0.8 to 2.0)	2/69	0.6 (0.1 to 2.3)	2/28	1.4 (0.3 to 6.7)
10	0.29	115/1115	46/400	1.1 (0.8 to 1.6)	3/68	0.5 (0.1 to 1.5)	2/28	0.7 (0.2 to 3.2)
90	22.62	123/1107	43/403	1.0 (0.7 to 1.4)	6/65	0.8 (0.3 to 1.9)	2/28	0.6 (0.1 to 2.7)
95	127.35	61/1169	23/423	1.0 (0.6 to 1.7)	2/69	0.5 (0.1 to 2.3)	2/28	1.4 (0.3 to 5.9)
98	458.44	24/1206	10/436	1.1 (0.5 to 2.4)	1/70	0.7 (0.1 to 5.4)	1/29	1.7 (0.2 to 13.1)

P indicates percentile; AntiTPO, anti-thyroid peroxidase antibodies; OR, odds ratio; CI95, 95% confidence interval; TTE <1 year, cases with thrombosis less than one year from blood draw and TTE <0.5 year, cases with thrombosis less than a half year from blood draw. *: For the lower percentiles the numbers of case and control subjects below the cut-off percentile are compared to numbers above this cut-off percentile. For the higher percentiles the opposite applies.

Table 5: Odds ratios for percentiles of FT4 and provoked or unprovoked cases (adjusted for age, sex and BMI).

		Provoked			Unprovoked		
P	**FT4 (pmol/l)**	**Cases***	**Controls***	**OR (CI95)**	**Cases***	**Controls***	**OR (CI95)**
2	10.7	5/233	21/1210	1.2 (0.4 to 3.2)	5/203	21/1210	1.5 (0.7 to 3.6)
5	11.1	7/231	54/1177	0.7 (0.3 to 1.5)	15/193	54/1177	1.5 (0.8 to 2.9)
10	11.6	27/211	122/1109	1.2 (0.7 to 1.8)	32/176	122/1109	1.5 (1.0 to 2.3)
90	15.3	31/207	120/1111	1.5 (1.0 to 2.4)	24/184	120/1111	1.4 (0.9 to 2.3)
95	16.0	18/220	58/1173	1.9 (1.0 to 3.3)	12/196	58/1173	1.5 (0.8 to 3.0)
98	16.9	10/228	24/1207	2.6 (1.1 to 5.8)	7/201	24/1207	2.5 (1.0 to 6.2)

P indicates percentile; FT4, free thyroxine; OR, odds ratio; CI95, 95% confidence interval. *: For the lower percentiles the numbers of case and control subjects below the cut-off percentile are compared to numbers above this cut-off percentile. For the higher percentiles the opposite applies.

Table 6: Incidence rates for higher levels of FT4

p	n	py	TBSE	cases>p	cases<p	Incidence >p (CI95)	Incidence <p (CI95)
90	65215	341853	all	55	391	1.6 (1.2 to 2.1)	1.3 (1.2 to 1.4)
95	65215	341853	all	30	416	1.8 (1.2 to 2.5)	1.3 (1.2 to 1.4)
98	65215	341853	all	17	429	2.5 (1.4 to 4.0)	1.3 (1.2 to 1.4)
90	65215	65215	1	14	57	2.1 (1.2 to 3.6)	1.0 (0.7 to 1.3)
95	65215	65215	1	9	62	2.8 (1.3 to 5.2)	1.0 (0.8 to 1.3)
98	65215	65215	1	5	66	3.8 (1.2 to 8.9)	1.0 (0.8 to 1.3)
90	65215	32608	0.5	6	24	1.8 (0.7 to 4.0)	0.8 (0.5 to 1.2)
95	65215	32608	0.5	5	25	3.1 (1.0 to 7.2)	0.8 (0.5 to 1.2)
98	65215	32608	0.5	4	26	6.1 (1.7 to 15.7)	0.8 (0.5 to 1.2)

P indicates percentile; n, number of participants; py, person years; TBSE, time between sampling and event; CI95, 95% confidence interval.

High levels of procoagulant factors mediate the association between free thyroxine and risk of venous thrombosis: the MEGA-study

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Abstract

Background

Thyroid hormone affects the coagulation system, but its effect on clinical disease is not clear. We determined the associations between levels of free thyroxine (FT4), thyroid stimulating hormone (TSH) and thyroid peroxidase antibodies (antiTPO) with levels of coagulation factors and the risk of venous thrombosis.

Methods

In a large population based case-control study (MEGA-study) into the etiology of venous thrombosis we determined levels of FT4, TSH and antiTPO and coagulation factors FII, FVII, FVIII, FIX, FX, VWF, antithrombin, protein C and S, and fibrinogen in 2177 cases and 2826 controls.

Results

High levels of FT4 were associated with increased concentrations of procoagulant factors, and not with levels of the anticoagulant factors. High levels of FT4 were also associated with the risk of venous thrombosis, up to an OR of 2.2 (CI95 1.0-4.6) for levels above 24.4 pmol/l relative to FT4 levels between 15.5 and 18.9 pmol/l. In 11 cases and 1 control clinical hyperthyroidism had been diagnosed within a year of the thrombotic event, leading to an OR of 17.0 (CI95 2.2-133.0) for thrombosis. The odds ratios approached unity after adjustment for FVIII and VWF, which suggests that the effect was mediated by these factors. Low TSH levels were also, but less evidently associated with thrombosis, while there was no association with antiTPO and venous thrombosis risk.

Conclusions

High levels of FT4 increase the concentrations of procoagulant proteins FVIII, FIX, fibrinogen and VWF and by this mechanism increase the risk of venous thrombosis.

Introduction

As early as 1913, a case-report suggested a relationship between thyroid disease and venous thrombosis [13,23]. This and subsequent case-reports described subjects with hyperthyroidism and rare types of venous thrombosis, such as cerebral venous thrombosis [14-18]. Later studies focused on alterations in coagulation factors in patients with thyroid disease and most confirmed that hyperthyroidism was associated with prothrombotic changes. These included a rise in several individual coagulation factors, with most consistent effects on levels of factor VIII (FVIII), von Willebrand factor (VWF) and fibrinogen [19,22,25,32-34]. However, studies assessing the effect of high levels of FT4 on the occurrence of venous thrombosis are scarce. In a recent case-control study a 2-fold increased risk for venous thrombosis was found for a free thyroxine (FT4) level above 17 pmol/L compared with a FT4 below this level, where relative risks increased with higher levels of FT4 [42]. Furthermore, in a case-cohort study performed within the HUNT2 cohort in Norway, the risk of venous thrombosis clearly increased gradually with increasing levels of FT4 (up to a 10-fold increased risk for FT4 levels >17.3 pmol/l relative to levels below this cut-off) [48]. The sample sizes of these studies allowed establishing the relation between FT4 and venous thrombosis, but no in depth analysis was possible. Also, no citrated blood was available for analysis of coagulation factors. While the associations with venous thrombosis risk in these studies, and with procoagulant factors in other studies suggest a mechanism for the prothrombotic effect of high levels of FT4, no studies have assessed both in a single sample, and hence no formal mediation analysis has been performed.

The aim of the current analysis was therefore to study in a large population-based case-control study the effect of thyroid hormone levels on levels of coagulation proteins and to assess the role of the latter in the association between FT4 and venous thrombosis.

Methods

The Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study is a large population-based case-control study. Data

collection and ascertainment of venous thrombotic events have been previously described in detail [4]. Briefly, patients aged 18 to 70 years and diagnosed with a first deep venous thrombosis (DVT) of the leg, a pulmonary embolism (PE) or a combination of both were asked to participate in the study. Patients were recruited between 1999 and 2004 from six regional Anticoagulation Clinics in the Netherlands, which all are sole providers in a well-defined geographical region. Partners of these patients and subjects recruited by Random Digit Dialling (RDD), frequency-matched for age and sex, were invited as control subjects. For both control-groups the same selection criteria as for patients were applied. The Medical Ethics Committee of the Leiden University Medical Center approved the study protocol. Written consent was obtained from all participants.

Of 6237 eligible patients with DVT and PE, 276 died before they could be included in the study. Of the remaining patients 4958 (83%) participated. Information regarding the diagnostic procedure was obtained via hospital records and general practitioners. Deep venous thrombosis (DVT) was considered definite when (Doppler) ultrasonography showed the presence of a thrombus in the deep veins. Pulmonary embolism was considered definite when confirmed with a high-probability ventilation-perfusion scan, positive spiral computed tomographic findings, or positive angiographic findings. Pulmonary embolism was considered probable when the diagnosis was based on a low- or intermediate-probability ventilation-perfusion scan, inconclusive spiral computed tomographic findings, or inconclusive angiographic findings. In all patients the diagnosis was considered definite or probable [72].

Of the 4958 participating patients, 3581 had an eligible partner of whom 2917 participated (81%). An additional 314 control subjects were included of whom the partner (case) was excluded for several reasons (non-Dutch speaking, severe psychiatric disorders), resulting in a total of 3231 partners. Of 4350 eligible RDD control subjects, 3000 participated (69%), for a total of 6231 controls. For patients and their partner controls, the date of the thrombotic event was used as the index date. For the RDD controls the date of completion of the questionnaire was used as index date. Participants were asked to complete a questionnaire on potential risk factors for venous thrombosis (including thyroid disease and thyroid medication) and

to donate a venous blood sample after an overnight fast. Blood was donated with a median of 10 months after the event (range: 3 to 36 months). Only when a participant was still using oral anticoagulant treatment with vitamin K antagonists more than one year after the index date, the blood sample was drawn during anticoagulant treatment. For logistic reasons, blood sampling was performed for patients included up to June 2002; after this date only DNA was collected via buccal swabs.

For the present analysis, participants with a history of cancer were excluded because of the effect of cancer on the coagulation system [4], leaving a total of 7717 remaining participants (2775 patients and 4942 controls). Participants who provided a positive answer to the question “did you have any thyroid disease in the past 12 months” or indicated use of thyroid related medication in the questionnaire (n=310) within one year from the index date received a second questionnaire requesting details about type and time of diagnosis of thyroid disease. These diagnoses were checked with the general practitioner.

Blood samples were drawn into vacuum tubes containing 0.1 volume 0.106 mol/l trisodium citrate as anticoagulant. Cells and plasma were separated by centrifugation. Plasma samples were stored at -80°C. A blood sample was available in 2179 patients. In 2177 of these samples FT4 and antiTPO could be measured; TSH was measured in 2142 samples. A total of 2826 blood samples were available from the controls. Levels of free thyroxine (FT4) could be assessed in 2826 samples, thyroid stimulating hormone (TSH) in 2769 samples, and anti-thyroid peroxidase antibodies (antiTPO) in 2820 samples of control subjects. Levels of FT4, TSH and antiTPO were measured in citrated plasma using commercially available assays (ADVIA Centaur® immunoassay system, Siemens Healthcare Diagnostics, Marburg, Germany). As these tests have not been validated by the manufacturer for use with citrated plasma, a serum/citrated plasma study was performed to characterize the difference. Systematic differences were small and linear regression analysis showed a strong association between serum and plasma levels for all thyroid hormones (regression coefficients: $\beta \geq 0.92$) [42]. From this analysis we recalibrated FT4 levels (correction factor 1.11). Concentrations of factor II, VII, VIII, X, XI, protein S and

antithrombin were measured as activity levels with a mechanical clot detection method on a STA-R coagulation analyzer (Roche Diagnostics, Almere, the Netherlands). All measurements were performed following the instructions of the manufacturer (Diagnostica Stago, Asnières, France). Levels of factor IX antigen were determined by enzyme-linked immunosorbent essay (ELISA). Von Willebrand factor antigen was measured with the immuno-turbidimetric method, using the STA liatest kit (rabbit anti human von Willebrand factor antibodies (Diagnostica Stago, Asnières, France)). Fibrinogen activity was measured on the STA-R analyzer according to the method of Clauss. Protein C was measured with a STA-R chomogen kit (Diagnostica Stago, Asnières, France). All tests were performed by technicians who were unaware of the status of the sample (i.e. whether it was from a case patient or control subject).

General characteristics are reported as medians and their 2.5- to 97.5- percentile or as total numbers and proportions (%). We calculated means and their 95% confidence intervals (CI95) for each coagulation factor for different FT4 levels (FT4<10 pmol/l, FT4=10 to 12, FT4=13 to 15, FT4=16 to 18, FT4=19 to 21, FT4=22 to 24, FT4>24). For the vitamin K dependent coagulation factors, vitamin K antagonist users were excluded from the analyses (n=31).

FT4 levels in citrate were only provided by the laboratory in rounded numbers, without decimals. Therefore, using percentiles was not possible, as there were too many subjects with the same FT4 level. Practically, a citrated FT4 level of 14 pmol/L can be read as all FT4 levels between 13.5 and 14.5 pmol/L. Citrated FT4 levels of 14 to 17 pmol/l (corresponding to serum FT4 15.5 to 18.9 pmol/l) were chosen as reference group, as this range consisted of about 60 % in the controls, and contrasted to citrated FT4 levels of 18, 19, 20, 21 and >22 pmol/l (respectively corresponding to serum FT4 20.0; 21.1; 22.2; 23.3 and >24.4 pmol/l). For TSH and AntiTPO (which results were provided in decimals) we determined percentiles for the distribution within the control subjects. For TSH and AntiTPO, participants with levels between the 20^{th} to the 80^{th} percentile of values within the controls constituted the reference category. TSH levels between the 10^{th} and 5^{th}, 5^{th} and 2.5^{th}, 1^{st} to 2.5^{th}, 0.5^{th} to 1^{st} and $<0.5^{th}$ percentile were compared with the reference category. For

AntiTPO, levels between the 80th and 90th, 90th and 95th, 85th and 97.5th 97.5th and 99th and >99th percentile were compared against the reference category. Laboratory values in patients diagnosed with hyperthyroidism between the thrombotic event and the blood draw will have been affected by treatment. Therefore, to study the effect of overt hyperthyroidism, patients with diagnosed hyperthyroidism at the time of the thrombotic event or FT4 levels >24pmol/l were combined and compared with those with normal laboratory values. Logistic regression was used to calculate odds ratios and their 95% confidence intervals. All analyses were adjusted for age and sex, in a conventional logistic model, to take the frequency matching into account. Adjustment for body mass index (BMI) and smoking was added because of the effect of BMI and smoking on both FT4 and on thrombosis. For body mass index, adjustment was made categorically (BMI: BMI<25 kg/m^2 BMI between 25 kg/m^2 and 30 kg/m^2; BMI >30 kg/m^2), and smokers were classified as current/former smoker and non-smoker. A restricted analysis with only the cases in whom blood was sampled within 6 months after the thrombotic event was performed to minimize the effect of natural variation in hormone levels over time.

