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Lascero tutti gli onori,
ma non l'onore di servire Te.
Lo dirò a tutti
il giorno in cui potrò trovare
un granello della polvere dei tuoi piedi.

Quando me ne starò, distratto,
alla finestra del mondo,
anche il viandante della strada
vedrà nel mio volto la tua parola.

Lascero tutti gli onori,
ma non l'onore di servire Te.

(Tagore)

Thyroid disease and haemostasis A relationship with clinical implications?

Alessandro Squizzato

Thyroid disease and haemostasis

A relationship with
clinical implications?

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clinical implications?**

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Thyroid disease and haemostasis

A relationship with clinical implications?

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
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ten overstaan van een door het college voor
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Promotores:	Prof. dr. H.R. Büller Prof. dr. W. Ageno
Co-promotores:	Dr. V.E.A. Gerdes Dr. D.P.M. Brandjes Dr. S.C. Cannegieter
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Faculteit der Geneeskunde

Voor Alessandra, Pietro en Elisabetta

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Chapter 1

General Introduction and
Outline of the Thesis

Alessandro Squizzato, Victor EA Gerdes,
Harry R Büller

GENERAL INTRODUCTION

Haemostasis is a well-balanced system that prevents us from bleeding without inducing thrombotic events. The complex regulation of haemostasis is very similar to a 'symphony orchestra', in which coagulation and fibrinolytic system interact simultaneously 'in concert' with cellular surfaces of platelets and the vascular endothelium^[1]. The endothelium plays a crucial role in maintaining the blood in a fluid state. Its surface has several anticoagulant properties: when there is a breach in the integrity of the vessel wall platelets adhere to subendothelial collagen and clot formation is initiated. Subsequently, platelets aggregate and fibrin strands are formed while anticoagulant proteins and the fibrinolytic system regulate and limit this clot formation^[2].

It is a spontaneous thought to believe that hormones, which exert their effects in all tissues and organs, interfere with the haemostatic balance. Apart the well-known interactions with sex hormones, oral contraceptives and hormonal replacement therapy, almost all hormones have been described to directly influence both primary and secondary haemostasis^[3,4]. A wide variety of endocrine disorders have been associated with both mild abnormalities in laboratory coagulation tests, and clinical thrombotic or bleeding manifestations^[4]. Among them, the first description of an interaction was with thyroid disorders, when both Kaliebe and Doyle reported an episode of cerebral vein thrombosis in a thyrotoxic patient in 1913 and 1927, respectively^[5]. Several elements of the process of thrombus formation may be involved^[6,7]. Both thyroid dysfunction and autoimmunity may modify physiological processes of primary and secondary hemostasis and lead to bleeding or thrombosis. Idiopathic thrombocytopenic purpura, secondary antiphospholipid syndrome, or acquired haemophilia have been associated in individual cases with autoimmune thyroid disorders^[8]. Thyroid hormones influence the coagulation and fibrinolytic system mainly by the interaction between the hormone and its receptors^[9]. Various abnormalities have been described, ranging from subclinical laboratory abnormalities to patients with major haemorrhages or fatal thromboembolic events^[7].

However, in clinical practice the relationship between thyroid hormones and the coagulation system is often ignored. One of the reasons could be that, although several *in vivo* abnormalities have been reported in patients with hypothyroidism and hyperthyroidism, most published studies focus on laboratory measurements, and good studies on the relationship between thyroid dysfunction and clinically manifest bleeding or thrombosis are lacking. In addition, most previous studies have important methodological drawbacks, such as lack of a control group, small study size, heterogeneity of cause and severity of thyroid dysfunction, and different laboratory assays.

The limited evidence has stimulated us to start a research program to improve the knowledge on the relationship between endocrine disorders and haemostasis, starting from thyroid dysfunctions. To understand both the scientific and clinical relevance of this relationship, it is important to stress that both cardiovascular disease and venous thromboembolism are multi-causal diseases in which usually more than one genetic or environmental condition coincides to produce clinically apparent thrombosis^[10]. For this reason, also weak thromboembolic risk factors may be clinically relevant, especially if treatable such as endocrine disorders.

OUTLINE OF THE THESIS

The thesis starts with an overview of the literature. **Chapter 2, 3, 4 and 5** were performed with the purpose to generate well-founded hypotheses and to give direction for future basic and clinical research on this topic. **Chapter 2** is a brief overview of the literature on the relationship between endocrine disorders and haemostasis. In **Chapter 3** we systematically review the studies on the effects of thyroid dysfunction on coagulation and fibrinolysis, while in **Chapter 4** the current evidence on relationship between hypothyroidism and acquired von Willebrand syndrome is reviewed. In **Chapter 5** we give an overview of the literature on thyroid disease and cerebrovascular disease.

In **Chapter 6** the results of a pilot study on the presence of overt and subclinical thyroid disorders in patients with venous thrombosis are described. We subsequently measured thyroid hormone concentrations and antibodies in a larger group of patients with venous thrombosis and control persons (**Chapter 7**). In addition, in a large database in which hospital admission for pulmonary embolism and the use of medication was registered we analysed whether treatment for thyroid disorders was associated with pulmonary embolism (**Chapter 8**). The effect of hyperthyroidism on coagulation and fibrinolysis markers is described in a randomized controlled crossover trial in healthy volunteers in **Chapter 9** and, in particular, the effect of hypothyroidism and hyperthyroidism on thrombin-activatable fibrinolysis inhibitor in **Chapter 10**. A young woman who attempted suicide and took a very high dose levothyroxine gave us the opportunity to study the effect of this on coagulation and fibrinolysis (**Chapter 11**).

The second part of the thesis is dedicated to the interactions of thyroid disease with vitamin K antagonist. **Chapter 12** is an editorial in which we commented the available evidence and highlighted the lack of published studies, in particular on subclinical hyper- and hypothyroidism. In two small pilot studies, **Chapter 13 and 14**, we tested the effect of subclinical hypothyroidism on vitamin K antagonist stability and sensitivity and the effect of thyroid auto-antibodies on vitamin K warfarin stability. In **Chapter 15** we describe a patient with hyperthyroidism and recurrent deep venous thrombosis during optimal anticoagulation. **Chapter 11 and 15** are case reports from the Netherlands and Italy, respectively. Besides to support the clinical relevance of the relationship between thyroid disease and haemostasis, these chapters reinforce and ‘definitely demonstrate’ that the scientific relationship of our two countries can help ‘the haemostatic orchestra’ playing *ad libitum*.

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Chapter 2

The coagulation system in
endocrine disorders
A narrative review

Alessandro Squizzato, Victor EA Gerdes, Walter
Ageno, Harry R Büller

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ABSTRACT

Endocrine disorders can influence the haemostatic balance. Abnormal coagulation test results have been observed in patients with abnormal hormone levels. Also unprovoked bleeding or thrombotic events have been associated with endocrine disease. The aim of the present review is to summarise the available evidence on the influence of common endocrine disorders on the coagulation system, and their possible clinical implications. We focus on thyroid dysfunction, hyper- and hypocortisolism and growth hormone disturbances, while other endocrine disorders are only briefly discussed. In the published literature a clear bleeding diathesis has only been associated with overt hypothyroidism, mainly mediated by an acquired von Willebrand syndrome. A clinically relevant hypercoagulable state may be present in patients with hyperthyroidism, hypercortisolism or abnormal growth hormone levels, but adequate prospective clinical studies are lacking. Also effects of pheochromocytoma, hyperprolactinaemia and hyperaldosteronism on the coagulation system have been described. It is apparent that unprovoked bleeding and thrombotic episodes can be secondary to endocrine disorders.

INTRODUCTION

Haemostasis is a complex biological process that prevents individuals from experiencing excessive bleeding or thrombosis. Many factors are responsible for maintaining the haemostatic balance, and, among them, hormones directly influence both primary and secondary haemostasis^[1]. A wide variety of endocrine disorders have been associated with both mild abnormalities in laboratory coagulation tests, and clinical thrombotic or bleeding manifestations. In this overview of the literature, the influence of common endocrine disorders on the coagulation system and their possible clinical effects are discussed. Results will be highlighted for thyroid dysfunction, hyper- and hypocortisolism and growth hormone (GH) abnormalities, while other endocrine disorders are briefly mentioned. The influence of sex hormones, oral contraceptives and hormonal replacement therapy is well known and has been reviewed recently by others^[2–5].

THYROID DYSFUNCTION

The link between the haemostatic system and thyroid disease has been investigated since the beginning of the last century. Several mechanisms are involved at different levels^[6]. In particular both thyroid dysfunction and autoimmunity may modify physiological processes of primary and secondary haemostasis, and lead to bleeding or thrombosis. Idiopathic thrombocytopenic purpura, secondary antiphospholipid syndrome (APS) and acquired haemophilia are sometimes associated with autoimmune thyroid disorders^[7]. The influence on the coagulation system is mainly mediated by the interaction between thyroid hormone and its receptors^[8].

Hypothyroidism

A bleeding tendency is frequently observed in hypothyroid patients. Mild mucocutaneous bleeding (epistaxis, gum bleeding, menorrhagia, bruising), but also, rarely, severe post-traumatic and post-surgical bleeding may occur^[9]. Several coagulation abnormalities have been reported in patients with hypothyroidism^[9,10]. Case reports describe hypothyroid patients with a bleeding complication who had low levels of von Willebrand factor (VWF), compatible with a diagnosis of acquired von Willebrand's syndrome type I^[11,12]. Franchini and colleagues studied this association in detail^[13–15]. First, they screened 131 consecutive subjects with low VWF levels for thyroid hormone levels^[13]. Eight of the 131 individuals (6.1%) with low VWF levels had a concomitant subclinical hypothyroidism as documented by normal thyroid hormone levels and raised TSH concentrations. Three of them (37.5%) had bleeding symptoms. Thyroid hormone replacement therapy showed that haemostatic parameters had returned to normal in all patients. Second, they studied 1342 consecutive patients with various thyroid diseases who were candidates for thyroid surgery^[15]. A preoperative screening including prothrombin time, activated partial thromboplastin time and platelet function analyser (PFA-100) identified 39 patients (2.9%) with abnormalities. Of these, 35 had von Willebrand's disease (type 1 in 33 cases and type 2A in 2 cases). However, all these patients were euthyroid at the time of the screening. Other coagulation test abnormalities have been described in hypothyroid patients. Egeberg, and Simone and colleagues, show a significant reduction in coagulation factors

(F) VIII, IX and XI activity^[16:17]. Other studies report decreased plasma levels of FVII, FX and FXII^[9:18]. These findings are in contrast with the observation that hypothyroid patients who use warfarin require higher dosages to obtain the target International Normalised Ratio (INR) compared to euthyroid anticoagulated patients. In fact, a prolonged half-life of all vitamin K-dependent coagulant factors (FII, FVII, FIX and FX) is reported^[9]. Recent studies suggest that the bleeding tendency is present only in clinically overt hypothyroidism. Muller and colleagues show an increased factor VII activity in subclinical hypothyroid patients, suggesting a possible mild hypercoagulable state^[19]. Chadaverian and colleagues obtain similar results^[20:21]. They demonstrate also that a different pattern of fibrinolytic abnormalities according to the severity of thyroid dysfunction can be distinguished: in overt hypothyroidism, low levels of tissue plasminogen activator (t-PA), low concentrations of plasminogen activator inhibitor (PAI-1) and high D-dimer levels suggest increased fibrinolytic activity. Conversely, in subclinical hypothyroidism, high t-PA, high PAI-1 and low D-dimer indicates decreased fibrinolytic activity. When combined, these results suggest that overt hypothyroidism leads to lower levels of VWF and other coagulation factors, but at the same time an increased halflife of these factors. In addition, fibrinolytic activity may be increased, altogether leading to a bleeding tendency. Unfortunately, most published studies on the relation between the coagulation system and thyroid hormones have important methodological drawbacks. Lack of a control group, small study size, heterogeneity of cause and of severity of thyroid dysfunction, and different laboratory assays hamper definitive conclusions. In summary, hypothyroidism may lead to acquired von Willebrand syndrome type I and to hypocoagulability, with sometimes unexplained bleeding in these patients.

Hyperthyroidism

As in hypothyroidism, the heterogeneity of the published studies obscures the real *in vivo* effects of elevated thyroid hormones on the haemostatic system. However, several coagulation perturbations suggest a hypercoagulable state^[6]. An increased activity of plasma FVIII and FIX, and a reduced fibrinolytic activity (low t-PA levels) potentially increase the risk of thromboembolic events^[22:23]. Conversely to hypothyroid patients, an increased turnover of FII, FVII and FX increase the effect of vitamin K antagonists, reducing the mean warfarin dosage required to reach the target INR^[24]. Rogers and Shane conducted a small but important study^[25]. They administered oral levothyroxine, 0.6 mg daily for 14 days, to 14 volunteers; nine of them continued levothyroxine for an additional 3 days along with oral propranolol, 20 mg four times a day. Moreover, they administered oral L-triiodothyronine, 50 µg three times daily for 7 days, to nine healthy male volunteers; subsequently, the same volunteers received both L-triiodothyronine and oral propranolol; four volunteers received dextrothyroxine, 2 mg daily for 16 days. A significant increase in resting pulse and factor VIII-related activities (FVIII activity, FVIII-related antigen and ristocetin co-factor activity) was detected in patients who received levothyroxine and L-triiodothyronine. No significant effect was seen during oral dextrothyroxine administration. The addition of oral beta-adrenergic blockade significantly reduced or prevented the elevation in resting pulse but not the increase in factor VIII-related properties. Published case reports support the hypothesis that hyperthyroidism is associated with a hypercoagulable state. Sinus and cerebral vein thrombosis have been reported in clinically overt hyperthyroidism, in particular during thyroid storm^[26]. This association is

more than expected, as sinus and cerebral vein thrombosis are rare diseases (4 per million inhabitants per year)^[27]. Several inherited and acquired thrombotic risk factors are also present in these patients. Thus far, no studies with clinical outcomes have been published. Prospective studies are therefore necessary before routinely checking the thyroid function status in patients with thromboembolic disorders. Nevertheless, long-standing hyperthyroidism is, besides the increased factor VIII levels, associated with, sometimes, severe cardiomyopathy with or without atrial fibrillation, which is a risk situation for systemic emboli.

HYPER- AND HYPOCORTISOLISM

Endogenous hypercortisolism Cushing's syndrome is uncommon. The estimated annual incidence of pituitary-dependent Cushing's disease is 0.1–1 per 100 000 inhabitants per year^[28]. The estimation of the true incidence and risk of haemostatic disorders is therefore difficult. Nevertheless several venous thromboembolic events, including cerebral sinus thrombosis, have been described in association with endogenous hypercortisolism, suggesting a prothrombotic state in Cushing's syndrome^[29–31]. To test this hypothesis, some published studies explore the *in vivo* abnormalities of the coagulation and fibrinolytic systems. Obviously, the small number of enrolled patients limits the strength of the evidence. Several coagulation factors (FIX, FXI, FXII) have been found to be increased, but mainly the increase of FVIII and von Willebrand antigen (VWF-Ag) was marked^[32]. High levels of plasma thrombin–antithrombin complexes and of plasmin–antiplasmin complexes further support the hypothesis of coagulation cascade activation^[33]. A reduced fibrinolytic capacity as a result of increase in plasma PAI-1 activity has been observed in one study^[34], but not confirmed in another^[35]. Recently, a retrospective observational study analysed the incidence of postoperative thromboembolic events in a large group of patients with Cushing's syndrome not receiving (group 1: 75 patients) or receiving (group 2: 232 patients) prophylactic heparin and warfarin, evaluated in the periods 1972–1981 and 1982–2000, respectively^[36]. Patients of the group 1 underwent routine haemostatic function testing, i.e., prothrombin time and activated partial thromboplastin time. Patients of the group 2 underwent a thorough investigation as to haemostatic parameters. Patients with Cushing syndrome showed various abnormalities of haemostatic parameters (e.g., VWF antigen and activity, PAI-1 activity, fibrinogen). A significant correlation between activated partial thromboplastin time and urinary free cortisol was observed. During follow-up, 15 patients (20%; mean follow-up, 9.4 ± 6.4 years) from the group 1 and 14 (6.0%; mean follow-up, 6.6 ± 4.2 years) from group 2 had thromboembolic complications. Of these patients, eight in the group 1 and one in the group 2 died. Survival analysis demonstrates a significantly higher morbidity and mortality due to thromboembolic events in patients in the group 1 than in the group 2, who were treated with anticoagulants in the perioperative period until cure of the disease and normalisation of clotting parameters. In conclusion, even if based on modestly strong evidence, in Cushing's syndrome whenever a condition of increased risk of venous thromboembolism (VTE) occurs, an adequate thromboembolic risk assessment should be performed to prescribe the best prophylaxis strategy. Conversely, physicians who treat thromboembolic disorder should not be surprised to find typical Cushing's stigmata (hypokalaemic hypertension, round facies, central obesity and depression) in a patient with VTE.

Exogenous hypercortisolism

Glucocorticoid therapy is extremely common for a wide range of inflammatory, autoimmune and neoplastic disorders. It is therefore important to correctly assess the risk/benefit of steroids. Clinical experience suggests that, considering the wide utilisation of glucocorticoids, the risk of venous thromboembolic events during steroid therapy is marginal^[37]. The effects of iatrogenic hypercortisolism on the haemostatic balance are probably often unappreciated. In fact, similar to endogenous hypercortisolism, administration of glucocorticoids modifies the coagulation balance towards a hypercoagulable state, increasing plasma FVIII and VWF levels^[38-40]. This prothrombotic state is probably counterbalanced by other mechanisms, such as the inhibition of platelet aggregation or the reduced tissue factor-mediated leucocyte procoagulant activity^[41;42]. Nonetheless, different types of venous thrombotic events have been observed in patients on steroid therapy: deep venous thrombosis, superficial vein thrombosis, pulmonary embolism, cerebral sinus thrombosis and also kidney intragraft thrombosis^[43-45]. Recently, Brotman and colleagues performed an *in vivo* controlled study to explore the effect of glucocorticoid on the coagulation system^[46;47]. They randomised 24 healthy men to receive either dexamethasone 3 mg twice daily vs. placebo for 5 days. Subjects were advised to maintain their usual sleep-wake schedule, exercise and dietary habits during the study. The mean ages were 23.3 years in the dexamethasone group and 24.9 in the placebo group. Fasting morning blood samples were drawn before and after the intervention for the following parameters: clotting factors VII, VIII and XI, VWF, D-dimer, PAI-1, soluble CD40-ligand and fibrinogen. Treatment with dexamethasone modestly increased clotting factors levels and fibrinogen without significantly affecting PAI-1, D-dimer or sCD40-ligand. Factor VII increased by a mean of 13% ($P=0.04$), factor VIII by 27% ($P<0.01$), factor XI by 6% ($P=0.01$) and fibrinogen by 13% ($p=0.05$).

Hypocortisolism

Adrenal insufficiency is not commonly complicated by bleeding or thrombotic episodes. Although a possible bleeding diathesis has been suggested in a patient who developed a spontaneous intracerebral haemorrhage, an extensive search of the literature reveals that no relevant *in vitro* or *in vivo* studies have been published^[48]. Nevertheless, an association with the APS has been described^[49;50]. Acute adrenal failure may be the first clinical manifestation of APS, whereas a few patients may have a history of Addison's disease. Adrenal failure is present in 10%–26% of patients with catastrophic APS^[51]. The pathophysiologic mechanism is not clearly understood, but an adrenal vein thrombosis could provoke an adrenal haemorrhagic infarction^[51]. APS should be considered as a possible cause of Addison's disease when the aetiology is unclear.

GROWTH HORMONE (GH) EXCESS AND DEFICIENCY IN ADULTS

GH is produced by the anterior pituitary, and acts by binding to receptors on liver and other cells leading to the initiation of a cascade of events that results in the secretion of insulin-like growth factor I (IGF-I), which mediates many of the biologic actions of GH^[52]. Abnormal GH levels are relatively rare (e.g., the incidence of GH-secreting adenomas is 3–4 cases per million each year)^[53].

For this reason few small studies are available on the possible interaction between abnormal GH, IGF-1 and the coagulation system. They mainly explore the fibrinolytic system, as an increased cardiovascular and cerebrovascular mortality risk has been associated with both GH-related pituitary dysfunctions^[54;55]. Twenty-three patients with active acromegaly (14 women and 9 men, mean age 49.8 years) were compared to a sex-, body mass index- and age-matched control group. An increased PAI-1 activity and t-PA concentration was reported in 30% and 66% of patients, respectively^[56]. There was a correlation between human GH and PAI-1 ($r=0.59$) and between IGF-1 and t-PA activity ($r=0.44$). High fibrinogen levels (4.0 g/dl) were decreased to 3.2 g/dl following treatment of acromegaly^[57]. GH-deficient patients seem to also have increased fibrinogen levels and PAI-1 activity. Seventeen patients with adult-onset GH deficiency were studied during two years of treatment with recombinant human GH (12 µg/kg body weight/day)^[58]. PAI-1 antigen and activity, t-PA antigen levels and α -2 antiplasmin decreased during replacement therapy ($p<0.05$). In contrast, FVII, FVIII and antithrombin were not modified^[58]. Even though apparently unprovoked thromboses have been described, the relation between GH, IGF-I and the coagulation system remains to be clarified^[59; 60].

OTHER ENDOCRINE DISORDERS

The aetiology of pathological hyperprolactinaemia is diverse: any process interfering with dopamine synthesis, its transport to the pituitary gland or its action on lactotroph dopamine receptors can lead to hyperprolactinaemia^[53]. Once drugs are excluded, prolactinomas are the most common causes of hyperprolactinaemia. In several conditions such as pregnancy, and oestrogen and antipsychotic therapy, hyperprolactinaemia and an increased risk of VTE co-exist. A direct effect of prolactin on the coagulation system has not been systematically investigated, but Wallaschofski and colleagues demonstrate that hyperprolactinaemia is a potent co-stimulator of platelet aggregation^[61]. In a subsequent study the same group shows that patients with VTE have significantly increased plasma prolactin levels in comparison to healthy subjects^[62]. The mean values of prolactin were 185 ± 60 and 165 ± 50 mU/l in 100 healthy controls and 96 patients with congenital risk for VTE, respectively. In contrast, in 98 patients with VTE but without congenital or acquired risk factors, the mean prolactin value was 285 ± 351 mU/l ($P<0.01$). Likewise, they found 6 patients with documented VTE in their series of 136 prolactinoma patients^[62]. The hypothesis that an overactivity of the sympathetic nervous system and increased catecholamine levels may have an influence on haemostasis (platelets, coagulation and fibrinolytic factors, and endogenous anticoagulants) is actually an old topic^[63]. Since the beginning of the last century investigators observed that parenteral administration of adrenaline hastened blood coagulation in animals and in man^[64-66]. As recently reviewed, adrenergic infusions increase FV activity, FVIII activity, VWF antigen, t-PA antigen and plasmin- α 2-antiplasmin complexes, mainly mediated by endothelial β -adrenoceptors (most likely β 2-receptors), leading to an activation of both the coagulation and fibrinolytic system^[63;67]. However, only a few published case reports report an association between pheochromocytoma and venous thrombosis, in which other mechanisms have been claimed, such as paraneoplastic polycythaemia and vascular compression^[68-70]. These apparently conflicting data could be explained by considering overactivity of the sympathetic nervous system as a physiological mechanism to protect the organism from deleterious bleeding in a 'fight or flight' situation^[63]. Recently, β -receptor blocker propranolol was shown to decrease FVIII:C levels in patients with

venous thrombosis and elevated fVIII:C^[71]. Two cohorts of patients with documented deep venous thrombosis and elevated fVIII:C (>175 IU/dl) and healthy volunteers were studied. One of the former and healthy volunteers received propranolol, 40 mg thrice daily, for 14 days. The mean baseline level of FVIII:C was 220 and 102 IU/dl. A significant 23% reduction of fVIII:C was shown, with a return to initial high levels after propranolol discontinuation. No effect was seen in healthy volunteers. Recent evidence suggests that the renin-angiotensinaldosterone system (RAAS) may participate in the regulation of fibrinolytic activity. Angiotensin II, and probably aldosterone, stimulates PAI-1 expression, whereas angiotensin-converting-enzyme inhibition decreases PAI-1 both *in vitro* and *in vivo*^[72-74]. Primary aldosteronism is usually secondary to Conn's adenoma or bilateral adrenal hyperplasia. Prevalence estimates vary from 0.5% to 2% of the hypertensive population^[75]. Low plasma renin is present, concomitant with elevated aldosterone levels. The effect of primary aldosteronism on the coagulation system has not been systematically investigated. An association with VTE has not been claimed. However, an increased risk of cardiovascular events has recently been shown in patients with primary aldosteronism in comparison to patients with essential hypertension^[76]. Overall, there are indications that hyperprolactinaemia, pheochromocytoma and primary aldosteronism may influence elements of the haemostatic balance, however, there is no clear evidence available to suggest that this is clinically relevant.

CONCLUSIONS

The main findings on the relationship between endocrine disorders and the coagulation system are summarised in Table 1. As we state in the table, the only demonstrated clinically relevant effect of endocrine disorders on the coagulation system is the acquired von Willebrand's syndrome type I in overt hypothyroidism. Laboratory studies confirm that low levels of VWF are responsible for bleeding episodes that frequently occur in these patients. In Table 2, we summarise the main published study for each endocrine disorder. As it is not a systematic review of the literature, we report the best available evidence.

Table I. Overall effect on the coagulation system in common endocrine disorders

Endocrine disorder	Coagulation imbalance	Certain clinical effect
Hyperthyroidism		
- Overt	Probable mild hypercoagulable state	
- Subclinical	Possible mild hypercoagulable state	
Hypothyroidism		
- Overt	Certain mild/moderate bleeding diathesis	Acquired von Willebrand syndrome type I
- Subclinical	Possible mild hypercoagulable state	
Hypercortisolim		
- Endogenous	Possible moderate hypercoagulable state	
- Exogenous	Probable mild hypercoagulable state	
Abnormal GH levels	Possible mild hypercoagulable state	

Table II. Main relevant published studies

Endocrine disorder	Type	Population	Main results	Ref.
<i>Hypothyroidism</i>				
	Observational	131 consecutive individuals with low VWF	6.1% had subclinical hypothyroidism VWF returned to normal after thyroid hormone replacement therapy	[13]
<i>Hyperthyroidism</i>				
	Drug interventional (L-thyroxine, 600 µg/day, and L-triiodothyronine, 50 µg x 3/day)	14 and 9 healthy volunteers	Increased in fVIII:C, VWF: antigen e activity	[25]
<i>Hypercortisolism</i>				
Endogenous	Observational, retrospective	Cushing's syndrome not (group 1) or receiving (group 2) prophylactic perioperative heparin and/or warfarin	Group 1: 20% thromboembolic episodes (mean follow-up: 9.4 years) Group 2: 6% thromboembolic episodes (mean follow-up: 6.6 years) Group 1: increased VWF antigen and activity, PAI-1 activity, fibrinogen	[36]
Exogenous	RCT (3 mg dexamethasone twice daily for 5 days vs placebo)	Healthy man volunteers	Increased factor VII, VIII, XI.	[46]
<i>Abnormal GH levels</i>				
Acromegaly	Case-control	23 patients with active acromegaly compared to sex, BMI, age-matched control group	Correlation between human GH and PAI-1 ($r=-0.59$) and IGF-1 and t-PA activity ($r=-0.44$).	[58]
GH deficiency in adults	Drug interventional (recombinant human GH 12 µg/Kg body weight/day)	17 patients with adult-onset GH deficiency during two years of treatment	During replacement therapy, decreased: PAI-1 antigen and activity t-PA antigen α-2 antiplasmin Not modified: fVII, fVIII, antithrombin	[58]
<i>Hyperprolactinaemia</i>				
	Case-control	Patients with VTE (96 with congenital risk factor, 98 without risk factor) and 100 healthy subjects	Mean value prolactin: VTE with risk f.: 165 ± 50 mU/l VTE without: 285 ± 351 mU/l Healthy subjects: 185 ± 60 mU/l	[62]

Based on these data and the briefly described others, we can undoubtedly conclude that hormones influence the haemostatic system, as each described hormone modifies several plasma coagulation and fibrinolysis markers. Several papers on the relation between the haemostatic system and endocrine dysfunction have important methodological drawbacks that do not allow definitive conclusions. Well-designed clinical studies are therefore necessary for a better definition of the interaction between hormones and the haemostatic system, and, in particular, to assess the clinical relevance of coagulation abnormalities. Therefore future studies should prospectively evaluate both *in vivo* and *in vitro* effects, exploring the molecular mechanism of interaction between each hormone and the synthesis, secretion and clearance of coagulation and fibrinolytic factors. In particular, *in vitro* studies will allow the definition of the level at which the hormones physiologically regulate the coagulation cascade. Moreover, clinical studies should prospectively evaluate the effect of a well-conducted treatment of each endocrine disorder on individual thromboembolic or bleeding risk, and evaluate the clinical relevance of introducing hormone screening tests in patients with a venous thromboembolic episode. Nowadays, VTE is best understood as a 'multi-causal' disease in which more than one genetic or environmental condition coincide to produce clinically apparent thrombosis[77]. For this reason, also combinations of weak thromboembolic risk factors could imbalance the coagulation system and provoke thrombotic episodes. Endocrine disorders could be clinically relevant for this reason. If further evidence becomes available, some endocrine disorders will need to be included in the checklist of venous thromboembolic risk factors. In conclusion, endocrine disorders are involved in the pathogenesis of apparently unprovoked bleeding or thrombotic episodes.

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Part 1

*Thyroid, coagulation and fibrinolysis,
and venous thromboembolism*

Chapter 3

Thyroid dysfunction and effects
on coagulation and fibrinolysis
a systematic review

Alessandro Squizzato, Erica Roumadi,
Harry R Büller, Victor EA Gerdes

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ABSTRACT

Introduction: Various changes in the coagulation-fibrinolytic system have been described in patients with an excess or deficiency of thyroid hormones. The purpose of this systematic review is to summarize the effects of hyperthyroidism and hypothyroidism on these systems.

Methods: All published case-control or interventional cohort studies that evaluated the effects of hyperthyroidism and hypothyroidism on the coagulation-fibrinolytic system *in vivo* were identified by a computer-assisted search of the MEDLINE and EMBASE electronic databases. A scoring system was used to divide studies into three quality categories: high, medium, and low quality.

Results: A total of 36 papers were included. Because in several papers more than one case-control study or both a case-control and intervention study were described, a total of 39 case-control studies and 24 interventional cohort studies were analyzed. No high quality study was identified. Three (7.7%) case-control and eight (33.3%) cohort studies were of medium quality. A total of 19 tests were investigated in the medium-quality studies. These tests revealed a hypocoagulable state for overt hypothyroidism and a hypercoagulable state for overt hyperthyroidism.

Conclusion: This analysis confirmed that clinically overt hyperthyroidism and hypothyroidism modify the coagulation-fibrinolytic balance, indicating that thyroid hormone excess or deficit is the probable main pathophysiological mechanism. Patients with overt hypothyroidism and overt hyperthyroidism appear to have an increased risk of bleeding and of thrombosis, respectively.