To assess mediation of the relation between FT4 and venous thrombosis via coagulation factors found to be related to FT4 levels, the odds ratios were additionally adjusted for the levels of these factors. To assess whether there was a difference in risk for venous thrombosis in types of thrombosis, analyses were split for DVT and PE patients. In the group with pulmonary embolism, patients with and without concurrent deep venous thrombosis were included. For the analysis of provoked thrombosis, patients were included who had had surgery, plaster cast, who were bed ridden for more than 4 days, who had a minor injury, were pregnant or used oral contraceptives or hormone replacement therapy with estrogens within one year before the thrombosis. Unprovoked thrombosis was defined as absence of the aforementioned factors. Statistical analyses were performed with statistical package SPSS 17.0 for OsX (SPSS Inc, Chicago, IL, USA).

Results

Table 1 shows the general characteristics of the 2177 case patients and the 2826 control subjects. The age of the participants ranged from 18 to 70 years with a

median of 49.3 years in the cases and a median of 49.9 years in the controls. Median level of FT4 was 16.6 pmol/l (P2.5 to P97.5: 12.2 to 21.1), median TSH level was 1.22 mU/l (P2.5 to P97.5: 0.35 to 4.06) and median antiTPO level was 16.7 U/ml (P2.5 to P97.5: 16.7 to 1444.4), all in controls. Levothyroxine was used by 47 cases (2.2%) and 86 controls (3.0%). Thyreostatic drugs were used by 9 cases (0.4%) and 7 controls (0.2%). 35 cases (1.6%) and 33 controls (1.2%) had overt hyperthyroidism, either based on levels exceeding 24 pmol/l (16 cases, 12 controls) or on a previous clinical diagnosis (19 cases, 21 controls)

A rise in mean level of FVIII, FIX, fibrinogen and VWF was observed with increasing FT4 levels in the control subjects (Figure 1). In controls with a FT4 <10 pmol/L mean VWF level was 111 U/dL (CI95 78 to 143) which gradually rose towards a mean VWF level of 178 U/dL (CI95 140 to 215) for FT4>24 pmol/L. FVIII activity also rose gradually from 110 U/dL (CI95 86 to 133) for FT4 levels of <10 pmol/L towards 154 U/dL (CI95 129 to 179) for FT4>24 pmol/L. FVIII antigen levels rose from 120 U/dl (CI95 87 to 154) at FT4 levels <10pmol/L towards 176 U/dL (CI95 146 to 207) for FT4>24 pmol/L. At a FT4 level <10 pmol/L the mean FIX level was 99 U/dl (CI95 84 to 113), whereas a mean FIX level of 121 U/dL (CI95 114 to 128) was found for FT4 levels >24 pmol/L. Fibrinogen levels were equal at the lowest and highest FT4 levels (3.8 g/L (CI95 3.3 to 4.2) and 3.7 g/L (CI95 3.4 to 4.0) respectively), while they were lower in between. No clear associations were observed between FT4 levels and concentrations of prothrombin, VII, X, XI, antithrombin, protein C or protein S (data not shown).

Individuals with the highest FT4 levels (over 24.4 pmol/l) had a two-fold increased risk of thrombosis, relative to those with levels in the reference category of less than 18.9 pmol/l (OR 2.2, CI95 1.0-4.6, adjusted for age, sex, BMI and smoking). When the analysis was restricted to cases in whom blood samples were taken within 6 months after the thrombosis (282 cases), the association was somewhat stronger, with an odds ratio of 2.6 (CI95 0.7 to 9.6) for FT4 of >24.4 pmol/L. The odds ratios increased gradually with FT4 levels between the reference category and the highest levels (Table 2). Those with a history of ever hyperthyroidism had a 1.6-fold increased risk of venous thrombosis (CI95 1.0 to 2.8). When only subjects were included in whom this was established, by clinical diagnosis or blood draw, less than

one year before or shortly after the index date, an odds ratio of 17.0 (CI95 2.2 to 133.0) was found. The relation between TSH and the risk of venous thrombosis was inverse to that of FT4, but less pronounced. No clear association was observed between levels of antiTPO and risk of venous thrombosis (Table 2).

The association between FT4 levels and venous thrombosis was present for both deep venous thrombosis and pulmonary embolism, although slightly stronger for the former, with an odds ratio of 2.7 (CI95 1.2 to 6.1) for DVT and 1.5 (CI95 0.5 to 4.5) for PE, for FT4 levels >24.4 pmol/L (Table 3). High FT4 levels were associated with a doubling of the risk of provoked thrombosis, with an OR of 2.0 (CI95 0.9 to 4.7) for FT4 > 24 pmol/L, and a 2.8-fold increased risk of unprovoked thrombosis (CI95 0.8 to 9.4) (Table 3). Since FT4 appeared to have a clear effect on levels of FVIII, VWF, FIX and fibrinogen, an adjustment for these factors was made in the analysis. Adjustment for FVIII and VWF (besides age, sex, BMI and smoking) led to an attenuation of odds ratios: at a FT4 level >24.4 pmol/L the OR went from 2.2 (CI95 1.0 to 4.6) to 1.2 (CI95 0.5 to 3.0). This attenuation was not seen when we adjusted for levels of FIX or fibrinogen. Because ABO blood group is a determinant of both FVIII/VWF and venous thrombosis, additional adjustment was made for ABO blood group (in addition to age, sex, BMI and smoking), which did not lead to any substantial changes in the results (data not shown).

Discussion

We studied the relation between thyroid hormones and venous thrombosis in a large population-based case-control study with approximately 2000 patients and 3000 controls. The risk of thrombosis was two-fold increased for individuals with the highest levels of FT4 (>24 pmol/l) compared with those with average values. Those with either clinical or biochemical diagnosis of hyperthyroidism shortly before the event had a 17-fold increased risk of thrombosis. Increased levels of FT4 led to concomitant increases in several procoagulant factors, notably VWF and FVIII, which appeared to fully explain the effect on thrombosis risk.

These findings are in line with previous studies on separate components of this causal pathway: For the first part, the relation between FT4 and procoagulant factors, several studies (including experimental ones) showed a shift in the direction of a prothrombotic state (mainly elevated levels of factor VIII and von Willebrand factor) [21,22,32,42,46-48,73]. For the second part, the causal effect of factor VIII and von Willebrand factor on risk of venous thrombosis, several studies show evidence, of which the strongest is the effect of ABO blood group, which is a genetic, and therefore unconfounded determinant of elevated VWF and FVIII levels and which increases (for non-0 groups) the risk of venous thrombosis about 1.5-fold [74-76]. Another indication that factor VIII is causally related to venous thrombosis risk is that patients with haemophilia A or von Willebrand disease (i.e. with low levels of factor FVIII), have a low thrombosis risk [77].

Three previous studies on the relation between FT4 and venous thrombosis also reported an elevated risk with high levels of FT4 [42,48,49] with a relative risk of 10 for FT4 levels of approximately 24 pmol/L measured less than 6 months before thrombosis. The latter finding is in line with the present study where an OR of 17 was found for overt hyperthyroidism.

There are several potential limitations to this study. First, clinical hyperthyroidism present at time of thrombosis could have been discovered and treated between the time of thrombosis and time of blood sampling (which was the case, as far as we know, in at least eight of our clinical cases, in six of whom FT4 levels were normalized at the time of blood sampling). This limitation would result in a misclassification leading to underestimation of the risk estimates. This explains why risk estimates are lower in this analysis than in a previous prospective study where blood sampling had taken place before the event [48]. Secondly, the case-control design of the MEGA study enabled us to explore mechanistic relationships between FT4 and venous thrombosis, via different coagulation factors. A drawback of this design is that we cannot explain in detail how high levels of FT4 lead to increased levels of FVIII and vWF. For this, experimental studies are required. Thirdly, although the MEGA study is a large case control study, the estimates at high FT4 levels were still rather imprecise, particularly in subgroups. However, our findings are in line with the literature and the dose-response relationship also supports accurate findings.

Fourthly, the FT4 levels measured after the thrombosis may not reflect FT4 levels at time of the event. However, as these are stable over time [64,65] it is likely that they are representative for those at the time of the event. Prolonged storage at -80 (as was used in the MEGA-study) does not substantially affect levels of factor VIII or von Willebrand factor [78]. Even if it would, such changes would result in random misclassification, underestimating the true effect.

How thyroxine influences the coagulation system is not well known. The effect has been linked to autoimmune processes and to a direct effect of thyroid hormone on gene transcription in the liver and endothelial cells [36,66,69,70,79,80]. In vitro studies have shown a direct effect of tri-iodothyronine (T3), the active form of thyroid hormone, on hepatocytes and endothelial cells causing an up-regulation of fibrinogen, factor II, factor X, von Willebrand factor and plasminogen [36,66,69]. This would support the hypothesis of a direct effect on coagulation gene transcription by thyroid hormone. Recently Hooper et al. focused on the influence of thyroxine on fibrin formation. Clots proved to be denser and resistant to fibrinolysis in hyperthyroid patients. They suggested that, partially, this relation could be mediated by an inflammation response [81]. Although the present study did not focus primarily on the pathophysiology of thyroid hormone induced thrombosis, the negative finding of risk modification by the presence of anti-thyroid peroxidase antibodies disagrees with the hypothesis of this specific autoimmune effect. The positive association between FVIII and VWF with levels of FT4 as well as the attenuation of the effect when adjusting for FVIII and VWF points toward an effect of FT4 on FVIII and VWF production or clearance. Whilst both FVIII/VWF and venous thrombosis are influenced by ABO blood group, adjustment for ABO blood group did not influence the results in our study.

What are the clinical implications of these findings? Most of the cut-off FT4 levels in the present study are within levels of FT4 that will be left untreated in clinical practice. Findings of these FT4 levels would in itself have no clinical consequences, but could be used in constructing an overall risk profile. For overt hyperthyroidism a 17-fold risk increase was found. Based on our and the above mentioned previous findings in three other studies [42,48,49], routine testing for hyperthyroidism of

patients with an unprovoked thrombosis may be considered, as the diagnosis is not always obvious clinically and testing is easily done and relatively cheap. A positive result will have a strong impact for the patient because an undetected condition can be treated.

Overall, this study provides further insights in the association between thyroid hormone and venous thrombosis. A gradually increasing risk of venous thrombosis for increasing levels of FT4 was found, which appeared to be mediated by levels of FVIII and VWF. The underlying mechanism and clinical implications of these findings should be further explored in future studies.

Table 1. Characteristics of patients with venous thrombosis and control subjects

	Cases (n=2177)	Controls (n=2826)
Male, n (%)	987 (45.3)	1352 (47.8)
Age at blood sampling, years, median (P2.5 to P97.5)	48.2 (23.3 to 69.4)	48.7 (23.7 to 68.8)
BMI, kg/m2, median, (P2.5 to P97.5)	26.3 (19.4 to 38.8)	24.9 (19.1 to 35.0)
DVT, n (%)	1283 (58.9)	
PE, n (%)	894 (41.1)	
Blood sampling within 6 months from VT, n, %	260 (11.9)	
Operation, n (%)	425 (19.5)	181 (6.4)
Plaster cast immobilisation, n (%)	129 (5.9)	35 (1.2)
Bed immobilization at home, n (%)	369 (16.9)	222 (7.9)
Bed immobilisation in hospital, n (%)	447 (20.5)	214 (7.6)
Minor sportsinjury, n (%)	520 (23.9)	429 (15.2)
Pregnany, n (%)	103 (4.7)	36 (1.2)
Oral contraceptives, n (%)	662 (30.4)	323 (11.4)
Hormone replacement therapy, n (%)	61 (2.8)	81 (2.9)

N indicates number; P, percentile; BMI, body mass index; DVT, deep venous thrombosis; PE, pulmonary embolism; VT, venous thrombosis; FT4, thyroxine; TSH, thyroid stimulating hormone and AntiTPO, thyro-peroxidase antibodies.

Table 2. Risk of venous thrombosis associated with different percentiles of FT4, TSH and antiTPO.

FT4 (pmol/L)	Controls	Cases	OR (CI95)	Cases*	OR* (CI95)
15.5 to 18.9	1844	1346	ref	196	Ref
20.0	135	108	1.2 (0.9 to 1.6)	11	0.9 (0.5 to 1.6)
21.1	44	44	1.5 (0.9 to 2.2)	5	1.2 (0.5 to 3.1)
22.2	20	25	1.8 (1.0 to 3.3)	3	1.3 (0.4 to 4.7)
23.3	8	7	1.3 (0.5 to 3.9)	1	1.3 (0.2 to 11.1)
>24.4	12	16	2.2 (1.0 to 4.6)	3	2.6 (0.7 to 9.6)
TSH (mU/L)	**Controls**	**Cases**	**OR (CI95)**	**Cases***	**OR* (CI95)**
0.79 to 1.89	1648	1217	ref	152	ref
<0.14	0	0	na	0	na
0.14 to 0.24	27	31	1.5 (0.9 to 2.6)	5	2.1 (0.8 to 5.6)
0.24 to 0.39	42	42	1.3 (0.8 to 2.0)	6	1.6 (0.7 to 3.9)
0.39 to 0.47	69	49	0.9 (0.6 to 1.3)	4	0.6 (0.2 to 1.8)
0.47 to 0.60	132	95	1.0 (0.7 to 1.3)	16	1.3 (0.7 to 2.2)
Anti-TPO (U/mL)	**Controls**	**Cases**	**OR (CI95)**	**Cases***	**OR* (CI95)**
18.9 to 41.1	2257	1696	Ref	216	ref
41.1 to 159.1	281	255	1.2 (1.0 to 1.5)	40	1.5 (1.0 to 2.2)
159.1 to 361.2	141	124	1.1 (0.9 to 1.5)	17	1.1 (0.7 to 2.0)
361.2 to 554.6	24	26	1.5 (0.8 to 2.7)	1	0.5 (0.1 to 3.4)
554.6 to 804.9	0	0	Na	0	na

P indicates percentile; FT4, free thyroxine; TSH, thyroid stimulating hormone; antiTPO, thyroid peroxidise antibodies; OR, odds ratio; 95% CI, 95% confidence interval; and na, not applicable.* restricted analysis for blood sampling within 6 months after the thrombotic event

Adjusted for age, sex, BMI and smoking

Table 3. Risk of venous thrombosis for different subgroups associated with different percentiles of FT4.

		DVT		PE	
FT4 (pmol/L)	**Controls**	**Cases**	**OR[2] (CI95)**	**Cases**	**OR[2] (CI95)**
15.5 to 18.9	1844	809	ref	537	Ref
20.0	135	63	1.2 (0.9 to 1.6)	45	1.2 (0.9 to 1.8)
21.1	44	30	1.7 (1.0 to 2.8)	14	1.1 (0.6 to 2.1)
22.2	20	10	1.2 (0.6 to 2.6)	15	2.5 (1.2 to 5.1)
23.3	8	4	1.3 (0.4 to 6.1)	3	1.3 (0.3 to 5.3)
24.4	12	11	2.7 (1.2 to 6.1)	5	1.5 (0.5 to 4.5)
		Provoked		**Unprokoved**	
FT4 (pmol/L)	**Controls**	**Cases**	**OR[2] (CI95)**	**Cases**	**OR[2] (CI95)**
15.5 to 18.9	1844	924	ref	364	Ref
20.0	135	61	1.1 (0.8 to 1.6)	44	1.6 (1.1 to 2.3)
21.1	44	30	1.7 (1.0 to 2.7)	12	1.4 (0.7 to 2.8)
22.2	20	10	1.0 (0.4 to 2.2)	13	3.0 (1.4 to 6.3)
23.3	8	4	1.2 (0.3 to 4.3)	3	2.2 (0.5 to 9.2)
24.4	12	11	2.0 (0.9 to 4.7)	4	2.8 (0.8 to 9.4)

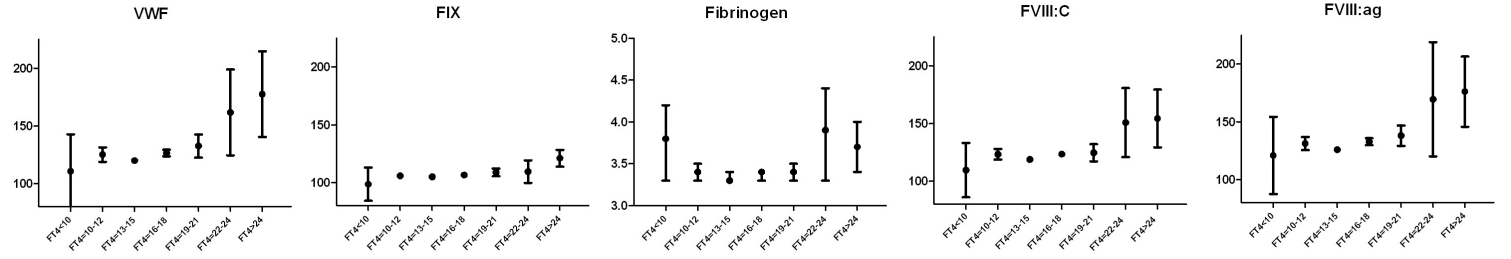

Figure 1. Effect of different levels of FT4 on individual coagulation factors in controls. (mean with CI95)

vWF indicates von Willebrand factor (U/dL); FIX, factor IX (U/dL); fibrinogen (g/L); FVIII:c, factor VIII activity (IU/mL); FVIIIag, factor VIII antigen (U/dL); and FT4, serum free thyroxine (pmol/l).

Low levels of thyroxine and the risk of venous thrombosis

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To be submitted

Abstract

Introduction

Recently a relationship between high levels of free thyroxine and venous thrombosis has been established. Low levels of thyroxine have been associated with both a decreased and an increased risk for venous thrombosis. The aim of this study was to evaluate the risk of venous thrombosis in patients with low levels of free thyroxine.

Methods

In a large population based case-control study (MEGA-study) into the aetiology of venous thrombosis we determined levels of FT4, TSH and antiTPO in 2177 cases and 2826 controls and estimated odds ratios to determine relative risks for different levels of these hormones.

Results and conclusion

The odds ratio was highest (OR 2.5 (CI95 0.9 to 6.7)) at the lowest FT4 levels (<10.0 pmol/l) compared with the reference category of 15.5 to 18.9 pmol/l. The odds ratio decreased with higher levels of FT4. Adjustment for BMI, smoking, HsCRP, cholesterol, HDL cholesterol, triglycerides and homocysteine levels attenuated the risk slightly, indicating involvement of these factors in the relation between low levels of thyroxine and venous thrombosis. Our study cannot determine the exact relation between hypothyroidism and risk of venous thrombosis as a protective association between low levels of FT4 and venous thrombosis may have been masked by misclassification and influence of other factors.

Introduction

The relation between hormones and the coagulation system has been a recent focus of interest [19,21,23,42,45,46,48,63,67,71]. Hormones such as cortisol, prolactine and thyroxine have been shown to have an influence on the coagulation system which also results in clinical events [42,82-85]. Generally, high levels of these hormones increase the risk of venous thrombosis [42,48,67,84]. With respect to thyroxine (FT4), higher levels are associated with higher levels of factor VIII (FVIII) and von Willebrand factor (VWF) [32-34,46,47]. Other coagulation factors (factor IX, fibrinogen) have also been shown to rise with the levels of thyroxine [67]. With lower FT4 levels, a decrease of levels of these coagulation factors has been described [19,34]. In line with this finding, lower levels of thyroxine were found to be related to an increased risk of major bleeding in patients using vitamin K antagonists (this thesis) and to acquired von Willebrand disease [34,45,86]. Theoretically, these phenomena could translate in a protective effect on the risk of thrombosis. However, the two studies on thyroxine and risk of venous thrombosis that have been published thus far show contradictory results. In the ACT study [42], a linear dose relationship was seen for the relationship between thyroxine and thrombosis risk, with a protective effect for lower levels and an increased risk for higher levels of thyroxine. In the TROL study [48], the analysis revealed a U-shaped relation with an increased risk for thrombosis at both highest and lowest levels of thyroxine. Since these studies included 190 and 446 patients with venous thrombosis, of whom only a fraction had low levels of thyroid hormone, sample sizes were too small to obtain reliable results. To shed more light on these contradictory findings, we assessed in detail the effect of low levels of thyroxine on the risk of venous thrombosis in a large case-control study designed to study risk factors for venous thrombosis (the MEGA study) [67]. Since there is little pathophysiological support for an increased thrombosis risk with lower levels of thyroxine, we hypothesized that low levels of thyroxine would have either no or a protective effect on the risk for venous thrombosis.

Methods

Study design

The Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study is a large population-based case-control study. Data collection and ascertainment of venous thrombotic events have been previously described in detail [4,67]. Briefly, patients aged 18 to 70 years and diagnosed with a first deep venous thrombosis (DVT) of the leg, a pulmonary embolism (PE) or a combination of both were asked to participate in the study. Patients were recruited between 1999 and 2004 from six regional Anticoagulation Clinics in the Netherlands, which all are sole providers in a well-defined geographical region. Partners of these patients and subjects recruited by Random Digit Dialling (RDD), frequency-matched for age and sex, were invited as control subjects. For both control-groups the same selection criteria as for patients were applied. During the first half of the inclusion period venepunctures were performed to obtain plasma and DNA, while in the second half DNA was obtained from buccal swabs. For the present analysis, participants with a history of cancer were excluded because of the effect of cancer on the coagulation system [4] (2775 cases and 4942 controls remaining). Blood samples were available in 2179 cases. In 2177 of these samples FT4 was measured. In 2826 controls FT4 levels could be measured. Levels of FT4 and TSH were measured in citrated plasma using commercially available assays (ADVIA Centaur® immunoassay system, Siemens Healthcare Diagnostics, Marburg, Germany). As these tests have not been validated by the manufacturer for use with citrated plasma, serum/citrated plasma studies were performed for validation [42]. Systematic differences were small and linear regression analysis showed a strong correlation between serum and plasma levels for all thyroid hormones (regression coefficients: $\beta \geq 0.92$. Therefore all citrated FT4 values are multiplied by 10/9 for correction in this study). The Medical Ethics Committee of the Leiden University Medical Center approved the study protocol. Written consent was obtained from all participants.

Statistical analysis

For patients and their partner controls, the date of the thrombotic event was used as the index date. For the random digit dialing controls the date of completion of the questionnaire was used as index date. FT4 levels were only provided by the laboratory in round numbers. Therefore, analyzing the results using percentiles would have required arbitrary choices when establishing percentiles. We chose FT4 levels of 15.5 to 18.9 pmol/l as reference group and compared them to levels of 14.4, 13.3, 12.2, 11.1 and <10.0 pmol/l. For TSH, we used the 20th to the 80th percentile as reference category. Further categories were made of the 90th to the 95th percentile, the 95th to the 97.5th percentile, the 97.5th to the 99th percentile, the 99th to the 99.5th percentile and higher than the 99.5th percentile. We used logistic regression to calculate odds ratios and their 95% confidence intervals. All analyses were adjusted for age and sex, to take the frequency matching into account. Furthermore, we adjusted categorically for body mass index (BMI: BMI<25 kg/m^2, BMI between 25 kg/m^2 and 30 kg/m^2; BMI >30 kg/m^2) and smoking status (current smoker vs former smoker or never smoker). High sensitivity CRP (HsCRP), triglyceride, cholesterol, high density lipid (HDL) cholesterol and homocysteine plasma concentrations were added as continuous variables. Statistical analyses were performed with statistical package SPSS 17.0 for OsX (SPSS Inc, Chicago, IL, USA).

Results and Discussion

A total of 2177 patients and 2826 control subjects were included in the analysis. Blood samples were collected with a median of 10 months after the event (range 3 to 36 months).

As shown in Table 1, we found an increase in the risk of venous thrombosis for levels of FT4 below 13.3 pmol/l, compared with the reference category of 15.5 to 18.9 pmol/l. The odds ratio was highest (OR 2.5 (CI95 0.9 to 6.7)) at the lowest FT4 levels (<10.0 pmol/l) compared with the reference category. Given the negative feedback of FT4 on TSH, it is expected that increased levels of TSH give similar risk estimates as reduced levels of FT4. This was indeed observed (see Table 2). In general, the

odds ratios increased with increasing TSH levels. ORs of 1.8 (CI95 0.9 to 3.5) were found for TSH levels from 5.54 to 8.96 mU/ml as well as for TSH > 8.97 mU/ml (OR 1.8 (CI95 0.8 to 3.6)). Additional adjustment for BMI, smoking, HsCRP, cholesterol, HDL cholesterol, triglycerides and homocysteine levels attenuated the risk slightly, especially in the FT4 analysis, indicating involvement of these factors in the relation between low levels of thyroxine and venous thrombosis.

As an increased risk of venous thrombosis at the hypothyroid end of the spectrum is difficult to explain pathophysiologically, how can our findings of an increased risk be understood? A first option is that this is due to chance, which is plausible, considering the rather wide confidence intervals. However, it seems unlikely that chance would reverse a truly protective effect. Alternatively, our findings can be explained by confounding, although we adjusted for putative confounders such as age, sex, BMI, and smoking. Several studies have described an association between hypothyroidism and cardiovascular risk factors, such as obesity, LDL cholesterol, hyperhomocysteinemia and endothelial dysfunction; hypothyroidism has also been associated with increases in diastolic blood pressure and C-reactive protein [59,60,62,63]. Recent studies have also suggested that some of these arterial cardiovascular risk factors are associated with an increased risk of venous thrombosis [87-89]. Adjustment for these factors led to some attenuation of the ORs, indicating a pathway between hypothyroidism and thrombosis via these factors. Another possibility is misclassification of the thyroid hormone measurements, i.e., that the measured FT4 levels did not reflect the actual levels at the time of the event. Although levels of FT4 generally do not tend to fluctuate very much [64,65], it is not unthinkable that at the time of the thrombosis high FT4 levels were present, caused by some form of thyroid disease, which levels, due to treatment or natural course of this condition dropped to low FT4 levels at the time of blood sampling, leading to a spurious association between low levels and risk of thrombosis.

Other studies reported low risks at the hypothyroid end of the spectrum depending on time of blood sampling. In the TROL study [48], the odds ratio for the lowest FT4 category (<10.7) was 1.3 (CI95 0.6 to 2.8). However, this OR decreased to 0.8 (CI95 0.1 to 6.0) when the analysis was restricted to cases in whom blood was drawn within 6 months before the thrombosis. In the ACT study [42], where blood

was sampled at the time of presentation of the event, a protective effect for low levels of FT4 was also seen up to a 10-fold protective effect for FT4 levels <12 pmol/L. Both studies indicate that blood sampling close to the event is associated with a protective effect of low levels of FT4, while this reverses with increasing time between sampling and the event.

Putting the information of these studies and our results together, we would propose the following relation between hypothyroidism and venous thrombosis: hypothyroidism is in its acute phase associated with a decreased risk of venous thrombosis. However, as hypothyroidism is also linked with some cardiovascular risk factors which in their turn are associated with increased risk of venous thrombosis, this could lead to a non-causal inverse association, i.e., of an increased risk. When misclassification in the measurement of FT4 is present, the protective relation between hypothyroidism and venous thrombosis will be obscured by the presence of these factors. This has probably been the case in our study where the median time between venous thrombosis and blood sampling was 10 months with a minimum of 3 months. We therefore conclude that our study cannot determine the exact relation between hypothyroidism and risk of venous thrombosis and that a protective association may have been masked by cardiovascular risk factors such as obesity, decreased endothelial function and inflammation which are associated with decreased thyroid function. Further knowledge on this issue is nevertheless important as physicians may find this of use in hypothyroid patients when they encounter high risk situations, such as a planned surgery.

Table 1. Risk of venous thrombosis associated with low levels of FT4

FT4 (pmol/L)	Controls	Cases	OR[1] (CI95)	OR[2] (CI95)	OR[3](CI95)
15.5 to 18.9	1844	1346	ref	ref	ref
14.4	454	318	1.0 (0.8 to 1.1)	0.9 (0.8 to 1.1)	0.9 (0.8 to 1.1)
13.3	206	202	1.3 (1.1 to 1.6)	1.3 (1.0 to 1.6)	1.3 (1.0 to 1.6)
12.2	76	72	1.3 (0.9 to 1.8)	1.3 (0.9 to 1.8)	1.2 (0.9 to 1.7)
11.1	21	28	1.8 (1.0 to 3.1)	1.6 (0.9 to 2.8)	1.7 (0.9 to 3.0)
<10.0	6	11	2.5 (0.9 to 6.7)	2.0 (0.7 to 5.7)	1.7 (0.6 to 4.9)

FT4 indicates free thyroxine; OR, odds ratio;CI95, 95% confidence interval and ref, reference category. OR[1]: odds ratio adjusted for age and sex; OR[2]: odds ratio adjusted for age, sex, BMI and smoking; OR[3]: odds ratio adjusted for age, sex, BMI, smoking, HsCRP, cholesterol, triglyceride, HDL cholesterol and homocysteine levels.

Table 2. Risk of venous thrombosis associated with increased TSH levels

TSH (mU/ml)	Controls	Cases	OR[1] (CI95)	OR[2] (CI95)	OR[3](CI95)
0.79 to 1.89	1658	1225	ref	ref	ref
2.42 to 3.00	138	103	1.0 (0.8 to 1.3)	1.0 (0.7 to 1.3)	1.0 (0.7 to 1.3)
3.01 to 4.02	70	77	1.4 (1.0 to 2.0)	1.4 (1.0 to 2.0)	1.4 (1.0 to 2.0)
4.03 to 5.53	41	37	1.2 (0.8 to 1.9)	1.2 (0.8 to 2.0)	1.2 (0.8 to 2.0)
5.54 to 8.96	15	20	1.8 (0.9 to 3.5)	1.7 (0.8 to 3.4)	1.7 (0.8 to 3.5)
>8.97	13	17	1.8 (0.8 to 3.6)	1.5 (0.7 to 3.1)	1.4 (0.7 to 3.0)

TSH, thyroid stimulating hormone: percentiles (p) reference is p20 to p80, p90 to p95, p95 to p97.5, p97.5 to p99, p99 to p.99.5 and >p99.5; OR, odds ratio, 95% CI, 95% confidence interval.