INTRODUCTION

The link between the hemostatic system and thyroid diseases has been known since the beginning of the past century. The first clinical association was described in 1913, when Kaliebe reported an episode of cerebral vein thrombosis in a thyrotoxic patient^[1]. Several elements of the process of thrombus formation may be involved^[2]. Both thyroid dysfunction and autoimmunity may modify physiological processes of primary and secondary hemostasis and lead to bleeding or thrombosis. Idiopathic thrombocytopenic purpura, secondary antiphospholipid syndrome, or acquired hemophilia have been associated in individual cases with autoimmune thyroid disorders^[3]. The influence of thyroid hormone on the coagulation-fibrinolytic system is mainly mediated by the interaction between the hormone and its receptors^[4]. Various abnormalities have been described, ranging from subclinical laboratory abnormalities to major hemorrhages or fatal thromboembolic events. The relationship between thyroid hormones and the coagulation system is, however, often ignored. One of the reasons could be that, although several *in vivo* abnormalities have been reported in patients with hypothyroidism and hyperthyroidism, most published studies focus on laboratory measurements, and good studies on the relationship between thyroid dysfunction and clinically manifest bleeding or thrombosis are lacking. In addition, a large number of studies have important methodological drawbacks, such as lack of a control group, small study size, heterogeneity of cause and severity of thyroid dysfunction, and different laboratory assays. Therefore, the purpose of this review is to summarize systematically the effects of excess or deficiency of thyroid hormone on the coagulation-fibrinolytic system *in vivo*, to generate well-founded hypotheses, and to give direction for future basic and clinical research on this topic.

PATIENTS AND METHODS

Study identification

A computer-assisted search of the MEDLINE and EMBASE electronic databases up to July 2006 was performed to identify published studies that evaluated the effect of hyperthyroidism and hypothyroidism on the coagulation-fibrinolytic system. The following search terms (medical subject heading terms and text words) were used for the MEDLINE search: hemostasis, blood coagulation tests, blood coagulation, blood coagulation factors, blood coagulation disorders, thyroid diseases, thyroid hormones, receptors thyroid hormone, hyperthyroidism, hypothyroidism. These terms were used for the EMBASE database search: hemostasis, blood clotting, blood clotting test, blood clotting factor, blood clotting disorders, thyroid disease, thyroid hormone, thyroid hormone receptor, hyperthyroidism, hypothyroidism. Reference lists of all included studies were manually searched for other potentially eligible studies.

Inclusion criteria

Two investigators (A.S. and E.R.) independently performed the study selection. Main inclusion criterion was that the study has to evaluate the effect of dysfunctional thyroid diseases, hypothyroidism and hyperthyroidism, overt and/or subclinical, on the coagulation-fibrinolytic

system *in vivo*. The following study designs were allowed: 1) case-control study or 2) cohort study in which an intervention was performed to correct hyperthyroidism or hypothyroidism and in which laboratory tests were performed before and after treatment. Case reports, case series, reviews, editorials, *in vitro*, and non-human studies were excluded. Moreover, studies on cancer patients (cancer itself influences the coagulation system) and studies without statistical analysis were excluded. Studies in which thyroid functional status was not determined by thyroid hormone measurement were also excluded. For this reason, we excluded studies that were published before 1980^[5]. No language restrictions were initially applied to the search strategy, but only papers written in English, French, Spanish, German, Dutch, and Italian were evaluated. The two investigators independently reviewed titles and/or abstracts from the initial search to determine whether the inclusion criteria were satisfied. The full text of the study was obtained when an article could not be excluded with certainty. Decisions regarding inclusion were made separately, results were compared, and any disagreement was resolved by discussion. When multiple papers for a single study had been published, it was decided to use the latest publication and to supplement it, if necessary, with data from the earlier publications.

Quality assessment

The same two investigators independently completed the assessment of study quality. Disagreement was resolved by consensus and by the opinion of a third reviewer, if necessary. Although the use of quality scoring systems or quality scales in observational studies is controversial^[6], the Newcastle-Ottawa Scale for assessing quality of non randomized studies was adapted for use^[7]. This assesses the three broad areas of selection, comparability, and outcome or exposure, for cohort or case control studies, respectively. For summarizing study validity, we adapted a simple Cochrane Collaboration approach for interventional studies^[8]. Three categories were therefore identified: high quality (low risk of bias), medium quality (moderate risk of bias), or low quality (high risk of bias). The main items for case-control studies and their scoring were as follows: 1) definition of type of thyroid dysfunction (1 point was given if degree of thyroid dysfunction and underlying cause were registered and if the interval between thyroid hormone measurement and study enrolment was <48 h); 2) selection of patients (1 point was given if they were consecutive or clearly a representative series of cases); 3) definition of control group (1 point was given if thyroid hormones were measured or if an explicit statement of no history of thyroid disease was reported); 4) selection of control group (1 point was given if it was a community control group); and 5) comparability on the basis of the design or analysis (1 point was given if controls were age and sex matched or if there was an adequate adjustment for age and sex in the statistical analysis). The main items for the cohort studies in which thyroid dysfunction was corrected and their scoring were as follows: 1) definition of type of thyroid dysfunction (1 point was given if degree of thyroid dysfunction and underlying cause were registered and if the interval between thyroid hormone measurement and study enrolment was <48 h); 2) selection of patients (1 point was given if they were consecutive or clearly a representative series of cases); 3) definition of euthyroid state after therapy (1 point was given if thyroid hormone measurement was repeated to define the euthyroid state; 2 or 1 points were given if 100% or >90% of patients were euthyroid, respectively, and interval between assessment of euthyroid state and coagulation

test measurement was <48 h). The scoring system defined the three quality categories as follows: a total of 5 points was for high-quality study; 4 points for a medium quality study; and 3 or less for a low-quality one. Three was used as cut-off value for low quality, because, in these studies, the definition and selection of the populations, either cases or controls, or both, were believed to be inaccurate. The quality assessment form is available on request. No attempts to mask for authorship, journal name, or institution were made.

Data extraction

The same two reviewers independently completed the data extraction form. Disagreement was resolved by consensus and by the opinion of a third reviewer, if necessary. The following characteristics were collected: 1) investigated thyroid dysfunction (hypothyroidism and/or hyperthyroidism, clinically overt and/or subclinical); 2) total number of enrolled cases and controls; 3) performed coagulation-fibrinolytic tests; 4) statistically decreased, increased, or no statistically difference of each test of cases vs. controls or before vs. after treatment.

Statistical analysis

Data for qualitative variables were presented as incidence rates (N, number, and percentage).

RESULTS

The initial search strategy identified 4895 papers. A total of 158 publications were considered potentially eligible based on the title and/or abstract. Of these, 41 were excluded for language, 64 were written before 1980, and four were not available. We retrieved full copies of 49 potentially appropriate studies. After excluding 13 articles not meeting the pre-specified inclusion criteria^[9–21], a total of 36 were included in the final analysis^[22–57]. As summarized in Tables 1 and 2, several studies were designed as both a case-control and intervention study and explored different types of thyroid dysfunction. A total of 39 case-control studies and 24 interventional cohort studies were analyzed. If the authors clearly defined that the enrolled patients had a subclinical degree of thyroid dysfunction, in the column “Type of dysfunction,” we reported “subclinical”; otherwise, if the authors declared that they enrolled hypothyroid or hyperthyroid patients or they specifically declared that they enrolled only overt hypothyroid or hyperthyroid patients, we reported “hypothyroidism” or “hyperthyroidism.” No high-quality study was identified. Three (7.7%) case-control and eight (33.3%) cohort studies were of medium quality. Table 3 summarizes the findings regarding the various elements of “Definition of thyroid dysfunction.” Only a few studies enrolled an optimal thyroid dysfunction population, mainly because most studies included a heterogeneous patient group with several underlying causes of thyroid dysfunction (autoimmune, postsurgical, *etc.*). Moreover, some authors did not enroll patients with the same degree of thyroid dysfunction (as reported above, some studies with overt hypothyroid or hyperthyroid patients included also patients with subclinical disease) and did not measure thyroid hormones at the same time of coagulation-fibrinolytic measurements. Few authors did not report the type of tested thyroid hormones, *i.e.* total or freeT4, total or freeT3, and TSH, on which they based laboratory diagnosis of thyroid dysfunctional disease. Table 4 summarizes the data on coagulation and fibrinolytic tests of

Table 1. Individual study quality assessment of case-control study design

First author		Patients definition	Patients selection	Controls definition	Controls selection	Sex-age com- parability	Score	Quality	Type of dysfunction	Ref.
Arnout	(A)	0	0	0	0	0	0	Low	hypo	[22]
	(B)	1	0	0	0	0	1	Low	hyper	
Burggraaf		1	0	1	0	1	3	Low	hyper	[23]
Canturk		0	0	1	1	1	3	Low	sub. hypo	[24]
Chaderevian		0	1	1	1	0	3	Low	hypo	[26]
Chaderevian		0	0	1	1	0	2	Low	hypo	[25]
Coban		1	0	1	1	1	4	Medium	sub. hyper	[28]
Dorr	(A)	0	1	1	1	1	4	Medium	hypo	[29]
	(B)	0	1	1	1	1	4	Medium	hyper	
	(C)	0	1	1	1	1	4	Medium	sub. hyper	
Erem		0	0	0	0	0	0	low	hypo	[32]
Erem		0	0	0	0	1	1	Low	hyper	[31]
Erem	(A)	0	0	1	0	1	2	Low	sub. hypo	[30]
	(B)	0	0	1	0	1	2	Low	sub. hyper	
Graninger		0	0	0	0	1	1	Low	hyper	[34]
Guldiken		0	0	1	1	0	2	Low	sub. hypo	[35]
Gullu	(A)	1	0	1	0	0	2	low	Hypo	[36]
	(B)	1	0	1	0	0	2	low	sub. Hypo	
Gursoy	(A)	0	0	1	0	1	2	low	hypo	[37]
	(B)	0	0	1	0	1	2	low	sub. hypo	
Jorde		0	0	1	1	1	3	low	sub. hypo	[38]
Kventy		0	1	1	1	0	3	low	sub. hypo	[39]
Li		1	0	0	0	0	1	low	hyper	[41]
Liu		0	1	1	0	0	2	low	hyper	[42]

First author	Patients definition	Patients selection	Controls definition	Controls selection	Sex-age com- parability	Score	Quality	Type of dysfunction	Ref.
Marongiu	0	0	0	0	1	1	low	hyper	[43]
Marongiu	0	0	0	0	1	1	low	hypo	[46]
Marongiu	0	0	0	0	1	1	low	hyper	[45]
Marongiu	0	0	0	0	0	0	low	hyper	[44]
Myrup (A)	0	1	0	0	0	1	low	hypo	[49]
(B)	0	1	0	0	0	1	low	hyper	
Morishita	0	0	1	0	0	1	low	hyper	[47]
Muller	0	0	1	0	0	1	low	sub. hypo	[48]
Ozcan (A)	0	0	1	0	1	2	low	hypo	[52]
(B)	0	0	1	0	1	2	low	hyper	
(C)	0	0	1	0	1	2	low	sub. hypo	
Rogers II	0	0	0	0	0	0	low	hyper	[54]
Rosc	0	0	1	0	0	1	low	hyper	[55]
Wahrenberg	1	0	0	0	0	1	low	hyper	[56]
Yong	0	0	1	0	0	0	low	hypo	[57]
Total (%)	7 (17.9)	8 (20.5)	24 (61.5)	11 (28.2)	19 (48.7)				

Ref, references; hypo, hypothyroidism; hyper, hyperthyroidism; sub, subclinical

Table 2. Individual study quality assessment of interventional cohort study design

First author	Patients definition	Patients selection	All pts euthyroid at control	>90% euthyroid at control	Hormone measurement for euthy.	Quality score	Study quality	Type of dysfunction	Ref.
Arnaout (A)	0	0	0	0	1	1	low	hypo	[22]
(B)	1	0	0	0	1	2	low	hyper	
Burggraaf	1	0	2	0	1	4	medium	hyper	[23]
Canturk	0	0	2	0	1	3	low	sub. hypo	[24]
Chadarevian	0	1	0	0	1	2	low	hypo	[27]
Erfurth	0	0	0	0	1	1	low	hypo	[33]
Gullu (A)	1	0	2	0	1	4	medium	hypo	[36]
(B)	1	0	2	0	1	4	medium	sub. hypo	
Levesque	0	0	0	0	1	1	low	hypo	[40]
Li	1	0	2	0	1	4	medium	hyper	[41]
Liu	0	1	2	0	1	4	medium	hyper	[42]
Marongiu	0	0	0	0	0	0	low	hyper	[45]
Morishita	0	0	0	0	1	1	low	hyper	[47]
Myrup (A)	0	1	2	0	1	4	medium	hypo	[49]
(B)	0	1	2	0	1	4	medium	hyper	
Nagasaki	0	1	2	0	1	4	medium	hypo	[50]
Nagasaki	0	1	2	0	1	4	medium	hypo	[51]
Ozcan (A)	0	0	0	0	0	0	low	hypo	[52]
(B)	0	0	0	0	0	0	low	hyper	
(C)	0	0	0	0	0	0	low	sub. hypo	
Palareti	0	0	0	0	0	0	low	hypo	[53]
Rogers II (A)	0	0	0	0	1	0	low	hypo	[54]
(B)	0	0	0	0	1	0	low	hyper	
Wahrenberg	1	0	0	0	1	2	low	hyper	[56]
Total (%)	6 (25)	6 (25)	10 (41.7)	0	19 (79.2)				

Pts indicates patients; euthy, euthyroidism; ref, references; hypo, hypothyroidism; hyper, hyperthyroidism; and sub, subclinical

Table 3. Study quality assessment on ‘Definition of dysfunctional thyroid patients’: fulfilled item and sub-items

	Case control (39)	Cohort (24)
<i>Patients’ definition</i>	7 (17.9%)	6 (25.0%)
<i>Reporting of:</i>		
- measured thyroid hormones’ assay	37 (94.9%)	21 (87.5%)
- degree of thyroid dysfunction	31 (79.5%)	16 (66.7%)
- underlying cause	13 (33.3%)	10 (41.7%)
- brief interval (<48 h) between thyroid hormone measurement and study enrollment	26 (66.7%)	16 (66.7%)

the medium-quality studies. Supplemental Table 1 (published as supplemental data on The Endocrine Society’s Journals Online website at <http://jcem.endojournals.org>) summarizes the coagulation and fibrinolytic effect for each thyroid dysfunction in all quality studies; also poor-quality ones can be provided. The activated partial thromboplastin time, prothrombin time, and clotting time are general coagulation tests that evaluate time necessary to clot *in vitro*, whereas the bleeding time is a test in which the interaction between platelets and the vessel wall is reflected *in vivo*. Therefore, a prolongation of these tests means a reduced hemostatic response and a bleeding tendency. Factor VIII and von Willebrand factors are procoagulant factors. A decreased plasma level or activity indicates a higher chance of bleeding, especially when levels are markedly reduced. The fibrinolytic system is important to counterbalance the coagulation cascade. In general, increased fibrinolysis, for instance, by fibrinolysis-stimulating factors or by reduced antifibrinolytic factors, will result in a increased chance of bleeding. An increase of inhibitory factors, especially plasminogen activator inhibitor 1 level, has been associated with an increased risk of thrombotic complications^[58].

In summary, the available evidence revealed four important changes: 1) thyroid dysfunction modifies the balance between coagulation and fibrinolysis; 2) coagulation tests revealed a hypocoagulable state for overt hypothyroidism and a hypercoagulable state for overt hyperthyroidism; 3) the hypothesis of a hypercoagulable state in subclinical hypothyroidism is not supported; and 4) few coagulation-fibrinolytic test abnormalities have been described in subclinical hyperthyroidism and hypothyroidism.

DISCUSSION

Many factors are responsible for maintaining the hemostatic balance, and, among them, hormones directly influence both primary and secondary hemostasis^[59]. In particular, a bleeding tendency is often observed in hypothyroid patients, and a possible increased risk of sinus and cerebral vein thrombosis has been reported in clinically overt hyperthyroidism^[1]. Several *in vivo* studies have been performed to elucidate the pathophysiology of bleeding and thrombotic events in

overt thyroid dysfunction. Recent research focused on subclinical disorders. We conducted this systematic review to better define which hypotheses on the relationship between dysfunctional thyroid disease and the coagulation-fibrinolytic system are well founded. For this purpose, we selected the better quality publications. Two arbitrary criteria were introduced for study selection and quality assessment. First, we excluded studies that were published before 1980. In 1963, Condliffe purified TSH, and, soon thereafter, Odell and Utiger both reported the first immunoassays for human TSH^[5]. In 1971, Mayberry and Hershman simultaneously described the TSH immunoassays for the diagnosis of hypothyroidism^[5]. Second, use of quality scoring in metaanalyses for observational studies is controversial^[6,60]. For this reason, we adapted the Newcastle-Ottawa Scale to identify main biases in case and control selection and comparability and a simple Cochrane Collaboration approach for summarizing study validity^[8]. However, no high-quality case-control or interventional cohort studies could be identified. Study quality assessment elucidated a number of methodological weaknesses in the published thyroid-coagulation studies.

Additional information about the influence of thyroid hormones on coagulation comes from two intervention studies performed in healthy volunteers^[9,34]. Unfortunately only a few tests were performed during administration of oral thyroid hormones in these studies, but a clear increase of factor VIII, von Willebrand antigen and activity, and tissue plasminogen activator antigen was observed. However, these studies only revealed the effect of relatively short period, 2 wk, of hyperthyroidism.

Given the large amount of studies performed on this topic, future studies should be of high quality to provide more definitive data. They should enrol consecutive patients with a well-defined degree of dysfunction (overt or subclinical), based on thyroid hormone measurement (TSH, free T4, and free T3), with the same underlying cause (autoimmune, postsurgical, *etc.*); blood for coagulation-fibrinolytic tests and thyroid hormone measurements should be drawn the same day, no more than 48 h after starting drug therapy; the control group should be of the same community as cases, should have no history of thyroid disease, and thyroid hormones should be measured; in case of an interventional cohort study, tests should be repeated when all patients have reached an euthyroid state, defined by hormones measurements; and sex- and age-matched control group would be the optimal, but any possible confounder may be easily adjusted for in the analysis (interventional cohort study has an intrinsic perfect comparability).

Four main conclusions can be drawn from the summarized coagulation-fibrinolysis results. First, thyroid dysfunctional diseases, hypothyroidism and hyperthyroidism, overt and subclinical, alter the coagulation-fibrinolytic balance. The overall coagulation and fibrinolytic effect in medium-quality studies is shown in Table 4. Hypothyroidism and hyperthyroidism modify each group of coagulation-fibrinolytic tests. The effect is consistent across the three groups and supports an increased risk of bleeding in hypothyroidism and an increased risk of thrombosis in hyperthyroidism. Even if some results are discordant, medium-quality studies may have a moderate risk of bias. No definitive data are available to assess the degree of the hypocoagulable and hypercoagulable state in overt hypothyroidism and hyperthyroidism, respectively. Published case reports suggest a clinical relevance, but prospective clinical studies are absolutely lacking. Second, clinically overt hypothyroidism and hyperthyroidism modify the hemostatic balance

Table 4. Overall coagulation and fibrinolytic changes in medium quality studies

	N° of studies	Hypo	Hyper	Sub.Hypo	Sub.Hyper
<i>General hemostatic tests</i>					
Bleeding time	4	↑	↓	=	
aPTT	2	↑		=	
PT	2	↑		=	
Clotting time	2	↑		=	
Prothrombin fragment 1-2	1		=		
<i>Coagulation tests</i>					
FVIII:C	4	↓ or =		↓	
VWF:Ag	6	↓	↑ or =		
VWF:C	2	↓			↑
VWF II:Ag	1		↑		
VWF ristocetin	2	↓		↓	
Fibrinogen	6	↓ or =	↑ or =		↑
Ristocetin agglutination	2	↓	=		
<i>Fibrinolytic tests</i>					
t-PA:Ag	2		↑ or =		
t-PA:C	1		=		
PAI-1	2		↑ or =		
Plasminogen	1		↓		
α 2 antiplasmin	1		↑		
Plasmin-antiplasmin complex	1		=		

aPTT indicates activated partial thromboplastin time; PT, prothrombin time; F, factor; C, activity, Ag, antigen; VWF, von Willebrand factor; t-PA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1; ↑, increased; ↓, decreased; and =, non statistical significant difference

in opposite directions. This supports the assumption that thyroid hormone excess and deficit are the main mechanisms of a hypercoagulable and hypocoagulable state, respectively. The complex hemostatic balance can be influenced by autoimmune mechanisms, such as idiopathic thrombocytopenic purpura, secondary antiphospholipid syndrome, or acquired hemophilia, but these occur rarely. The hypercoagulable and hypocoagulable states are probably independent of the underlying pathophysiology of thyroid disease. In the past, other hypotheses have been postulated to explain coagulation abnormalities in thyroid patients, such as endogenous arginine vasopressin and adrenergic system imbalance, but these have never been proven^[9;61]. Third, the concept of a hypercoagulable state in subclinical hypothyroidism cannot be supported by the present analysis. A possible increased risk of myocardial infarction in patients with subclinical hypothyroidism suggested a prothrombotic effect^[25;26]. Some of the studies in which this was suggested were also included in our review, but they were of low quality^[25;26]. Moreover, the role of the coagulation-fibrinolytic system in the pathophysiology of atherosclerosis and arterial thrombosis is still a matter of debate^[62;63]. Fourth, few coagulation test abnormalities have been described in subclinical hyperthyroidism and hypothyroidism. Only one study, of low quality, extensively investigated coagulation-fibrinolytic abnormalities in subclinical hyperthyroidism^[30].

Many physicians still ignore the existing relationship between thyroid hormones and the coagulation system. It is important for clinicians to realize that thyroid dysfunction, as well as hepatic, renal, and other systemic diseases can affect hemostatic balance.

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Chapter 4

Hypothyroidism and acquired
von Willebrand's syndrome:
a systematic review

Elisa Manfredi, Bregje van Zaane, Victor EA
Gerdes, Dees PM Brandjes, Alessandro Squizzato

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ABSTRACT

Acquired von Willebrand's syndrome type 1 is the supposed main underlying cause of a bleeding tendency in hypothyroid patients. The purpose of this systematic review was to summarise the published evidence on the association between hypothyroidism and acquired von Willebrand's syndrome. All published clinical epidemiological and interventional studies, case reports, and *in vitro* studies that investigated the association between hypothyroidism and acquired von Willebrand's syndrome were identified by a computer-assisted search of the MEDLINE and EMBASE electronic databases. A quality assessment was performed for clinical epidemiological studies. A total of 41 papers were included. A total of 22 epidemiological *in vivo* studies, 2 *in vitro* studies, and 47 case reports were finally analysed. No high quality *in vivo* study was identified. Almost all bleeding episodes described in the case reports were mucocutaneous. Von Willebrand factor (VWF) antigen value was available for 23 patients: median value 28 U/dL (range 4–45). VWF activity was available for 24 patients: median value 28.5 U/dL (range <3–55). Factor VIII activity was available for 16 patients: median value 47 U/dL (range 9–74). Acquired von Willebrand's syndrome may be the main factor responsible for a bleeding diathesis in overt hypothyroid patients. Even if bleeding episodes are mainly mild and mucocutaneous, blood transfusion, drug administration, or surgical procedure may be required.

INTRODUCTION

A bleeding tendency is frequently observed in overt hypothyroid patients. Mild mucocutaneous bleeding (epistaxis, gum bleeding, menorrhagia or bruising), and also, rarely, severe posttraumatic and postsurgical bleeding may occur^[1,2]. Several alterations in haemostatic mechanisms may be responsible for this bleeding tendency. However, it has been suggested that acquired von Willebrand's syndrome type 1 is the most contributing cause^[3]. The International Society on Thrombosis and Haemostasis retrospective registry on acquired von Willebrand's syndrome identified hypothyroidism as cause of acquired von Willebrand's syndrome in 2% of the patients compared with an 8% of all cases already published in the literature^[4]. The purpose of this review was to systematically summarise published literature on the association between hypothyroidism and acquired von Willebrand's syndrome, in order to provide useful clinical advices for physicians who treat patients with a bleeding tendency or patients with hypothyroidism.

METHODS

Study identification

A computer-assisted search of the MEDLINE and EMBASE electronic databases up to July 2007 was performed to identify published studies on the association between hypothyroidism and acquired von Willebrand's syndrome. The following search terms (textwords and MeSH or Emtree terms, respectively) were used for the MEDLINE search: Von Willebrand Factor, Von Willebrand Disease, Thyroid Diseases, Thyroid Hormones, Thyroid, Hyperthyroidism and Hypothyroidism; and for the EMBASE database search: Von Willebrand Factor, Von Willebrand Disease, Von Willebrand, Thyroid Disease, Thyroid Hormone, Thyroid, Hyperthyroidism, and Hypothyroidism. No language restrictions were initially applied to the search strategy. Reference lists of all studies included in the present systematic review were searched for potential additional eligible studies. All *in vivo* case-control and cohort studies were identified using a previous systematic review^[5].

Study selection

Two investigators (EM and AS) concomitantly performed the study selection. Main inclusion criterion was that the paper had to investigate the association between hypothyroidism, both clinically overt and subclinical, and acquired von Willebrand's syndrome or von Willebrand factor (VWF) levels. Case-control studies and cohort studies in which laboratory tests were performed before and after treatment to correct hypothyroidism, as well as case reports and *in vitro* studies were included. Reviews, editorials, and non-human studies were excluded. No attempt was made to translate papers written in a language uncommon and unknown to the reviewers (other than English, French, Spanish, German, Dutch, and Italian). The two investigators concomitantly reviewed titles and/or abstracts from the initial search to determine whether inclusion criteria were satisfied. Full text of the study was obtained when an article could not be excluded with certainty.

Quality assessment

Quality assessment of the selected studies was performed for clinical studies^[5,6]. In particular, the Newcastle-Ottawa Scale (NOS scale) for assessing quality of non-randomised clinical studies in meta-analysis was adapted for use^[5,6]. This assesses three broad areas: selection, comparability, and outcome or exposure, for cohort or case-control studies. For summarising study validity, we adapted a simple Cochrane Collaboration approach for interventional studies^[7]. Three categories were therefore identified: high quality (low risk of bias), medium quality (moderate risk of bias), or low quality (high risk of bias). To the best of our knowledge, no quality assessment scale for *in vitro* studies has been well validated. Therefore, quality assessment on these studies was not performed.

Data extraction

One reviewer (EM) independently completed the data extraction form. A second reviewer (AS) checked the extracted data. The following characteristics were collected for case reports: (i) age; (ii) gender; (iii) degree of thyroid dysfunction (clinically overt or subclinical); (iv) plasma antigen levels of VWF; (v) plasma activity level of VWF; (vi) other coagulation tests or plasma levels of coagulation/fibrinolytic factors (e.g. activated partial thromboplastin time (APTT), prothrombin time (PT), bleeding time, factor VIII antigen and activity, platelet levels); (vii) site of haemorrhage; (viii) degree of haemorrhage (minor, major or fatal haemorrhage; major haemorrhage was defined as cerebral, retroperitoneal, pericardial, peritoneal, or intraocular bleeding, as haemorrhage requiring hospitalisation, surgery, or blood transfusion with ≥ 2 units, or as haemorrhage causing a haemoglobin drop of ≥ 2 g/dL); (ix) bleeding tendency after restoration of euthyroidism; (x) concomitant diseases that are well recognised associated with acquired von Willebrand's syndrome (e.g. monoclonal gammopathies, lymphoproliferative disorders, myeloproliferative disorders, solid tumours, autoimmune disorders, congenital and acquired cardiac defects, drugs such as ciprofloxacin, griseofulvin, valproic acid, hydroxyethyl starch, or infectious diseases such as hydatid cyst, Epstein Barr Virus infection); and (xi) presence of inherited or acquired (e.g. hepatic or renal insufficiency) bleeding disorders. For case-control and cohort studies, as previously described, the following characteristics were collected: (i) degree of hypothyroidism (clinically overt and/or subclinical); (ii) total number of enrolled cases and controls; (iii) performed coagulation/fibrinolytic tests; and (iv) statistically decreased, increased or no statistically difference of each coagulation test of cases vs controls, or pre- vs post-treatment^[4].

Statistical analysis

Data for qualitative variables were presented as incidence rates (i.e. number and percent). The data of continuous variables were summarised using measures of central tendency (i.e. mean and median) and dispersion (i.e. standard deviation, range).

RESULTS

Of the 185 papers identified with the initial search strategy, 33 were considered potentially eligible based on the title and/or abstract. We retrieved full copies of 28 potentially appropriate studies and 4 abstracts (of 1 paper neither manuscript nor abstract could be retrieved)^[8]. After excluding 5 articles for not meeting the prespecified inclusion criteria, a total of 23 articles plus 4 abstracts were included in the final analysis^[3,9-34]. In addition, we report on an analysis of a further 14 articles discussing 22 *in vivo* studies as previously reviewed (2 articles were newly retrieved)^[5,35-48].

Epidemiology

In hypothyroidism, the frequency of both laboratory diagnosis of von Willebrand's syndrome and bleeding events are unclear. Only one cross-sectional study has really investigated the association between these two disorders and its symptoms^[20]. Franchini et al tested 131 consecutive individuals with low VWF levels, found during coagulation screening tests, for serum levels of thyroid hormones. Eight of the 131 individuals with low VWF levels (6.1%) had a concomitant subclinical hypothyroidism as defined by normal thyroid hormone levels and elevated thyrotropin (TSH) concentrations. Three of them (37.5%) had experienced bleeding symptoms (2 patients had menorrhagia and the third experienced prolonged bleeding after dental extraction). In all patients, normalisation of haemostatic parameters was present after thyroid hormone replacement therapy.

Tani et al provided only indirect data^[31]. They measured activated partial thromboplastin time (APTT) and lupus anticoagulant in 532 patients with autoimmune thyroid disease (327 with Graves' disease and 205 with Hashimoto's thyroiditis). Prolonged APTT was observed in 33 (6.2%) blood samples. However, the samples with prolonged APTT showed no abnormality in coagulation factors (factors VIII, IX, XI, XII, and VWF).

Pathophysiology: *in vitro* studies

The molecular mechanisms of interaction between thyroid hormones, thyroid receptors and VWF have been investigated in 2 *in vitro* studies^[12,17]. Differently to other acquired von Willebrand's syndrome, decreased VWF levels in hypothyroidism are mainly secondary to a reduction in synthesis and/or secretion, and not to a reduced half-life due to autoantibodies or to molecular conformational changes. Baumgartner-Parzer et al explored the effect of thyroid hormones on endothelium-derived proteins such as VWF, fibronectin and endothelin-1^[12]. In particular, VWF secretion and mRNA expression were measured in human umbilical vein endothelial cells (HUVECs) exposed to tri-iodothyronine (T3) for 13±1 days, using ELISA, Western blot, RIA and Northern blot analysis, respectively. Exposure of HUVECs to T3 significantly increased VWF secretion (50 ng T3/mL: 117±5%, $p < 0.01$; 100 ng T3/mL: 127±26%, $p < 0.01$) as well as VWF mRNA expression (50 ng/mL: 116±13%, $p < 0.01$; 100 ng/mL: 136±30%, $p < 0.01$ Wilcoxon signed rank test). Protein kinase C (PKC) isoforms epsilon and beta-II were not significantly affected by T3, whereas PKC alpha was increased in whole cell lysates and in membrane fractions of cells incubated with 100 ng T3/mL but not in those incubated with 50 ng T3/mL. Data indicate thyroid hormone-induced upregulation of mRNA expression and protein synthesis of VWF by PKC alpha-, beta-II and epsilon-independent pathways.

Table 1A. Individual study quality: Case-control studies.

First author	Study quality	Type of dysfunction
Arnout ^[37]	Low	Hypothyroidism
Erem ^[38]	Low	Hypothyroidism
Erem ^[39]	Low	Subclinical hypothyroidism
Gullu ^[40] (A)	Low	Hypothyroidism
(B)	Low	Subclinical hypothyroidism
Homoncik ^[36] (A)	Low	Hypothyroidism
(B)	Medium	Subclinical hypothyroidism
Kventy ^[41]	Low	Subclinical hypothyroidism
Myrup ^[42]	Low	Hypothyroidism
Muller ^[43]	Low	Subclinical hypothyroidism
Nagasaki ^[35]	Medium	Hypothyroidism

Indirect data comes from Diekman et al who investigated the gene expression of thyroid hormone receptors (TR) in ECRF24 immortalized HUVECs at both the mRNA and the protein level^[17]. They also measured VWF production in response to short-term T3 administration (4, 8, 12 and 24 h) in doses of 0 M, 10⁻⁶ M and 10⁻¹⁰ M. VWF was measured in cell supernatants with a two-site immunoenzymatic assay. VWF secretion did not increase upon addition of T3. Failure to replicate Baumgartner-Parzer data is probably due to low levels of TR expression in immortalized ECRF24 HUVECs. The number of TRs per endothelial cell appears to be insufficient to find an increase in VWF production after addition of T3.

Pathophysiology: in vivo studies

The *in vivo* effects of hyperthyroidism and hypothyroidism on the coagulation and fibrinolytic system have been previously reviewed^[5]. Individual study quality and the effect of hypothyroidism on VWF parameters are summarised in Tables 1 and 2. No high quality studies have been identified.

Table 1B. Individual study quality: Interventional cohort studies.

First author	Study quality	Type of dysfunction
Arnaout ^[37]	Low	Hypothyroidism
Chadarevian ^[44]	Low	Hypothyroidism
Erfurth ^[45]	Low	Hypothyroidism
Gullu ^[40] (A)	Medium	Hypothyroidism
(B)	Medium	Subclinical hypothyroidism
Homoncik ^[36]	Low	Hypothyroidism
Myrup ^[42]	Medium	Hypothyroidism
Nagasaki ^[46]	Medium	Hypothyroidism
Nagasaki ^[47]	Medium	Hypothyroidism
Nagasaki ^[35]	Medium	Hypothyroidism
Palareti ^[48]	Low	Hypothyroidism

Acquired von Willebrand's syndrome: laboratory diagnosis and clinical manifestations

A total of 47 cases of acquired von Willebrand's syndrome associated with hypothyroidism, both overt and subclinical, have been described in the literature, as summarised in Tables 3 and 4. Mean age at diagnosis was 43.6 years and 30 of 43 patients were women (69.8%). Data on bleeding, as summarised in Table 5, were available for only 30 patients (63.8%). Almost all bleeding episodes were mucocutaneous. Data on concomitant diseases were available in only 29 patients (61.7%). Relevant medical conditions as possible causes of acquired von Willebrand's syndrome were as follows: vasculitis (n=1), lymphoma (n=1), Waldenstrom's macroglobulinaemia (n=3), chronic lymphatic leukaemia (n=1), multiple myeloma (n=3), pernicious anaemia (n=2) and monoclonal gammopathy of uncertain significance (MGUS) (n=1). In only 11 patients, other coagulation factor measurements were described: in 6 of these patients, no other coagulation factor abnormalities were detected; reduced FV levels were present in 2 patients; reduced FIX levels in 3 patients; and reduced FXI levels in 1 patient. The bleeding time was available for 19 patients with a mean value of >14 min. APTT and PT were reported in 12 and 11 patients, respectively; mean value was >50 s for APTT and 15 s for PT. Platelet count was reported in only 7 patients with a mean value of >250 000/mm³; von

Table 2A. Overall effect of hypothyroidism (overt and subclinical) on von Willebrand factor parameters: All studies combined (high risk of bias).

Coagulation tests	N. of studies	Hypothyroidism	N. of studies	Subclinical Hypothyroidism
VWF antigen	6	↓	3	=
VWF activity	7	↓ or =	2	=
VWF ristocetin cofactor activity	6	↓ or = or ↑	3	↓ or =
Ristocetin induced platelet agglutination	2	↓		

N indicates number; VWF, von Willebrand factor; ↓, decreased levels; ↑, increased levels; and =, non statistical significant difference.

Table 2B. Overall effect of hypothyroidism (overt and subclinical) on von Willebrand factor parameters: Medium quality studies (moderate risk of bias).

Coagulation tests	N. of studies	Hypothyroidism	N. of studies	Subclinical Hypothyroidism
VWF antigen	1	↓	1	=
VWF activity	1	↓	1	=
VWF ristocetin cofactor activity	4	↓ or ↑	1	↓
Ristocetin induced platelet agglutination	1	↓		

N indicates number; VWF, von Willebrand factor; ↓, decreased levels; ↑, increased levels; and =, non statistical significant difference.

Willebrand factor antigen value was available for 23 patients (median value 28 U/dL, range 4–45); VWF activity was available for 24 patients (median value 28.5 U/dL, range <3–55); and FVIII activity was available for 16 patients (median value 47 U/dL, range 9–74). Finally, Arjane et al described a case of overt hypothyroidism having predisposed to a haemorrhagic tendency aggravating an unsuspected von Willebrand disease^[9].

Therapy

Erfurth et al investigated the acute haemostatic effect of desmopressin (DDAVP) and the long-term effect of thyroxine replacement therapy on the plasma concentrations of coagulation factors in 10 hypothyroid patients^[34]. They administered DDAVP, given intravenously over 10 min at a dosage of 0.3 mcg/kg. Thyroxine was given orally as tablets, at a daily dose slowly increasing from 0.05 mg to 0.10–0.15 mg per day after 3 months of treatment until the concentration of TSH was normalised. Before treatment, 5 patients had bleeding

Table 3A. Published case reports on acquired von Willebrand's syndrome and hypothyroidism: Laboratory diagnosis.

Patient	Age	Gender	Degree of hypothyroidism	Von Willebrand factor antigen	Von Willebrand factor activity (Ristocetin Cofactor)	Factor VIII activity	Ref
1	17	F	Subclinical	H: 45 U/dL T: 144 U/dL (n.v. 50-146 U/dL)	H: 27 U/dL T: 120 U/dL (n.v. 50-172 U/dL)	H: 67 U/dL T: 122 U/dL (n.v. 57-122 U/dL)	[3]
2	53	F	Subclinical	H: 17 U/dL T: 74 U/dL (n.v. 50-146 U/dL)	H: 17 U/dL T: 68 U/dL (n.v. 50-172 U/dL)	H: 18 U/dL T: 85 U/dL (n.v. 57-122 U/dL)	[3]
3	29	F	Subclinical	H: 34 U/dL T: 93 U/dL (n.v. 50-146 U/dL)	H: 55 U/dL T: 139 U/dL (n.v. 50-172 U/dL)	H: 45 U/dL T: 126 U/dL (n.v. 57-122 U/dL)	[3]
4	34	F	Overt	NR	H: 0.19 U/mL T: 0.6 U/mL (n.v. 0.50-1.80 U/mL)	H: 0.31 U/mL T: 1.03 U/mL (n.v. 0.55-1.85 U/mL)	[29]
5	29	F	Overt (clinical diagnosis)	NR	NR	NR	[27]
6	13	F	Mild (clinical diagnosis)	NR	NR	NR	[27]
7	34	F	Overt	H: 25 U/dL T: 80 U/dL (n.v. 50-146 U/dL)	H: 50 U/dL (n.v. 50-172 U/dL)	H: 38 U/dL T: 59 U/dL (n.v. 57-122 U/dL)	[27]
8	17	F	Overt	H: 36 U/dL T: 79 U/dL (n.v. 50-146 U/dL)	H: 31 U/dL T: 71 U/dL (n.v. 50-172 U/dL)	H: 36 U/dL T: 120 U/dL (n.v. 50-190 U/dL)	[13]
9	15	F	Overt	NR	H: 44% T: 144% (n.v. 58-166%)	H: 70% T: 105% (n.v. 50-150%)	[15]
10	29	F	Overt	H: 30% T: 56% (n.v. 50-150%)	H: 30% T: 75% (n.v. 50-100%)	H: 38% T: 70% (n.v. 60-150%)	[32]

Patient	Age	Gender	Degree of hypothyroidism	Von Willebrand factor antigen	Von Willebrand factor activity (Ristocetin Cofactor)	Factor VIII activity	Ref
11	14	F	Overt	H: 36% T: 51% (n.v. 43-150%)	H: 0.5 U/mL T: 1.6 U/mL (n.v. 0.52-1.6 U/mL)	H: 47% T: 71% (n.v. 65-150%)	[14]
12	14	F	Overt	NR	RiCo H: 0.5 U/mL T: 1.1 U/mL (n.v. 0.52-1.6 U/mL)	H: 47% T: 90% (n.v. 65-150%)	[14]
13	34	M	Overt	H: 42% T: 90% (n.v. 50-150%)	NR	H: 47% T: 189% (n.v. 50-150%)	[10]
14	30	F	Overt	RiCo/VWF:Ag H: 0.38/0.39 U/mL (n.v. 0.50-1.5 U/mL)	NR	H: 42 U/dL (n.v. 50-150 U/dL)	[11]
15	55	M	NR	NR	NR	NR	[25]
16	67	M	NR	H: 22 U/dL	H: 12 U/dL	H: 11 U/dL	[24]
17	58	F	NR	H: 8 U/dL	H: 7 U/dL	H: 23 U/dL	[24]
18	22	F	Overt (clinical diagnosis)	H: 42 U/dL	H: 55 U/dL	H: 58 U/dL	[24]
19	34	F	Overt (clinical diagnosis)	H: 24 U/dL	H: 27 U/dL	H: 36 U/dL	[24]
20	55	M	NR	H: 28 U/dL	H: 33 U/dL	H: 47 U/dL	[24]
21	45	F	NR	H: 22 U/dL	H: 18 U/dL	H: 44 U/dL	[24]
22	64	M	NR	H: <10 U/dL	H: <3 U/dL	H: <10 U/dL	[24]
23	50	M	NR	H: 15 U/dL	H: 10 U/dL	H: 30 U/dL	[24]

24	64	F	NR	H: 41 U/dL	H: 11 U/dL	H: 25 U/dL	[24]
25	58	F	NR	H: 8 U/dL	H: 7 U/dL	H: 15 U/dL	[24]
26	9	M	Overt hypothyroidism secondary to pituitary hypoplasia with associated growth hormone deficiency	H: 28% T: 68% (n.v. 50-150%)	NR	H: <1% T: 6.25% (n.v. 50-150%)	[26]
27	13	F	Mild (clinical diagnosis)	H: 37% T: 95% (n.v. 50-146%)	H: 37% (n.v. 50-72%)	H: 50% T: 80% (n.v. 57-122%)	[16]
28	11	F	Mild	H: 27% T: 86% (n.v. 50-146%)	NR	NR	[16]
29	42	M	Overt	H: 43%	H: 48%	H: 74%	[28]
30	60	F	NR	H: 0.04 U/mL	H: 0.26 U/mL	H: 0.09 U/mL	[33]
31-42 ^a	NR	NR	NR	NR	NR	NR	[23]
43-46 ^a	NR	NR	NR	NR	NR	NR	[22]
47 ^a	73	M	Overt	NR	NR	NR	[30]

Ref indicates reference; F, female; M, male; H, during hypothyroidism; T, after thyroid replacement therapy; n.v., normal value; NR, not reported; RiCo, ristocetin cofactor; VWF, von Willebrand factor; and Ag, antigen.

^aOnly abstract available.

Table 3B. Published case reports on acquired von Willebrand's syndrome and hypothyroidism: Clinical manifestations.

Patient	Bleeding episodes	Degree of bleeding	Bleeding diathesis after euthyroidism	Concomitant diseases (possible causes of AVWS)	Associated coagulation factors deficiency	Ref
1	Gum bleeding	NR	No	NR	Factor IX:C H: 39 u/dL (n.v. 50-175 u/dL)	[3]
2	Easy bruising and bleeding after extraction of tooth	Minor	No	No	No	[3]
3	Spontaneous bruising and menorrhagia for 6 months	NR	No	No	NR	[3]
4	Frequent and prolonged gum bleeding	Minor	No	No	Factor V:C H: 0.27 U/mL T: 0.96 U/mL (n.v. 0.65-1.27 U/mL).	[29]
5	Mild menorrhagia	Minor	No	NR	NR	[27]
6	Prolonged bleeding after dental extraction	NR	No	NR	No	[27]
7	Spontaneous bruising, menorrhagia, and recurrent epistaxis	Minor (in the past major bleedings after delivery and miscarriage)	No bruising; persistent menorrhagia due to local factors	NR	NR	[27]
8	Vaginal bleeding	Major (transfused)	No	No	No	[13]
9	Vaginal bleeding	Major (transfused for Hb 5.0 g/dL)	Only one heavy menses after 9 months	No	No	[15]
10	Prolonged bleeding after dental extraction, gum bleeding	Minor	No	No	No	[32]
11	Heavy menorrhagia (10 days), past spontaneous knee haemarthrosis	Major (transfused)	No	Positive ANA 1:180 (n.v. <1:40)	NR	[14]

12	-	-	-	-	NR	[14]
13	Persistent bleeding 3 weeks after a tooth extraction	NR	No	NR	Factor IX:C H: 55% (n.v. 50-150%)	[10]
14	Easy bruising, menorrhagia and gingival bleeding	Minor	No	Pernicious anaemia	NR	[11]
15	Severe epistaxis (nasal packing and balloon tamponade)	Major (transfused for haematocrit 30%)	NR	Monoclonal IgGκ	NR	[25]
16	Recurrent gastrointestinal bleeding	Major (transfused)	NR	MGUS, gastric cancer, and positive ANCA	NR	[24]
17	Bruising and epistaxis	Minor (treated with DDAVP and TA)	NR	No	NR	[24]
18	Menorrhagia and prolonged bleeding after tonsillectomy	Major	No	No	NR	[24]
19	Easy bruising and prolonged bleeding after dental procedure	Minor	No	No	Factor IX:C H: 42%	[24]
20	Epistaxis	Major (transfused)	NR	Multiple myeloma	Factor XI:C H: 65% Factor IX:C H: 47%	[24]
21	Haematoma after hip surgery	Minor (treated with DDAVP)	NR	Waldenstrom's macroglobulinaemia	NR	[24]
22	Haematoma after femoral bypass	NR	NR	Multiple myeloma	NR	[24]
23	Easy bruising	Minor	NR	No	NR	[24]

Patient	Bleeding episodes	Degree of bleeding	Bleeding diathesis after euthyroidism	Concomitant diseases (possible causes of AVWS)	Associated coagulation factors deficiency	Ref
24	Epistaxis	Minor (treated with factor VIII concentrate)	NR	B-cell lymphoma	NR	[24]
25	Haematoma after surgery	Minor (treated with factor VIII concentrate, TA, and DDAVP)	NR	Multiple myeloma	NR	[24]
26	Prolonged bleeding after tooth extraction	NR	NR	Growth hormone deficiency	Factor V:C H: 12% T: 10% (n.v. 50-150%)	[26]
27	Recurrent epistaxis and low gastrointestinal bleeding	Minor	No	No	NR	[16]
28	Recurrent epistaxis	Minor	No	No	NR	[16]
29	Recurrent gum bleeding and easy bruising	Minor (treated with DDAVP)	No	NR	NR	[28]
30	Melaena and telangiectasia	-	NR	Chronic lymphatic leukaemia	NR	[33]
31-42 ^a	-	-	NR		NR	[23]
43-46 ^a	-	-	NR	2 patients with Waldenström's macroglobulinaemia and 1 with MGUS	NR	[22]
47 ^a	Subcutaneous haemorrhages after venipuncture	-	NR	Pernicious anaemia	NR	[30]

AVWS indicates acquired von Willebrand's syndrome; NR, not reported; C, activity; H, during hypothyroidism; n.v., normal value; T: after thyroid replacement therapy; Hb; haemoglobin; ANA, anti-nuclear antibodies; ANCA, anti-nuclear cytoplasmatic antibodies; DDAVP, desmopressin; TA, tranexamic acid; and MGUS, monoclonal gammopathy of uncertain significance.

^aOnly abstract available.

Table 4. Bleeding episodes in 30 patients with acquired von Willebrand's syndrome and hypothyroidism.

Bleeding episode	Number of patients (%)
Bleeding gums	4 (13.3)
Bruising	8 (26.7)
Epistaxis	6 (20)
Menorrhagia	6 (20)
Gastrointestinal bleeding	3 (10)
Prolonged bleeding after tooth extraction	6 (20)
History of bleeding	2 (6.7)
Haemarthrosis	1 (3.3)
Vaginal bleeding	1 (3.3)
Subcutaneous haemorrhages after venipuncture	1 (3.3)
Prolonged bleeding after surgery	4 (13.3)

manifestations; only 1 had prolonged bleeding time and 1 had low plasma concentrations of VWF antigen. DDAVP virtually immediately reduced bleeding time, enhanced platelet adhesiveness and significantly increased plasma concentrations of FVIII and VWF. The plasma concentrations of FVIII and VWF showed a significant increase after 4 months of thyroxine treatment, whereas 7 months were needed to significantly reduce bleeding time.

Conflicting data

Acquired von Willebrand's syndrome has also been associated with thyroid diseases during a euthyroid state^[18;19]. Franchini et al described 14 patients undergoing thyroid surgery for a thyroid disease with an acquired von Willebrand's syndrome type 1^[18]. At diagnosis, 12 patients were euthyroid and 2 were hyperthyroid. All patients received desmopressin at a standard dose of 0.3 lg/kg subcutaneously, 1 h before operation and then once daily until the end of the second postoperative day. No bleeding complications were recorded during or after surgery. In 10 of these 14 patients, coagulation parameters were checked 6 months after surgery demonstrating coagulation parameters within normal range in all cases. Recently, the same group published data of 1342 consecutive patients with various thyroid diseases who were candidates for thyroid surgery^[19]. A preoperative screening including prothrombin time, activated partial thromboplastin time and platelet function analyser (PFA-100), identified

39 patients (2.9%) with abnormalities. Of these, 35 had von Willebrand's syndrome (type 1 in 33 cases and type 2A in 2 cases). However, all these patients were euthyroid at the time of screening.

DISCUSSION

The link between the haemostatic system and thyroid disease has been investigated since the beginning of the last century^[1;2]. Several mechanisms are involved at different levels. In particular, both thyroid dysfunction and autoimmunity may modify physiological processes of primary and secondary haemostasis and lead to bleeding or thrombosis^[49;50].

A bleeding diathesis may be secondary to idiopathic thrombocytopenic purpura or acquired haemophilia, which are sometimes associated with autoimmune thyroid disorders. However, thyroid hormones, irrespective of associated diseases, have a direct influence on the coagulation system that is mainly mediated by the interaction with its receptors^[5]. In particular, overt hypothyroidism is associated with a bleeding diathesis secondary to a defect of primary haemostasis: acquired von Willebrand syndrome seems to be the main pathophysiological mechanism^[5]. Laboratory and clinical data of this review support the hypothesis of a pivotal role of VWF: bleeding episodes were mainly mucocutaneous, bleeding tendency was ameliorated by DDAVP administration, and laboratory tests did not reveal any relevant associated coagulation abnormalities other than low levels of VWF (antigen and activity) and low FVIII levels. A defect of primary haemostasis may be secondary to thrombocytopenia or platelet dysfunction. However, no low platelet levels were found in the retrieved case reports. In the literature, apart from associated idiopathic thrombocytopenic purpura, inhibited megakaryocytopoiesis is rarely described in severe hypothyroidism with bone marrow myxoedema^[1;51]. Platelet function tests were unfortunately not performed in the retrieved case reports, but in further literature qualitative platelet abnormalities, in particular impaired platelet reactivity to ristocetin, collagen, adrenalin, have indeed been reported^[1;48]. However, only patients with markedly elevated thyrotropin levels displayed an increased haemostatic sensitivity to aspirin as measured by bleeding time prolongation, and platelet sensitivity to some agonists and to prostacyclin as well as thromboxane production did not differ from control subjects^[52;53]. A systematic quality assessment of the literature on the effect of thyroid hormones on platelet function is desirable. Platelet dysfunction certainly contributes to a bleeding diathesis, but it is probably a secondary pathophysiological mechanism.

Several limitations of our systematic review may affect the validity of our findings. First, no high quality studies have been published and several studies do not directly address the topic. Second, many clinical data is based only on case reports. Third, conflicting data have been published. Fourth, bleeding tendency in subclinical hypothyroid patients is not well defined: an increased risk of thrombosis has also been advocated^[54].

As scientists, these drawbacks perfectly fit with the main purpose of our systematic review to stimulate well-designed clinical studies on this topic. Many issues are still to be addressed: in overt hypothyroidism, the clinical impact of bleeding and the involved pathophysiological mechanisms; in subclinical hypothyroidism, whether a bleeding diathesis still persists. To collect all new cases of acquired von Willebrand's syndrome, an international registry of the subcommittee on VWF of the Scientific Standardization Committee of the International Society on Thrombosis and Haemostasis is available at the web site <http://www.intreavws.com>. The Steering Committee encouraged the organization of prospective studies on the frequency of acquired von Willebrand's syndrome within specific underlying disorders^[5,55-57]. Collaboration with endocrinologists and their scientific societies may be desirable for developing appropriate protocols and improving recruitment of the patients.

As physicians, published literature is sufficient to draw attention on this neglected complication of hypothyroidism. It is common experience that many physicians who daily treat thyroid disease and bleeding events still ignore the existing relation between thyroid hormones and the haemostatic system. It is important for clinicians to realise that not only kidney or liver diseases affect the haemostatic balance increasing the risk of bleeding, but also in patients with severe hypothyroidism VWF activity may be too low.

In conclusion, acquired von Willebrand's syndrome may be the main factor responsible for a bleeding diathesis in overt hypothyroid patients. Even if bleeding episodes are mainly mild and mucocutaneous, blood transfusion, drug administration, or surgical procedures may be required. A prompt recognition of the underlying mechanism could be life saving, even if rarely, for the patient.

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Chapter 5

Thyroid diseases and cerebrovascular disease

Alessandro Squizzato, Victor EA Gerdes,
Dees PM Brandjes, Harry R Büller, Jan Stam

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ABSTRACT

Introduction: Acute cerebral ischemia has been described in different diseases of the thyroid gland, and not only as a result of thyrotoxic atrial fibrillation and cardioembolic stroke. The purpose of this review is to summarize the studies on the relationship between thyroid diseases and cerebrovascular diseases, discussing the main findings for overt hyperthyroidism and hypothyroidism, as well as for subclinical thyroid dysfunction

Summary: In overt hyperthyroidism, cardioembolic stroke is clearly associated to thyrotoxic atrial fibrillation, and in subclinical hyperthyroidism with serum thyroid-stimulating hormone levels <0.1 mU/L, the incidence of atrial fibrillation is increased. Although in vitro and in vivo studies indicate a hypercoagulability state in hyperthyroidism, there is insufficient evidence to prove that this state leads to an increased risk of cardiac emboli. However, the hypothesis that overt hyperthyroidism may cause acute cerebral venous thrombosis is intriguing. Possible associations between hyperthyroidism and Moyamoya or Giant cell arteritis have only been described in case reports. There is enough evidence that overt hypothyroidism is associated with several traditional and newer atherosclerotic risk factors, especially hypertension, hyperlipidemia, and hyperhomocysteinemia. For subclinical hypothyroidism, these associations are less certain. Hypothyroidism has been associated with signs of aortic or coronary atherosclerosis, but no case-control or cohort studies have ever investigated hypothyroidism as a possible risk factor for atherothrombotic stroke.

Conclusions: Hyperthyroidism is associated with atrial fibrillation and cardioembolic stroke. Hypothyroidism is associated with a worse cardiovascular risk factor profile and leads to progression of atherosclerosis. Associations between hyperthyroidism and acute cerebral venous thrombosis, Moyamoya, and Giant cell arteritis have been suggested, but sound evidence is lacking. Additional studies are needed to clarify these issues

INTRODUCTION

Acute ischemic stroke is a well-described manifestation of thyrotoxic atrial fibrillation (AF). However, AF and cardioembolic stroke are not the only underlying pathological mechanisms of acute cerebral ischemia in thyroid disease. The purpose of this review is to summarize the studies on the relationship between thyroid disease and cerebrovascular diseases. We reviewed the published literature from *in vivo* studies through case reports to epidemiological and prospective studies. The main findings are highlighted and discussed for overt thyrotoxicosis and hypothyroidism, as well as for subclinical thyroid dysfunction.

HYPERTHYROIDISM

Thyrotoxicosis was originally a clinical term that described the appearance of affected patients and is commonly used to refer to any condition in which there is an excessive amount of circulating thyroid hormone, irrespective of the origin. The term hyperthyroidism was restricted to the diseases in which the thyroid gland synthesizes and secretes excessive hormones. However, the distinction in the literature between thyrotoxicosis and hyperthyroidism is not always clear and does not necessarily clarify the pathophysiology of each condition^[1]. Therefore, the terms will be used interchangeably. Subclinical hyperthyroidism is defined as a serum thyroidstimulating hormone (TSH) concentration below the statistically defined lower limit of the reference range when serum free thyroxine (fT4) and free triiodothyronine (fT3) concentrations are within their reference ranges^[2:3].

AF and Cardioembolic Stroke

AF occurs frequently in patients with hyperthyroidism and may be the presenting symptom^[4]. Various studies suggest a prevalence of 10% to 15% in patients with hyperthyroidism, and it is more common in men than in women^[5]. The prevalence increases with age (rare at < 40 years of age; > 25% of those > 60 years of age)^[6:7]. In comparison, in the general population, the prevalence increases from <1% in persons <60 years of age to >8% in those >80 years of age^[8]. The clinical diagnosis of thyrotoxicosis is not always obvious in elderly patients, in whom AF may be the dominant feature^[6:9:10]. The prevalence of thyrotoxicosis in patients with AF is 2% to 5%^[11-14]. Although an abnormal TSH level is common in patients with recent-onset AF, only <1% of cases of new-onset AF are caused by overt hyperthyroidism^[14]. So routine TSH screening of patients who had AF may be better applied to those patients at higher risk of having undiagnosed clinical thyroid disease. Otherwise, AF may be the initial manifestation of subclinical thyroid disease. In the Framingham Study, a low serum TSH level in subjects >60 years of age was associated with a 3.1-fold increased risk for AF after 10 years of follow-up: 21% of subjects with definitely low serum thyrotropin concentrations (<0.1 mU/L) compared with 12% of those with slightly low concentrations (0.2 to 0.4 mU/L) and 8% of those with normal concentrations^[15]. Also, 2 other studies reported an increased risk of AF in patients with subclinical hyperthyroidism compared with euthyroid persons (5-fold and 2.8-fold increased risk, respectively)^[16:17]. There is only limited evidence that the treatment of subclinical hyperthyroidism facilitates spontaneous reversion or cardioversion of AF to normal sinus rhythm^[16:18]. It is still controversial

whether the frequency of stroke and systemic embolism is increased in thyrotoxic AF or not. Some studies^[19–21] have reported a high frequency of stroke and systemic embolism in patients with thyrotoxic AF, but all these studies have methodological flaws. In another study, there was no statistically significant difference between AF patients and age- and sex-matched patients with normal sinus rhythm^[22]. No studies demonstrated an increased incidence of arterial embolism in patients with subclinical hyperthyroidism. However, in a recent 10-year cohort study, a single measurement of low serum thyrotropin was associated with increased mortality from circulatory and cardiovascular diseases^[23]. In patients with hyperthyroidism, AF is frequently of acute onset and will spontaneously revert to sinus rhythm without associated side effects. Spontaneous reversion within 6 weeks after the return to the euthyroid state is the expected outcome in patients <60 years of age who do not have pre-existing heart disease and in whom thyrotoxicosis is of short duration. When AF does not resolve after >3 to 4 months after reaching a euthyroid state, spontaneous reversion to sinus rhythm is unlikely^[24]. Whether patients with hyperthyroidism who have AF should receive anticoagulant therapy is controversial. In each patient, the risk of bleeding must be weighed against the risk of systemic embolization. Some authors conclude that the epidemiological data suggest that the rate of thromboembolism in patients with thyrotoxic AF exceeds that for nonthyrotoxic nonvalvular AF^[25], but the majority agree that that thyrotoxic AF is not a more potent risk factor for stroke than other causes of AF. Among major randomized control trials of stroke prevention with warfarin^[7], only the Copenhagen AFASAK Study included patients with hyperthyroidism: 16 (5%) in the warfarin arm, 12 (4%) in the aspirin arm, and 13 (4%) in the placebo group^[11]. These data appear insufficient to draw a conclusion. Therefore, because the incidence of thromboembolic events in patients with thyrotoxic AF appears to be similar to other etiologies of AF^[5], antithrombotic therapies should probably be chosen based on associated risk factors and the risk of bleeding, as stated by international guidelines^[7]. Patients with hyperthyroidism are particularly sensitive to anticoagulant effects of warfarin, and lower-than-normal warfarin doses are usually required because hyperthyroidism is associated with increased clearance of vitamin K-dependent clotting factor^[13;26].

A Hypercoagulability State?

We conducted an extensive search in all-language literature up to April 2005 with the databases MEDLINE and EMBASE using the text words 'hyperthyroidism' or 'thyrotoxicosis' and 'stroke,' 'cerebral vein thrombosis,' or 'cerebrovascular disease' to identify all case reports of cerebral venous and arterial thromboembolism during a noncancer (benign) thyroid disease. We retrieved articles and reviewed the references cited. We identified 2 main groups of patients other than the ones reported before: patients with cerebral venous thrombosis (CVT) and patients with acute ischemic stroke without cardiac arrhythmia.