* restricted analysis for blood sampling within 6 months after the thrombotic event. OR[1]: odds ratio adjusted for age and sex; OR[2]: odds ratio adjusted for age, sex, BMI and smoking; OR[3]: odds ratio adjusted for age, sex, BMI, smoking, HsCRP, cholesterol, triglyceride, HDL cholesterol and homocysteine levels.

The influence of thyroid hormone on the risk of recurrent venous thrombosis

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Abstract

Background

High levels of the thyroid hormone thyroxine are associated with an increased risk of first venous thrombosis. The risk of recurrent venous thrombosis in relation to thyroxine levels has not been studied.

Methods

We performed a nested case-control study in a large cohort of patients with a first venous thrombosis (MEGA study), in whom blood samples were taken some months after their first event. Information on recurrent venous thrombosis was retrieved over a mean period of 5.8 years. Patients with a recurrence (381 cases) were matched (1:2) with patients without a recurrence (761 controls) on time from first venous thrombosis to blood sampling. Odds ratios (OR) and 95% confidence intervals (CI95) were calculated for the risk of venous thrombosis for different cut-off points of free thyroxine (FT4) levels. A restricted analysis was performed in subgroups where the time between blood sampling and recurrent event was less than 0.5, 1 or 1.5 years.

Results

The risk of recurrent venous thrombosis was not associated with levels of FT4. The OR for recurrent venous thrombosis for FT4 levels <15.5 pmol/l was 0.8 (CI95 0.6-1.1), relative to FT4 levels of 15.5 to 18.9 pmol/L; for FT4 >24.4 pmol/l the OR was 0.6 (CI95 0.1-3.8). Similar odds ratios were found in the restricted analyses.

Conclusions

High levels of FT4 are not associated with an increased risk of recurrent venous thrombosis.

Introduction

While the annual incidence of a first venous thrombosis is approximately 1-2 per 1000 person-years [3], the recurrence risk of a venous thrombotic event is much higher. The cumulative incidence of recurrent venous thrombosis is 4-11% within the first year and 12-30% in the five years after the first event, with incidence rates of recurrence varying between 25 to 46 per 1000 person-years [7-10].

Many risk factors for first venous thrombosis are known, but the determinants of recurrent venous thrombosis are less clear[8]. Furthermore, the risk profile for recurrent venous thrombosis is different from that of first venous thrombosis. Many prothrombotic abnormalities do not play an important role in the risk of recurrence [7,9]. Age, an important risk factor for first venous thrombosis, does not influence risk of recurrence [11,12].

In recent years, the link between endocrine disorders and venous thrombosis has been extensively studied [19,22,42,46,48,83-85,90,91]. High levels of endogenous hormones, such as the stress hormone cortisol and thyroid hormone (thyroxine), have been found to be associated with an increased risk of venous thrombosis [42,83]. One case-control study reported a 2-fold increased risk for venous thrombosis for free thyroxine (FT4) levels above 17 pmol/L compared with a FT4 level of 15 pmol/L [42]. In another case-cohort study there was a positive relation between increasing levels of free thyroxine and risk of venous thrombosis (up to a 10-fold increased risk for FT4 levels >17.3 pmol/l relative to levels below this cut-off) [48]. Furthermore, a 2.3-fold increased risk for venous thrombosis was seen in patients with clinical hyperthyroidism compared to non-thyroid disease patients [49]. The effect of thrombosis seemed mainly mediated by increased levels of von Willeband factor and factor VIII [this thesis].

So far, to our knowledge, the risk of recurrent venous thrombosis in relation to high levels of thyroxine has not been studied. The aim of our study was therefore to investigate the role of thyroid hormones in recurrent venous thrombosis, in a large population based cohort of patients with a first thrombotic event.

Methods

Study design

The MEGA-study is a large population-based case-control study into risk factors for a first venous thrombosis. Data collection and ascertainment of venous thrombotic events have been previously described in detail [4]. Briefly, patients aged 18 to 70 years and diagnosed with a first deep venous thrombosis of the leg, a pulmonary embolism or a combination of both were asked to participate in the study. Patients were recruited between 1999 and 2004 from six regional Anticoagulation Clinics in the Netherlands. Blood samples were obtained with a median of 10 months after the event. Only when a participant was still using oral anticoagulant treatment with vitamin K antagonists more than one year after the index date, the blood sample was drawn during anticoagulant treatment. For logistic reasons, blood sampling was performed for patients included up to June 2002; after this date only DNA was collected via buccal swabs.

Blood samples were available in 2179 patients. In 2177 of these samples FT4 and antiTPO could be measured; TSH was measured in 2142 samples. The Ethics Committee of the Leiden University Medical Center approved the study protocol. Written consent was obtained from all participants.

Possible recurrent events were retrieved in two ways. Firstly, from the patients via a short questionnaire inquiring about whether a patient had had a recurrent event and secondly from the anticoagulation clinics. Questionnaires were sent by mail between June 2008 and July 2009. When questionnaires were not returned, the questions were asked by telephone interview [Flinterman, LE et al. submitted for publication]. Subsequently, additional information was requested from physicians to adjudicate the diagnosis.

A recurrence was considered certain when a discharge letter was present concluding a diagnosis of recurrence. The event should be in a different vein or in a different part of the body than the first event. If the location of either the first or second

thrombosis was unclear, an event was still classified as a certain recurrence when at least three months had passed since the first thrombosis. An event was also classified as certain when a discharge letter was not available but both the anticoagulation clinic and the patient reported a recurrence at a clearly different location than the first event (contralateral leg, or DVT after PE or vice versa) or when a time period of more than a year had passed between the two events. A third option was a registered cause of death from pulmonary embolism or venous thrombosis at least six months after the first event.

Laboratory assessments

Blood samples were drawn into vacuum tubes containing 0.1-volume 0.106-mol/l trisodium citrate as anticoagulant. The blood sample was separated into plasma and cells through centrifugation. Plasma samples were stored at -80°C. We measured levels of FT4, TSH and antiTPO in citrated plasma using commercially available assays (ADVIA Centaur® immunoassay system, Siemens Healthcare Diagnostics, Marburg, Germany). As these tests have not been validated by the manufacturer for use with citrated plasma, serum/citrated plasma studies were performed to characterize the correlation [42]. Only small systematic differences were detected and linear regression analysis showed a strong association between serum and plasma levels for all thyroid hormones (regression coefficients: $\beta \geq 0.92$). The levels of FT4 were returned from the routine laboratory rounded to whole numbers. TSH was provided as 2 decimal numbers. The samples were measured before the recurrence status of the patients was known.

Statistical analyses

We determined percentiles for the distribution of FT4 and TSH within the control subjects. However, as citrated FT4 levels were rounded to whole numbers, the data distribution was more categorical than continuous. Therefore the data were analyzed with citrated FT4 (CFT4) of 14 to 17 pmol/l as reference category (resembling the 20^{th} to 80^{th} percentile). This resulted in a reference category of 15.5-18.9 for the recalculated plasma FT4 levels which was compared with levels of FT4 of 20, 21.1, 22.2, 23.3 and >24.4 pmol/l. A category for a FT4 of < 15.5 pmol/l was also included. For TSH the reference category was the 20th to the 80th percentile. These data

were further divided in categories containing the 20th to 10th percentile, 10th to 5th percentile, 5th to 2.5th percentile, 2.5th to 1st percentile and <1st percentile.

In the MEGA study, blood was sampled a few months after the first venous thrombosis. Since thyroid disease can develop relatively acutely and is a treatable disease, levels of FT4 are likely to change over time and so the risks will similarly vary. Furthermore, the timing of the measurement differed between patients (i.e., a median of 10 months after the first event (range: 6 to 21)), as well as the duration of the subsequent follow-up. To better take these variations into account, we decided to use a nested case-control design. All patients with a certain recurrence (cases) were matched with patients without a recurrence (controls), in a 1:2 ratio, first of all on time from first venous thrombosis to blood sampling; furthermore, controls had to be recurrence free at the time their corresponding case had a recurrence. This way both cases and controls had the same exposure duration and the same time (opportunity) to develop a recurrent event, and hence the odds ratio can be considered as an incidence rate ratio [54]. A conditional logistic regression analysis was performed and all analyses were additionally adjusted for age and sex. Subsequently, analyses were performed in subgroups where the time between blood sampling and recurrent event was restricted to a maximum of 1 and 1.5 years. All matching and analyses were performed using R 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria) [55-57].

Results

A total of 2209 patients with a first venous thrombosis from the MEGA study in whom blood samples were available were followed for recurrence [67]. Of these 2209 patients, 423 (19%) experienced a recurrent thrombosis. With a total follow-up of 16 021 person-years, this led to an incidence rate of 26.4 (95% CI 24.0-29.0) events per 1000 person-years. Median follow up time was 1128 days (3 years) for the patients with recurrence and 2976 days (8 years) for the patients without. In 381 of the 423 recurrence cases, all information needed to perform the matching procedure was available. Matching in a 1:2 ratio resulted in 761 patients without a recurrent venous thrombosis. Of the 381 cases, FT4 levels had been measured in 343 cases and TSH

levels in 335 cases. The median time from first venous thrombosis to blood sampling was 310 days in both groups. Median time from blood sampling to recurrent event or end of follow-up was 757 days in patients with recurrent venous thrombosis and 1725 days in patients without recurrence (Table 1). Median age distribution was almost equal in patients with recurrence as in patients without (Table 1). Of patients with recurrent venous thrombosis 236 (62%) were men compared with 332 (44%) of patients without recurrence.

At time of blood sampling, the median FT4 level was 16.7 pmol/l both in patients with and without recurrence (p2.5 to p97.5: 12.2 to 21.1 pmol/l for both groups). For TSH, the median level was 1.31 mU/l (p2.5 to p97.5: 0.38 to 4.65 mU/l) in the patients with recurrent venous thrombosis and 1.36 mU/l (p2.5 to p97.5: 0.35 to 4.77 mU/l) in the patients without recurrent venous thrombosis.

In the matched analysis, the odds ratios (OR) for risk of recurrent thrombosis varied between 0.8 (CI95 0.6 to 1.1) for FT4 levels below 15.5 pmol/l compared with the reference category (FT4 levels of 15.5 to 18.9 pmol/l) to an OR of 0.6 (CI95 0.1 to 3.8) at a cut-off of >24.4 pmol/l. For TSH, the ORs varied between OR: 0.9 (CI95 0.7 to 1.3) for a cut-off level of >2.03 mU/l and OR 0.4 (CI95 0.1 to 1.8) for a level of <0.22 mU/l compared with the reference category of TSH levels of 0.84 to 2.03 mU/l (Table 2). An analysis restricted to cases who had a recurrent venous thrombosis within one year after blood sampling and their matched controls, resulted in similar risk estimates (Table 3). When time between blood sampling and recurrent thrombosis was extended to 1.5 year, an OR of 0.5 (CI95 0.1 to 3.8) for the highest FT4 levels (>24.4 pmol/L) was found.

An effect of FT4 on recurrence is expected to be most evident in the group with a combination of hyperthyroidism (a FT4>23 pmol/l) at time of blood measurement and an unprovoked thrombosis, since their first venous thrombosis is most likely to have been caused by elevated levels of FT4. This was the case in 4 patients. Three of these four patients, who all were men, suffered from recurrent venous thrombosis (153, 729 and 771 days after the first venous thrombosis) over a total follow-up of 13.18 person-years, leading to an incidence rate of 227.6 per 1000 person-years

(CI95 46.9 to 665.2). One of the three unprovoked patients reported that hyperthyroidism had been diagnosed between the first venous thrombosis and the recurrent event. At the time of their first event they had not been classified as possible thyroid patients based on medication or disease history reported in the primary questionnaire.

Discussion

In line with the pattern observed for many other risk factors for first venous thrombosis (e.g. thrombophilia, age), high levels of FT4, while a risk factor for a first event, do not play a major role in the occurrence of recurrent venous thrombosis. High levels of FT4 at the time of blood sampling even seem to decrease the risk of recurrence. Similar findings have been reported for other transient risk factors for a first event, such as surgery or plaster cast immobilization [9,92].

As thyroid disease can occur acutely and will be, when treated, of a transient nature, the analysis of the relation was not straightforward, particularly since only one blood sample between first and recurrent venous thrombosis was available. Several scenarios influencing risk calculations could have occurred, either leading to an accurate or to an underestimation of the relative risk (Table 4). In some patients with first venous thrombosis, hyperthyroidism was discovered in the workup for venous thrombosis. If the FT4 levels were normalized at the time of blood sampling, no effect on the risk for recurrence is expected. However, if the FT4 levels normalized after blood sampling and before recurrence, the risk estimate would be an underestimation of the true risk because high FT4 levels were used in the calculations while the actual FT4 levels were low most of the observation time. If FT4 levels would remain high over time, the point estimate would not be different since the levels of FT4 at blood sampling would reflect the level of FT4 at the recurrent event. If hyperthyroidism had developed after the first venous thrombosis and no treatment was given, there is no error in the risk estimate. If there was treatment after blood sampling, misclassification of the exposure (high FT4 levels instead of normal levels) would have occurred which would have led to an underestimation of the risk for recurrence for high FT4 levels. For completeness, if FT4 levels would be

normal over time, there would be no misclassification of the risk estimate for recurrence. To minimize the effect of such misclassifications, we performed the matched case-control analysis and, additionally, calculated ORs for different time windows between blood sampling and recurrent event. Shorter time between the two did not influence the relation that was found. However, despite all these measures to reduce misclassification, some residual misclassification cannot be excluded.

Apart from these misclassification issues, there are several limitations that need to be addressed. Although the group of recurrent venous thrombosis was large compared with other studies, sub-analysis within the FT4 strata was difficult due to small numbers. We noted a high incidence of recurrent venous thrombosis in participants with a combination of FT4>23 and an unprovoked first thrombosis, which could be of interest and should be further examined in cohorts of patients in whom thyroid function is measured in more detail.

Despite these limitations, we conclude that hyperthyroidism present at a first venous thrombotic event is not a risk factor for recurrence. In line with other transient risk factors for a first event, it was even associated with a reduced risk of recurrence. This emphasizes the need for awareness of possible hyperthyroidism in patients who present with an unprovoked thrombotic event as well as in patients with such a history, so that the patient will not only benefit from recovery of thyroid disease but also from a reduced risk of recurrent venous thrombosis.

Table 1. General characteristics

	Recurrence (n=381)	No recurrence (n=761)
Age (median years; p2.5 to p97.5)	50.5 (21.7 to 69.2)	48.1 (22.9 to 68.6)
Male (%)	236 (61.9)	332 (43.6)
Follow up (median days; p2.5 to 97.5)	1128 (147 to 3064)	2976 (642 to 3358)
Time from first VT to blood sampling (median days; p2.5 to 97.5)	310 (169 to 641)	310 (169 to 641)
TBSI (median days; p2.5 to p97.5)	757 (-265 to 2771)	1725 (68 to 2868)
FT4 (median; p2.5 to 97.5) pmol/l	16.7 (12.2 to 21.1)	16.7 (12.2 to 22.1)
TSH (median; p2.5 to 97.5) mU/l	1.31 (0.38 to 4.65)	1.36 (0.35 to 4.77)

n indicates number; p, percentile; VT, venous thrombosis; DVT, deep venous thrombosis; TBSI, Time from blood sampling to recurrence or end of follow-up; FT4, free thyroxine; TSH, thyroid stimulating hormone.

Table 2. The risk for recurrent venous thrombosis for different strata of free thyroxine and thyroid stimulating hormone.

CFT4	**FT4 (pmol/l)**	**Controls**	**Cases**	**OR[1] (CI95)**	**OR[2] (CI95)**
<14	<15.5	210	86	0.8 (0.6 to 1.1)	0.8 (0.6 to 1.1)
14 to 17	15.5 to 18.9	407	220	Ref	Ref
18	20.0	41	20	0.9 (0.5 to 1.5)	0.6 (0.4 to 1.2)
19	21.1	11	9	1.8 (0.7 to 4.9)	1.8 (0.7 to 4.8)
20	22.2	9	4	0.7 (0.2 to 2.3)	0.6 (0.2 to 2.0)
21	23.3	5	2	0.7 (0.1 to 3.5)	0.5 (0.1 to 2.5)
22	>24.4	3	2	0.7 (0.1 to 4.6)	0.6 (0.1 to 3.8)
Percentile	**TSH (mU/l)**	**Controls**	**Cases**	**OR[1] (CI95)**	**OR[2] (CI95)**
>80	>2.03	143	66	0.9 (0.6 to 1.2)	0.9 (0.7 to 1.3)
20 to 80	0.84 to 2.03	395	203	Ref	Ref
10 to 20	0.63 to 0.84	61	33	1.0 (0.6 to 1.6)	0.9 (0.5 to 1.5)
5 to 10	0.49 to 0.63	32	19	1.1 (0.6 to 2.0)	0.9 (0.5 to 1.6)
2.5 to 5	0.36 to 0.49	16	8	0.9 (0.4 to 2.1)	0.8 (0.3 to 1.9)
1 to 2.5	0.22 to 0.36	11	4	0.8 (0.2 to 2.5)	0.6 (0.2 to 2.0)
<1	<0.22	9	2	0.4 (0.1 to 2.0)	0.4 (0.1 to 1.8)

CFT4 indicates free thyroxine measured in citrate; FT4, free thyroxine; OR, odds ratio and TSH, thyroid stimulating hormone. OR[1]: crude odds ratio; OR[2]: odds ratio adjusted for age and sex.

Table 3. Risk for recurrent venous thrombosis for different strata of FT4 with varying time intervals between blood sampling and recurrent thrombosis.

CFT4	FT4 (pmol/l)	Controls	Cases	OR[1] (CI95)	Controls	Cases	OR[2] (CI95)
<14	<15.5	136	54	0.8 (0.6 to 1.2)	193	80	0.8 (0.6 to 1.1)
14 to 17	15.5 to 18.9	291	149	Ref	386	209	Ref
18	20.0	22	12	0.7 (0.3 to 1.5)	38	19	0.6 (0.3 to 1.2)
19	21.1	8	4	1.1 (0.3 to 4.0)	10	7	1.4 (0.5 to 3.9)
20	22.2	5	2	0.5 (0.1 to 2.9)	9	4	0.6 (0.2 to 2.0)
21	23.3	3	2	0.8 (0.1 to 5.3)	5	2	0.4 (0.1 to 2.4)
22	>24.4	0	1	na	3	2	0.5 (0.1 to 3.8)
Percent	**TSH (mU/l)**	**Controls**	**Cases**	**OR[1] (CI95)**	**TSH (mU/l)**	**Controls**	**Cases**	**OR[2] (CI95)**
>80	>2.10	91	42	1.0 (0.6 to 1.5)	>2.03	129	63	1.0 (0.7 to 1.4)
20 to 80	0.86 to 2.10	276	131	Ref	0.83 to 2.03	370	191	Ref
10 to 20	0.64 to 0.86	40	22	1.0 (0.5 to 1.8)	0.62 to 0.83	69	32	0.7 (0.4 to 1.2)
5 to 10	0.50 to 0.64	20	10	0.8 (0.4 to 1.9)	0.48 to 0.62	24	18	0.9 (0.5 to 1.8)
2.5 to 5	0.35 to 0.50	13	8	0.9 (0.3 to 2.2)	0.36 to 0.48	18	6	0.5 (0.2 to 1.4)
1 to 2.5	0.24 to 0.35	6	3	1.0 (0.2 to 4.5)	0.22 to 0.36	8	4	1.0 (0.3 to 3.4)
<1	<0.24	6	1	0.3 (0.0 to 2.9)	<0.22	9	1	0.2 (0.0 to 1.5)

CFT4 indicates free thyroxine measured in citrate; FT4, free thyroxine; OR, odds ratio; percent, percentile and TSH, thyroid stimulating hormone. OR[1]: odds ratio adjusted for age and sex, blood sample within 1 year for recurrent VT; OR[2]: odds ratio adjusted for age and sex, blood sample within 1.5 year for recurrent VT.

Table 4. Hypothetical types of misclassification within the study design.

FT4 levels at first VT	Treatment	FT4 levels at blood sampling	Treatment after blood sampling	Recurrence risk*	Effect of misclassifcation on true OR
high	+	Normal	-	not affected	**none**
high	-	High	+	not affected	**lower**
high	-	High	-	increased	**none**
normal	-	High	-	increased	**none**
normal	-	High	+	not affected	**lower**
normal	-	Normal	-	not affected	**none**

FT4 levels at first VT indicates levels of FT4 at blood sampling shortly after first venous thrombosis; Treatment yes (+) or no (-); FT4 levels at blood sampling, FT4 levels at the time of blood sampling; Recurrence risk, Risk of recurrence at the time of recurrence and misclassification of true OR, the direction the OR will go if a this situation would happen.

*based on the hypothesis that high levels of FT4 will increase recurrence risk

Summary: Thyroid hormone, haemorrhage and venous thrombosis: solved and unsolved matters

Jan Debeij

Overview

At the end of this thesis we present an overview incorporating the literature and the papers presented in this thesis. This chapter focuses on the associations between hyperthyroidism and venous thrombosis, hypothyroidism and bleeding, hypothyroidism and venous thrombosis, the pathophysiology and clinical implications. In this overview we distinguish between case reports and controlled studies, and between studies of clinically diagnosed thyroid disease and those of levels of thyroid hormones.

On PubMed the following search terms were used to identify studies on the relation between thyroid hormones and haemorrhage or venous thrombosis: venous thrombosis, thrombosis, embolism, pulmonary embolism, thyroid, thyroxine, haemorrhage and hemorrhage. Studies were included up to December 2013 and cross referenced. All controlled studies on both FT4 levels or clinical thyroid disease and symptomatic venous thrombosis were included leading to seven studies(table 1) [20,42,48,49,67,71,93]. No studies on thyroid disease and asymptomatic venous thrombosis were found. Three of the studies considered clinically diagnosed thyroid disease: the study by Lin et al. [49] and Kootte et al. [93] looked at patients with hyperthyroidism, while the study by Danescu et al [71] included both hypo- and hyperthyroidism. Four studies concerned the full spectrum of FT4 levels, rather than diagnoses of thyroid disease [20,42,48,67]. There was only one study on the risk of bleeding with low levels of free thyroxine (this thesis).

Hyperthyroidism

The relation between high levels of thyroid hormone or thyrotoxicosis and venous thrombosis was first described in case reports in 1913 by Kaliebe et al. [13] and in 1928 by Doyle et al. [94]. Other case reports appeared

Thyroid disease	Author	Year	Exposure	Outcome	Study result
Hyperthyroidism	Squizzato et al.	2007	FT4 levels	Venous thrombosis	no effect
	Debeij et al.	2012	FT4 levels	Venous thrombosis	elevated risk
	van Zaane et al.	2010	FT4 levels	Venous thrombosis	elevated risk
	Debeij et al.	submitted	FT4 levels	Venous thrombosis	elevated risk
	Lin et al.	2010	Hyperthyroidism	Venous thrombosis	elevated risk
	Kootte et al.	2012	Hyperthyroidism	Venous thrombosis	elevated risk
	Danescu et al.	2009	Hyperthyroidism	Venous thrombosis	no effect
Hypothyroidism	Debeij et al.	2014	FT4 levels	Haemorrhage	elevated risk
	Michiels et al.	2001	Hypothyroidism	Haemorrhage	elevated risk
	Danescu et al.	2009	Hypothyroidism	Venous thrombosis	elevated risk
	Squizzato et al.	2007	FT4 levels	Venous thrombosis	elevated risk
	Debeij et al.	2012	FT4 levels	Venous thrombosis	elevated risk
	van Zaane et al.	2010	FT4 levels	Venous thrombosis	reduced risk
	Debeij et al.	submitted	FT4 levels	Venous thrombosis	elevated

Table 1: *summary of identified studies and their exposures and outcomes.*

from time to time, mainly focusing on the relation between thyroid dysfunction and cerebral venous thrombosis [14-18]. In a review of case reports published between 1990 and 2009, Franchini et al. identified 34 case reports on hyperthyroidism and venous thrombosis [21]. Twenty-five of these reported on a case of cerebral vein thrombosis (CVT). It is likely that this rare form of thrombosis (1 per 100 000 person years [95]), rather than the occurrence of deep venous thrombosis or pulmonary embolism, raised questions on the aetiology and was subsequently deemed interesting to report on.

High FT4 levels and venous thrombosis risk

A first controlled study was performed in 2007 on the risk of deep venous thrombosis in 50 patients with provoked, 50 patients with unprovoked and 50 controls with no venous thrombosis [20]. No effect of hyperthyroidism on the risk for venous thrombosis was found in this small study.

Subsequently, three larger studies on the relation between thyroid hormone and risk of venous thrombosis have been performed: the ACT-study [42], the MEGA-study [67] and the TROL study [48], all using different study designs. All three studies collected blood samples and data on venous thrombosis. The most obvious difference in the design of the studies was the timing of the blood draw: In the TROL

study blood was sampled before the thrombosis, in the ACT-study blood was drawn at the time of thrombosis and in the MEGA study blood was drawn with a minimum of 3 months after the thrombosis. In addition, the MEGA study has substantially more power than the other two studies due to a large number of patients with thrombosis.

The TROL study [48] is a nested case control study within the HUNT-2 cohort (n=66140). This is a Norwegian cohort study where participants were included between 1995 and 1997. With follow-up untill 2002, cases of venous thrombosis (DVT and PE) in this cohort were retrieved and matched with controls in a 1:3 ratio. This nested case control study included 515 cases with venous thrombosis and 1476 controls. In these subjects FT4 and TSH were measured. In the overall analysis an association between levels of FT4 and the risk of venous thrombosis was found with a relative risk of 1.3 (CI95 0.6 to 3.0) at the 90th percentile (FT4>15.5 pmol/l) rising to a relative risk of 2.5 (CI95 1.3 to 5.0) at the 98th percentile (FT4>17.3 pmol/l) comparing subjects with FT4 levels above the cut-off with subjects with FT4 levels below this cut-off. Because the blood could have been sampled between 1 day to several months or years before the thrombosis, there could be misclassification of the FT4 level when this time period was very long because of variations in FT4 levels (regression dilution bias), masking a true effect. Indeed, at the higher percentiles of FT4, such an effect of time between blood sampling and the event was found. When only including cases in whom blood was drawn within 1 year before thrombosis, the OR at the 98th percentile rose from 2.5 to 4.8 (CI95 1.7 to 14.0). Restricting this time to half a year, this OR was 9.9 (CI95 2.9 to 34.0), indicating that the closer the blood sampling to the event, the lesser the misclassification and the stronger the effect.

In the ACT study [42] FT4 levels and triiodothyronine (T3) levels were measured at the time of thrombosis in 190 cases and 379 controls. The authors reported that higher levels of FT4 gave rise to an increased risk of venous thrombosis, also in a dose response relation. A 1.7 fold increased risk (CI95 1.0 to 2.9) of venous thrombosis was observed in patients with FT4 above the 90th percentile (>18 pmol/L) compared with patients with a FT4 level beneath the 90th percentile. At the 99th percentile (>22 pmol/L) this risk increased to 4.7 (CI95 1.2 to 18.6). This effect was also seen for levels of T3, although less pronounced.

In the MEGA study [67], 2177 cases with venous thrombosis and 2826 controls were included in the analysis. Blood was drawn several months after the venous thrombosis. Here also a dose response relation was found with an OR of 1.2 (CI95 0.9 to 1.6) at the 90th percentile, gradually rising to an OR of 2.2 (CI95 1.0 to 4.6) at FT4 levels >24.4 pmol/L compared with FT4 levels between 15.5 pmol/L and 18.9 pmol/L (reference category). In the MEGA study, blood could have been drawn at various time points from the thrombotic events (3 months to 36 months). When only participants with blood drawn within 6 months after the event were included, the OR was 2.6 (CI95 0.7 to 9.6) at FT4 levels >24.4 pmol/L compared with the reference category. When only subjects were included in whom hyperthyroidism was established, by clinical diagnosis or blood draw, less than one year before or shortly after the index date, an odds ratio of 17.0 (CI95 2.2 to 133.0) was found.

Importantly, in all three studies, the increased risks for thrombosis were found for FT4 levels within the reference range.

Clinical hyperthyroidism and venous thrombosis risk

According to our search, only three studies reported on the relationship between clinically diagnosed hyperthyroidism and venous thrombosis. In a population based cohort from Taiwan, only focusing on pulmonary embolism, Lin et al. [49] included participants with hyperthyroidism over a 5 year period. A total of 8903 patients were found. They were compared to 44515 participants without hyperthyroidism. Fourteen patients with hyperthyroidism developed a pulmonary embolism (0.16%) as compared with 27 euthyroid participants (0.06%). They found that patients with hyperthyroidism had a 2.3-fold increased risk for pulmonary embolism compared with euthyroid subjects (CI95 1.2 to 4.5). The study by Kootte et al. [93] estimated the incidence of venous thrombosis in hyperthyroid patients in three hospitals in the Netherlands and found an incidence rate for first venous thrombosis of 5.3 per 1,000 person-years (CI95 1.1 to 15.6), which was considered to be higher than the population incidence of 1 per 1000 person-years [93]. A study by Danescu [71] et al was performed in the National Hospital Discharge Survey USA from 1979 to 2005. They looked at the discharge codes for hypothyroidism, hyperthyroidism, pulmonary

embolism and deep venous thrombosis. No increased risk for venous thrombosis with a relative risk of 0.98 (CI95 0.96 to 1.01) was found in patients with a diagnosis of hyperthyroidism compared to patients without a diagnosis of thyroid dysfunction in the database.