Cerebral Vein Thrombosis

CVT is a very uncommon disease with an estimated incidence of 4 per 1 000 000 per year and with mortality rates between 5% and 30%^[27]. CVT has been associated with several causes and risk factors, such as inherited thrombophilia, oral contraceptives, pregnancy, and puerperium. However, ~25% of cases of CVT are still considered to be idiopathic^[27]. A possible association between thyrotoxicosis and CVT was already described by Kaliebe in 1913 and Doyle in 1927^[28]. The other case reports are summarized in Table 1^[29–39]. As described by Verberne et al, it is

unlikely that the association between CVT and thyrotoxicosis is explained by chance, and in addition to thyrotoxicosis, additional procoagulant influences are probably required for CVT to develop^[36;40]. As suggested by different authors, it is necessary to stress that hemodynamic factors, dehydration, and stasis of venous blood flow attributable to goiter may also contribute to the multifactorial pathogenesis of CVT.

Ischemic Stroke Without Cardiac Arrhythmias

In thyrotoxic patients without cardiac arrhythmia, only 7 cases of acute cerebrovascular ischemic disease have been identified (references available on request), and even in some of these cases, paroxysmal AF or vasculitis were not excluded entirely. Because acute cerebral ischemia is less rare than CVT, these case reports do not prove that there is an increased rate in thyrotoxic patients without cardiac arrhythmia.

Antiphospholipid Syndrome

The antiphospholipid syndrome (APS) is defined as a combination of clinical manifestations entailing arterial or venous thrombosis and recurrent fetal loss, accompanied by abnormal laboratory tests, namely, the lupus anticoagulant or anticardiolipin antibodies^[41]. Primary or secondary APS is defined by the presence or absence of concomitant viral infections, or hematologic, malignant, or autoimmune diseases^[41]. Ischemic stroke and CVT are neurological manifestations accepted as clinical diagnostic criteria for the APS. We found only 3 case reports of patients with Graves disease and APS^[42–44]. Conversely, patients experiencing primary APS reveal an increased prevalence of thyroid autoantibodies^[45;46]. Of course, these associations may be merely coincidental. However, there are indications that genetic predisposition can explain the presence of APS in patients with autoimmune thyroid disorders^[47], and it has been suggested that anticardiolipin antibodies may act as thyrotropin receptor-stimulating antibodies^[48]. However, this latter hypothesis is only speculative.

Cerebral Vasculitis

Giant cell (temporal) arteritis (GCA) is a chronic vasculitis of large and medium-sized vessels, which occurs among individuals >50 years of age. Although it may be generalized, vessel inflammation most prominently involves the cranial branches of the arteries originating from the aortic arch^[49]. Cranial ischemic complications are the most dreaded manifestations of GCA. The incidence of visual loss in most recent studies is ~15%. Stroke occurs in 3% to 4% of the patients^[50;51]. Ischemic damage in GCA is usually attributed to an occlusive vasculopathy caused by intimal proliferation (and usually not by thrombosis) in carotid and vertebrobasilar arteries^[49] or as a result of aortic dissection^[52]. The association between GCA and thyroid dysfunction is controversial^[53;54] but because giant cells are a possible feature of Graves' disease, a common pathway has been suggested. In 2 series, of 101 and 98 patients, respectively, the prevalence of hyperthyroidism was reported to be 6 times higher in cases of GCA than in controls^[55;56], which was not confirmed in a multicenter case-control study^[57]. An association between hyperthyroidism and Takayasu arteritis was described in 2 patients in the literature^[58].

Table 1. Case reports of acute cerebral vein thrombosis associated with hyperthyroidism reported in the literature.

Pt	Sex	Age	Site of thrombosis	Thyroid disease	Thyroid hormone state	Associated risk factors for thrombosis	Ref
1	M	24	SSS	Graves' disease	ftT4: 35.5 pmol/L TSH: <0.05 mU/L	No thrombophilia. No AF	[29]
2	F	32	SSS Transverse sinus sx	Graves' disease	ftT4: 80 pmol/L TSH: <0.05 mU/L	No thrombophilia. No AF	[29]
3	F	28	Superior sagittal vein Transverse sinus dx	Graves' disease	ftT4: 57.3 pmol/L TSH: <0.05 mU/L	No oral contraceptive pill. Factor V Leiden heterozygous	[30]
4	F	18	Deep cerebral veins Inferior sagittal sinus Straight sinus Left lateral sinus.	Graves' disease	ftT4: 130 pmol/L TSH: <0.05 mU/L	No thrombophilia. Low-dose oral contraceptive pill.	[31]
5	F	39	Lateral sinus sx Jugular vein sx	Graves' disease	ftT4: 28.8 pmol/L TSH: <0.02 mU/L	Oral contraceptive. Increased FVIII	[32]
6	M	32	Sigmoid sinus sx	Not specified	ftT4: 65 pmol/L TSH: <0.06 mU/L	Protein C deficiency.	[33]
7	F	32	SSS Lateral sinus sx	Not specified	Not reported	Obesity. Oral contraceptives. APC-R. No family history of VTE	[34]
8	M	34	Dural sinuses Internal jugular veins	Not specified	T4 uptake by radioimmunoassay 17.8	Plasminogen deficiency. Chronic inflammatory disease.	[35]
9	F	28	Transverse sinus sx Sigmoid Sinus Rectus sinus Internal jugular vein sx	Graves' disease	ftT4 >70 pmol/L TSH <0.01 mU/L	Oral contraceptives. aCL IgG FVIII 190%	[36]
10	M	60	SSS Transverse sinus dx	Graves' disease.	ftT4: >77 pmol/L TSH: 0.002 mU/L	Low protein C activity 52%. Increased fibrinogen. AF.	[37]
11	F	42	SSS Transverse sinus dx	Subacute De Quervain's thyroiditis	ftT4: 76,2 pmol/L TSH: <0.01 mU/L	Prothrombin G20210A heterozygous mutation	[38]
12	F	38	Lateral sinus sx	Graves' disease	ftT4: >75 pmol/L TSH: <0.10 mU/L	No thrombophilia	[39]
13	F	44	Longitudinal superior sinus Lateral sinus dx	Not specified	ftT4: >49 pmol/L TSH: <0.15 mU/mL	No deficit protein C and S	[39]

SSS indicates superior sagittal sinus; AF, atrial fibrillation; FVIII, factor VIII; APC-R, activated protein C-resistance; VTE, venous thromboembolism; aCL, anticardiolipin; sx, left; and dx, right.

Moyamoya

Moyamoya disease is a rare cerebrovascular disorder that is characterized by bilateral stenosis or occlusion of the distal segments of the internal carotid arteries accompanied by typical collaterals vessels^[59;60]. The pathogenesis of Moyamoya disease is unknown, but hyperactivity of cervical sympathetic nerves may contribute to the stenosis of the cerebral arteries. In general, transient ischemic attacks and infarction are more common in the juvenile group, whereas intracranial hemorrhage is a more prevalent clinical manifestation in adult cases^[60]. A total of 10 cases of Graves’ disease associated with Moyamoya disease or Moyamoya variant have been described^[61–67]. In most cases, cerebral infarcts occurred when the patients were thyrotoxic. There is no clear pathogenetic relationship between Graves disease, an autoimmune disorder, and Moyamoya.

Table 2. Thyrotoxicosis and cerebrovascular disease.

	Possible pathogenesis	Frequency of association	Evidence
Cardioembolic stroke	AF (certain)	25% of AF in patients >60 ys.	A*
Non cardioembolic stroke	Hypercoagulability state, APS	Unknown	D
Cerebral vein thrombosis	Hypercoagulability state, APS, vascular compression	Rare	D
Giant Cell Arteritis	Autoimmunity	From 0 to 6-fold increase	D
Moyamoya	Unknown	Rare	D

APS indicates antiphospholipid syndrome
 *Insufficient evidence of an increased risk compared to non-thyrotoxic AF patients

Evidence for Tables 2, 3, 4:
Grade A: well-designed controlled cohort and/or case control studies with concordant results.
Grade B: one well designed controlled cohort or case control studies.
Grade C: cohort or case control studies with one or two of the following limits: not concordant results; not well designed (not controlled study, limited number of patients, etc.); insufficient statistical power.
Grade D: only case reports or case series; or more than two limits in Grade C; or indirect evidence from studies with other primary and secondary objectives.

Vascular Compression

A goiter may lead to venous stasis or a reduction in arterial cerebral blood flow attributable to carotid compression. At least 1 patient with a large goiter that directly compressed the brachiocephalic vessels and no other risk factors have been reported in the literature. This patient had a goiter and a left hemiparesis attributable to right temporoparietal infarction. Cerebral arteriography showed a brachiocephalic and right subclavian stenosis secondary to compression by a thyroid nodule^[68].

HYPOTHYROIDISM

Overt hypothyroidism can be classified on the basis of its time of onset, severity, and pathogenesis^[69]. In patients with primary hypothyroidism, in whom serum thyrotropin is elevated, the distinction between overt and subclinical hypothyroidism can be defined biochemically by whether the serum free thyroxine concentration is below or within the reference range^[2;3]. The term myxedema is now usually reserved for cases of overt hypothyroidism that are severe or complicated.

Atherosclerosis

In 1878, Greenfield found diffuse atherosclerosis in a 58-year old woman with myxedema at autopsy: 'There was edema of the skin . . . much serous effusion in the pericardium . . . the heart was large . . . the arteries were everywhere thickened, the larger ones atheromatous'^[70;71]. In 1883, Kocher noted that arteriosclerosis occurred commonly after thyroid extirpation and raised the hypothesis of a causal relationship between hypothyroidism and atherosclerosis^[72]. Since then, a body of clinical case reports, epidemiological studies, biochemical observations, and case-control and cohort studies have linked hypothyroidism and atherosclerosis^[71]. Coronary artery atherosclerosis is twice as common in patients with hypothyroidism compared with sex and age-matched controls, and adequate thyroid hormone replacement therapy may protect against progression^[73;74]. The frequency of atherosclerosis in other arteries is less studied. Early arterial structural and functional alterations involve the muscular arteries more than the elastic arteries^[75]. A raised serum TSH has been proposed as one of the risk factors for the development of peripheral arterial disease in women^[76]. A decrease in carotid intima-media thickness as a result of thyroid hormone replacement therapy has been described for overt hypothyroidism and for subclinical hypothyroidism^[77;78]. There are no randomized studies that have assessed the impact of L-thyroxine replacement therapy on important cardiovascular outcomes^[3]. Subclinical hypothyroidism certainly influences cardiovascular risk factors; however, the relationship with atherosclerosis is still a matter of debate. Some studies showed a relationship^[79-82] that was not confirmed in other studies^[83-85]. These inconsistent results can be explained by differences in design and the relatively small size of most studies. Also in euthyroid patients, a correlation between levels of thyroid hormones and atherosclerosis has been suggested. One study showed that low fT4 is a risk factor for atherosclerosis in male euthyroid hyperlipidemic patients measuring carotid atherosclerotic lesions^[86], and an angiographic study in 100 patients suggested that a variation of thyroid function within the normal range might influence the presence and severity of coronary atherosclerosis^[87]. We performed an extensive search through MEDLINE and EMBASE databases in the all-languages literature using the text words 'hypothyroidism' and 'stroke' or 'cerebrovascular disease'. We did not identify any cohort or case-control studies that investigated hypothyroidism as a possible risk factor for atherothrombotic stroke. Nonetheless, hypothyroidism has a clear influence on atherosclerotic risk factors, and this may lead to cerebrovascular disease^[88].

Cardiovascular Risk Factors

The increased cardiovascular morbidity in hypothyroid patients has been attributed to the traditional cardiovascular risk factors: elevated low-density lipoprotein (LDL) cholesterol levels

and diastolic hypertension^[89]. Important associations have been identified for other risk factors for atherosclerosis (eg, hyperhomocysteinemia and endothelial dysfunction)^[90] in individuals with overt hypothyroidism and, in some cases, subclinical hypothyroidism.

Lipids

The composition and the transport of lipoproteins are disturbed in thyroid diseases. Overt hypothyroidism is characterized by hypercholesterolemia^[91], and conversely, 4% to 14% of hypercholesterolemic patients have been reported to have hypothyroidism^[92], a relationship that is often unsuspected. The lipid profile is presented as a marked increase in LDL and apolipoprotein B because of a decreased fractional clearance of LDL by a reduced number of LDL receptors in the liver^[93]. The high-density lipoprotein (HDL) levels are normal or even elevated in severe hypothyroidism because of decreased activity of cholesteryl-ester transfer protein and hepatic lipase, which are regulated by thyroid hormones. The low activity of cholesteryl-ester transfer protein, and more specifically of hepatic lipase, results in reduced transport of cholesteryl esters from HDL to very low-density lipoproteins, and intermediate LDLs and reduced transport of HDL^[2] to HDL^[3,93]. Moreover, hypothyroidism increases the oxidation of plasma cholesterol^[94]. However, thyroxine therapy, in a thyrotropin (TSH)-suppressive dose, usually leads to a considerable improvement of the lipid profile. The changes in lipoproteins are correlated with changes in free thyroxine levels. The effects of subclinical hypothyroidism on serum lipid levels remain controversial, probably because of a great individual variability. Some^[95], in contrast to other^[96], cross-sectional studies have demonstrated that serum levels of total cholesterol and LDL cholesterol are higher in patients with subclinical hypothyroidism than in euthyroid controls. A recent meta-analysis on the effect of therapy for subclinical hypothyroidism on serum lipid levels demonstrated a very limited mean reduction in the total cholesterol level (7.9 mg/dL [0.2 mmol/L]) and in the LDL cholesterol level (10 mg/dL [0.26 mmol/L]), and no clear effect on HDL cholesterol [97]. Patients with higher cholesterol levels (>240 mg/dL [6.21 mmol/L]) and patients with subclinical hypothyroidism as a result of inadequately treated overt hypothyroidism had greater reductions in cholesterol levels. Small studies^[92] have suggested that patients whose serum thyrotropin level is <10 mU/L may have no reduction in cholesterol levels with thyroxine replacement, but the meta-analysis did not directly address this issue. Two population-based studies have added to the uncertainty in this area. In the first, based on a 20-year follow-up of the Whickham cohort, there was no relationship between subclinical hypothyroidism, cardiovascular outcome, and lipids after adjustment for age^[85]. In the second study, a cross-sectional cohort study of middle-aged Dutch women, those with subclinical hypothyroidism were approximately twice as likely as euthyroid control women to have calcification of the aorta on a chest X-ray film and a history of myocardial infarction after adjustment for known cardiovascular risk factors^[82]. Remarkably and unexplained, at baseline, women with subclinical hypothyroidism had age-adjusted serum cholesterol levels that were lower than those of the euthyroid control women. In conclusion, overt hypothyroidism has a clear influence on lipids, especially on LDL cholesterol, whereas mild thyroid hormone deficiency may have a limited effect.

Hypertension

Overt hypothyroidism may alter blood pressure, in particular diastolic values^[89,98]. In a study of 169 women with overt hypothyroidism, the prevalence of hypertension was nearly 3 times higher

Table 3. Influence of hypothyroidism on cardiovascular risk factors.

	Effect	Evidence
Lipids	Increased total and LDL cholesterol	A
Blood pressure	Diastolic hypertension	A
Smoking	Impaired thyroid hormone actions	B
Homocysteinemia	Mild increase	A
Endothelium	Endothelial dysfunction	C
CRP	Mild increase	C
Coagulation abnormalities	Different coagulation profile in moderate and severe hypothyroidism	C

Table 4. Subclinical diseases and risk factors.

	Effect	Evidence
<i>Sub. hyper</i>		
AF	Increased incidence (2.8- 5-fold)	Serum TSH <0.1 mU/L: A Serum TSH 0.1-0.45 mU/L: C
<i>Sub. hypo</i>		
Lipids	Mild increased in total and LDL cholesterol	Serum TSH > 10 mU/L: A Serum TSH 4.5-10 mU/L: C
Blood pressure	Mild increase in diastolic pressure	C
Homocysteine	None	B

than in a euthyroid control group (14.8% versus 5.5%)^[89], and euthyroid normotensive patients had an increase in diastolic blood pressure after thyroidectomy-induced hypothyroidism^[99]. In a survey of consecutive hypertensive outpatients, 3.6% were found to be hypothyroid, and in this subset, diastolic blood pressure fell significantly after adequate thyroid replacement therapy^[100]. Regarding subclinical hypothyroidism and hypertension, there is less published evidence. Luboshitzky et al observed in 2 small case-control studies that mean diastolic blood pressure was higher in 57 and 44 women with subclinical hypothyroidism than in euthyroid controls^[101;102]. Potential mechanisms for reversible diastolic and systolic hypertension in hypothyroidism include increases in peripheral vascular resistance^[103] and arterial stiffness^[104]. Vasoconstriction

may reflect the absence of demonstrated vasodilatory T3 effects on vascular smooth muscle^[105] or be the result of a higher circulating noradrenaline level and a decrease in the number of vascular β -adrenergic receptors^[89]. More than half of hypothyroid hypertensive patients display low plasma renin activity^[106]. Low angiotensin levels have also been reported in hypothyroidism^[107], and prohormone natriuretic peptide and atrial natriuretic factor are decreased^[108]. Finally, vasopressin plasma levels are mildly increased and improve after replacement therapy^[109].

Smoking

Synergistic effects between smoking and hypothyroidism have been reported. Smokers with overt hypothyroidism have been shown to have higher serum concentrations of total and LDL cholesterol, higher clinical symptom scores, more prolonged ankle-reflex times, and higher creatine kinase concentrations than nonsmokers with hypothyroidism. These differences were noted despite similar concentrations of TSH, fT4, and triiodothyronine, suggesting that cigarette smoking may impair thyroid hormone action in target tissue^[71;110].

Endothelial Dysfunction

Endothelial dysfunction occurs early in the atherosclerotic process and may be a key initiating event^[111]. Endothelial dysfunction is present in hypothyroid patients, thereby providing an additional link between hypothyroidism and vascular disease^[112]. Nevertheless, its presence in hyperlipemic subjects makes it difficult to discern which abnormality is responsible for the endothelial dysfunction. Recently, 2 studies showed that endothelial function improved with thyroid replacement therapy, in agreement with animal studies suggesting that thyroid hormone exerts part of its vascular effect through an endothelial mediated mechanism^[90;113]. In one study, the improvement occurred without change in medications, blood pressure, serum lipids, homocysteine levels, or high-sensitive C-reactive protein (CRP) levels. In thyrotoxic patients, increased levels of soluble endothelial molecules such as thrombomodulin, endothelin, and adhesion molecules have been observed as well^[114;115].

Homocysteine

Homocysteine is an independent risk factor for cardiovascular disease, premature atherosclerotic vascular disease, and venous thrombosis^[116]. It is still controversial whether mild hyperhomocysteinemia is a causal factor. Plasma homocysteine concentration is controlled by genetic (MTHFR C677T mutation), nutritional (vitamins folate, B12, or B6), and acquired factors (renal function, malignancies, inflammation, and cigarette smoking^[116]). Thyroid hormones act by modulating gene expression and by influencing a multitude of enzyme systems, including several enzymes involved in homocysteine metabolism^[117], thereby modulating homocysteine levels^[118;119]. Other studies indicated that the increase in homocysteine concentrations during hypothyroidism might also be explained by changes in folate status^[120] or by concurrent changes in renal function^[121]. Mildly elevated total plasma homocysteine levels (mean value $<20 \mu\text{mol/L}$) have been reported in patients with overt hypothyroidism and mildly decreased levels in overt hyperthyroidism^[91;120;122]. Plasma fT4 is an independent determinant of homocysteine concentrations. In fact, lower folate levels, lower creatinine clearance in hypothyroidism, and a higher creatinine clearance in hyperthyroidism only partially explain the changes in homocysteine^[122]. A normalization

of hyperhomocysteinemia has been achieved after treatment for hyperthyroidism and for hypothyroidism^[120;123;124]. Clearly, clinical relevance of these alterations in homocysteine levels and the response on L-thyroxine treatment is uncertain. Different from overt hypothyroidism, subclinical hypothyroidism has not been found to be associated with hyperhomocysteinemia, and L-thyroxine supplementation has no influence on homocysteine levels^[101;125].

CRP, Insulin Resistance, and Coagulation Abnormalities

CRP is an acute-phase protein that circulates in higher concentrations in a variety of acute and chronic diseases. Increased concentrations have been measured in overt and subclinical hypothyroid patients compared with controls^[125]. However, CRP levels did not decrease with T4 treatment of subclinically hypothyroid patients^[102]. Insulin resistance or metabolic syndrome is an independent risk factor for cardiovascular disease even in individuals without diabetes. Hypothyroidism does not seem to cause insulin resistance^[126], but LDL cholesterol concentrations were higher in insulin-resistant subjects with high normal TSH levels compared with insulin-sensitive individuals^[127]. Hypothyroidism may have procoagulant and anticoagulant influences. It leads to lower von Willebrand factor levels, a decreased fibrinolytic activity in patients with moderate hypothyroidism, and, conversely, a tendency toward increased fibrinolytic activity in subjects with severe hypothyroidism. These results suggest possible different coagulation profiles in moderate and severe hypothyroidism^[48;128–130].

THYROID DISEASES AND OTHER CEREBROVASCULAR MANIFESTATIONS

We searched specifically for a possible association between thyroid diseases and other types of cerebrovascular disease. We focused especially on cerebral small vessel disease (or white matter lesions), cerebral aneurysms, and subarachnoidal bleeding, but we did not find relevant studies indicating such an association.

CONCLUSION

The main findings on the relationship between thyroid disease and cerebrovascular diseases are summarized in Tables 2, 3, and 4. In subclinical hyperthyroidism with serum TSH levels <0.1 mIU/L, the incidence of AF is increased, and in overt hyperthyroidism, cardioembolic stroke is clearly associated to thyrotoxic AF. There is insufficient evidence to support the concept of an increased cardioembolic risk attributable to a hypercoagulability state. Only in vivo and in vitro studies suggest an increased thrombotic risk in thyrotoxicosis^[48]. Acute CVT is a relatively rare disorder that has been observed in patients with hyperthyroidism. The high rate of case reports suggests that there may be a causal relationship^[36]. Hyperthyroidism has also been associated with Moyamoya and GCA in some case reports. Although these associations are not definitively proven, physicians should be aware of these rare conditions. In hypothyroidism, enough evidence supports the association of several traditional and newer atherosclerotic risk factors with overt disease, such as increased LDL and endothelial

dysfunction. Nonetheless a clear influence on atherosclerotic risk factors, which may lead to cerebrovascular disease, no case-control or cohort studies have ever investigated hypothyroidism as a possible risk factor for atherothrombotic stroke. Nowadays, the usual presentation of hypothyroidism is the subclinical form, and no good evidence exists to demonstrate its association with cardiovascular risk factors. Subclinical hypothyroidism could also be a risk factor itself, but no definite evidence confirms this. Because sound evidence is lacking, well-designed case-control or prospective studies are therefore warranted to confirm the association between atherothrombotic stroke and overt and subclinical hypothyroidism. It is not likely that subclinical hypothyroidism has a very strong influence on stroke risk. To detect an increase of 20% in stroke risk, and accepting a type I error of 0.05 and a power of 0.80, more than 2000 stroke patients and control persons have to be included in a case-control study, given a prevalence of subclinical hypothyroidism of 15% to 20% in the elderly. Even if definitive conclusion cannot be drawn, physicians must be aware of the possible role of hypothyroidism in atherothrombotic cerebrovascular disease as well.

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Chapter 6

Subclinical hypothyroidism and
deep venous thrombosis
A pilot cross-sectional study

Alessandro Squizzato, Erica Romualdi,
Elia Piantanida, Victor EA Gerdes, Harry
R Büller, Maria Tanda, Luigi Bartalena,
Achille Venco, Walter Ageno.

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ABSTRACT

Introduction: Several in-vivo studies have shown a procoagulant state in both overt and subclinical hyperthyroidism and in subclinical hypothyroidism. Insofar, no clinical studies have ever evaluated the relationship between thyroid dysfunction and clinically deep venous thrombosis (DVT). A pilot cross-sectional study aimed at assessing the frequency of overt and subclinical thyroid dysfunction patients with DVT was carried out.

Methods: Fifty consecutive adult outpatients with a previous diagnosis of provoked DVT (pDVT), and 50 consecutive adult outpatients with a previous diagnosis unprovoked DVT (unDVT), both of the lower legs, who were followed at the Thrombosis Unit of the University Hospital of Varese, Italy, were enrolled after written informed consent. Fifty subjects, in whom such a diagnosis could be ruled out, served as controls. In each patient serum free thyroxine (FT4), free triiodothyronine (FT3), thyrotropin (TSH), anti-thyroid peroxidase (AbTPO), and anti-thyroglobulin (AbTg) antibodies were assayed.

Results: Previously unrecognised subclinical hypothyroidism was diagnosed in seven (14.0%) unDVT patients, one (2%) pDVT patient, and one (2%) control (odds ratio at multivariate analysis, 5.54; 95% confidence interval, 0.6–52.6); one new case of subclinical hyperthyroidism was diagnosed in each group; only one case (in the control group) of clinical overt hyperthyroidism was observed. The prevalence of thyroid autoantibodies, including both euthyroid and subclinical hypothyroid patients, did not differ in the three groups.

Conclusions: The results of this pilot study suggest an increased prevalence of subclinical hypothyroidism in patients with unDVT. The clinical relevance of these preliminary findings needs to be addressed in larger prospective studies.

INTRODUCTION

The link between the haemostatic system and thyroid disease has been investigated since the beginning of the last century^[1]. Both thyroid dysfunction and autoimmunity are the advocated pathophysiological mechanisms leading to primary and secondary haemostasis imbalance^[2]. In particular, the effects of thyroid hormone on the coagulation system are mainly mediated by hormone interaction with its receptors^[3]. Several studies investigated *in vivo* the effect of overt and subclinical thyroid dysfunction on blood coagulation and fibrinolytic factors, suggesting that a procoagulant state is present in both overt and subclinical hyperthyroidism and in subclinical hypothyroidism^[2,4–10]. Insofar, no clinical studies evaluating the relationship between thyroid dysfunction and clinically manifest deep venous thrombosis (DVT) have ever been reported. Aim of this pilot cross-sectional study was to explore the prevalence of thyroid dysfunction, both overt and subclinical, in DVT patients.

SUBJECTS AND METHODS

Subjects

In the period from October 2004 to August 2006, 50 consecutive adult outpatients with provoked DVT, and 50 consecutive adult outpatients with unprovoked DVT, referred to the Thrombosis Unit of the Department of Clinical Medicine of the University Hospital of Varese, Italy, were enrolled (Table 1). Most patients with clinically suspected DVT presenting to the general practitioners or to the Department of Emergency Medicine are referred to our Unit. In all patients, diagnosis of DVT of the lower limbs was made by compression ultrasound of the legs using a 3–11 MHz linear probe (Esaote Technos Mpx). Patients were examined in the supine position for the femoral veins and in the sitting position for the popliteal vein and the confluence of deep calf veins. Patients were not routinely screened for concomitant pulmonary embolism and for inherited and acquired thrombophilia. DVT had to be diagnosed no more than two years prior to enrolment. DVT was defined as provoked if one of the following criteria was present: recent surgery (<3 months), recent trauma and/or bone fracture (<3 months), immobilisation (>7 days), pregnancy, puerperium, oral contraceptives, active cancer, known hereditary or acquired thrombophilia, severe medical disease. Already diagnosed concomitant or past thyroid disorders were not considered an exclusion criterion. Drugs and clinical conditions potentially causing non-thyroidal illness syndrome (NTIS) were checked^[11]. Potentially eligible cases were identified by the electronic database of our Thrombosis Unit. During the first visit, patients were informed about the design and purpose of the study, provided written informed consent, and underwent blood testing. Fifty patients referred to our Thrombosis Unit from general practitioners or the emergency department for a suspicion of deep or superficial vein thrombosis, in whom this diagnosis could be excluded by objective methods, were enrolled as a control group if they had no history of deep and superficial venous thrombosis (Table 1). All controls signed an informed consent form. The local ethical committee approved the study protocol.

Assays

Serum free thyroxine (FT4; FT4 RIA, Brahms, Germany), free triiodothyronine (FT3; FT3 SPART RIA, Brahms), thyrotropin (TSH; TSH-CTK-3, DiaSorin, Italy), anti-thyroid peroxidase (AbTPO; DYNOtest® anti-TPOn, Brahms), anti-thyroglobulin (AbTg; anti-Tgn RIA, Brahms) were assayed by commercial methods. Reference values in our laboratory are as follows: FT4, 0.75 – 1.90 ng/dl; FT3, 1.50 – 5.30 pg/ml; TSH, 0.31 – 4.50 mU/l; AbTPO, <60 U/ml; AbTG, <60 U/ml.

Definition of thyroid status

Hyperthyroidism was biochemically defined by reduced/suppressed serum TSH concentration in the presence of normal (subclinical hyperthyroidism) or increased (overt hyperthyroidism) serum FT4 and/or FT3 concentrations^[12;13]. Hypothyroidism was biochemically defined by increased serum TSH concentration in the presence of normal (subclinical hypothyroidism) or increased (overt hypothyroidism) serum FT4 and/or FT3 concentrations^[11;13].

Statistical analysis

Because the prevalence of thyroid dysfunction in DVT patients was unknown, we planned a pilot study enrolling 50 unprovoked DVT patients, 50 provoked DVT patients, and 50 controls. Differences were tested by Chi2-test (or Fisher's test) for proportions. For continuous variables, normal distribution was explored with Kolmogorov-Smirnov and Shapiro-Wilk tests. If normally distributed, the Student's t-test for mean value was applied; if not, the non-parametric Wilcoxon-Mann-Whitney test was used. A multivariate binary logistic regression was performed, and odds ratio (OR) and 95% confidence interval (CI) were reported. A two-sided P-value of less than 0.05 was required to consider the findings as statistically significant. Statistical analyses were performed with statistical package SPSS 12.0.1 (Chicago, IL, USA).