Hypothyroidism

In 25 case reports, as summarized by Galli-Tsinopoulou et al. [86] from 1987 to 2006, an association was reported between acquired von Willebrand disease, i.e., low levels of von Willebrand factor, and hypothyroidism. Of these case reports 10 patients showed only laboratory abnormalities, and 15 patients presented with clinical symptoms as menorrhagia, epistaxis, bruising and excessive bleeding after dental extraction. A similar evaluation was made by Manfredi et al. further investigating the association between bleeding, acquired von Willebrand disease and hypothyroidism [45].

Low FT4 levels and haemorrhage

Apart from these case-reports, only two controlled studies addressed the question on the risk of bleeding with low levels of free thyroxine. In the Factors study (this thesis), a 1:2 matched case-control study with 330 participants using vitamin K antagonists, a 5-fold increased risk for major bleeding was found for patients with FT4 levels <13.3 pmol/l and a 3-fold increased risk for FT4 levels <14.4 pmol/l. The other paper reports on patients with a combination of acquired von Willebrand disease and hypothyroidism. By normalizing levels of FT4 using levothyroxine, the low levels of VWF and FVIII also returned to normal [34].

Clinical hypothyroidism and venous thrombosis

If the effect on bleeding of low FT4 levels is causal, low levels of FT4 could translate in a protective effect against venous thrombosis. However, both a pro- and an anti-thrombotic effect of low levels of FT4 have been described. Peralta et al. claimed a role for hypothyroidism in the aetiology of cerebral vein thrombosis, by describing two cases with hypothyroidism concomitantly diagnosed with cerebral venous thrombosis [96]. The study by Danescu et al. reported no risk for venous thrombosis

with the diagnosis of hyperthyroidism as mentioned before, but did report on an increased risk for venous thrombosis with the diagnosis of hypothyroidism. An increased risk of 1.60 (CI95 1.59 to 1.60) was reported for patients diagnosed with hypothyroidism compared with participants without thyroid dysfunction.

Low FT4 levels and venous thrombosis

The first controlled study on risk of venous thrombosis was done by Squizzato et al[20]. In patients with unprovoked deep venous thrombosis, a 5.5-fold increased risk (CI95 0.6 to 52.6) for subclinical hypothyroidism was found. In provoked DVT patients a 6.8-fold higher chance to find subclinical hypothyroidism was found compared with controls. However, this study was small, so that chance findings were plausible.

In contrast, in the ACT study, where blood was sampled at the time of the event [42], a linear relationship between the risk of venous thrombosis and FT4 levels was found with a protective effect of low levels of thyroid hormone (FT4 < 15 pmol/l) . At the 10th percentile (FT4<13 pmol/l), there was a 2-fold decreased risk (OR 0.5 CI95 0.2 to 1.0) for venous thrombosis, while at the 5th percentile (FT4<12 pmol/l) a 10-fold decreased risk was found (OR 0.1 CI95 0.0 to 0.9).

In the MEGA [67] and the TROL study [48], the results were more difficult to interpret. In the MEGA study we studied the effect of low levels of FT4 on the risk of venous thrombosis (this thesis). In the overall analyses, we found an OR of 1.8 (CI95 1.0 to 3.1) comparing FT4 levels of 11 pmol/L to the reference category of levels between 15 and 19 pmol/L. At FT4 levels <10 pmol/L an OR of 2.5 (CI95 0.9 to 6.7) was seen. Similar observations were made in the TROL study[48]. In the overall analysis, a mildly increased thrombosis risk of 1.4 (CI95 1.0 to 1.9) was found at the 10th percentile cut-off of FT4 (<11.7 pmol/l) comparing subjects below with subjects above this cut-off. At the 1st percentile, using the same comparison with a cut-off of 10.7 pmol/l an OR of 1.3 (CI95 0.7 to 2.8) was found. Summarizing, the slightly increased risk that was found in the TROL and the MEGA study for low levels of FT4, reversed or attenuated to unity in the TROL study when only a short period between thrombosis and blood sampling was taken.

Pathological considerations

There is not much known on the pathophysiological mechanisms underlying the effect of thyroid hormones on the coagulation system. Thyroid hormone affects gene transcription, which may form the basis of changes in pro- and anticoagulant proteins. Since thyroid hormone influences the basal rate of the carbohydrate, lipid but also the protein metabolism, it is likely that thyroid hormone also affects the production and clearance of coagulations proteins. In vitro studies have shown a direct effect of tri-iodothyronine (T3) on hepatocytes and endothelial cells. T3 causes an up-regulation of fibrinogen, factor II (prothrombin), factor X, von Willebrand factor and plasminogen [36,66,69]. Also in vivo, a quick response of the coagulation system was seen with increased production of nearly all coagulation factors, but most notably VWF and FVIII, on increasing levels of FT4 caused by restarting levothyroxine substitution after withdrawal [46,47]. Additional proof comes from a clinical study [67], where we found a positive association between FVIII and VWF with levels of FT4. Also the risk for venous thrombosis attenuated upon adjustment for FVIII and VWF, which suggests that VWF and FVIII are in the causal pathway between thyroid hormone and venous thrombosis. Although not much is known about the relation between thyroid specific auto-immune antibodies and the effect on the coagulation system, some claims of an effect via this pathway have been made. In this thesis, we have looked in 3 different studies whether there was an effect of anti-thyroid peroxidase antibodies on the risk of thrombosis. None of the studies were able to find a relation between these antibodies and venous thrombosis. A third mechanism was recently proposed by Hooper et al. [81] who studied the influence of thyroxine on fibrin formation. A cross-over design was applied with two arms: Blood was sampled from 19 patients with hyperthyroidism en from 19 euthyroid matched controls. Another blood sample was gathered after treatment of the hyperthyroid patients, i.e., when they returned to a euthyroid state. Levothyroxine was added to the blood samples of the euthyroid participants. In hyperthyroidism, a dense clot structure with impaired fibrinolysis was found. The clot structure partly returned to normal when a euthyroid state was restored. Short term exogenous hyperthyroidism was not associated with alterations in clot structure. Inflammation may also play a

role because complement C3 levels were also associated with clot formation in hyperthyroid patients. The denser clot structure and higher resistance to fibrinolysis might, in itself or in combination with faster clot formation as hypothesized earlier, also be a plausible explanation for the clinical effects found.

The last pathophysiological explanation is the association between hypothyroidism and cardiovascular risk factors. These cardiovascular risk factors include obesity, LDL cholesterol, mild increases of homocysteinemia and endothelial dysfunction; hypothyroidism also increases diastolic blood pressure and induces a mild increase in C-reactive protein [59,60,62,63]. Since recent reports state that arterial cardiovascular risk factors also tend to increase the risk for venous thrombosis to some extent [87-89], it might be possible that these cardiovascular parameters are somewhere in the pathway between hypothyroidism and venous thrombosis.
The effect of low levels of free thyroxine via cardiovascular risk factor would result in a pro-thrombotic effect, while the direct effect of hypothyroidism on the coagulation system is anti-coagulant.

There are several directions for further research. First, the finding of an increased risk of bleeding in patients with low levels of FT4 using VKA needs to be confirmed in larger cohorts, including patients treated with other anticoagulants. Second, since the relation between high levels of FT4 and venous thrombosis risk is now well established, research needs to be aimed at the clinical implications of this relation. Does FT4 need to be included in the standard workup protocol for unprovoked venous thrombosis? Would treatment of hyperthyroidism levels lead to modification of the risk of venous thrombosis? Is anti-coagulation needed in patients with high FT4? Or is anti-coagulation therapy needed in hyperthyroid patients before surgery? It is also interesting to see what the risk of venous thrombosis is in clinical hyperthyroid patients compared to euthyroid persons in a larger patient sample. Furthermore, more work needs to be done to uncover the specific pathways in which FT4 influences the coagulation system.

In conclusion, in the studies in this thesis we found strong evidence that higher than average levels of FT4 increase the risk of venous thrombosis. Weak evidence is

provided that lower than average levels of FT4 give an increased risk of bleeding in patients using vitamin K antagonists. A hypothyroid state possesses probably both pro- and anti-coagulant properties. The effect of FT4 on venous thrombosis is mediated at least by coagulation factors factor VIII and von Willebrand factor.

Clinical implications

Most of the FT4 levels where an increased risk for venous thrombosis is found are within the FT4 range that will be left untreated in clinical practice. Thus, findings of these FT4 levels would in itself have no clinical consequences, but could be used in constructing an overall risk profile.

The relation between free FT4 and the risk of venous thrombosis has implications for both physicians treating venous thrombosis and physicians treating endocrine disorders. The most important clinical implication is that physicians should be aware of the relation. When treating patients with hypothyroidism, physicians must be aware of symptoms such as easy bruising, gum bleeding etcetera, suggesting aqcuired von Willebrand disease. Although the evidence is not very strong, it suggests that with levothyroxine substitution therapy, coagulation factors return to normal [34,46,47]. At the other end of the spectrum, when treating patients with hyperthyroidism, physicians need to be aware of symptoms of deep venous thrombosis and pulmonary embolism. Especially in situations with an elevated risk for venous thrombosis such as surgery or pregnancy, patients with hyperthyroidism should be monitored closely. Early referral for treatment will be beneficial for the patient.

For physicians treating patients with thrombosis, there are several clinical implications. In patients with venous thrombosis, it is advisable to look for signs of thyroid disease, such as goiter, sweating, heat or cold intolerance etc. Based on our and the above mentioned previous findings in three other studies [42,48,49], routine testing for hyperthyroidism of patients with an unprovoked thrombosis may be considered, as the diagnosis is not always obvious clinically and testing is easily done and relatively cheap. A positive result will have a strong impact for the patient:

not only will an undetected condition be treated, but also can the patient receive anticoagulant treatment for a shorter period (3 instead of 6 months (ACCP guidelines [97])), with hence a lower bleeding risk.

Nederlandse samenvatting

Jan Debeij

Nederlandse samenvatting

Veneuze trombose is een ziekte die veel voorkomt. Met een incidentie van ongeveer 1 per 1000 persoonsjaren, is het een ziekte met een hoge morbiditeit en mortaliteit. Een trombose vormt zich vaak in het diepe veneuze systeem van het been, het wordt dan een diepe veneuze trombose genoemd. Een trombose kan zich echter ook in de meer centrale vaten vormen of in de arm. Als een veneus gevormd stolsel losschiet, loopt het vast in het eerste vernauwde vaatsysteem op zijn route: het longbed. Een stolsel in het longbed wordt een longembolie genoemd. Aangezien een longembolie een obstructie geeft van de bloedstroom door de longen, is het een belemmering voor de zuurstofuitwisseling tussen bloed en buitenlucht. Hierdoor ontstaat er een disbalans tussen ventilatie en perfusie en kan een patiënt te weinig zuurstof opnemen. Veel risicofactoren voor het krijgen van veneuze trombose zijn bekend. Deze zijn gemakshalve onder te verdelen in genetische risicofactoren en verworven risicofactoren. Bij genetische risicofactoren moet bijvoorbeeld gedacht worden aan een protrombine-mutatie of de factor V Leiden-mutatie. Mensen met deze mutaties hebben een verhoogd risico op het krijgen van een veneuze trombose. Verworven risicofactoren zijn onder anderen onderbeensgips, kanker, immobilisatie, een recente operatie en zwangerschap. Als therapie ter voorkoming van een recidief veneuze trombose, danwel vanwege een andere indicatie zoals atriumfibrillatie, een pacemaker of bypass chirurgie, kan antistollingsmedicatie gegeven worden. Deze medicatie remt het stollingssysteem op bepaalde punten teneinde de vorming van stolsels te voorkomen. Een voor de hand liggende bijwerking is een verhoogd risico op bloeding. Dit kunnen kleine bloeduitstortingen zijn, maar dit kan zich ook manifesteren als bloedingen in gewrichten of in het brein. Dit kan resulteren in onomkeerbare schade en zelfs overlijden.

De laatste jaren is er meer aandacht gekomen voor de invloed van endocriene hormonen op het krijgen van een veneuze trombose. Daarbij moet gedacht worden aan cortisol, groeihormoon, prolactine en schildklierhormoon. In dit proefschrift wordt gekeken naar de invloed van schildklierhormoon op de individuele stollingsfactoren van het stollingssysteem en het daaruit volgende risico op het ontwikkelen van een veneuze trombose.

De eerste beschrijving van een relatie tussen schildklierhormoon en veneuze trombose stamt uit 1913. In dit artikel wordt een patiënt beschreven die een cerebrale veneuze trombose heeft tezamen met een ernstige hyperthyreoïdie. In de loop der tijden zijn er meerdere van deze studies beschreven. Opmerkelijk genoeg worden vaak cerebrale veneuze tromboses beschreven. Waarschijnlijk vanwege het zeldzame karakter van deze aandoening, in combinatie met de desastreuze gevolgen die dit ziektebeeld met zich mee brengt. Naast deze patiëntrapportages is er ook nog gekeken naar het effect van schildklierhormoon op de verschillende stollingsfactoren van het stollingssysteem. Hierbij wordt gezien dat een verhoging van het schildklierhormoon gepaard gaat met een verhoging van in ieder geval stollingsfactoren factor VIII, von Willebrand factor en fibrinogeen. Een individuele verhoging van deze factoren leidt tot een hogere activiteit van het stollingssysteem, hetgeen kan leiden tot een verhoogd risico op vorming van een veneuze trombose. Pas in 2007 werd het eerste klinisch onderzoek verricht naar de relatie tussen schildklierhormoon en veneuze trombose. In deze studie werd geen associatie gevonden tussen beiden. Opgemerkt dient te worden dat er zo weinig patiënten in het onderzoek zaten dat er alleen een effect gevonden kon worden als dit heel groot bleek te zijn. Kort daarop zijn drie verschillende grotere studies gepubliceerd om meer te weten te komen over de relatie tussen schildklierhormoon en veneuze trombose. Het grote verschil tussen deze drie studies is het moment waarop bloed bij de patiënt werd afgenomen in verhouding tot het ontstaan van de trombose. In het grote Noorse HUNT cohort werd bloed afgenomen bij ongeveer 66.000 mensen. Gekeken werd welke mensen later een trombose kregen. Daarbij werden controles gezocht. Er werd gevonden dat personen met de hoogste schildklierhormoon-waarden een ongeveer 2,5 keer zo hoog risico hadden op het krijgen van veneuze trombose. Bij een korte tijd tussen het afnemen van bloed en het optreden van een trombose (waarbij de gemeten waarde van het schildklierhormoon meer waarschijnlijk de waarde ten tijde van trombose is), nam dit toe tot een ongeveer 10 keer verhoogd risico. In de ACT studie werd bloed afgenomen op het moment dat patiënten een trombose hadden. Hier werd een 5 keer verhoogd risico gevonden op het optreden van een veneuze trombose bij hoge schildklierhormoonwaarden. In de MEGA studie werd het bloed ter bepaling van schildklierhormoonwaarde enkele maanden na de trombose afgenomen. Hier werd een 2 keer verhoogd risico

gevonden. Als ook hier alleen patiënten meegenomen werden met weinig tijd tussen de trombose en de bloedafname, nam het risico toe tot ongeveer 2,5. Als enkel de groep werd bekeken met hoge schildklierhormoonwaarden ten tijde van de trombose werd zelfs een 17-voudig verhoogd risico gevonden. Al deze 3 studies vinden dus een verhoogd risico op het krijgen van een veneuze trombose bij verhoogde waarden van schildklierhormoon. Hoog schildklierhormoon, of hyperthyreoïdie, kan dus beschouwd worden als een risicofactor voor veneuze trombose.