RESULTS

As shown in Table 1, unprovoked DVT patients were significantly older than provoked DVT patients, but there was no significant age difference with the control group; in addition there was a higher prevalence of women among controls than in the unprovoked DVT group (Table 1). Time from diagnosis of DVT did not differ between unprovoked DVT and provoked DVT groups (Table 1). Table 2 illustrates the transient (64%) or permanent (36%) risk factors identified in pDVT patients. Median serum FT4 and FT3 concentrations did not differ between the three groups, whereas median serum TSH concentration was significantly higher in unprovoked DVT and provoked DVT than in controls (Table 3). Few patients (5 in unprovoked DVT, 3 in provoked DVT, 4 in controls) had known thyroid disorders at the time of enrolment: one unprovoked DVT patient with subclinical hyperthyroidism and two provoked DVT patients with subclinical hypothyroidism were still not corrected by appropriate treatment. Thyroid dysfunction was newly diagnosed in eight unprovoked DVT patients, two provoked DVT patients, and three controls. One patient in each group had subclinical hyperthyroidism, due to nonautoimmune subclinical hyperthyroidism or to excess L-thyroxine replacement for hypothyroid chronic autoimmune (Hashimoto's) thyroiditis. In the control group, one patient had autoimmune overt hyperthyroidism. In the remaining patients with thyroid dysfunction (7 unprovoked

Table 1. Clinical features of study groups

	Unprovoked DVT	Provoked DVT	Control group
Enrolled patients (N)	50	50	50
Mean ($\pm$ SD) age (yr)	69 $\pm$ 14 ^b	57 $\pm$ 16 ^{b,c}	63 $\pm$ 17 ^c
Men (N)	27 ^b	20	12 ^b
Women (N)	23 ^b	30	38 ^b
Concomitant PE (N)	13 ^c	4 ^c	-
Recurrent episode (N)	2	5	-
Distal DVT (N)	3	5	-
Time from DVT diagnosis (months) ^a			
- Median	9.5	6	
- Interquartile range	13	13	
Drugs/conditions causing NTIS (N)			
- Corticosteroids	2	4	1
- Methotrexate	1	0	1
- Chronic renal insufficiency	0	1	0
- Alcohol abuse	0	0	1
- Thrombocytaemia	0	0	1
- Diabetes mellitus type 2	1	0	0
- COPD	0	1	3
- Chronic hepatic C virus infection	0	0	2

N indicates number; yr, years; DVT, deep venous thrombosis; PE, pulmonary embolism; NTIS, non-thyroidal illness syndrome; and COPD, chronic obstructive pulmonary disease;

^aIn a patient in the unprovoked group and in two patients in the provoked group, time from diagnosis was 27 months.

^bP<0.01

^cP<0.05.

DVT, 1 provoked DVT, 1 control), this was represented by had subclinical hypothyroidism (Fig. 1); while thyroid autoantibodies were positive in the provoked DVT patient and the control subject, thyroid autoantibody tests were positive only in two out of seven unprovoked DVT patients. Thyroid ultrasound was not carried out in this pilot study to verify the likely possibility of thyroid autoantibody-negative Hashimoto's thyroiditis, but other causes of

spontaneous hypothyroidism, including drugs (lithium, amiodarone, interferon- α , iodine load) were ruled out. No DVT patient had either overt hyperthyroidism or overt hypothyroidism. By univariate binary logistic regression, newly diagnosed subclinical hypothyroidism was associated with unprovoked DVT, as compared with provoked DVT or controls (OR, 7.97; 95% CI, 0.9–67.5); this association persisted after multivariate analysis, considering age, gender, and time from DVT diagnosis (OR, 5.54; 95% CI, 0.6–52.6 vs. provoked DVT; OR, 6.8; 95% CI, 0.7–64.5 vs. controls). Euthyroid Hashimoto's thyroiditis, as assessed by positive AbTPO and/or AbTg tests, was newly diagnosed in four unprovoked DVT, seven provoked DVT, and 10 control subjects (Fig. 1). These differences did not reach statistical significance. As a whole, Hashimoto's thyroiditis, associated with either euthyroidism or subclinical hypothyroidism, was found in six unprovoked DVT, eight provoked DVT, and 11 control subjects (Fig. 1), with no differences among groups.

Table 2. Causes of provoked deep vein thrombosis

Transient risk factors^a (%)	34 (64)
Recent surgery (%)	6 (17.6)
Trauma and/or bone fracture (%)	7 (20.6)
Immobilisation (%)	13 (38.2)
Pregnancy/puerperium (%)	0
Oral contraceptives (%)	11 (32.4)
Severe medical disease (%)	4 (11.8)
Permanent risk factors (%)	16 (32)
Active cancer (%)	14 (87.5)
Inherited thrombophilia (%)	3 (18.8)
- Factor II G20210 – hyperhomocysteinemia	1
- MTHFR homozygous	1 ^b
- Protein S deficiency – factor V Leiden – MTHFR heterozygous	1

^aSeven patients had two concomitant transient risk factors

^bThis patient has also an active cancer

Table 3. Serum thyroid hormone and TSH concentrations in the study groups

	Unprovoked DVT	Provoked DVT	Control group
<i>TSH, mU/L ^a</i>			
median	2.13 ^b	2.04 ^c	1.61 ^{b,c}
interquartile range	1.17-2.99	1.44-2.96	0.99-2.18
<i>FT3, pg/mL</i>			
median	3.20	3.16	3.05
interquartile range	2.92-3.58	2.95-3.60	2.74-3.52
<i>FT4, ng/dL</i>			
median	1.20	1.18	1.20
interquartile range	1.11-1.35	1.05-1.34	1.06-1.42

DVT indicates deep venous thrombosis; TSH, thyrotropin; FT3, free triiodothyronine; and FT4, free thyroxine.
^aAn outlier TSH value (91.38 µU/mL) of a patient in the control group was excluded from analysis
^bP=0.05
^cP<0.05;

Figure 1: Subjects with subclinical hypothyroidism (Sub Hypo, black columns) or positive thyroid autoantibody tests (TAB +ve, grey columns), both hypo- or euthyroid. unDVT: unprovoked deep venous thrombosis; pDVT: provoked deep venous thrombosis; Con: controls

DISCUSSION

Studies *in vivo* and case reports indicated that hyperthyroidism is associated with a hypercoagulable state. Sinus and cerebral vein thrombosis occurring, in particular, during severe hyperthyroidism^[1], and coagulation abnormalities, such as an increased activity of plasma FVIII, and FIX, and a reduced fibrinolytic activity (low tissue plasminogen activator, t-PA, levels)^[7,14] in hyperthyroid patients, suggest a potential increase in the risk of venous thromboembolic events in clinically overt hyperthyroidism^[9]. On the other hand, mild mucocutaneous bleeding (epistaxis, gum bleeding, menorrhage, bruising) is frequently observed in overt hypothyroid patients, mainly due to an acquired type I von Willebrand syndrome. However, recent studies postulated that also subclinical hypothyroidism might be associated with a hypercoagulable state. Muller et al. showed an increase in factor VII activity in patients with subclinical hypothyroidism^[10]. Chadaverian et al. reported that, while patients with overt hypothyroidism have low t-PA, low plasminogen activator inhibitor (PAI-1), and high D-dimer levels indicating an increased fibrinolytic activity, in patients with subclinical hypothyroidism a decreased fibrinolytic activity is present, associated with high t-PA, high PAI-1, and low D-dimer levels^[5,6].

No studies with clinical outcomes have ever tested these two hypotheses. To address this issue, the present pilot cross-sectional study was carried out, aimed at exploring the association between DVT and thyroid dysfunction. Our results showed an increased prevalence of newly diagnosed subclinical hypothyroidism in unprovoked DVT. Although differences with provoked DVT and control groups did not reach statistical significance, likely owing to the relatively low number of enrolled patients, by multivariate analysis OR was 5.54 (95% CI, 0.6–52.6), as compared with provoked DVT group, and 6.8 (95% CI, 0.7–64.5), as compared with controls. No difference among groups was detected as to the prevalence of newly diagnosed overt thyroid dysfunction or subclinical hyperthyroidism. Interestingly enough, there was no difference in the prevalence of newly diagnosed thyroid autoimmunity in the different groups when euthyroid subjects with positive thyroid autoantibody tests were considered. This suggests that thyroid dysfunction rather than thyroid autoimmunity is associated with unprovoked DVT. Needless to say, a pilot study, such as the present one, is only explorative by definition. However, our data lend support to the hypothesis of a hypercoagulable state in subclinical hypothyroidism. The latter condition may affect cardiovascular risk factors, and, although this is still controversial, it has been suggested that it might increase the risk of myocardial infarction just like overt hypothyroidism, in which coronary artery atherosclerosis is two-fold increased compared to euthyroidism^[4]. Indeed, several studies in subclinical hypothyroid patients supported the idea of an increased risk of *arterial* thrombosis in this condition^[2,5,6,8,10]. The results of the present provide the first evidence that subclinical hypothyroidism may also be a potential risk factor for *venous* thromboembolism.

This study has some limitations that need to be considered. Because of the time lag from DVT diagnosis to assessment of thyroid function, transient thyroid dysfunction might have been missed. Moreover, thyroid dysfunction might have developed after DVT diagnosis. However, should any thyroid dysfunction have been missed, this would have biased both unprovoked

and provoked DVT groups and thus just the correct estimate of the frequency of association. In addition, time lag was considered in the multivariate analysis. Finally, provoked DVT and control groups had a very similar detected frequency of old and new diagnoses of thyroid dysfunction, thus suggesting a possible mild effect of time bias. Age and gender in the control group were significantly different from provoked and unprovoked groups. Thyroid diseases are more frequent in older age and in females^[11;12]. However, multivariate analysis took into account these differences, limiting their bias effect. Moreover, control patients were not consecutively selected. But, as previously reported, the similar frequencies of detected outcomes in provoked DVT and control group suggest a mild influence. The lack of differences with regard to TSH levels between patients with unprovoked DVT and provoked DVT has several reasons: TSH values were not normally distributed, TSH values were < 10 UI mU/l in subclinical hypothyroid patients, TSH reference range is relatively large, and the absolute number of new diagnosis of subclinical hypothyroidism is low.

In conclusion, our data suggest an increased frequency of subclinical hypothyroidism in patients with unprovoked DVT. The clinical relevance of our preliminary findings needs to be addressed in larger case-control or cohort studies.

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Chapter 7

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

Bregje van Zaane, Alessandro Squizzato,
Roeland Huijgen, Anton P van Zanten,
Eric Fliers, Suzanne C Cannegieter,
Harry R Büller, Victor EA Gerdes,
Dees PM Brandjes

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ABSTRACT

Introduction: There is a hypercoagulable state in hyperthyroidism, but the association with venous thrombosis (VT) is not fully explored. We aimed to investigate VT risk for different plasma levels of thyroid hormones and thyroid antibodies.

Methods: We used a case-control study on leg vein thrombosis conducted between September 1999 and August 2006 at the Academic Medical Centre, Amsterdam, the Netherlands. Parameters of thyroid function were assessed in 190 cases (mean age 57 years, range 19-90) and 379 gender-matched controls (mean age 56 years, range 18-93). Odds ratios (ORs) and 95% confidence intervals (CIs) for VT risk were estimated according to several cut-off levels derived from plasma levels observed in controls.

Results: We found the risk of venous thrombosis to gradually rise with increasing levels of free thyroxine (FT4). In the absence of traditional acquired risk factors, FT4 levels above 17 pmol/L yielded a gender- and age-adjusted OR of 2.2 (95% CI 1.2-4.2) for deep venous thrombosis, which further increased up to an OR of 13.0 (95% CI 1.1-154.1) for FT4 levels above reference range.

Conclusions: Our data suggest increasing levels of free thyroxine to be a risk factor for venous thrombosis and may have implications for both the prevention and management of this disease.

INTRODUCTION

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

Hyperthyroidism has been associated with a hypercoagulable state, and is thus hypothesised to increase the risk of venous thrombosis^[5]. Although there have been several reports on sinus, cerebral or deep venous thrombosis following thyrotoxicosis, the relation between thyroid function and the risk of venous thrombosis is not fully explored^[6-10]. While high concentrations of factor VIII and von Willebrand factor contribute to a hypercoagulable state in overt hyperthyroidism, lower von Willebrand factor concentrations found in overt hypothyroidism may, at least in part, protect against venous thrombosis^[5;11]. Regarding these alterations in coagulation factors, similar findings have been described for subclinical thyroid disease^[9]. Subclinical thyroid disease has also been linked with arterial vascular disease and there are good indications that variations in thyroid hormone levels within the physiological range can modify the function of several organs^[12-15]. Therefore, we hypothesised that increasing levels of thyroid hormone may be a risk factor for venous thrombosis.

In a case-control design, we aimed to clarify the associations between different plasma levels of free thyroxine (FT4), thyrotropin (TSH), thyroid peroxidase antibodies (antiTPO), and the presence of venous thrombosis. Since acute illness such as venous thrombosis may in itself affect thyroid hormone concentrations by altered protein binding or by inhibition of T4-to-T3 conversion, tri-iodothyronine (T3) levels were subsequently analysed to explore whether our findings were influenced by this so-called non-thyroidal illness syndrome (NTIS).

PATIENTS, MATERIALS AND METHODS

Study population

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

The study was approved by the institutional review board, and all patients provided written informed consent.

Data collection and diagnosis of venous thrombosis

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

All patients underwent routine work-up for diagnosis of DVT according to an algorithmic management strategy combining clinical pretest probability and D-dimer testing (Tinaquant, Roche Diagnostics, Basel, Switzerland), followed by compression ultrasound (CUS) of the lower extremities if indicated^[16;17]. Failure to fully collapse the lumen of the deep or superficial veins during compression testing was the main criterion for the presence of venous thrombosis, including DVT, calf vein thrombosis and superficial thrombophlebitis. DVT was defined as proximal thrombosis of the iliac or superficial femoral vein, or thrombosis of at least the upper third part of the deep calf veins. Thrombosis was considered provoked if at least one of the following criteria was present: use of oestrogen- or progesterone containing agents, malignancy, pregnancy or puerperium, paralysis of the symptomatic leg, recent trauma (within last 60 days), surgery within the last 4 weeks or bedridden for more than 3 days, hospitalisation within the previous 6 months, or long distance travel (6 hours or more) within the previous 3 months. In the absence of these acquired risk factors, venous thrombosis was considered unprovoked. Six patients with a combination of high pretest probability and a positive D-dimer assay, but normal initial ultrasonographic findings, failed to return for repeated CUS after 1 week. In total, 211 patients were diagnosed with venous thrombosis (cases), whereas in 537 patients diagnosis of venous thrombosis was objectively ruled out using the above-mentioned diagnostic management strategy (controls) (Figure 1).

Laboratory assay of thyroid function

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

Levels of FT4, T3, TSH and antiTPO were assessed in citrated plasma using commercially available assays (ADVIA Centaur® immunoassay system, Siemens Healthcare Diagnostics, Marburg, Germany). The intra- and interassay coefficients of variations (CVs) were below or equal to 4.7% and 4.6% for FT4, 3.2% and 1.3% for T3, 9.0% and 4.4% for TSH, and 4.1% and 8.0% for antiTPO, respectively. As these tests have not been validated by the manufacturer for use with citrated plasma, serum/citrated plasma studies were performed to characterise the correlation. Results in plasma were corrected for dilution with citrate. Based on the analysis of at least 21 samples, small systematic differences between serum and citrated plasma were observed, but linear regression analysis showed a significant association between serum and plasma levels of thyroid hormones (regression coefficient $\beta \geq 0.92$; $P < 0.01$).

Overt and subclinical primary hyperthyroidism were biochemically defined as TSH below the lower limit of the local reference range (0.32-4.32 mU/L) combined with a FT4 concentration above or within the local reference range (10-24 pmol/L), respectively^[18;19].

Figure 1. Flow chart of selection procedure and analysis.

CUS indicates compression ultrasound; DVT, deep venous thrombosis; and VT, venous thrombosis.

Primary hypothyroidism, overt and subclinical, was biochemically defined as elevated TSH and a FT4 concentration below or within the local reference range, respectively^[19,20]. Thyroid peroxidase antibodies above 80 U/mL were considered elevated.

Statistical analysis

In one plasma sample, thyroid hormones could not be assessed due to insufficient volume. After exclusion of this control subject, FT4, TSH and antiTPO levels of 190 cases and 379 controls were used for final analysis (Figure 1). Since levels of T3 were analysed only after the initial measurements of FT4, TSH and antiTPO had been performed, T3 levels were available for 186 cases and 375 control subjects; in 8 plasma samples the volume was insufficient for this additional measurement. Subjects under thyroid medication or with known thyroid dysfunction were not excluded from the analyses.

Categorical variables measured in this study were expressed as number and percent. FT4 and T3 levels were normally distributed in both cases and controls and were presented as means (95% confidence interval). Distributions of TSH and antiTPO levels were skewed and therefore presented as medians (95% confidence interval). Between-group comparisons were performed using t-tests or non-parametric tests, depending on the distribution of the data. Odds ratios and 95% confidence intervals for the risk of venous thrombosis at different levels of FT4, TSH and antiTPO were calculated using binary logistic regression, taking different percentiles of the values observed in the control subjects as cut-off levels. For each cut-off level below the

50th percentile, we compared subjects below the cut-off to subjects above this level using the latter as reference. Vice-versa for cut-off levels above the 50th percentile. Subsequently, the same analyses were performed for levels of T3, but mostly to support any findings on FT4 levels. A multivariate model was used to adjust for gender to take the frequency matching on gender into account, and for age as a possible confounding factor. In the analysis for TSH, we additionally adjusted for FT4 to explore the causal relation. Separate analysis was performed for patients with deep venous thrombosis, excluding those with calf vein thrombosis or superficial thrombophlebitis. Chi-square tests, or Fisher's exact test in case cells had a count less than five, were employed to compare the two study groups with respect to the presence of thyroid abnormalities in comparison with euthyroidism. Statistical analysis was performed with the use of SPSS 15.0 software package (SPSS Inc, Chicago, IL, USA).

RESULTS

Patient characteristics

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

At inclusion, 5 patients with provoked DVT, 1 with thrombophlebitis and 7 control subjects were on thyroxine substitution therapy for previously diagnosed hypothyroidism. Only 1 patient, diagnosed with thrombophlebitis, was on thiamazole treatment for known hyperthyroidism. All patients with known hypo- or hyperthyroidism had plasma FT4 levels within the reference range combined with either TSH levels within reference range, or marginally decreased or marginally elevated TSH levels at the time of inclusion. Baseline characteristics for cases and controls are summarised in Table 1.

FT4 and risk of venous thrombosis

Mean FT4 in all patients with venous thrombosis was 16.0 pmol/L (95% CI 15.6-16.4), in patients with DVT 16.2 pmol/L (95% CI 15.8-16.7) and in control subjects 15.4 pmol/L (95% CI 15.1-15.6) (Table 2). We found odds ratios for each cut-off level that clearly increased with the percentiles used (adjusted for the matching factor gender). These odds ratios were below unity for cut-off levels below the 50th percentile (i.e. indicating a protective effect of lower FT4 levels) but above unity for higher cut-off levels (i.e. indicating an increased risk for higher FT4 levels). Further adjustment for age slightly reduced the crude odds ratios. Similar results were found when we analysed only patients with DVT of the leg. In the absence of traditional acquired risk factors for venous thrombosis, FT4 above reference range (>24 pmol/L) yielded an odds ratio of 9.6 (95% CI 0.8-109.1) for VT and 13.0 (95% CI 1.1-154.1) for DVT, adjusted for gender and age (Figure 2 and Table 3). Mean T3 levels were higher in patients with venous thrombosis (1.94 nmol/L, 95% CI 1.87-2.00) than in control subjects (1.79 nmol/L, 95% CI 1.75-1.82), indicating that the association between higher FT4 and venous thrombosis as found in the present study is very unlikely to

reflect the non-thyroidal illness syndrome (NTIS) as low serum T3 is the hallmark of NTIS (Table 2). Similar to FT4, we found the risk of venous thrombosis to gradually rise with increasing levels of T3, although the relation was slightly less linear (Figure 2).

TSH and risk of venous thrombosis

Median TSH in all patients with venous thrombosis was 1.41 mU/L (95% CI 1.22-1.51), in those with DVT 1.37 mU/L (95% CI 1.17-1.51) and in control subjects 1.21 mU/L (95% CI 1.11-1.30) (Table 2). TSH levels were not associated with the overall risk of venous thrombosis. The risk of unprovoked DVT was almost 3-fold increased for TSH below 0.02 mU/L (OR 2.9; 95% CI 0.7-12.0), but returned to unity after adjustment for FT4 (OR 0.9; 95% CI 0.2-5.2) (data not shown).

AntiTPO and risk of venous thrombosis

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

Thyroid function at the time of thrombotic event

Three patients with DVT had thyroid hormone concentrations consistent with primary hyperthyroidism (1.6% of all patients with venous thrombosis; 1.9% of DVT patients only), whereas this was not observed in any of the control subjects. Statistical analysis confirmed that hyperthyroidism and (deep) venous thrombosis co-occurred more frequently than expected by chance ($p=0.04$, all patients with venous thrombosis; $p=0.02$, DVT patients only, two-sided Fisher's exact test). No such association was found for subclinical hyperthyroidism, nor for overt or subclinical primary hypothyroidism (Table 2).

All three patients with biochemical hyperthyroidism appeared to have clinically manifest hyperthyroidism during follow-up that was diagnosed 2, 54 and 65 months after presentation for DVT, respectively. For a description of these patients, see Appendix 1.

Figure 2. Risk of venous thrombosis for different levels of FT4 and T3 (adjusted for gender and age). FT4 indicates free thyroxine; T3, tri-iodothyronine; and OR, odds ratio. Reference range for FT4 10-24 pmol/L; reference range for T3 1.2-2.8 nmol/L.

Table 1. Patient characteristics.

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

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

^aAll patients with venous thrombosis (deep venous thrombosis, calfvein thrombosis or thrombophlebitis).

Table 2. Thyroid function.

	All cases ^a (n=190) A	DVT only (n=155) B	Controls (n=379) C	p-value (2-sided) A vs C	p-value (2-sided) B vs C
FT4, pmol/L, mean (95% CI)	16.0 (15.6-16.4)	16.2 (15.8-16.7)	15.4 (15.1-15.6)	<0.01	<0.01
T3, nmol/L, mean (95% CI) ^b	1.94 (1.87-2.00)	1.90 (1.83-1.97)	1.79 (1.75-1.82)	<0.01	<0.01
TSH, mU/L, median (95% CI)	1.41 (1.22-1.51)	1.37 (1.17-1.51)	1.21 (1.11-1.30)	0.03	0.09
AntiTPO, U/mL, median (95% CI)	29.0 (27.3-30.8)	28.9 (27.2-30.7)	29.8 (29.0-30.7)	0.48	0.47
Hyperthyroidism, n (%)	3 (1.6)	3 (1.9)	0 (0)	0.04 ^c	0.02 ^c
Sub. Hyperthyroidism, n (%)	9 (4.7)	7 (4.5)	18 (4.7)	0.96 ^c	0.97 ^c
Euthyroidism, n (%)	170 (89.5)	138 (89.0)	348 (91.8)	NA	NA
Sub. Hypothyroidism, n (%)	8 (4.2)	7 (4.5)	12 (3.2)	0.50 ^c	0.43 ^c
Hypothyroidism, n (%)	0 (0.0)	0 (0.0)	1 (0.3)	1.00 ^c	1.00 ^c

N indicates number; DVT, deep venous thrombosis; FT4, free thyroxine; CI, confidence interval; T3, tri-iodothyronine; TSH, thyrotropin; antiTPO, thyroid peroxidase antibodies; sub, subclinical, and NA, not applicable.

^aAll patients with venous thrombosis (deep venous thrombosis, calfvein thrombosis or thrombophlebitis).

^bMean T3 levels involved 186 patients with venous thrombosis, of whom 151 patients were diagnosed with deep venous thrombosis, and 375 control subjects.

^cIn comparison with euthyroidism.

DISCUSSION

Using laboratory assessment of thyroid function in 190 patients with confirmed venous thrombosis of the lower extremities and 379 control subjects, we were able to show a clear gradual relation between plasma FT4 levels and the risk of venous thrombosis. Notably, the thrombotic risk was substantially increased for FT4 levels well within the physiological range. Moreover, FT4 levels were particularly associated with the risk of unprovoked DVT, indicating FT4 as a potential novel risk factor. Similar to FT4, the risk of venous thrombosis also increased with higher levels of T3, but the relation was slightly less linear. No clear association was found for TSH or antiTPO.

The present analysis is to our knowledge the first to study the effect of increasing levels of thyroid hormones on the risk of venous thrombosis. Over the last decades we have witnessed

Table 3. Risk of venous thrombosis for different FT4 levels (adjusted for gender and age).

		Controls (n=379)				VT ^c (n=190)				DVT (n=155)				Unprovoked VT (n=69)				Unprovoked DVT (n=51)			
FT4 ^a (pmol/L)	Percentile ^b	n		n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)				
<10	LoRef	2		0	NA	0	NA	0	NA	0	NA	0	NA	0	NA	0	NA				
<11	1	3		0	NA	0	NA	0	NA	0	NA	0	NA	0	NA						
<12	5	16		1	0.1 (0.0-0.9)	1	0.2 (0.0-1.2)	1	0.4 (0.0-2.7)	1	0.5 (0.1-4.0)										
<13	10	36		9	0.5 (0.2-1.0)	6	0.4 (0.2-1.0)	5	0.8 (0.3-2.2)	2	0.4 (0.1-1.9)										
<14	30	85		36	0.8 (0.5-1.3)	28	0.8 (0.5-1.3)	14	1.0 (0.5-1.8)	8	0.8 (0.3-1.7)										
>16	60	121		76	1.4 (1.0-2.0)	69	1.7 (1.1-2.4)	25	1.2 (0.7-2.0)	23	1.6 (0.9-3.0)										
>17	80	68		46	1.4 (0.9-2.2)	41	1.6 (1.0-2.5)	18	1.5 (0.8-2.7)	18	2.2 (1.2-4.2)										
>18	90	35		28	1.7 (1.0-2.9)	27	2.0 (1.1-3.4)	13	2.0 (1.0-4.1)	13	2.8 (1.4-6.0)										
>19	95	12		16	2.8 (1.3-6.1)	15	3.1 (1.4-6.9)	9	3.9 (1.6-9.9)	9	5.4 (2.1-13.8)										
>20	97.5	6		9	3.1 (1.1-8.8)	9	3.7 (1.3-10.6)	5	4.2 (1.2-14.5)	5	5.8 (1.7-20.3)										
>22	99	3		7	4.7 (1.2-18.6)	7	5.7 (1.5-22.5)	5	8.9 (2.0-38.5)	5	12.5 (2.8-55.4)										
>24	UpRef	1		3	5.9 (0.6-57.3)	3	6.9 (0.7-67.7)	2	9.6 (0.8-109.1)	2	13.0 (1.1-154.1)										

N indicates number; VT, venous thrombosis; DVT, deep venous thrombosis; FT4, free thyroxine; OR, odds ratio; CI, confidence interval; LoRef, lower limit of reference range; NA, not applicable; and UpRef, upper limit of reference range.

^aReference range 10-24 pmol/L.

^bAnalysis was performed for the 1st, 2.5th, 5th, 10th, 20th, 30th, 40th, 50th, 60th, 70th, 80th, 90th, 95th, 97.5th and 99th percentile, as well as for FT4 levels below or above reference range. Due to laboratory reportage of FT4 results in round figures, the 1st percentile corresponded with the 2.5th percentile, the 10th percentile with the 20th percentile, the 50th percentile with the 60th percentile, and the 70th percentile with the 80th percentile. For each cut-off level below the 50th percentile we compared subjects below the cut-off to subjects above this level, thus setting the latter as reference. Vice versa for each cut-off level above the 50th percentile.

^cDeep venous thrombosis, calfvein thrombosis or thrombophlebitis.

a multiplicity of genetic or acquired risk factors for venous thrombosis to be identified. The estimated magnitude of each varies widely. To illustrate, thrombosis risk following surgery is 6- to 15-fold increased; hospitalisation is associated with an 8- to 11-fold increased risk, whereas in pregnancy the VT risk is 1- to 5-fold increased, in puerperium 14- to 60-fold, in users of oral contraceptives 1.4- to 5-fold, and in heterozygotes of factor V Leiden 3- to 8-fold^[21-23]. Generally, newly identified genetic or acquired risk factors tend to confer less thrombotic risk than the well-established ones. Although confirmation is required, the here reported risk of increasing FT4 levels, varying from 1.7- to 13-fold, appears to be of a similar magnitude.

It is likely that the risk of venous thrombosis associated with FT4 levels reflects thyroid hormone-induced alterations in factor VIII synthesis and secretion, high concentrations of which are an independent risk factor for venous thrombosis, and possibly other procoagulant yet unknown changes^[24]. Indeed, our findings corroborate clinical data suggesting both high concentrations of factor VIII in thyroid hormone excess and low factor VIII concentrations in overt hypothyroidism^[5;11]. However, the reduced risk of venous thrombosis for lower FT4 levels, yet still within the physiological range, is in contrast with some studies, including one of our own, that suggest the hypercoagulable state to extend into subclinical hypothyroidism^[25-27]. In a previous pilot study, we found an increased frequency of subclinical hypothyroidism in patients with unprovoked DVT (OR 6.8; 95% CI 0.7-64.5). The different outcome is best explained by both the limited sample size and the fact that, in this pilot study, thyroid function was assessed only several months after the thrombotic event^[27]. Although, in the present study, we did not observe any association between biochemical diagnosis of subclinical thyroid disease and thrombotic risk, it is now clearly demonstrated that FT4 levels and risk of venous thrombosis constitute a continuum.

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

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

Several potential limitations should be addressed. First, the present analysis was limited to a population suspected of deep venous thrombosis. As mentioned, acute or chronic disease

is known to affect thyroid function, and multiple alterations in thyroid hormone levels have been observed in patients with this non-thyroidal illness syndrome^[32]. The most common and earliest change is inhibition of T4-to-T3 conversion, with a resulting decrease in the circulating T3 level. In general, levels of FT4 are usually less affected by NTIS than T3, but values may be higher than normal in mild or moderate forms of the syndrome^[32,33]. T4 is present in the circulation either free or bound to thyroxine-binding globulin, thyroxine-binding prealbumin or albumin. As such, changes in protein binding are likely to decrease the total amount of T4 (free and bound), whereas levels of the unbound hormone may increase. If, in the present study, decreased protein binding was more present in the patients with venous thrombosis than in control subjects, this would have resulted in higher FT4 levels in cases compared to controls, and thus to an overestimation of our effect measures. However, to discriminate between NTIS (low T3) or a direct association between high FT4 (and T3) and the risk of venous thrombosis, we subsequently performed additional assessment of T3 levels. Although a certain degree of NTIS was present in some patients with venous thrombosis, or in some control subjects (e.g. those with erysipelas), the higher T3 levels in cases compared to controls, as well as the clear association between T3 and venous thrombosis, make it highly unlikely that our findings are solely a reflection of NTIS. Nonetheless, prospective epidemiological studies are needed to further confirm our findings.

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

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

In conclusion, our data suggest that increasing levels of FT4 are a risk factor for venous thrombosis, whereas lower FT4 levels are protective of venous thrombosis. Future studies are needed to further broaden our knowledge on this issue and to evaluate whether implementation of this novel risk factor in daily practice can improve our ability to prevent and manage venous thrombosis in terms of reducing its risks. In particular, epidemiological studies in which thyroid hormone levels are measured before or after the thrombotic event may provide definitive answers on the influence of NTIS. Furthermore, it would be interesting to investigate whether VT patients with higher FT4 levels also have an increased risk of recurrent thrombosis, or, vice versa, whether subjects who have been treated for hyperthyroidism have a lower risk. In addition, further insights in the clinical relevance of our findings could be obtained by assessing the relation between thyroid hormone levels and venous thrombosis in high risk patients, such as those undergoing surgery.

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APPENDIX 1.

DESCRIPTION OF PATIENTS WITH BIOCHEMICAL HYPERTHYROIDISM.