Ook is er gekeken naar het effect van laag schildklierhormoon, hypothyreoïdie, op het stollingssysteem. In een review van de beschikbare literatuur werd gezien dat globale stollingstesten zoals de aPTT en de PT(INR) verhoogd zijn bij lagere waarden van schildklierhormoon. Hogere waarden van deze stollingstesten houden in dat bloed moeilijker stolt. Daarnaast werd ook gezien dat er, in tegenstelling tot de stijging bij hyperthyreoïdie, een daling optreedt van stollingsfactoren factor VIII, von Willebrand factor, fibrinogeen en factor IX. Een daling van deze waarden zou kunnen leiden tot een verhoogde bloedingsneiging. Deze verhoogde bloedingsneiging kon teniet gedaan worden door het normaliseren van de schildklierhormoonwaarden met medicatie. Er is maar één klinische studie verschenen over de relatie tussen lage schildklierhormoonwaarden en een verhoogde bloedingsneiging. Bij lage schildklierhormoon waarden werd een 5 keer verhoogd risico gevonden op het ontwikkelen van een ernstige bloeding (bijvoorbeeld een gewrichtsbloeding of een hersenbloeding) in patiënten die al anti-stollingsmedicatie gebruikten. Met een verhoogde bloedingsneiging gaat een verminderde stollingsneiging gepaard, hetgeen een beschermend effect kan hebben op het ontwikkelen van een veneuze trombose. Ook hiernaar is gekeken. Uit patiëntrapportages blijkt dat hypothyreoïdie geduid wordt als zowel een risicofactor alsook een beschermende factor voor een veneuze trombose. Ook uit de recente klinische studies waar gekeken is of laag schildklierhormoon een beschermende factor danwel een risico factor is, volgt geen eenduidig antwoord. In het geval dat laag schildklierhormoon een risicofactor is voor het optreden van een bloeding en een beschermende factor voor een veneuze trombose, zou dit in lijn zijn met lage stollingsfactoren die worden gevonden bij lage schildklierhormoonwaarden. Als laag schildklierhormoon een risicofactor blijkt te zijn voor het optreden van een trombose, zou het beschermende effect van de lage

stollingsfactoren tegengewerkt moeten worden. Hypothyreoïdie is, behalve met lage stollingsfactoren, ook geassocieerd met cardiovasculaire risicofactoren zoals obesitas, hoog LDL cholesterol, diastolische hypertensie, verminderde endotheelfunctie en inflammatie. Via deze factoren zou het beschermende effect van lage stollingsfactoren teniet kunnen worden gedaan en zelfs een trombose neiging kunnen ontstaan.

Hoewel is aangetoond dat er een relatie bestaat tussen schildklierhormoon en veneuze trombose is het nog te vroeg om er strikte klinische consequenties aan te verbinden. Wel is van belang dat zowel haematologen als endocrinologen op de hoogte zijn van de relatie. Haematologen zouden een aan schildklierhormoon toegeschreven trombose na behandeling van de schildklierziekte kunnen beschouwen als een passagère risicofactor. Hierdoor zou de preventieve antistollingsbehandeling kunnen worden ingekort, waardoor het risico op bijwerkingen, met als belangrijkste bloeding, afneemt. Endocrinologen zouden door kennis van de associatie bij een patiënt met hyperthyreoïdie sneller een veneuze trombose kunnen diagnostiseren.

Concluderend is er een effect van hoge schildklierhormoonwaarden op het risico van een veneuze trombose. Op basis van de huidige literatuur en (basale) onderzoeken lijkt het er op dat lage schildklierhormoonwaarden een beschermend effect hebben op het ontstaan van trombose en een hoger risico op bloeding geven in patiënten die anti-stollingsmedicatie gebruiken. Welk mechanisme ten grondslag ligt aan het gevonden effect is nog niet duidelijk. Naar dit pathofysiologisch mechanisme moet dan ook nog meer onderzoek gedaan worden. Verdere richtingen voor verder onderzoek zijn: het repliceren van de verhoogde bloedingsneiging bij lage schildklierhormoonwaarden bij anti-stollingsmedicatie in een groter cohort, de klinische implicaties van de gevonden relatie en het onderzoeken van het risico op veneuze trombose in een groep patiënten met klinische hyperthyreoidie.

Resumerend:
1) Er is sterk bewijs dat hoge schildklierhormoonwaarden een verhoogd risico geven op veneuze trombose.

2) Er is zwak bewijs dat lage schildklierhormoonwaarden een verhoogd risico geven op bloeding in patiënten die anti-stolling gebruiken.
3) Het effect van schildklierhormoon op het stollingssysteem loopt voornamelijk via stollingsfactoren factor VIII en von Willebrand factor.

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Curriculum Vitae

Jan Debeij werd geboren op 22 november 1981 te Margraten. In 2000 behaalde hij zijn VWO diploma aan het Eurocollege te Maastricht. In 2006 behaalde hij aan de Universiteit van Tilburg zijn doctoraal Psychologie met als aandachtsgebied neuro- en revalidatiepsychologie. Vervolgens kreeg hij in 2006 de mogelijkheid om via een zij-instroom traject Geneeskunde te studeren aan de Universiteit Leiden en in 2010 slaagde hij voor zijn artsexamen. Tijdens zijn studie is hij begonnen met onderzoek naar de effecten van schildklierhormoon op het stollingsysteem en veneuze trombose bij de afdeling Klinische Epidemiologie van het Leids Universitair Medisch Centrum. De resultaten van dit onderzoek zijn in dit proefschrift beschreven. Tijdens dit onderzoek volgde hij tevens de opleiding tot epidemioloog B. Hij is vanaf 2012 in opleiding tot plastisch chirurg. Zijn vooropleiding heeft hij genoten bij de afdeling heelkunde van het Rijnland ziekenhuis te Leiderdorp. Hij vervolgt nu zijn opleiding tot plastisch chirurg bij de afdeling plastische en reconstructieve chirurgie en handchirurgie in het Erasmus Medisch Centrum te Rotterdam.

References

1 Tagalakis V, Patenaude V, Kahn SR, Suissa S. Incidence of and mortality from venous thromboembolism in a real-world population: the Q-VTE Study Cohort. *AmJMed* **2013;** 126: 832.e13–21.

2 Cushman M, Tsai AW, White RH, Heckbert SR, Rosamond WD, Enright P, Folsom AR. Deep vein thrombosis and pulmonary embolism in two cohorts: the longitudinal investigation of thromboembolism etiology. *AmJMed* **2004;** 117: 19–25.

3 Rosendaal FR. Venous thrombosis: a multicausal disease. *Lancet* **1999;** 353: 1167–73.

4 Blom JW, Doggen CJM, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. *JAMA* **2005;** 293: 715–22.

5 Anderson FA, Wheeler HB, Goldberg RJ, Hosmer DW, Patwardhan NA, Jovanovic B, Forcier A, Dalen JE. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. *ArchInternMed* **1991;** 151: 933–8.

6 Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost* **2007;** 5: 692–9.

7 Christiansen SC, Cannegieter SC, Koster T, Vandenbroucke JP, Rosendaal FR. Thrombophilia, clinical factors, and recurrent venous thrombotic events. *JAMA* **2005;** 293: 2352–61.

8 Kyrle PA, Rosendaal FR, Eichinger S. Risk assessment for recurrent venous thrombosis. *Lancet* **2010;** 376: 2032–9.

9 Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. *Lancet* **2003;** 362: 523–6.

10 Hansson PO, Sörbo J, Eriksson H. Recurrent venous thromboembolism after deep vein thrombosis: incidence and risk factors. *ArchInternMed* **2000;** 160: 769–74.

11 Eischer L, Eichinger S, Kyrle PA. Age at first venous thromboembolism and risk of recurrence: a prospective cohort study. *Medicine (Baltimore)* **2009;** 88: 366–70.

12 Christiansen SC, Lijfering WM, Helmerhorst FM, Rosendaal FR, Cannegieter SC. Sex difference in risk of recurrent venous thrombosis and the risk profile for a second event. *J Thromb Haemost* **2010;** 8: 2159–68.

13 Kaliebe H. Autochthone Sinusthrombose bei einem Falle von Morbus Basedow und Tabes. *Med Klinik* **1913**.

14 De Schryver EL, Hoogenraad TU, Banga JD, Kappelle LJ. Thyrotoxicosis, protein C deficiency and lupus anticoagulant in a case of cerebral sinus thrombosis. *Neth J Med* **1999;** 55: 201–2.

15 Molloy E, Cahill M, O'Hare JA. Cerebral venous sinus thrombosis precipitated by Graves' disease and Factor V Leiden mutation. *Ir Med J* **2003;** 96: 46–7.

16 Mouton S, Nighoghossian N, Berruyer M, Derex L, Philippeau F, Cakmak S, Honnorat J, Hermier M, Trouillas P. Hyperthyroidism and cerebral venous thrombosis. *Eur Neurol* **2005;** 54: 78–80.

17 Pekdemir M, Yilmaz S, Ersel M, Sarisoy HT. A rare cause of headache: cerebral venous sinus thrombosis due to hyperthyroidism. *Am J Emerg Med* **2008;** 26: 383–3.

18 Siegert CE, Smelt AH, de Bruin TW. Superior sagittal sinus thrombosis and thyrotoxicosis. Possible association in two cases. *Stroke* **1995;** 26: 496–7.

19 Squizzato A, Romualdi E, Büller HR, Gerdes VE. Clinical review: Thyroid dysfunction and effects on coagulation and fibrinolysis: a systematic review. *JClinEndocrinolMetab* **2007;** 92: 2415–20.

20 Squizzato A, Romualdi E, Piantanida E, Gerdes VE, Büller HR, Tanda M, Bartalena L, Venco A, Ageno W. Subclinical hypothyroidism and deep venous thrombosis. A pilot cross-sectional study. *Thromb Haemost* **2007;** 97: 803–6.

21 Franchini M. Hemostatic changes in thyroid diseases: haemostasis and thrombosis. *Hematology* **2006;** 11: 203–8.

22 Hofbauer LC, Heufelder AE. Coagulation disorders in thyroid diseases. *EurJEndocrinol* **1997;** 136: 1–7.

23 Squizzato A, Gerdes VE, Brandjes DP, Büller HR, Stam J. Thyroid diseases and cerebrovascular disease. *Stroke* **2005;** 36: 2302–10.

24 Erem C, Kavgaci H, Ersoz HO, Hacihasanoglu A, Ukinc K, Karti SS, Deger O, Telatari M. Blood coagulation and fibrinolytic activity in hypothyroidism. *IntJClinPract* **2003;** 57: 78–81.

25 Homoncik M, Gessl A, Ferlitsch A, Jilma B, Vierhapper H. Altered platelet plug formation in hyperthyroidism and hypothyroidism. *JClinEndocrinolMetab* **2007;** 92: 3006–12.

26 Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, Davies TF, Zaidi M. TSH is a negative regulator of skeletal remodeling. *Cell* **2003;** 115: 151–62.

27 de LA, Bursell J, Gregory JW, Rees DA, Ludgate M. TSH receptor activation and body composition. *JEndocrinol* **2010;** 204: 13–20.

28 Mazziotti G, Sorvillo F, Piscopo M, Cioffi M, Pilla P, Biondi B, Iorio S, Giustina A, Amato G, Carella C. Recombinant human TSH modulates in vivo C-telopeptides of type-1 collagen and bone alkaline phosphatase, but not osteoprotegerin production in postmenopausal women monitored for differentiated thyroid carcinoma. *JBone MinerRes* **2005;** 20: 480–6.

29 Heemstra KA, Burggraaf J, van der Klaauw AA, Romijn JA, Smit JW, Corssmit EP. Short-term overt hypothyroidism induces sympathovagal imbalance in

thyroidectomized differentiated thyroid carcinoma patients. *ClinEndocrinol(Oxf)* **2010;** 72: 417–21.

30 Heemstra KA, Hamdy NA, Romijn JA, Smit JW. The effects of thyrotropin-suppressive therapy on bone metabolism in patients with well-differentiated thyroid carcinoma. *Thyroid* **2006;** 16: 583–91.

31 Pacini F, Castagna MG. Diagnostic and therapeutic use of recombinant human TSH (rhTSH) in differentiated thyroid cancer. *Best Pract Res Clin Endocrinol Metab* **2008;** 22: 1009–21.

32 Erem C, Ersoz HO, Karti SS, Ukinc K, Hacihasanoglu A, Deger O, Telatar M. Blood coagulation and fibrinolysis in patients with hyperthyroidism. *JEndocrinolInvest* **2002;** 25: 345–50.

33 Gullu S, Sav H, Kamel N. Effects of levothyroxine treatment on biochemical and hemostasis parameters in patients with hypothyroidism. *EurJEndocrinol* **2005;** 152: 355–61.

34 Michiels JJ, Schroyens W, Berneman Z, van der Planken M. Acquired von Willebrand syndrome type 1 in hypothyroidism: reversal after treatment with thyroxine. *Clin Appl Thromb Hemost* **2001;** 7: 113–5.

35 Squizzato A, Gerdes VE. Thyroid disease and haemostasis - a relationship with clinical implications? *Thromb Haemost* **2008;** 100: 727–8.

36 Shih CH, Chen SL, Yen CC, Huang YH, Chen CD, Lee YS, Lin KH. Thyroid hormone receptor-dependent transcriptional regulation of fibrinogen and coagulation proteins. *Endocrinology* **2004;** 145: 2804–14.

37 Frigo A, Dardano A, Danese E, Davi MV, Moghetti P, Colato C, Francia G, Bernardi F, Traino C, Monzani F, Ferdeghini M. Chromosome translocation frequency after radioiodine thyroid remnant ablation: a comparison between recombinant human thyrotropin stimulation and prolonged levothyroxine withdrawal. *JClinEndocrinolMetab* **2009;** 94: 3472–6.