The first patient with undetected biochemical hyperthyroidism (FT4 32 pmol/L, TSH <0.02 mU/L, and antiTPO >1300 U/mL), a woman of age 83, was diagnosed with an unprovoked DVT of the left leg. Screening for inherited or acquired thrombophilia revealed a heterozygous factor V Leiden mutation. Two months after the thrombotic event, the patient was referred to the department of internal medicine for excessive sweating, nocturnal unrest and rapid aggravation of pre-existing dementia. Physical examination showed a multinodular goiter with left-sided enlargement of the thyroid gland. Subsequent laboratory testing revealed a serum FT4 of 26 pmol/L and a suppressed TSH (<0.02 mU/L). She was initially treated with methimazole alone and later received radioactive iodide therapy for a toxic multinodular goiter.

The second hyperthyroid patient (FT4 26 pmol/L, TSH 0.13 mU/L, antiTPO 28.3 U/mL) was a 76 year old woman with severe disabling Parkinson's disease, who had experienced a DVT following trauma of the symptomatic left leg. Positive family history suggested the presence of thrombophilia, but besides a heterozygous factor V Leiden mutation no other thrombophilic factors were found. She was treated with anticoagulant therapy for three months. Follow-up was uneventful. Yet, five years later she returned with a recurrent DVT of the right leg. Another five months later, routine laboratory testing of thyroid function revealed a FT4 of 30 pmol/L with a TSH of 0.03 mU/L. Unfortunately, we were unable to retrieve further data on underlying cause, treatment, or follow-up.

The third patient with undetected hyperthyroidism (FT4 26 pmol/L, TSH 0.02 mU/L, and antiTPO 29.3 U/mL) was a man of 75 years old, diagnosed with an unprovoked DVT of the right popliteal vein continuing in the common femoral vein. Laboratory screening for acquired or inherited thrombophilia showed no abnormalities. Unfortunately, routine measurement of thyroid function was not performed until 4.5 years later, only after the patient presented with an acute severe occlusion of the left axillary artery evoked by cardiac embolism and atrial fibrillation (FT4 47.5 pmol/L, TSH <0.01 mU/L). The patient underwent thrombectomy of the left arm from 5 centimeters below the clavicle until wrist level. He was later diagnosed with toxic multinodular goiter and treated with methimazole.

Chapter 8

Thyroid disease, antithyroid or
thyreomimetic agents, and the
risk of pulmonary embolism

Bregje van Zaane, Alessandro Squizzato,
Sara Biere-Rafi, Patrick C Souverein, Dees PM
Brandjes, Harry R Büller, Victor EA Gerdes,
Anton de Boer

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ABSTRACT

Introduction: There are indications that thyroid disease is associated with the risk of venous thromboembolism. We therefore aimed to evaluate the association between pulmonary embolism (PE) and the start of treatment for thyroid disease.

Methods: A nested case-control study was conducted using the PHARMO Record Linkage System, a Dutch population-based pharmacy registry. Cases were patients hospitalised for PE and the date of hospitalisation was set as index date. Controls were gender- and age-matched subjects without a history of PE prior to this index date. Odds ratios (ORs) and their 95% confidence intervals (95% CI) were estimated for new use of antithyroid or thyreomimetic agents, or hospitalisation for thyroid disease from 12 months *before* to 12 months *after* the index date.

Results: The study population consisted of 3479 cases and 11830 controls. New use of antithyroid agents or hospitalisation for thyrotoxicosis within 6 months *after* the index date (i.e. untreated hyperthyroidism at the index date) was significantly associated with PE (adjusted OR 3.22; 95% CI 1.12-9.22), whereas a relation between thyreomimetic agents and PE was observed for new use *before* the index date, especially within the first 3 months after treatment onset (adjusted OR 4.58; 95% CI 1.28-16.43). No association was found for new use of thyreomimetic agents *after* the index date or for new use of antithyroid agents *before* the index date.

Conclusions: Our findings suggest that both patients with untreated hyperthyroidism and patients who have recently started with thyreomimetic agents for hypothyroidism are at an increased risk for PE.

INTRODUCTION

Thyroid hormone has many effects on the heart and vascular system, and both hyper- and hypothyroidism have been associated with an increased cardiovascular morbidity^[1-3]. Among this, several case reports have pointed towards a possible relationship between thyroid disease and venous thromboembolism^[4-6]. Thyroid hormone excess has been reported to induce prothrombotic changes, as witnessed by high levels of von Willebrand factor, factor VIII, fibrinogen and plasminogen activator inhibitor-1 in hyperthyroid patients^[7]. It is therefore hypothesised that hyperthyroidism may be a risk factor for venous thromboembolism. Inversely, reduced levels of von Willebrand factor and factor VIII have been observed during hypothyroidism^[7,8]. These effects may, at least in part, protect against venous thromboembolism. Indeed, we have recently shown that high levels of FT4 are associated with an increased risk of venous thrombosis, whereas a reduced risk of venous thrombosis was observed for low levels FT4; all within the normal reference range^[9].

To date, only two published studies, including one of our own, have assessed the effect of thyroid disease on the occurrence of venous thromboembolism^[10,11]. Remarkably, both studies found that (subclinical) hypothyroidism, but not hyperthyroidism, was associated with an increased risk of venous thromboembolism. These conflicting findings may result from the fact that the impact of treatment was not disentangled in the analysis. In general, thyroid hormone levels gradually decrease to normal levels within 4 to 12 weeks of treatment with antithyroid agents^[12]. A simultaneous reduction in procoagulant and antifibrinolytic factors may therefore rapidly attenuate the risk of venous thromboembolism in patients treated for hyperthyroidism^[13]. In hypothyroidism, treatment entails thyroid hormone substitution therapy with thyreomimetic agents. While normalisation of thyroid hormone levels may occur within the first months after treatment onset, the vascular consequences of the increased body weight, hypertension and dyslipidaemia, predominantly seen in prolonged thyroid hormone deficiency, may persist^[13,14]. It is therefore uncertain to what extent the transition in coagulation factors during the first months of substitution therapy may affect the risk of venous thromboembolism.

In a nested case-control design, we set out to evaluate the risk of pulmonary embolism associated with thyroid disease, while at the same time analysing the influence of treatment.

METHODS

Study design and population

Patients with a first hospitalisation for pulmonary embolism and control subjects without a history of pulmonary embolism were derived from a large database constructed via the PHARMO Record Linkage System (Pharmo Institute, Utrecht, the Netherlands, available at <http://www.pharmo.nl>). This system includes demographic details and complete medication histories of Dutch community pharmacies. Data of more than 2 million community-dwelling residents, residing in 25 geographic areas in the Netherlands are available from 1985 onwards. The medication histories are linked to hospital admission records. Because virtually all patients in the Netherlands are registered with a single community pharmacy, pharmacy records are essentially complete insofar

as prescription drug use is concerned. For assembling of the database, drug prescribing and hospitalisation data were used. Drugs were coded according to the *Anatomical Therapeutic Chemical Classification (ATC)*. The hospital admission and discharge codes were coded according to the *International Classification of Diseases Ninth Revision (ICD 9), Clinical Modification*.

Cases were all patients with a first hospitalisation for pulmonary embolism (ICD-9 code 415.1). Verification of whether the diagnosis had been confirmed by objective tests, including computed tomography or ventilation perfusion scanning, was performed in a random sample of 10% of the cases by retrieving data on confirmation of the diagnosis. Diagnosis was correctly confirmed in more than 95% of the cases.

The date of the first hospitalisation for pulmonary embolism was considered the index date. For each case patient, up to 4 control subjects, matched for gender, age (± 5 years) and geographic region, were randomly selected using risk-set sampling, meaning controls did not have a venous thromboembolism prior to the index date of the matched case. For this study, the population was restricted to cases and controls of whom data on prescription use and hospitalisations were available for at least 12 months before and after the index date.

Definition of exposure

For all cases and controls, we identified dispensings for antithyroid (ATC codes H03B and H03C) or thyreomimetic agents (ATC code H03A), as well as admissions for thyrotoxicosis (ICD 9 code 242) or hypothyroidism (ICD 9 code 244). In order to identify individuals with either active (untreated) thyroid disease or recent start of treatment for thyroid disease, new use of thyroid-related drugs or hospitalisations within 12 months *before* and 12 months *after* the index date was analysed. New use was defined as first ever use, or recurrent use if the time interval between the preceding treatment episode and the new prescription exceeded 12 months.

For hyperthyroidism, this meant that those individuals who were identified as new users of antithyroid agents or those who were hospitalised for thyrotoxicosis *before* the index date, had experienced hyperthyroidism prior to the date of treatment onset or hospitalisation, and were treated for hyperthyroidism at the index date (i.e. decreasing or already normal levels of thyroid hormone prior to the index date). Those individuals identified as new users of antithyroid agents, or hospitalised for thyrotoxicosis *after* the index date, especially in the first months, were likely to have active and untreated hyperthyroidism at the index date.

For hypothyroidism, those individuals who were identified as new users of thyreomimetic agents without previous use of antithyroid drugs or those who were hospitalised for hypothyroidism *before* the index date, had experienced hypothyroidism prior to the date of treatment onset or hospitalisation, and were treated for hypothyroidism at the index date (i.e. increasing levels of thyroid hormone relative to prior to treatment). Those individuals identified by treatment onset or hospitalisation *after* the index date, were likely to have untreated hypothyroidism at the index date.

By using ICD codes for hospitalisation, information on conditions potentially associated with a higher risk of venous thromboembolism (i.e. pregnancy, malignancy, trauma, acute infections, surgery, cardiovascular disease, heart failure, inflammatory bowel diseases) occurring in the 3 months prior to the index date, was retrieved. This 3-month period was used since hospitalisations (co-morbidity) prior to this period were less likely to have influenced the

occurrence of pulmonary embolism on the index date. In addition, use of platelet inhibitors (i.e. aspirin, clopidogrel bisulfate, dipyridamole), vitamin K antagonists and heparin, as well as all other medication that may influence the occurrence of pulmonary embolism (i.e. statins, NSAID's, antidiabetics), were identified using medication histories.

Statistical analysis

Continuous variables were reported as means or medians (range), depending on the distribution of the data. Categorical variables were presented as numbers (percent). Odds ratios (ORs) and their 95% confidence intervals (CIs) for the risk of pulmonary embolism associated with new use of thyroid agents and hospitalisation for thyroid disease were estimated using conditional logistic regression. Individuals without thyroid disease, i.e. those who had never been treated with antithyroid or thyreomimetic agents, or hospitalised for thyroid disease, up to 12 months after the index date, were used as reference. The analysis was stratified for treatment onset or hospitalisation within 12 months *before* and 12 months *after* the index date in order to evaluate the effect of untreated thyroid disease and treatment onset. To investigate a time relationship, the analysis was further stratified for different time intervals between the date of new prescription or hospitalisation, and the index date (≤ 3 months, ≤ 6 months, and ≤ 12 months).

The influence of potential confounding factors was analysed by evaluating the influence of a potential confounder on the effect estimate of the exposure of interest. If a change in crude OR of more than 5% was found for any of the independent factors, these variables were added to the multivariate model. Statistical analysis was performed with the use of SPSS 15.0 software package (SPSS Inc, Chicago, IL, USA).

RESULTS

Patient characteristics

Patient characteristics are summarised in Table 1. The database consisted of 4494 cases and 16802 control subjects. Drug prescription data and hospitalisation data of at least 12 months before and 12 months after the index date were available for 3479 cases and 11830 controls. These subjects formed the basis of the present analysis. Matching ensured that age and gender distribution in cases and controls were comparable; mean age in the cases was 59 (18-94) years and 57.6% were women.

New use of antithyroid agents or hospitalisation for thyrotoxicosis

In total, 12 (0.3%) case patients and 23 (0.2%) control subjects were identified as new users of antithyroid agents during the study period (Table 2). One case patient and 3 control subjects were hospitalised for thyrotoxicosis in the 12 months *after* the index date; no individuals were hospitalised in the 12 months *before* the index date. The number of individuals without thyroid disease was 3303 (94.9%) in the cases and 13391 (96.3%) in the control subjects.

New use of antithyroid agents *before* the index date was not associated with pulmonary embolism (OR 1.11; 95% CI 0.35-3.52). Also, no clear difference was observed between the different time intervals. Adjustment for potential confounders did not substantially alter

Table 1. Patient characteristics.

	Cases n=3479	Controls n=11830
Age, mean (range)	59 (18-94)	58 (18-96)
Gender, female, n (%)	2003 (57.6)	6903 (58.4)
Previous hospitalisations, n (%) ^a		
- Acute Infection	58 (1.7)	16 (0.1)
- Cardiovascular disease	22 (0.6)	11 (0.1)
- Heart failure	12 (0.3)	5 (0.0)
- Inflammatory bowel disease	13 (0.4)	5 (0.0)
- Malignancy	118 (3.4)	38 (0.3)
- Pregnancy	12 (0.3)	6 (0.1)
- Surgery	224 (6.4)	113 (1.0)
- Trauma	36 (1.0)	13 (0.1)
Medication use, n (%) ^a		
- Heparins	89 (2.6)	24 (0.2)
- Platelet inhibitors	519 (14.9)	1334 (11.3)
- Coumarins	124 (3.6)	329 (2.8)

N indicates number.

^aHospitalisations and medication use ≤ 90 days and ≥ 1 day prior to index date.

the odds ratios. When new use of antithyroid agents or hospitalisation for thyrotoxicosis *after* the index date was analysed, a clear time-dependent relation was observed. Treatment onset or hospitalisation within the first three months *after* the index date resulted in a crude odds ratio of 3.46 (95% CI 0.57-21.10), which gradually decreased with extension of the time interval. After adjustment for potential confounding factors, a significant association between pulmonary embolism and new use of antithyroid agents or hospitalisation within 6 months *after* the index date was observed (OR 3.22; 95% CI 1.12-9.22).

New use of thyreomimetic agents or hospitalisation for hypothyroidism

A total of 35 (1.0%) case patients and 69 (0.6%) control subject were identified as new users of thyreomimetic agents during the study period (Table 3). No individuals were hospitalised for hypothyroidism during this period.

New use of thyreomimetic agents *before* the index date was associated with pulmonary embolism, in a time-dependent fashion. The risk of pulmonary embolism was highest for treatment onset within 3 months *before* the index date (crude OR 4.98, 95 CI 1.39-17.82), and gradually decreased when the time interval between the date of treatment onset and the index date increased. No clear association was found between pulmonary embolism and new use of thyreomimetic agents *after* the index date, but the risk was below unity in the analysis of treatment onset within the first 3 months *after* the index date. Adjustment for potential confounding factors slightly altered the crude odds ratios.

Table 2. Risk of pulmonary embolism associated with new use of antithyroid agents or hospitalisation for thyrotoxicosis.

	Cases n=3479 (%)	Controls n=11830 (%)	Crude OR (95% CI)	Adjusted OR ^a (95% CI)
No thyroid disease	3303 (94.9)	11391 (96.3)	Reference	Reference
<i>Start of antithyroid agents before the index date^b</i>				
≤90 days	1 (0.0)	3 (0.0)	0.90 (0.09-8.68)	0.89 (0.09-8.59)
≤180 days	3 (0.1)	5 (0.0)	1.74 (0.41-7.34)	1.60 (0.38-6.82)
≤365 days	4 (0.1)	11 (0.1)	1.11 (0.35-3.52)	0.94 (0.28-3.17)
<i>Start of antithyroid agents after the index date^c</i>				
≤90 days	3 (0.1)	2 (0.0)	3.46 (0.57-21.10)	3.57 (0.58-21.83)
≤180 days	7 (0.2)	8 (0.1)	2.74 (0.98-7.65)	3.22 (1.12-9.22)
≤365 days	9 (0.3)	15 (0.1)	1.75 (0.75-4.07)	1.81 (0.75-4.35)

N indicates number; OR, odds ratio; and CI, confidence interval.

^aAdjusted for hospitalisations (for malignancy, trauma, surgery, pregnancy or heart failure) and use of anticoagulants within 90 days prior to the index date.

^bNo individuals were hospitalised for thyrotoxicosis in the year before the index date.

^cOne case patient was hospitalised for thyrotoxicosis 118 days after the index date; the three control subject were hospitalised 42, 97, and 292 days after the index date.

Table 3. Risk of pulmonary embolism associated with new use of thyreomimetic agents^a.

	Cases n=3479 (%)	Controls n=11830 (%)	Crude OR (95% CI)	Adjusted OR ^b (95% CI)
No thyroid disease	3303 (94.9)	11391 (96.3)	Reference	Reference
<i>Start of thyreomimetic agents before the index date^a</i>				
≤90 days	6 (0.2)	4 (0.0)	4.98 (1.39-17.82)	4.58 (1.28-16.43)
≤180 days	10 (0.3)	14 (0.1)	2.62 (1.14-5.99)	2.57 (1.10-6.01)
≤365 days	17 (0.5)	28 (0.2)	2.05 (1.11-3.78)	2.07 (1.11-3.85)
<i>Start of thyreomimetic agents after the index date^a</i>				
≤90 days	4 (0.1)	14 (0.1)	0.87 (0.28-2.70)	0.95 (0.30-2.96)
≤180 days	10 (0.3)	19 (0.2)	1.68 (0.77-3.66)	1.81 (0.83-3.97)
≤365 days	18 (0.5)	41 (0.3)	1.41 (0.80-2.48)	1.54 (0.86-2.75)

N indicates number; OR, odds ratio; and CI, confidence interval.

^aNo individuals were hospitalised for hypothyroidism during the study period.

^bAdjusted for hospitalisations (for malignancy, trauma, surgery or pregnancy) and use of anticoagulants within 90 days prior to the index date.

DISCUSSION

In this nested case-control population-based study, consisting of over 15000 individuals, we found that new use of antithyroid agents or hospitalisation for thyrotoxicosis *after* the index date (i.e. untreated hyperthyroidism) was associated with an increased risk of pulmonary embolism, in a time-dependent manner. In addition, a significant association between pulmonary embolism and new use of thyreomimetic agents in the 12 months *before* the index date was found. The risk was highest for treatment onset within three months prior to the index date, again indicating a time-dependent relationship.

Our finding of an association between pulmonary embolism and new use of antithyroid agents, or hospitalisation for thyrotoxicosis, *after* the index date, i.e. untreated hyperthyroidism at the index date, is in line with our previous report on the relation between high levels of FT4 (mostly within reference range) and venous thrombosis as well as studies describing a hypercoagulable and hypofibrinolytic state in hyperthyroid patients^[7-9]. This association only reached statistical significance when it concerned treatment onset or hospitalisation within 6 months after the

index date. However, the time-dependent relationship is indicative of hyperthyroidism as risk factor for venous thromboembolism, considering that those individuals identified as new users of antithyroid agents or hospitalised for thyrotoxicosis within the first months *after* the index date were most likely to have experienced hyperthyroidism at the index date. Also, this association was not present for patients who were already treated for hyperthyroidism at the index date, which strengthens the hypothesis that treatment with antithyroid agents may rapidly reduce the risk of venous thromboembolism in hyperthyroid patients.

As for hypothyroidism, our findings confirm that patients suffering from this condition are at increased risk for venous thromboembolism, but only after institution of thyroid hormone substitution therapy. Several studies have described an increase in coagulation factor levels after treatment with thyreomimetic agents in hypothyroid patients^[7;15;16]. Moreover, thyreomimetic agents have been reported to induce prothrombotic changes in euthyroid subjects^[17-19]. Taken together with the fact that almost one fifth of the hypothyroid patients is given an excessive amount of medication^[14], it is conceivable that these haemostatic changes may result in an increased risk of venous thromboembolism.

In general, three major causes of thrombosis have been identified: changes in the vessel wall, changes in the blood flow, and changes in the blood composition^[20]. Weight gain or obesity, hypertension, and altered lipid metabolism are present in prolonged thyroid hormone deficiency; all affect one or more of these components of thrombosis risk^[16;21-23]. Overweight and obesity have been reported to increase the risk of venous thrombosis by 1.7- to 2.4-fold^[24;25]. It is, therefore, most likely that the combination of both weight gain and a transition in coagulation factors is responsible for the increased risk of venous thromboembolism in hypothyroid patients in the first months of thyroid hormone substitution therapy.

Some methodological aspects of this study require comments. This study concerns a large sample size of a well-defined population, with precise documentation of drug prescription. The study design has also some obvious limitations, inherent to all population based cohort studies. For example, some concern could be raised with regards to the accuracy of diagnosis of pulmonary embolism, given that diagnosis was derived from *ICD*-codes rather than hospital records. However, verification of a 10% random sample of the cases showed that pulmonary embolism was nearly always confirmed by objective diagnostic tests. Also, since this analysis was performed using drug prescription and hospitalisation data as indicators for the presence of thyroid disease, no information was available on the exact thyroid status at the index date. Treatment onset or hospitalisation *after* the index date could not ensure that thyroid disease was already present at the time of the index date, and diagnosis of thyroid disease may also have been delayed until after the study period causing misclassification. However, these biases have probably equally affected both cases and controls. Last, the small number of individuals identified as new users of thyroid medication or hospitalised for thyroid disease limits the robustness of our findings. With the incidence of hyperthyroidism in the general population being so low, even in this large registry study there was insufficient power to efficiently report on this topic. Nevertheless, the present observations consistently point towards an increased risk of pulmonary embolism for increasing levels of thyroid hormone and therefore provide

valuable insights in the relation between thyroid disease, antithyroid or thyreomimetic agents, and pulmonary embolism. Although this study only concerned pulmonary embolism and not deep venous thrombosis of the lower extremities (as this usually does not lead to hospital admission), we believe the association applies for both manifestations of venous thrombosis^[9].

Overall, our findings suggest that both patients with untreated hyperthyroidism and patients who have recently started with thyreomimetic agents for hypothyroidism are at increased risk of pulmonary embolism. Physicians should be aware of this relationship and adequately assess the presence of additional risk factors for venous thromboembolism when prescribing thyreomimetic agents.

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Chapter 9

Alterations in coagulation and
fibrinolysis after levothyroxine
exposure in healthy volunteers:
a controlled randomised
crossover study

Bregje van Zaane, Alessandro Squizzato,
Jan Debeij, Olaf M Dekkers, Joost CM Meijers,
Anton P van Zanten, Harry R Büller,
Victor EA Gerdes, Suzanne C Cannegieter,
Dees PM Brandjes

Submitted for publication

ABSTRACT

Introduction: Several haemostatic abnormalities have been reported in hyperthyroidism, but the overall effect of thyroid hormone excess on coagulation and fibrinolysis is unclear. Our aim was to assess whether the use of supraphysiological doses of levothyroxine leads to coagulation activation and inhibition of fibrinolysis.

Methods: Healthy volunteers were randomised to receive levothyroxine or no medication for 14 days with a wash-out period of at least 28 days in a crossover design. To study the effects of different degrees of thyroid hormone excess, 16 participants received levothyroxine in a dose of 0.3 mg per day, and 12 received levothyroxine 0.45 or 0.6 mg per day depending on body weight.

Results: Levels of von Willebrand factor antigen (VWF:Ag) and activity (VWF:RiCo), factor (F) VIII, plasminogen activator inhibitor-1 (PAI-1) and clot-lysis time were slightly higher after levothyroxine 0.3 mg per day than after the control situation, but only levels of VWF were significantly increased compared to baseline values. After levothyroxine 0.45 or 0.6 mg per day, levels of fibrinogen increased by 17%, VWF antigen by 26%, VWF activity by 24%, factor VIII by 19%, factor IX by 14%, factor X by 7%, PAI-1 by 116%, clot-lysis time by 14%, and activated partial thromboplastin time decreased by 3%; all significant changes compared to after the control situation. We did not observe clear evidence of coagulation activation.

Conclusions: Our data suggest that thyroid hormone excess increases coagulation factor levels and inhibits fibrinolysis, in a dose-dependent fashion. This implies an increased risk of venous thrombosis during hyperthyroidism.

INTRODUCTION

Thyroid dysfunction was first linked with haemostatic abnormalities in the beginning of the past century when a case of cerebral venous thrombosis during thyrotoxicosis was described^[1]. Several other cases have since been reported^[2-6], however, the issue as to whether thyroid hormone excess enhances the risk of venous thrombosis, and to which extent, is controversial^[7-8]. Indirect evidence comes from an increasing number of studies suggesting a hypercoagulable and hypofibrinolytic state during hyperthyroidism^[8-9]. Compared to euthyroid controls, increased levels of von Willebrand factor (VWF) and factor VIII (FVIII) have repeatedly been reported in patients with overt or subclinical hyperthyroidism, but results on other coagulation and fibrinolytic parameters are less consistent. Importantly, the majority of these studies were of low study quality, which may have lead to biased results^[9].

To assess the influence of thyroid hormone on coagulation and fibrinolysis, two intervention studies have been performed in healthy subjects^[10,11]. In these studies, thyroid hormones significantly increased factor VIII, von Willebrand factor, fibronectin and tissue-type plasminogen activator (t-PA). Unfortunately, neither study included a control situation and only a limited number of haemostatic parameters were determined, leaving the net effect on coagulation and fibrinolysis still unresolved.

The aim of the present study was to assess whether the induction of mild as well as more pronounced thyroid hormone excess by supraphysiological doses of levothyroxine leads to activation of coagulation and inhibition of fibrinolysis in healthy volunteers. This design enabled us to investigate the effects of different degrees of thyroid hormone excess independent of the accompanying changes in blood pressure, arterial wall stiffness and cardiac output during prolonged thyroid dysfunction. Moreover, because healthy volunteers were included, changes in coagulation factors are supposed to reflect the effect of high levels of thyroid hormone *per se*, and not of factors otherwise associated with hyperthyroidism such as autoimmunity.

METHODS

Study design

Healthy volunteers were randomised to receive levothyroxine or no medication for 14 days with a wash-out period of at least 28 days in a single-blinded, crossover design. Fasting venous blood samples were drawn at baseline and day 14 of each study period, at the same time of the day. For safety reasons, the study was conducted in two stages, characterised by a difference in the dose of levothyroxine that was administered. At the first stage, participants received levothyroxine 0.3 mg per day (*Study A*). Only after this dose had been proven to be safe, participants were asked to continue with the second stage of the study. Those unable or unwilling to further participate were replaced by new volunteers. Participants now received levothyroxine 0.45 mg per day if a body weight below 80 kg, or levothyroxine 0.6 mg per day if a body weight of 80 kg and more (*Study B*).

The study was performed between December 5, 2008 and September 3, 2009 at the department of Internal Medicine of the Slotervaart Hospital, Amsterdam, the Netherlands, and was

approved by the institutional medical ethics committee. All participants provided written informed consent.

Participants

We recruited healthy volunteers aged 18 to 40 years, regardless of gender or race, by local advertisements. Exclusion criteria applied were: a history of thyroid disease or venous thrombosis, ongoing medical or psychiatric illnesses, regular use of medications including oral contraceptive agents, illicit drugs or excessive alcohol use, surgery or hospitalisation in the previous 3 months, or non-traditional sleep/wake habits (e.g. night shift work or frequent travel across time zones). Potentially eligible participants were invited for a first screening visit during which a physical examination (including measurement of height, weight, heart rate, blood pressure and body temperature), an electrocardiogram (ECG) and blood sampling for routine laboratory tests (including blood count, renal, hepatic and thyroid function) was performed. In case of any relevant abnormalities, volunteers were excluded from further study participation.

Study procedures

The same study procedures were applied in *Study A* and *Study B*. Study visits were scheduled at baseline (day 0) and day 14 of each study period, between 8 and 10 am. Venous blood sampling was performed, and an independent physician collected anthropometric parameters and recorded in a standardised fashion whether any signs or symptoms associated with thyroid hormone excess had occurred. For safety reasons, an ECG was performed to check for any cardiac arrhythmias. Study medication was provided after screening and randomisation. Participants were instructed to start drug intake on day 1 of either the first or second study period, depending on randomisation. Levothyroxine was to be taken every morning, on awakening, 30 minutes prior to ingesting any food or drinks. Participants were advised to maintain their usual sleep-wake schedule, exercise and dietary habits during the study. Apart from the required visits per protocol, a telephone call was scheduled at day 7 of, and 14 days after each study period, allowing participants to further report any changes in their health status or perceived adverse effects of the study medication.

Randomisation, compliance and withdrawal

Randomisation was performed by an independent physician, who had no information about the participants included in the trial. Treatment allocation was concealed in sequentially numbered, sealed and opaque envelopes, which were handed to the participants at the end of the first visit. Laboratory personnel were unaware of treatment assignments, and participants were asked not to tell the investigator whether they were taking levothyroxine or not.

In case of any adverse drug reaction, an independent physician evaluated the clinical urgency for disclosure of randomisation or discontinuance of study participation. In all cases, the reasons for withdrawal were recorded. Participants who withdrew, or who were withdrawn, were replaced by new volunteers and were not included in the analysis.

At study completion, all participants were asked to report the number of levothyroxine doses missed.

Laboratory assays

Fasting morning blood samples were collected in 4.5 mL plain tubes for assessment of thyroid function, and in 5 mL 0.109 mol/L sodium citrated tubes for tests of coagulation and fibrinolysis (BD Vacutainer, Plymouth, UK). Citrated blood was immediately centrifuged, and the supernatant re-centrifuged, for 15 minutes at 2500 x g at 15 °C to obtain platelet-poor plasma. Plasma was aliquoted and stored at -80 °C until further use.

Levels of free thyroxine (FT4), tri-iodothyronine (T3) and thyroid stimulating hormone (TSH) were assessed at the local laboratory of the Slotervaart Hospital on the day of blood sampling, using commercially available assays (ADVIA Centaur® immunoassay system, Siemens Healthcare Diagnostics, Marburg, Germany).

Tests of coagulation and fibrinolysis were performed in batch after all participants had completed the study. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and functional assays of coagulation factors II (FII:C), VII (FVII:C), VIII (FVIII:C), IX (FIX:C), X (FX:C) and von Willebrand factor ristocetin cofactor activity (VWF:RiCo) were performed using an automated coagulation analyser (Behring Coagulation System, Dade Behring, Marburg, Germany) with reagents and protocols of the manufacturer (Siemens Healthcare Diagnostics). D-dimer (Innovance, Siemens Healthcare Diagnostics) was also determined on the Behring Coagulation System. For the measurements of von Willebrand factor antigen (VWF:Ag), and total and free protein S (PS), we used in-house enzyme linked immunosorbent assays (ELISA) with antibodies from DAKO (Glostrup, Denmark). Free protein S was measured in the supernatant after precipitating the C4b-binding protein-bound fraction with polyethylene glycol 8000 and measuring the concentration of free protein S in the supernatant. Prothrombin fragment 1+2 (Enzygnost monoclonal F1+2, Dade Behring), plasmin-antiplasmin complexes (PAP, DRG diagnostics, Marburg, Germany) and plasminogen activator inhibitor type-1 antigen (PAI-1:Ag, Hyphen BioMed, Andrésey, France) were determined by ELISA. Protein C was determined using the Coamatic protein C activity kit from Chromogenix (Mölnal, Sweden). Endogenous thrombin potential (ETP) was determined with a calibrated automated thrombogram (CAT) The CAT assays the generation of thrombin in clotting plasma using a microtiter plate reading fluorometer (Fluoroskan Ascent, ThermoLab systems, Helsinki, Finland) and Trombinoscope software (Thrombinoscope BV, Maastricht, the Netherlands) as previously described^[12]. Three parameters were derived from the thrombin generation curve: lag time, peak height, and ETP (area under the curve). Resistance to activated protein C (APC) was determined by testing the effect of APC on the ETP. The sensitivity of APC (APC-SR) of each plasma sample was determined in both the presence and absence of approximately 4 nM APC (f.c. Enzyme Research Laboratories). The APC concentrations used were adjusted to maintain a residual thrombin generation activity of approximately 10% in normal pooled plasma. Normal pooled plasma was run in parallel on each plate. The normalised APC-SR was determined by dividing the APC-SR of an individual by the APC-SR of the pooled plasma. A normalised APC-SR >1.0 reflects an APC resistant phenotype. The overall fibrinolytic activity was established by measuring clot-lysis times in citrated plasma as described previously with some minor modifications^[13]. Briefly, 47 µL plasma was activated by a mixture of 175 IU/mL Actilyse (Biopool AB, Umeå, Sweden), 17mM CaCl₂, 10 µM phospholipids and recombinant human tissue factor (10⁵ dilution, Innovin, Marburg, Germany). Volumes were adjusted to 100 µL with hepes-buffered saline. Turbidity was measured in time at 37 °C at 405 nm for 3

hours in a Thermomax microplate reader (Molecular Devices Corporation, Menlo Park, CA, USA). The clot-lysis time was defined as the time difference between half-maximal lysis and half-maximal clotting. Fibrinogen concentrations were derived from the maximal turbidity in the clot-lysis assay, and were expressed as percentage of normal pooled plasma (set at 100%). Reference values for thyroid hormones, and coagulation and fibrinolytic parameters are shown in Table 2 and 3.

Statistical analysis

The main outcome of the study was the difference in relative change from baseline between the two crossover periods. We expected higher doses of levothyroxine to result in greater effects on coagulation and fibrinolytic factors. Power analysis indicated that 16 participants in *Study A*, and 12 participants in *Study B*, would provide 80% power to detect a standardised difference of at least 1.0 and 1.2, respectively.

Analyses were performed separately for *Study A* and *Study B*. The general characteristics of the participants are reported as means and ranges. For parameters of thyroid function, coagulation and fibrinolysis, we calculated median levels with their 95% confidence intervals (95% CI) at baseline and day 14 of each study period. We compared baseline and day 14 levels of each parameter with the Wilcoxon signed rank test. We calculated relative changes per parameter for each individual by subtracting the baseline value from the day 14 value, dividing it by the baseline value and multiplying the result by 100%. We calculated the medians (95% CI) of these individual relative changes, and performed between-exposure comparisons using the Wilcoxon signed rank test. The same analyses were performed in a subgroup of participants, derived from both studies, in whom endogenous thyroid stimulating hormone production was fully suppressed (TSH<0.02 IU/mL) after 14 days of levothyroxine exposure. Last, to assess the relation between T3 and parameters of coagulation and fibrinolysis, Spearman rank correlation coefficients were computed.

Statistical analysis was performed with the use of SPSS 15.0 software package (SPSS Inc, Chicago, IL, USA). Confidence intervals of the medians were calculated using the bootstrap method in R version 2.8.1 computer software (The R Foundation for Statistical Computing, 2008).

RESULTS

Participants, protocol completion and compliance

Characteristics of participants are shown in Table 1.

Study A

Sixteen participants, 9 men and 7 women, with a mean age of 30.2 (range 25-39) years were enrolled. All participants completed both study periods. Three participants self-reported to have missed at least one of the daily levothyroxine doses.

Study B

For the second stage of the study, we enrolled 12 participants; 6 men and 6 women, with a mean age of 29.2 (range 26-40) years. Five of the 16 participants in *Study A* were willing and

able to further participate in *Study B*, and 7 new volunteers were recruited. One participant withdrew after the first screening visit for personal reasons. Two others were unable to finish the regular visits required per protocol due to illness (fever and flue-like symptoms), and were therefore withdrawn. They were replaced by new volunteers, maintaining a total of 12 participants who completed the protocol. Three participants had a body weight of 80 kg or more; they received levothyroxine in a dose of 0.6 mg per day. The remaining participants received levothyroxine 0.45 mg per day. One participant reported to have missed a dose of levothyroxine.

Table 1. Characteristics of participants.

	Study A (n=16)	Study B (n=12)
Age, years, mean (range)	30.2 (25-39)	29.3 (26-40)
Male, n (%)	9 (56.3)	6 (50.0)
Body-mass index, kg/m ² , mean (range)	24.2 (19.2-33.0)	23.3 (21.1-26.2)
Systolic blood pressure, mmHg, mean (range)	113.7 (93.0-130.0)	117.3 (99.0-130.0)
Diastolic blood pressure, mmHg, mean (range)	72.3 (58.0-90.0)	72.3 (61.0-83.0)

N indicates number.

Thyroid hormones

Study results are shown in Table 2 (*Study A*) and Table 3 (*Study B*). Median levels of FT4 and T3 markedly increased, and TSH levels decreased after levothyroxine exposure, whereas these values remained unchanged during the control period. Administration of levothyroxine 0.3 mg per day induced a mild increase in thyroid hormone levels (median FT4 28.5 pmol/L, T3 2.50 nmol/L); 11 participants were still in the subclinical range according to post-exposure T3 levels. Levothyroxine 0.45/0.6 mg per day induced a more pronounced thyroid hormone excess (median FT4 40.0 pmol/L, T3 3.80 nmol/L), with all participants becoming hyperthyroid. TSH levels were fully suppressed in 5 participants who received a dose of 0.3 mg per day, in another five who received a dose of 0.45 mg per day, and in all 3 participants who received levothyroxine 0.6 mg per day.

Coagulation and fibrinolytic parameters

Table 2 and 3 summarise the effects on coagulation and fibrinolytic parameters in *Study A* and *Study B*, respectively.

Study A

Median baseline values were similar for both study periods. Median levels of von Willebrand factor (both activity and antigen), factor VIII, PAI-1 and clot-lysis time were slightly higher after levothyroxine exposure than after the control situation, but only levels of von Willebrand

Table 2. Parameters of thyroid function, coagulation, and fibrinolysis before and after both study periods (Study A).

Parameter (reference range)	No medication			Levothyroxine			p-values for between group comparison	
	Baseline	Day 14	Relative change (%)	Baseline	Day 14	Relative change (%)	Day 14	Change
Thyroid function								
FT4 (10-23 pmol/L)	15.0 (13.0; 16.0)	15.0 (14.0; 16.0)	0.0 (-12.5; 10.5)	16.0 (14.0; 18.0)	28.5 (20.0; 33.0)	82.5 (20.0; 115.8)	<0.01	<0.01
T3 (1.2-2.8 nmol/L)	1.85 (1.75; 1.95)	1.80 (1.80; 2.10)	0.0 (-5.3; 6.7)	2.05 (1.70; 2.20)	2.50 (2.30; 3.10)	36.1 (4.2; 56.3)	<0.01	<0.01
TSH (0.35-4.7 mIU/L)	1.75 (1.21; 2.02)	2.15 (1.73; 2.27)	20.7 (8.6; 35.5)	1.74 (1.32; 2.73)	0.09 (0.02; 0.22)	-96.4 (-98.5; -92.2)	<0.01	<0.01
Coagulation								
VWF:Ag (50-150%)	87.0 (77.9; 102.0)	90.0 (75.0; 103.0)	3.0 (-13.9; 12.5)	88.5 (77.0; 113.5)	106.0 (87.0; 118.0)	10.0 (1.7; 20.5)	<0.01	0.03
VWF:RiCo (58-172%)	110.0 (92.0; 118.0)	104.5 (83.0; 126.0)	-1.3 (-9.5; 9.4)	117.0 (83.5; 138.1)	125.5 (115.5; 148.5)	7.1 (4.5; 15.8)	<0.01	0.03
Factor VIII (63-173%)	106.5 (96.0; 120.0)	102.5 (95.0; 116.0)	-2.0 (-10.4; 6.3)	114.0 (92.0; 132.0)	121.5 (107.5; 133.0)	1.4 (-7.6; 12.5)	0.01	0.23
Fibrinolysis								
PAI-1 (<100 ng/mL)	35.5 (23.0; 54.0)	33.5 (19.0; 49.0)	-17.1 (-29.1; 62.1)	29.0 (21.0; 65.0)	42.0 (26.0; 63.0)	10.6 (-1.8; 89.1)	0.06	0.30
Clot-lysis time (40-85 min)	48.0 (45.9; 50.2)	49.4 (46.1; 55.6)	1.5 (-3.2; 3.0)	48.9 (44.1; 52.3)	54.6 (49.5; 55.5)	6.6 (2.4; 12.7)	0.01	0.14

FT4 indicates free thyroxine; T3, triiodothyronine; TSH, thyrotropin; VWF, von Willebrand factor; Ag, antigen; RiCo, ristocetin cofactor activity; and PAI-1, plasminogen activator inhibitor-1. Data are presented as medians (95% confidence intervals).

factor were significantly increased compared to baseline values. The median relative individual changes of von Willebrand factor antigen and activity were 10.0% (95% CI 1.7 to 20.5%) and 7.1% (95% CI 4.5 to 15.8%) after levothyroxine exposure, and 3.0 (95% CI -13.9 to 12.5%) and -1.3% (95% CI -9.5 to 9.4%) after the control situation. No significant changes were observed for the remaining parameters (data not shown).

Study B

Median baseline values were similar for both study periods. Median levels of fibrinogen, von Willebrand factor, coagulation factors II, VIII, IX and X, protein S and PAI-1 were higher after levothyroxine exposure than after the control situation. Median aPTT was shortened, and median lag time of the ETP assay and clot-lysis time were prolonged after levothyroxine exposure relative to the control situation. The remaining coagulation and fibrinolytic parameters were not significantly affected by levothyroxine exposure.

We also found a clear difference in the individual relative change between the two study periods for aPTT (median individual relative change -3.4% after levothyroxine exposure vs 0.6% after the control situation), fibrinogen (17.2% vs -11.1%), von Willebrand factor antigen (25.7% vs 2.2%), von Willebrand factor activity (23.9% vs -1.1%), factor VIII (19.2% vs 0.6%), factor IX (14.0% vs 0.0%), factor X (7% vs -7%), PAI-1 (116.0% vs 29.5%), and clot-lysis time (14.3% vs 1.0%), indicating a strong effect on an individual level. This individual effect was confirmed, when analysing the relative changes after both study periods per individual, as displayed in Figure 1. This figure also shows that the magnitude of the individual relative changes in coagulation and fibrinolytic parameters was not clearly related to the magnitude of the relative change in T3 (or FT4). However, a significant correlation between these parameters and T3 (and FT4) was found for absolute values (data not shown).

Subgroup analysis

Similar results were found in the subgroup-analysis of those individuals in whom TSH levels were fully suppressed after levothyroxine exposure, but the differences between groups were slightly more pronounced (data not shown). Additionally, median values of the peak height of the ETP assay were modestly, but significantly higher after levothyroxine exposure (319 nM, 95% CI 279 to 358) than after the control situation (296 nM, 95% CI 277 to 357) ($p < 0.01$). The median individual relative changes in peak height of the ETP assay was 5.3% (95% CI 1.1 to 8.5) after levothyroxine exposure and 1.0% (95% CI -7.7 to 2.5) after the control situation ($p = 0.02$).

Adverse events

No serious adverse events occurred during the study. Heart rate slightly increased after levothyroxine exposure and two participants experienced palpitations. Other reported symptoms associated with thyroid hormone excess included insomnia and fatigue, increased appetite, muscle weakness, sweating, nervousness, and diarrhoea. One participant experienced severe headache during levothyroxine exposure, which ceased several days after intake of the last dose.

Table 3. Parameters of thyroid function, coagulation, and fibrinolysis before and after both study periods (Study B).

Parameter (reference range)	No medication			Levothyroxine			p-values for between group comparison	
	Baseline	Day 14	Relative change (%)	Baseline	Day 14	Relative change (%)	Day 14	Change
Thyroid function								
FT4 (10-23 pmol/L)	15.0 (12.5; 15.0)	14.0 (13.5; 15.0)	0.0 (-6.7; 8.0)	14.5 (14.0; 16.0)	40.0 (37.5; 47.5)	185.7 (142.5; 215.5)	<0.01	<0.01
T3 (1.2-2.8 nmol/L)	1.90 (1.70; 2.20)	2.10 (1.95; 2.40)	2.8 (-4.8; 28.6)	2.35 (2.15; 2.60)	3.80 (3.35; 4.05)	58.0 (47.9; 83.3)	<0.01	<0.01
TSH (0.35-4.7 mIU/L)	1.64 (1.19; 3.07)	1.65 (1.49; 2.97)	7.8 (-26.9; 28.8)	1.86 (1.49; 3.08)	0.02 (0.02; 0.03)	-98.9 (-99.3; -98.4)	<0.01	<0.01
Coagulation								
PT (10.7-12.9 sec)	12.45 (1.55; 12.85)	12.75 (11.40; 13.00)	0.8 (-0.8; 1.7)	12.40 (11.75; 12.90)	12.35 (11.70; 12.65)	-2.4 (-3.6; -0.4)	0.18	0.08
aPTT (25.0-38.0 sec)	32.40 (30.30; 36.95)	33.25 (31.85; 36.90)	0.6 (-1.8; 5.5)	32.70 (31.70; 38.60)	31.65 (29.80; 35.65)	-3.4 (-8.4; -1.3)	0.01	0.01
Fibrinogen (51-149%)	80.54 (69.95; 91.13)	74.49 (69.85; 80.03)	-11.1 (-15.6; 3.4)	79.87 (69.30; 87.89)	91.54 (78.53; 108.54)	17.2 (9.6; 20.4)	<0.01	0.02
VWF:Ag (50-150%)	77.5 (63.0; 98.5)	78.0 (65.5; 92.5)	2.2 (-17.4; 19.7)	77.0 (71.0; 92.5)	102.5 (85.0; 121.5)	25.7 (13.8; 32.9)	<0.01	0.02
VWF:RiCo (58-172%)	82.5 (57.5; 116.5)	78.5 (61.0; 102.5)	-1.1 (-22.7; 18.4)	91.5 (74.0; 102.5)	112.5 (98.0; 131.0)	23.9 (11.6; 40.1)	<0.01	0.04
Factor II (80-130%)	78.5 (73.0; 84.5)	81.0 (72.0; 84.5)	5.3 (-9.8; 9.6)	79.0 (74.0; 86.5)	85.5 (74.5; 91.0)	6.1 (0.0; 11.6)	0.02	0.64

Factor VII (71-152%)	77.0 (69.9; 81.0)	74.8 (69.5; 83.0)	-0.3 (-7.8; 3.2)	76.0 (70.0; 86.5)	78.5 (72.5; 88.0)	8.0 (0.1; 9.0)	0.26	0.10
Factor VIII (63-173%)	101.0 (84.0; 110.5)	102.6 (84.5; 108.5)	0.6 (-17.7; 9.6)	102.0 (95.0; 105.0)	123.5 (106.0; 135.5)	19.2 (10.0; 27.1)	<0.01	0.02
Factor IX (80-145%)	95.5 (83.0; 101.0)	90.0 (79.0; 100.0)	0.0 (-7.8; 1.7)	91.5 (83.5; 103.5)	105.0 (101.0; 107.0)	14.0 (5.2; 18.2)	<0.01	<0.01
Factor X (66-125%)	73.0 (68.5; 80.5)	68.0 (65.0; 82.5)	-7.0 (-11.4; 1.1)	76.5 (67.0; 89.0)	80.5 (69.0; 90.5)	7.0 (-0.5; 12.0)	<0.01	0.05
Anticoagulation								
Protein C (70-120%)	106.0 (85.5; 118.0)	101.0 (87.0; 114.0)	-5.6 (-7.1; -1.2)	95.5 (88.5; 114.0)	97.5 (86.5; 111.0)	-2.6 (-9.4; 1.2)	0.17	0.88
APC-SR (< 1.6 NR)	1.51 (1.03; 1.90)	1.59 (1.15; 2.02)	2.3 (-6.1; 17.5)	1.40 (1.03; 1.82)	1.32 (0.90; 1.88)	1.1 (-22.5; 22.0)	0.11	0.59
Total protein S (58-130%)	93.0 (81.5; 96.0)	87.0 (76.0; 96.0)	-5.8 (-10.3; -0.5)	89.5 (81.0; 101.0)	96.5 (77.5; 99.0)	-1.9 (-4.4; 5.7)	0.01	0.18
Free protein S (63-137%)	97.5 (88.5; 112.5)	94.0 (84.5; 105.5)	-5.2 (-10.1; -2.2)	97.0 (87.0; 107.0)	98.5 (90.5; 107.0)	-0.5 (-5.2; 6.6)	0.03	0.31
Thrombin Generation								
F1+2 (53-271 pmol/L)	109.0 (85.5; 129.5)	105.5 (75.0; 134.0)	1.1 (-15.6; 11.0)	97.5 (85.5; 140.0)	114.5 (81.5; 150.0)	9.6 (-4.2; 14.5)	0.42	0.70
Lag time of ETP (1.5-3.2 min)	2.00 (1.98; 2.25)	2.00 (2.00; 2.23)	0.0 (0.0; 0.0)	2.30 (2.00; 2.33)	2.33 (2.30; 2.33)	0.0 (0.0; 13.4)	0.01	0.06
Peak height of ETP (194-503 nM)	292.0 (261.5; 305.5)	282.5 (258.5; 299.5)	-2.9 (-7.5; 2.9)	281.5 (263.5; 298.5)	304.5 (274.0; 318.0)	5.5 (-2.6; 8.3)	0.08	0.07
ETP (area under curve) (1155-2606 nM.min)	1518.0 (1307.0; 1687.0)	1443.0 (1292.0; 1623.5)	-3.3 (-6.5; -1.5)	1476.0 (1368.5; 1688.5)	1498.0 (1321.0; 1639.5)	-0.5 (-4.6; 3.9)	0.24	0.31

No medication			Levothyroxine			p-values for between group comparison		
Parameter (reference range)	Baseline	Day 14	Relative change (%)	Baseline	Day 14	Relative change (%)	Day 14	Change
<i>Fibrinolysis</i>								
PAI-1 (<100 ng/mL)	27.5 (20.5; 36.5)	37.7 (28.0; 58.0)	29.5 (6.2; 68.6)	25.0 (17.5; 45.5)	63.5 (34.0; 100.5)	116.0 (76.6; 227.0)	0.05	0.04
PAP (47-563 µg/L)	367.0 (303.0; 542.0)	359.5 (242.5; 438.5)	-14.9 (-27.5; -6.8)	427.5 (339.5; 492.0)	342.5 (288.5; 442.5)	-12.5 (-20.1; -4.4)	0.94	0.64
D-dimer (<1.00 mg/L FEU)	0.17 (0.17; 0.23)	0.18 (0.17; 0.21)	0.0 (-11.7; 0.0)	0.19 (0.17; 0.27)	0.19 (0.18; 0.28)	2.9 (0.0; 9.5)	0.08	0.26
Clot-lysis time (40-85 min)	49.9 (46.0; 52.5)	50.2 (46.2; 53.4)	1.0 (-3.7; 3.2)	51.9 (46.7; 56.4)	57.0 (53.7; 64.5)	14.3 (9.0; 17.4)	<0.01	<0.01

FT4 indicates free thyroxine; T3, tri-iodothyronine; TSH, thyroid stimulating hormone (thyrotropin); PT, prothrombin time; aPTT, activated partial thromboplastin time; VWF, von Willebrand factor; Ag, antigen; RiCo, ristocetin cofactor activity; APC-SR, activated protein C sensitivity ratio; NR, normalised ratio; F1+2, prothrombin fragment 1+2; ETP, endogenous thrombin potential; PAI-1, plasminogen activator inhibitor-1; and PAP, plasmin-antiplasmin complexes. Data are presented as medians (95% confidence intervals).

Figure 1. Relative change in parameters of coagulation and fibrinolysis, by individual and ordered by change in T3. Order of bars (individuals) consistent across panels. T3 indicates tri-iodothyronine; aPTT, activated partial thromboplastin time; VWF, von Willebrand factor; Ag, antigen; RiCo, ristocetin cofactor activity; F, factor; and PAI-1, plasminogen activator inhibitor-1.

DISCUSSION

Our study showed that high circulating levels of thyroid hormone in healthy volunteers increase levels of fibrinogen, von Willebrand factor, coagulation factors VIII, IX and X, and plasminogen activator inhibitor-1, resulting in shortening of aPTT and increased clot-lysis times. Changes in von Willebrand factor antigen and activity were most pronounced. These findings suggest a hypercoagulable and hypofibrinolytic state in hyperthyroidism.

The present study is the first to assess the relation between thyroid hormone excess, coagulation, and fibrinolysis in a controlled randomised crossover study. Previous reports have mainly focussed on patients with overt or subclinical hyperthyroidism. These reports uniformly showed increased levels of von Willebrand factor and factor VIII, but the results on other parameters of coagulation and fibrinolysis are conflicting^[9]. When patients with thyroid dysfunction are simply compared with healthy controls, it is impossible to differentiate between the effects of thyroid hormone excess and the influence of possible confounding factors such as autoimmunity. Also, discrepancies between studies can be the result of variations in the severity of thyroid disease in the population under study. To evaluate the effects of different degrees of thyroid hormone excess, we administered both a moderate and high dose of levothyroxine in healthy volunteers. We noted that thyroid hormone influences parameters of coagulation and fibrinolysis in a dose-dependent fashion, with modestly increased levels of von Willebrand factor already occurring within the subclinical range, whereas significant changes in fibrinogen, coagulation factors VIII, IX, and X, and plasminogen activator inhibitor-1 were observed in more pronounced thyroid hormone excess. Absolute levels of these coagulation and fibrinolytic factors were significantly correlated with absolute values of T3, further suggesting that the spectrum of haemostatic abnormalities in hyperthyroid patients may be dependent on the severity of the disease.

Generally, these prothrombotic changes are thought to infer an increased risk of venous thrombosis by an enhanced capacity of thrombus formation. Therefore, it is at least remarkable that we did not find clear evidence of enhanced thrombin generation after levothyroxine exposure. In contrast to earlier reports, we found levels of prothrombin fragment 1+2, a fragment that is released during thrombin generation, to be unaffected by levothyroxine^[14-16]. While prothrombin fragment 1+2 measures the extent of thrombin generation *in vivo*, the ETP assay reflects the potential thrombin-forming capacity of the plasma *ex vivo* and is therefore considered a better instrument to identify individuals at risk of venous thrombosis^[12]. However, even this assay was hardly affected by levothyroxine exposure, apart from a very modest increase in its peak height in participants who experienced the most pronounced thyroid hormone excess (according to fully suppressed TSH levels). This suggests that the increase in procoagulant factors may be counterbalanced by a simultaneous rise in anticoagulant factors. However, we did not find marked differences in protein C and protein S. Another explanation may involve the sensitivity of the ETP assay. There is some evidence indicating that the ETP (area under the curve and peak height) is hardly affected by variations in coagulation factors VIII, IX and X, especially when these levels are still within the normal range^[9;17;18]. As such,

this *ex vivo* test of thrombin generation may have underestimated the potential thrombin forming capacity *in vivo*.

With respect to fibrinolytic activity, both high levels of tissue-type plasminogen activator (t-PA) and its primary inhibitor PAI-1 have been reported in hyperthyroid patients^[9;14;15]. The net effect may result in a functionally unchanged fibrinolytic state, which explains why, in our study, levels of plasmin-antiplasmin complexes and D-dimer were not significantly altered after levothyroxine exposure^[9;14;15]. However, as with thrombin generation, these parameters indicate the ongoing fibrinolytic process in the body, whereas clot-lysis time represents the overall plasma fibrinolytic potential. Impaired fibrinolytic activity, as measured by increased clot-lysis times, is associated with an increased risk of venous thrombosis^[19;20]. Our finding of higher clot-lysis time even during mild thyroid hormone excess implicates that increasing levels of thyroid hormone may be a risk factor for venous thrombosis. Since venous thrombosis is a multicausal disease in which more than one genetic or environmental condition is needed to overcome the thrombosis threshold, this may especially be true when exposure to an additional trigger, such as surgery or use of oral contraceptive agents, further imbalances the haemostatic system^[21].

Several pathophysiological mechanisms have been suggested to underlie the relation between thyroid hormone excess and haemostasis, including autoimmunity, indirect effects of endothelial dysfunction and atherosclerosis, increased peripheral sensitivity to catecholamines, and direct upregulation of protein synthesis^[22-27]. Although, in the present study, we did not attempt to unravel this complex issue, our findings are compatible with *in vitro* data showing thyroid hormone-mediated upregulation of gene transcription at both the hepatic and endothelial level^[28-30]. Moreover, our study was controlled for autoimmunity, and participants were free of any vascular changes observed in prolonged hyperthyroidism, making these factors less likely to determine the relation.

Our results may be limited due to the relatively small sample size in both study parts. However, even despite these small samples sizes, we were able to detect marked differences between groups. Nevertheless, we can not exclude that modest changes in, for example, endogenous thrombin generation went undetected due to the small number of participants in this study. Also, the partially unblinded nature of the study may be of some concern. Since we expected participants to notice, by associated symptoms and signs, the difference between the use of levothyroxine or placebo, participant blinding by use of placebo seemed unfeasible. However, since the endpoints of the study were laboratory values of coagulation and fibrinolytic parameters, these are not likely to be biased by not having performed a double-blinded study.

Overall, our study shows that thyroid hormone excess increases coagulation factors levels and inhibits fibrinolysis, in a dose-dependent fashion. Due to the combined effects on procoagulant, anticoagulant, and fibrinolytic factors in particular, patients with hyperthyroidism may be at increased risk of venous thrombosis. However, epidemiological and clinical studies are needed to further assess the association between thyroid hormone excess and venous thrombosis, and to evaluate the clinical relevance of our findings.

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Chapter 10

Thrombin-activatable fibrinolysis
inhibitor in hyperthyroidism and
hypothyroidism

Chantal JN Verkleij, Bregje van Zaane,
Alessandro Squizzato, Dees PM Brandjes,
Harry R Büller, Joost CM Meijers,
Victor EA Gerdes

ABSTRACT

Introduction: Endocrine disorders affect both the coagulation and fibrinolytic systems, and have been associated with the development of cardiovascular diseases. Thrombin-activatable fibrinolysis inhibitor (TAFI) is a link between the coagulation and fibrinolytic system. The aim of this study was to determine the effect of hyperthyroidism and hypothyroidism on TAFI levels and function.

Methods: The effect of hyperthyroidism on TAFI was studied in healthy volunteers who were treated with levothyroxine for 14 days. The effect of hypothyroidism on TAFI was studied in a multicentre observational cohort study. Blood was drawn before treatment of patients with newly diagnosed hypothyroidism and when euthyroidism was achieved. Plasma clot-lysis times, TAFIa-dependent prolongation of clot-lysis and TAFI levels were measured.

Results: Hyperthyroidism resulted in a hypofibrinolytic condition and in an enhanced TAFIa-dependent prolongation of clot-lysis. A trend towards decreased plasma TAFI levels was observed in healthy volunteers who used levothyroxine. Hypothyroidism resulted in hyperfibrinolysis and a reduced TAFIa-dependent prolongation of clot-lysis.

Discussion: Alterations of TAFIa-dependent prolongation of clot-lysis in patients with thyroid disorders may cause an impaired haemostatic balance. The disturbed haemostatic balance in patients with hyperthyroidism might make them more prone for thrombosis, while the risk for bleeding may increase in patients with hypothyroidism.

INTRODUCTION

Endocrine disorders are common diseases in the general population and affect both the coagulation and fibrinolytic systems. Patients with hypothyroidism as well as hyperthyroidism have an increased risk for cardiovascular diseases, however it is related to different underlying mechanisms^[1;2]. The mechanism for the development of cardiovascular disease in patients with hypothyroidism is mainly based on the development of atherosclerosis caused by weight gain, hypertension and increased lipid levels^[3;4], while patients with hyperthyroidism are more prone to atrial fibrillation and have increased plasma levels of procoagulant factors^[5;6]. The prothrombotic condition in patients with hyperthyroidism is illustrated by elevated plasma levels of von Willebrand factor (VWF), fibrinogen, factor VIII, factor IX and factor X, and possibly by enhanced platelet function^[6-11]. Moreover, a reduced fibrinolytic activity was observed in these patients due to increased levels of plasminogen activator inhibitor-1 (PAI-1)^[9].

In contrast, patients with overt hypothyroidism have an increased bleeding tendency. The most common coagulation disorder in these patients is acquired von Willebrand's syndrome, which is characterised by decreased levels of factor VIII and von Willebrand factor^[6;12;13]. Evidence on other coagulation parameters is less robust, but reduced levels of the coagulation factors IX, X and XI have been reported in hypothyroid patients^[6;14;15]. In addition, several changes in plasma levels of antiplasmin, t-PA, PAI-1 and D-dimer have been described, which suggests that thyroid hormone deficiency affects the fibrinolytic system as well^[6;16]. However, the net effect on fibrinolytic activity is still unclear^[6;17].

A protein that links the coagulation and fibrinolytic system is thrombin-activatable fibrinolysis inhibitor (TAFI)^[18]. TAFI is a glycoprotein that is synthesised in the liver and circulates in plasma as a carboxypeptidase B-like proenzyme. Thrombin activates TAFI and activated TAFI (TAFIa) acts as a fibrinolysis inhibitor. The antifibrinolytic function of TAFI is based on cleavage of C-terminal lysine residues of partly degraded fibrin, the binding sites for plasminogen and its activator tissue-type plasminogen activator. This results in less plasmin formation and thus TAFI attenuates fibrinolysis. Besides its antifibrinolytic function, TAFI is also involved in wound healing, angiogenesis and inflammation^[19-21].

Recently, plasma levels of TAFI were measured in patients with thyroid disorders. Elevated plasma TAFI levels were observed in patients with mild and overt hypothyroidism^[22-25], while both decreased as well as increased plasma TAFI levels were reported in patients with hyperthyroidism^[26;27]. Elevated TAFI levels in patients with hypothyroidism suggest a hypofibrinolytic activity. Given the uncertainty about the fibrinolytic activity in patients with hypothyroidism and contradictory results on TAFI levels in patients with hyperthyroidism, the present study was performed to assess the effects of both hyperthyroidism and hypothyroidism on plasma TAFI levels, and on TAFIa-dependent prolongation of clot-lysis.

MATERIALS AND METHODS

Study design

The effects of hyperthyroidism on TAFI were studied in two single-blinded, crossover, randomised controlled trials in healthy volunteers. Participants received both no medication or levothyroxine

to induce hyperthyroidism for 14 days and, based on the half-life of levothyroxine, exposures were separated by a wash-out of at least 28 days. Randomisation was performed by an independent physician who had no information about the participants, nor responsibility for determining eligibility of the participants.