38 Schobersberger W, Fries D, Mittermayr M, Innerhofer P, Sumann G, Schobersberger B, Klingler A, Stollnberger V, Fischbach U, Gunga HC. Changes of biochemical markers and functional tests for clot formation during long-haul flights. *Thromb Res* **2002;** 108: 19–24.

39 Schreijer AJ, Cannegieter SC, Meijers JC, Middeldorp S, Büller HR, Rosendaal FR. Activation of coagulation system during air travel: a crossover study. *Lancet* **2006;** 367: 832–8.

40 Stricker H, Colucci G, Alberio L, Mombelli G. Variation in coagulation inhibitors during prolonged sitting: possible pathogenetic mechanisms for travel-associated thrombosis. *J Thromb Haemost* **2006;** 4: 900–2.

41 Fender D, Harper WM, Thompson JR, Gregg PJ. Mortality and fatal pulmonary embolism after primary total hip replacement. Results from a regional hip register. *JBone Joint SurgBr* **1997;** 79: 896–9.

42 van Zaane B, Squizzato A, Huijgen R, van Zanten AP, Fliers E, Cannegieter SC, Büller HR, Gerdes VE, Brandjes DP. Increasing levels of free thyroxine as a risk factor for a first venous thrombosis: a case-control study. *Blood* **2010;** 115: 4344–9.

43 Flaherty ML. Anticoagulant-associated intracerebral hemorrhage. *SeminNeurol* **2010;** 30: 565–72.

44 Landefeld CS, Beyth RJ. Anticoagulant-related bleeding: clinical epidemiology, prediction, and prevention. *AmJMed* **1993;** 95: 315–28.

45 Manfredi E, van Zaane B, Gerdes VE, Brandjes DP, Squizzato A. Hypothyroidism and acquired von Willebrand's syndrome: a systematic review. *Haemophilia* **2008;** 14: 423–33.

46 Debeij J, Cannegieter SC, van Zaane B, Smit JWA, Corssmit EPM, Rosendaal FR, Romijn JA, Dekkers OM. The effect of changes in thyroxine and thyroid-stimulating hormone levels on the coagulation system. *J Thromb Haemost* **2010;** 8: 2823–6.

47 Yango J, Alexopoulou O, Eeckhoudt S, Hermans C, Daumerie C. Evaluation of the respective influence of thyroid hormones and TSH on blood coagulation parameters after total thyroidectomy. *EurJEndocrinol [Internet]* **2011;** 164: 599–603.

48 Debeij J, Dekkers OM, Asvold BO, Christiansen SC, Naess IA, Hammerstrom J, Rosendaal FR, Cannegieter SC. Increased levels of free thyroxine and risk of venous thrombosis in a large population-based prospective study. *J Thromb Haemost* **2012;** 10: 1539–46.

49 Lin HC, Yang LY, Kang JH. Increased risk of pulmonary embolism among patients with hyperthyroidism: a 5-year follow-up study. *J Thromb Haemost* **2010;** 8: 2176–81.

50 Garcia AA, van der Heijden JF, Meijers JC, Rosendaal FR, Reitsma PH. The relationship between ABO blood group and the risk of bleeding during vitamin K antagonist treatment. *J Thromb Haemost* **2006;** 4: 1418–20.

51 Kellett HA, Sawers JS, Boulton FE, Cholerton S, Park BK, Toft AD. Problems of anticoagulation with warfarin in hyperthyroidism. *QJMed* **1986;** 58: 43–51.

52 Kurnik D, Loebstein R, Farfel Z, Ezra D, Halkin H, Olchovsky D. Complex drug-drug-disease interactions between amiodarone, warfarin, and the thyroid gland. *Medicine (Baltimore)* **2004;** 83: 107–13.

53 van der Heijden JF, Rekke B, Hutten BA, van der Meer FJ, Remkes MG, Vermeulen M, Büller HR, Reitsma PH. Non-fatal major bleeding during treatment with vitamin K antagonists: influence of soluble thrombomodulin and mutations in the propeptide of coagulation factor IX. *J Thromb Haemost* **2004;** 2: 1104–9.

54 Miettinen O. Estimability and estimation in case-referent studies. *Am J Epidemiol* **1976;** 103: 226–35.

55 Team RDC. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; **2011**.

56 R-core members SDRB. Foreign: Read Data Stored by Minitab, S, SAS, SPSS, Stata, Systat, dBase. R package version 0.8-43; **2011**.

57 Lumley TTAOS->PBT. Survival analysis, including penalised likelihood. R package version 2.36-5; **2011**.

58 Gumieniak O, Perlstein TS, Hopkins PN, Brown NJ, Murphey LJ, Jeunemaitre X, Hollenberg NK, Williams GH. Thyroid function and blood pressure homeostasis in euthyroid subjects. *JClinEndocrinolMetab* **2004;** 89: 3455–61.

59 Rodondi N, Elzen den WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, Asvold BO, Iervasi G, Imaizumi M, Collet TH, Bremner A, Maisonneuve P, Sgarbi JA, Khaw KT, Vanderpump MP, Newman AB, Cornuz J, Franklyn JA, Westendorp RG, Vittinghoff E, et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. *JAMA* **2010;** 304: 1365–74.

60 Asvold BO, Bjoro T, Nilsen TI, Vatten LJ. Association between blood pressure and serum thyroid-stimulating hormone concentration within the reference range: a population-based study. *JClinEndocrinolMetab* **2007;** 92: 841–5.

61 Asvold BO, Bjøro T, Vatten LJ. Association of thyroid function with estimated glomerular filtration rate in a population-based study: the HUNT study. *EurJEndocrinol* **2011;** 164: 101–5.

62 Asvold BO, Vatten LJ, Nilsen TI, Bjoro T. The association between TSH within the reference range and serum lipid concentrations in a population-based study. The HUNT Study. *EurJEndocrinol* **2007;** 156: 181–6.

63 Asvold BO, Bjoro T, Nilsen TI, Gunnell D, Vatten LJ. Thyrotropin levels and risk of fatal coronary heart disease: the HUNT study. *ArchInternMed* **2008;** 168: 855–60.

64 Bremner AP, Feddema P, Leedman PJ, Brown SJ, Beilby JP, Lim EM, Wilson SG, O'Leary PC, Walsh JP. Age-related changes in thyroid function: a longitudinal study of a community-based cohort. *JClinEndocrinolMetab* **2012;** 97: 1554–62.

65 Maes M, Mommen K, Hendrickx D, Peeters D, D'Hondt P, Ranjan R, De Meyer F, Scharpé S. Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. *ClinEndocrinol(Oxf)* **1997;** 46: 587–98.

66 Lin KH, Lee HY, Shih CH, Yen CC, Chen SL, Yang RC, Wang CS. Plasma protein regulation by thyroid hormone. *JEndocrinol* **2003;** 179: 367–77.

67 Debeij J, van Zaane B, Dekkers O, Smit J, Romijn J, Gerdes V, van Zanten A, Brandjes D, Büller H, Rosendaal F, Cannegieter S. Hyperthyroidism is associated with an increased risk of venous thrombosis. *Journal of Thrombosis and Haemostasis* **2009;** 7.

68 Christiansen SC, Naess IA, Cannegieter SC, Hammerstrom J, Rosendaal FR, Reitsma PH. Inflammatory cytokines as risk factors for a first venous thrombosis: a prospective population-based study. *PLoSMed* **2006;** 3: e334.

69 Baumgartner-Parzer SM, Wagner L, Reining G, Sexl V, Nowotny P, Muller M, Brunner M, Waldhausl W. Increase by tri-iodothyronine of endothelin-1, fibronectin and von Willebrand factor in cultured endothelial cells. *JEndocrinol* **1997;** 154: 231–9.

70 Franchini M, Lippi G, Manzato F, Vescovi PP. Thyroid-associated autoimmune coagulation disorders. *JThrombThrombolysis* **2010;** 29: 87–91.

71 Danescu LG, Badshah A, Danescu SC, Janjua M, Marandici AM, Matta F, Yaekoub AY, Malloy DJ, Stein PD. Venous thromboembolism in patients hospitalized with thyroid dysfunction. *Clin Appl Thromb Hemost* **2009;** 15: 676–80.

72 van Stralen KJ, Rosendaal FR, Doggen CJ. Minor injuries as a risk factor for venous thrombosis. *ArchInternMed* **2008;** 168: 21–6.

73 Squizzato A, Gerdes VE, Ageno W, Büller HR. The coagulation system in endocrine disorders: a narrative review. *Intern Emerg Med* **2007;** 2: 76–83.

74 Wiggins KL, Smith NL, Glazer NL, Rosendaal FR, Heckbert SR, Psaty BM, Rice KM, Lumley T. ABO genotype and risk of thrombotic events and hemorrhagic stroke. *J Thromb Haemost* **2009;** 7: 263–9.

75 Ohira T, Cushman M, Tsai MY, Zhang Y, Heckbert SR, Zakai NA, Rosamond WD, Folsom AR. ABO blood group, other risk factors and incidence of venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE). *J Thromb Haemost* **2007;** 5: 1455–61.

76 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: 152–5.

77 Ruiz-Sáez A. Thrombosis in rare bleeding disorders. *Hematology* **2012;** 17 Suppl 1: S156–8.

78 Woodhams B, Girardot O, Blanco MJ, Colesse G, Gourmelin Y. Stability of coagulation proteins in frozen plasma. *Blood Coagul Fibrinolysis* **2001;** 12: 229–36.

79 Hoylaerts MF, Thys C, Arnout J, Vermylen J. Recurrent arterial thrombosis linked to autoimmune antibodies enhancing von Willebrand factor binding to platelets and inducing Fc gamma RII receptor-mediated platelet activation. *Blood* **1998;** 91: 2810–7.

80 Takamura N, Akilzhanova A, Hayashida N, Kadota K, Yamasaki H, Usa T, Nakazato M, Maeda T, Ozono Y, Aoyagi K. Thyroid function is associated with carotid intima-media thickness in euthyroid subjects. *Atherosclerosis* **2009;** 204: e77–e81.

81 Hooper JMW, Stuijver DJF, Orme SM, van Zaane B, Hess K, Gerdes VE, Phoenix F, Rice P, Smith KA, Alzahrani SH, Standeven KF, Ajjan RA. Thyroid dysfunction and fibrin network structure: a mechanism for increased thrombotic risk in hyperthyroid individuals. *JClinEndocrinolMetab* **2012;** 97: 1463–73.

82 Dekkers OM, Horváth-Puhó E, Jørgensen JOL, Cannegieter SC, Ehrenstein V, Vandenbroucke JP, Pereira AM, Sørensen HT. Multisystem morbidity and mortality in Cushing's syndrome: a cohort study. *JClinEndocrinolMetab* **2013;** 98: 2277–84.

83 Stuijver DJF, van Zaane B, Feelders RA, Debeij J, Cannegieter SC, Hermus AR, van den Berg G, Pereira AM, de Herder WW, Wagenmakers MAEM, Kerstens MN, Zelissen PMJ, Fliers E, Schaper N, Drent ML, Dekkers OM, Gerdes VEA. Incidence of venous thromboembolism in patients with Cushing's syndrome: a multicenter cohort study. *JClinEndocrinolMetab* **2011;** 96: 3525–32.

84 Stuijver DJF, Debeij J, van Zaane B, Dekkers OM, Smit JWA, Büller HR, Rosendaal FR, Gerdes VEA, Cannegieter SC. Levels of prolactin in relation to coagulation factors and risk of venous thrombosis. Results of a large population-based case-control study (MEGA-study). *Thromb Haemost* **2012;** 108.

85 Van Zaane B, Nur E, Squizzato A, Dekkers OM, Twickler MTB, Fliers E, Gerdes VEA, Büller HR, Brandjes DPM. Hypercoagulable state in Cushing's syndrome: a systematic review. *JClinEndocrinolMetab* **2009;** 94: 2743–50.

86 Galli-Tsinopoulou A, Stylianou C, Papaioannou G, Nousia-Arvanitakis S. Acquired von Willebrand's syndrome resulting from untreated hypothyroidism in two prepubertal girls. *Haemophilia* **2006;** 12: 687–9.

87 Lijfering WM, Flinterman LE, Vandenbroucke JP, Rosendaal FR, Cannegieter SC. Relationship between venous and arterial thrombosis: a review of the literature from a causal perspective. *Semin Thromb Hemost* **2011;** 37: 885–96.

88 van Schouwenburg IM, Gansevoort RT, Mahmoodi BK, Visser MM, Kluin-Nelemans HC, Lijfering WM, Veeger NJGM. Increased risk of arterial thromboembolism after a prior episode of venous thromboembolism: results from the Prevention of REnal and Vascular ENd stage Disease (PREVEND) Study. *Br J Haematol* **2012;** 159: 216–22.

89 Roach REJ, Lijfering WM, Flinterman LE, Rosendaal FR, Cannegieter SC. Increased risk of CVD after VT is determined by common etiologic factors. *Blood* **2013;** 121: 4948–54.

90 van Zaane B, Squizzato A, Debeij J, Dekkers OM, Meijers JCM, van Zanten AP, Büller HR, Gerdes VEA, Cannegieter SC, Brandjes DPM. Alterations in Coagulation and Fibrinolysis after Levothyroxine Exposure in Healthy Volunteers: A Controlled Randomized Crossover Study. *J Thromb Haemost* **2011**.

91 Van Zaane B, Squizzato A, Reuwer AQ, van Zanten AP, Twickler MTB, Dekkers OM, Cannegieter SC, Büller HR, Gerdes VEA, Brandjes DPM. Prolactin and venous thrombosis: indications for a novel risk factor? *Arterioscler Thromb Vasc Biol* **2011;** 31: 672–7.

92 Cannegieter SC, van Hylckama Vlieg A. Venous thrombosis: understanding the paradoxes of recurrence. *J Thromb Haemost* **2013;** 11 Suppl 1: 161–9.

93 Kootte RS, Stuijver DJF, Dekkers OM, Van Zaane B, Fliers E, Cannegieter SC, Gerdes VEA. The incidence of venous thromboembolism in patients with overt hyperthyroidism: a retrospective multicentre cohort study. *Thromb Haemost* **2012;** 107: 417–22.

94 Doyle JB. Obstruction of the longitudinal sinus. *Arch NeurPsych* American Medical Association; **1927;** 18: 374–82.

95 Coutinho JM, Zuurbier SM, Aramideh M, Stam J. The Incidence of Cerebral Venous Thrombosis: A Cross-Sectional Study. *Stroke* **2012**.

96 Peralta AR, Canhao P. Hypothyroidism and cerebral vein thrombosis--a possible association. *JNeurol* **2008;** 255: 962–6.

97 Guyatt GH, Norris SL, Schulman S, Hirsh J, Eckman MH, Akl EA, Crowther M, Vandvik PO, Eikelboom JW, McDonagh MS, Lewis SZ, Gutterman DD, Cook DJ, Schünemann HJ, American College of Chest Physicians. Methodology for the development of antithrombotic therapy and prevention of thrombosis guidelines: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed:

American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* **2012;** 141: 53S–70S.