For safety reasons, we started with inducing mild hyperthyroidism by treating the participants with levothyroxine 0.3 mg per day. After performing this study, and none of the participants had shown important side effects, we were allowed to induce more pronounced hyperthyroidism. Participants with a body weight below 80 kg received levothyroxine 0.45 mg per day, while participants with a body weight of 80 kg or more received levothyroxine 0.6 mg per day. Levothyroxine was taken every morning, on awakening, 30 minutes prior to food intake. Blood was drawn at baseline and day 14 of each study period, between 8 and 10 am. These studies were performed at the department of Internal Medicine of the Slotervaart Hospital in Amsterdam, the Netherlands.

The effect of hypothyroidism on TAFI was studied in a multicentre observational cohort study. Patients with a new diagnosis of overt hypothyroidism were included, before or within the first 48 hours of replacement therapy. Overt hypothyroidism was defined as an increase of serum thyrotropin (TSH) above 15 mU/L, with serum free thyroxine (FT4) concentration below the lower limit of the reference range. Blood sampling was performed at inclusion (during hypothyroidism) and repeated after reaching euthyroidism (usually after 3-4 months). Samples were collected at the Slotervaart Hospital and at the Academic Medical Centre, both in Amsterdam, the Netherlands.

Study protocols were approved by the Medical Ethical Review Board of both the Slotervaart Hospital and the Academic Medical Centre in Amsterdam. Written informed consent was obtained in all participants.

Participants and plasma

For the hyperthyroidism studies, healthy volunteers aged 18 to 40 years, regardless of gender or race, were recruited by local advertisements. Volunteers were excluded if they had any of the following: history of thyroid disease or venous thrombosis, ongoing medical or psychiatric illnesses, regular use of prescription or non-prescription medications including oral contraceptive agents, illicit drugs or excessive alcohol use, surgery or hospitalisation in the previous 3 months, or non-traditional sleep/wake habits (e.g. night shift work or frequent travel across time zones).

For the hypothyroidism study, patients with a new diagnosis of primary overt hypothyroidism, with a minimum age of 18 years, were enrolled before or within the first 48 hours of replacement therapy. Patients were excluded if they had any of the following; secondary hypothyroidism, subclinical hypothyroidism, more than 48 hours of thyroid hormone replacement therapy, known congenital or acquired von Willebrand's syndrome, or presence of severe inflammatory disease (e.g. active inflammatory bowel disease, pneumonia).

For all studies, blood was collected into trisodium citrate containing tubes and tubes were centrifuged twice (15 min, 2500 g at 15°C). Plasma was aliquoted and stored at -80°C until further use.

Reagents

Rabbit lung thrombomodulin was obtained from American Diagnostica (Greenwich, CT) and potato carboxypeptidase inhibitor from Calbiochem (La Jolla, CA). Hippuryl-Arginine and H-D-Phe-Pro-Arg-Chloromethylketone (PPACK) was purchased from Bachem (Bubendorf, Switzerland) and phosphoenolpyruvate (PEP), ATP, NADH, pyruvate kinase/dehydrogenase (PK/LDH), and recombinant t-PA (Actilyse) from Biopool AB (Umeå, Sweden). Recombinant human tissue factor (Innovin) was from Siemens Healthcare Diagnostics (Marburg, Germany) and thrombin was a gift from Dr. W. Kiesel (University of New Mexico, NM).

Thyroid hormones

Serum levels of thyroid hormones were measured at the local laboratory of the Slotervaart Hospital on the day of sampling. Levels of FT₄, tri-iodothyronine (T₃) and TSH were assessed using commercially available assays (ADVIA Centaur[®] immunoassay system, Siemens Healthcare Diagnostics, Marburg, Germany). Levels of thyroid hormones in samples of patients in the hypothyroidism study, recruited at the Academic Medical Centre in Amsterdam, were measured at the local laboratory of the Slotervaart Hospital after storage at -80°C.

Clot-lysis times

The overall fibrinolytic activity was determined by measuring clot-lysis times in citrated plasma as previously described with some minor modifications^[28]. Briefly, 47 µL plasma was activated by a mixture of 175 U/mL Actilyse, 17 mM CaCl₂, 10 µM phospholipids and recombinant human tissue factor (Innovin, 10⁵ dilution). Volumes were adjusted to 100 µL with hepes-buffered saline. Turbidity was measured in time at 37°C at 405 nm for 3 hours in a Thermomax microplate reader (Molecular Devices Corporation, Menlo Park, CA, USA). The clot-lysis times were defined as the time difference between half-maximal lysis and half-maximal clotting. Normal pool plasma that was used as a reference, was derived from a donor pool of at least 200 healthy volunteers.

TAFIa-dependent prolongation of clot-lysis time

TAFIa-dependent prolongation of clot-lysis time was determined by measuring clot-lysis times in the absence or presence of potato carboxypeptidase inhibitor (CPI, 12 µM). The TAFIa-dependent prolongation was defined as the difference in clot-lysis time in the absence and presence of CPI.

TAFIa activity

Plasma TAFI levels were determined in a TAFIa activity assay as previously described^[29], except that plasma was diluted 30 times.

All assays were performed by technicians who were unaware of the thyroid status of the samples.

Statistical analysis

Statistical analysis was performed using SPSS software, version 15.0 (SPSS Inc, Chicago, IL, USA). In the hyperthyroidism studies, relative changes per parameter for each individual

were calculated by subtracting the baseline value from the day 14 value, dividing it by the baseline value and multiplying the result by 100%. Change from baseline values were compared between levothyroxine treatment and no medication by Wilcoxon tests. In the hypothyroidism study, values during hyperthyroidism and euthyroidism were compared by Wilcoxon tests. Results are presented as medians with 95% confidence intervals (95% CI).

RESULTS

Hyperthyroidism: levothyroxine 0.3 mg per day

Sixteen participants (9 men and 7 women) completed the study with 0.3 mg per day levothyroxine. The mean age was 30 (range 25 to 39) years, with a body mass index of 24 (range 19 to 33) kg/m². (Table 1).

Treatment with levothyroxine 0.3 mg per day for 14 days resulted in significantly increased levels of FT4 and T3, and reduced levels of TSH, compared to the control situation. Median thyroid hormone values in participants treated with levothyroxine 0.3 mg per day were 28.5 pM (95% CI 20.0 to 33.0 pM) for FT4, 2.50 nM (95% CI 2.20 to 3.10 nM) for T3 and 0.09 mU/L (95% CI 0.02 to 0.22 mU/L) for TSH. A considerable number of individuals was still in the subclinical range (T3 levels within the reference range and TSH below the lower limit of the reference range). In the control situation, without levothyroxine treatment, thyroid hormone values remained within the normal reference range. (Table 2).

Although a number of individuals was still in the subclinical range, a trend towards prolonged clot-lysis times was observed after levothyroxine exposure compared to the control situation; median change 7% (95% CI 2 to 12%) vs 2% (95% CI -2 to 3%), *p*=0.14. In addition, plasma TAFI levels and TAFIa-dependent prolongation of clot-lysis were determined. No effect of levothyroxine 0.3 mg per day on plasma TAFI levels was observed (*p*=0.54). However, a trend towards increased TAFIa-dependent prolongation of clot-lysis was found after levothyroxine exposure compared to no medication; median change 12% (95% CI 4 to 24%) vs 1% (95% CI -7 to 10%), *p*=0.09. (Table 2).

Table 1. Baseline characteristics.

	Mild hyperthyroidism (n=16)	More pronounced hyperthyroidism (n=12)	Hypothyroidism (n=20)
Age (years)	30 (25; 39)	29 (26; 40)	52 (25; 86)
Gender (% male)	56.3	50.0	35.0
Body-mass index (kg/m2)	24.2 (19.2; 33.0)	23.3 (21.1; 26.2)	27.4 (17.9; 40.0)

N indicates number. Data are presented as mean (range).

Table 2. Healthy volunteers: mild hyperthyroidism.

	No medication			Levothyroxine 0.3 mg per day			
	t=0 days	t=14 days	Change (%)	t=0 days	t=14 days	Change (%)	p-value (change)
FT4 (pM)	15.0 (13.0; 16.0)	15.0 (14.0; 16.0)	0 (-13; 11)	16.0 (14.0; 18.5)	28.5 (20.0; 33.0)	83 (20; 116)	<0.01
T3 (nM)	1.85 (1.70; 1.90)	1.80 (1.80; 2.10)	0 (-5; 7)	2.05 (1.70; 2.20)	2.50 (2.20; 3.10)	36 (4; 56)	<0.01
TSH (mU/L)	1.75 (1.21; 1.94)	2.15 (1.73; 2.27)	21 (9; 35)	1.74 (1.32; 2.72)	0.09 (0.00; 0.22)	-96 (-98; -92)	<0.01
Clot-lysis time (% pooled plasma)	94 (88; 105)	97 (86; 109)	2 (-2; 3)	95 (85; 106)	109 (98; 111)	7 (2; 12)	0.14
TAFIa-dependent prolongation of clot-lysis (% pooled plasma)	96 (84; 113)	100 (83; 125)	1 (-7; 10)	94 (74; 115)	109 (91; 128)	12 (4; 24)	0.09
TAFI concentration (% pooled plasma)	89 (79; 97)	87 (75; 101)	-3 (-8; 7)	91 (79; 101)	89 (77; 103)	-3 (-9; 4)	0.54

FT4 indicates free thyroxine; T3, tri-iodothyronine; TSH, thyrotropin; and TAFI, thrombin-activatable fibrinolysis inhibitor. Data are presented as medians (95% confidence intervals).

Hyperthyroidism: levothyroxine 0.45 mg / 0.6 mg per day

To induce more pronounced hyperthyroidism, a second study was performed including 12 participants, 6 men and 6 women. Based on their body weight, 3 participants received levothyroxine 0.6 mg per day, while 9 participants received levothyroxine 0.45 mg per day. The mean age was 29 (range 26 to 40) years, with a body mass index of 23 (range 21 to 26) kg/m². Five of these participants had also participated in the first hyperthyroidism study. (Table 1). As expected, treatment with these higher of doses of levothyroxine resulted in more pronounced hyperthyroidism. Median thyroid hormone values after levothyroxine exposure were 40.0 pM (95% CI 37.5 to 47.0 pM) for FT4, 3.80 nM (95% CI 3.35 to 4.05 nM) for T3 and 0.02 mU/L (95% CI 0.02 to 0.03 mU/L) for TSH. In the control situation, without treatment of levothyroxine, thyroid hormone values remained within the normal reference range. (Table 3). Levothyroxine exposure significantly prolonged clot-lysis times compared to the control situation; median change 14% (95% CI 9 to 17%) vs 2% (95% CI -4 to 4%), $p<0.01$. Although not statistically significant, a trend towards reduced plasma TAFI levels was observed after levothyroxine exposure compared to the control situation; median change -3% (95% CI -10 to 0%) vs 4% (95% CI -2 to 13%), $p=0.11$. In addition, levothyroxine exposure significantly enhanced TAFIa-dependent prolongation of clot-lysis times compared to no medication; median change 19% (95% CI 5 to 33%) vs 7% (95% CI -6 to 13%), $p=0.03$. (Table 3).

Table 3. Healthy volunteers: more pronounced hyperthyroidism.

	No medication			Levothyroxine 0.45 / 0.6 mg per day			
	t=0 days	t=14 days	Change (%)	t=0 days	t=14 days	Change (%)	p-value (change)
FT4 (pM)	15.0 (12.5; 15.0)	14.0 (13.5; 15.0)	0 (-7; 8)	14.5 (14.0; 16.0)	40.0 (37.5; 47.0)	186 (143; 216)	<0.01
T3 (nM)	1.90 (1.70; 2.20)	2.10 (1.95; 2.40)	3 (-5; 29)	2.35 (2.15; 2.60)	3.80 (3.35; 4.05)	58 (48; 83)	<0.01
TSH (mU/L)	1.64 (1.19; 3.07)	1.65 (1.49; 2.97)	8 (-27; 29)	1.86 (1.49; 3.08)	0.02 (0.02; 0.03)	-99 (-99; -98)	<0.01
Clot-lysis time (% pooled plasma)	89 (79; 95)	88 (83; 95)	2 (-4; 4)	94 (82; 99)	104 (93; 113)	14 (9; 17)	<0.01
TAFIa-dependent prolongation of clot-lysis (% pooled plasma)	82 (70; 106)	94 (74; 108)	7 (-6; 13)	97 (79; 111)	108 (90; 128)	19 (5; 33)	0.03
TAFI concentration (% pooled plasma)	85 (66; 90)	86 (73; 90)	4 (-2; 13)	89 (75; 97)	81 (78; 90)	-3 (-10; 0)	0.11

FT4 indicates free thyroxine; T3, tri-iodothyronine; TSH, thyrotropin; and TAFI, thrombin-activatable fibrinolysis inhibitor. Data are presented as medians (95% confidence intervals).

Hypothyroidism

To evaluate the effect of hypothyroidism on TAFI, 20 patients with newly diagnosed overt hypothyroidism were studied, of whom 7 men and 13 women. All patients reached euthyroidism after substitution therapy. Patients had a mean age of 52 (range 25 to 86) years, with a body mass index of 27 (range 18 to 40) kg/m². (Table 1).

The median thyroid hormone levels at inclusion were consistent with hypothyroidism; 6.5 pM (95 % CI 5.0 to 8.0 pM) for FT4 and 58.37 nM (95% CI 45.76 to 91.09 nM) for TSH. Levels returned to normal values after treatment with levothyroxine. (Table 4).

Patients showed significantly reduced clot-lysis times during hypothyroidism compared to euthyroidism; median 93% (95% CI 88 to 102%) vs 106% (95% CI 96 to 110%), $p<0.01$. Plasma TAFI levels did not differ between the hypothyroid and the euthyroid state; median 98% (95% CI 93 to 115%) vs 96% (95% CI 90 to 105%), $p=0.20$, while TAFIa-dependent prolongation of clot-lysis was reduced during hypothyroidism compared to euthyroidism; median 89% (95% CI 71 to 108%) vs 114% (95% CI 92 to 125%), $p<0.01$. (Table 4).

Table 4. Hypothyroidism.

	Hypothyroidism	Euthyroidism	Change	p-value
FT4 (pM)	6.5 (5.0; 8.0)	17.0 (16.0; 18.0)	146 (113; 208)	<0.01
TSH (mU/L)	58.37 (45.76; 91.09)	1.94 (0.87; 3.29)	-96 (-99; -95)	<0.01
Clot-lysis time (% pooled plasma)	93 (88; 102)	106 (96; 110)	10 (5; 16)	<0.01
TAFIa-dependent prolongation of clot-lysis (% pooled plasma)	89 (71; 108)	114 (92; 125)	20 (9; 24)	<0.01
TAFI concentration (% pooled plasma)	98 (93; 115)	96 (90; 105)	-7 (-14; 3)	0.20

FT4 indicates free thyroxine; TSH, thyrotropin; and TAFI, thrombin-activatable fibrinolysis inhibitor. Data are presented as medians (95% confidence intervals).

DISCUSSION

This study showed that hyperthyroidism results in hypofibrinolysis and enhanced TAFIa-dependent prolongation of clot-lysis times, while a trend towards reduced plasma TAFI levels was observed. A hyperfibrinolytic condition with reduced TAFIa-dependent prolongation of clot-lysis times was found in hypothyroidism, despite unaltered TAFI levels.

Our findings corroborate previous reports describing reduced plasma TAFI levels in patients with hyperthyroidism or in patients receiving thyroid hormone substitution therapy^[22;23;26]. Reduced plasma TAFI levels suggest a hyperfibrinolytic state, however, this is inconsistent with the overall hypofibrinolytic condition observed in hyperthyroid patients. Although the fibrinolytic condition in patients with hyperthyroidism is best explained by simultaneous alterations in levels of plasminogen activator inhibitor-1^[9;26], TAFI activity may play an additional role.

To our knowledge, this study investigated for the first time a role of TAFIa in an *in vitro* fibrinolysis assay during hyper- or hypothyroidism. In these experiments, coagulation was initiated by tissuefactor and thereby TAFI activation occurs only via thrombin that has been generated by the coagulation cascade. We observed increased TAFIa-dependent prolongation of clot-lysis times in individuals with hyperthyroidism, indicating that TAFI may shift the fibrinolytic balance towards a hypofibrinolytic condition. A hypofibrinolytic condition is a risk factor for cardiovascular disease or thrombosis, and TAFI may thereby contribute to the development of thrombotic events in patients with hyperthyroidism. Conversely, patients with hypothyroidism showed a reduced TAFIa-dependent prolongation of fibrinolysis, suggesting that TAFI may be involved in the increased bleeding tendency as is observed in these patients.

Interestingly, in individuals with thyroid hormone excess or deficiency, the changes in plasma TAFI levels are conflicting with respect to the antifibrinolytic activity of TAFI. This might be explained by TAFI activation via thrombin. Since the procoagulant changes observed in patients with hyperthyroidism may result in enhanced thrombin generation, this may subsequently give rise to increased TAFI activation, independent of the plasma TAFI concentration, and vice-versa in patients with hypothyroidism. An alternative explanation for the paradoxical combination of TAFI levels and its antifibrinolytic function may be that other biological mechanisms are affected by TAFI. Since TAFI is known to play a role in inflammation processes^[19,30], it would be interesting to investigate inflammatory markers in future studies.

In conclusion, alterations of TAFI-dependent prolongation of clot-lysis in patients with thyroid disorders may cause an impaired haemostatic balance. The disturbed haemostatic balance in patients with hyperthyroidism might make them more prone for thrombosis, while the risk for bleeding may increase in patients with hypothyroidism.

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Chapter 11

**The effects of an extremely
high dose of levothyroxine on
coagulation and fibrinolysis**

Danka JF Stuijver, Bregje van Zaane,
Alessandro Squizzato, Joost CM Meijers,
Hans-Martin Otten

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ABSTRACT

Several haemostatic abnormalities have been reported in thyroid hormone excess. However, the overall effect on coagulation and fibrinolysis is still unclear. We were presented the unique opportunity to further explore the effects of excessive levels of thyroid hormone in the case of a young woman who attempted suicide by auto-intoxication with levothyroxine. Levels of thyroid hormones and parameters of coagulation and fibrinolysis were assessed in the days following the auto-intoxication. We found a marked increase in levels of coagulation factors VIII, IX and X, von Willebrand factor and plasminogen activator inhibitor-1, resulting in enhanced thrombin generation after intoxication with 25 mg of levothyroxine. These findings suggest that thyroid hormone excess shifts the haemostatic balance towards a hypercoagulable and hypofibrinolytic state, and is therefore likely to reflect an increased risk of venous thrombosis.

INTRODUCTION

Although the link between the haemostatic system and thyroid dysfunction has been known for many years, controversy remains whether thyroid hormone excess leads to an increased risk of venous thrombosis^[1,2]. In patients with hyperthyroidism, a hypercoagulable and hypofibrinolytic state has been suggested^[3]. This was further supported by the observation of increased levels of factor VIII (FVIII) and von Willebrand factor (VWF) after administration of oral thyroid hormones in healthy volunteers^[3,4]. However, the overall effect of thyroid hormone on coagulation activation and inhibition of fibrinolysis remains unclear. A unique situation was presented to us in the case of a young woman who attempted suicide using an extremely high dose of levothyroxine. We assessed levels of thyroid hormones and parameters of coagulation and fibrinolysis in the days following the auto-intoxication and in the succeeding months until restoration of euthyroidism.

CASE REPORT

A 23-year-old woman, who was on levothyroxine treatment for iatrogenic hypothyroidism after subtotal thyroidectomy for Morbus Graves, was brought to the emergency room on October 17, 2008, three hours after ingestion of 25 mg levothyroxine (250 tablets of 100 µg). Apart from levothyroxine, the patient did not use concomitant medication. After gastric lavage, she was treated with active charcoal and magnesium sulphate. Propanolol was given to prevent cardiac arrhythmias. No clinical adverse effects of the levothyroxine occurred during hospital admittance; the patient was discharged after 6 days.

During her full hospital stay, daily blood sampling was performed for assessment of thyroid hormones, i.e. serum free thyroxine (FT4), total tri-iodothyronine (T3), and thyroid stimulating hormone (TSH), as well as coagulation and fibrinolytic parameters, with permission of the patient. Citrated blood was processed two times by centrifugation at 2500 g for 15 minutes at 15 degrees, and stored at -80 °C until further use.

The course of thyroid hormones, and parameters of coagulation and fibrinolysis is shown in Figure 1. Serum free thyroxine levels are a direct reflection of the ingested levothyroxine, whereas total tri-iodothyronine is the active thyroid hormone. T3 levels slowly increased until a maximum at day 2, 24 hours after ingestion, was reached. Subclinical hypothyroidism was noted at day 40, and levothyroxine 0.15 mg once daily was started. Euthyroidism was restored at day 244.

Levels of factor VIII (FVIII), factor X (FX), von Willebrand factor activity (VWF:RiCo) and antigen (VWF:Ag) slowly increased during the first days after intoxication until a maximum was reached at day 5 (FVIII 172%, FX 81%, VWF:RiCo 148%, VWF:Ag 131%), whereas levels of plasminogen activator inhibitor-1 (PAI-1) peaked shortly after intake (140 ng/ml). Levels of factor X (FIX) were highest at day 19 (103%). As a result of these alterations, enhanced thrombin generation (ETP, area under the curve) was observed during the first days after intoxication, with a maximum at day 3. No clear alterations were observed for coagulation factors II and VII, prothrombin time, activated partial thromboplastin time (aPTT), protein C activity and protein S antigen (total and free), and prothrombin fragment (data not shown).

Figure 1. Biochemical and haemostatic parameters after ingestion of a high dose of levothyroxine. T3 indicates total tri-iodothyronine; FT4, serum free thyroxine; TSH, thyroid stimulating hormone; FVIII, factor VIII; VWF:RiCo, von Willebrand factor activity; VWF:Ag, von Willebrand factor antigen; FIX, factor IX; FX, factor X; PAI-1, plasminogen activator inhibitor-1; and ETP, endogenous thrombin potential. Local reference ranges: T3 1.2-2.8 nmol/L; FT4: 10-23 pmol/L; TSH 0.31-4.5 mIU/L; FVIII 63-173%; VWF:RiCo 58-172%; VWF:Ag 50-150%; FIX 80-145%; FX 66-125%; PAI-1 <100 ng/ml; ETP 1155-2606 nM.min.

Levels of T3 positively correlated with levels of ETP (Spearman's R 0.83) and PAI-1 (R 0.89). A negative correlation was found for TSH and FVIII (R -0.92), FIX (R -0.73), FX (R -0.80), VWF:RiCo (R -0.85), and VWF:Ag (R -0.98) (all p -values <0.01).

DISCUSSION

In this case report, we found that levothyroxine activates the coagulation system and inhibits fibrinolysis. In line with the present literature, levels of coagulation factors tended to normalise once euthyroidism was restored. Moreover, levels of FVIII, FIX and VWF even further decreased during subclinical hypothyroidism as previously described^[5,6].

Although in the present case most coagulation factors remained within the upper range of the local reference range, the combination of an increase of several coagulation factors, ETP and PAI-1 indicates a shift towards a procoagulant and hypofibrinolytic state in this patient. In the absence of well-designed epidemiological studies on the association between thyroid hormone excess and venous thrombosis, the measurement of endogenous thrombin generation (ETP), i.e. the thrombin forming capacity of the plasma, is one way of further elucidating the risk of venous thrombosis^[7]. In the present case, we observed a clear relation between T3 and ETP suggesting the risk of thrombosis to gradually rise with increasing levels of thyroid hormone. Although the increase of FVIII, FIX and FX was likely to induce enhanced thrombin generation, we cannot explain the earlier normalisation of ETP levels compared to the coagulation factors. Future studies are needed to clarify the relation between ETP and thyroid hormone excess.

The underlying mechanisms by which thyroid hormones may affect coagulation remain ill-defined. However, the observed delay in alterations of these coagulation factors is best explained by enhanced synthesis at hepatic or endothelial level, whereas the rapid increase in PAI-1 may be due to enhanced secretion^[8,9].

Our results may be influenced by the fact that the patient received concomitant medication at the emergency room, which can obscure the effects of levothyroxine. However, additional administration of propranolol was found not to influence factor VIII levels in healthy volunteers receiving levothyroxine, whereas magnesium sulphate has been reported to have potential antithrombotic effects^[4,10]. It is therefore unlikely that this would have affected our findings.

In conclusion, these data suggest that thyroid hormone excess shifts the haemostatic balance towards a hypercoagulable and hypofibrinolytic state, and is therefore likely to reflect an increased risk of venous thrombosis. Further prospective studies should focus on the influence of hyperthyroidism on the risk of venous thrombosis, and subsequently on the mechanisms behind this presumed relationship.

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Part 2

Thyroid and vitamin K antagonists

Chapter 12

Thyroid disease and haemostasis
A relationship with clinical
implications?

Alessandro Squizzato, Victor EA Gerdes

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The association between thyroid diseases and the haemostatic system has been investigated since the beginning of the past century, but underlying mechanisms and their clinical impact have not been definitely elucidated^[1]. There are indications that thyroid diseases modify primary and secondary haemostasis and lead to bleeding and thrombosis. The possible pathophysiological mechanisms are thyroid hormone excess or deficiency, autoimmunity or a direct mechanical effect of an enlarged thyroid gland. While some patients with overt hypothyroidism have a bleeding tendency due to an acquired von Willebrand syndrome^[2], patients with overt hyperthyroidism do have increased factor VIII and von Willebrand Factor levels. However, an increased thrombotic risk for patients with hyperthyroidism has not been proven.

In addition, an influence of overt thyroid dysfunction on oral vitamin K antagonist sensitivity has been described^[3]. Although not recognised by several clinicians, the effect of vitamin K antagonists is potentiated in thyrotoxicosis and attenuated in hypothyroidism. This disease-drug interaction has been described in a number of case reports and in animal studies, in particular for overt hyperthyroidism, in which a change in thyroid function was associated with a change in warfarin sensitivity, irrespective of the nature of the underlying thyroid disease^[3,4]. As the pharmacokinetic parameters – plasma clearance, volume of distribution, plasma protein binding – were unaffected by thyrotoxicosis, warfarin potentiation was thought to reflect an enhanced pharmacodynamic effect^[3]. This means that hypothyroid patients require higher dosages to obtain the target international normalised ratio (INR) compared to euthyroid anticoagulated patients and, conversely, hyperthyroid patients require a reduced dosage to reach the target INR.

Data on subclinical thyroid dysfunction and anticoagulation are lacking. In this issue of *Thrombosis and Haemostasis*, Bucerius and colleagues present the first published study on the influence of subclinical hyperthyroidism on oral vitamin K antagonist therapy^[5]. Retrospectively, they studied 178 patients with subclinical hyperthyroidism undergoing iodine-131 therapy for a benign thyroid disease that were on vitamin K antagonists with an INR target range of 2 to 3. The patients with subclinical hyperthyroidism were compared with two small control groups (overt hyperthyroidism, 15 patients; euthyroidism, 40 patients). The main outcome variable was an INR value within or out of target range during hospital stay for iodine-131. In a subgroup of 40 patients with subclinical hyperthyroidism, in which also INR values during euthyroidism were available, these INR values were compared. Multivariate analysis and intraindividual comparison did not show any effect. This study shows that a strong interaction between subclinical hyperthyroidism on oral vitamin K antagonists is unlikely.

However, some aspects require comment. Sensitivity and stability are the main characteristics of treatment with oral vitamin K antagonist that can be modified by any disease or external factors (drugs, food, seasonal variations, etc.). A different mean dosage of oral vitamin K antagonist required to maintain INR within the target range in the presence or in the absence of any agents is per definition a modified sensitivity of oral vitamin K antagonist. This is relevant for clinical practice because physicians should measure INR more frequently at the occurrence and at the disappearance of any clinical status influencing oral vitamin K antagonist sensitivity, such as in case of overt hyperthyroidism and hypothyroidism. Influence

on oral vitamin K antagonist stability is also clinically relevant. Instability is associated with a greater risk of haemorrhage and thrombotic complications. Of the various methods used to assess the stability, the time spent in the therapeutic range is the most commonly used and widely accepted parameter because it is easy to calculate when INR data are available^[6]. This time span is estimated by linear interpolation between successive INR measurements, calculating the portion of time during each interval that was spent in-range, summing across all intervals, and then dividing by the total duration of therapy. Unfortunately, whether subclinical hyperthyroidism influences oral vitamin K antagonist sensitivity has not been analysed by Bucerius and colleagues. Their analysis suggests a lack of effect on oral vitamin K antagonist stability, but the calculation of the time spent in the therapeutic range would have provided more convincing evidence. Regarding sensitivity, no data have been provided on mean oral vitamin K antagonist dosage: the subgroup of 40 patients would have been an optimal population. Finally, the moderate sample size, especially of the control group, for a study of patients with a subclinical disease limits the statistical power and makes it impossible to draw strong conclusions. In spite of these limitations, the authors' effort is still relevant for its potential clinical and research impact. As they correctly underlined in the manuscript, subclinical hyperthyroidism may cause atrial fibrillation that often requires oral anticoagulation. Therefore, an adequately powered prospective study on the sensitivity and stability of vitamin K antagonists in patients with subclinical hyperthyroidism is warranted. Even if routine thyroid hormone screening in a patient with new atrial fibrillation detects a hormonal cause of atrial fibrillation, patients may be at risk of complications until euthyroidism is reached. Moreover, thyroid-blocking agents, such as methimazole, and antiarrhythmic drugs for atrial fibrillation modify oral vitamin K antagonist sensitivity. In particular, amiodarone causes an increased sensitivity to warfarin that causes an increased INR at the beginning of therapy^[3]. Beyond the predictable pharmacokinetic drug-drug interaction requiring a significant warfarin dose reduction, the iodine-rich amiodarone affects the thyroid gland, causing overt hypothyroidism or thyrotoxicosis in 14%-18% of cases^[3]. Many physicians are not familiar with these complex drug-drug disease interactions. More research on this topic will definitely clarify the relationship and may, in the meantime, improve the awareness of this relationship among clinicians.

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Chapter 13

The effect of subclinical
hypothyroidism on
vitamin K stability and sensitivity

Alessandro Squizzato, Luca Galli,
Bregje van Zaane, Francesco Dentali,
Achille Venco, Walter Ageno,
Harry R Büller, Victor EA Gerdes

ABSTRACT

Introduction: The effect of vitamin K antagonists (VKAs) is potentiated in overt hyperthyroidism and attenuated in overt hypothyroidism. Data on subclinical thyroid dysfunction and anticoagulation are lacking, in particular on subclinical hypothyroidism. The aim of this study is to explore the effect of subclinical hypothyroidism on VKAs sensitivity and stability.

Methods: Among 996 patients followed at the Anticoagulation Clinic of the Department of Clinical Medicine of the University Hospital of Varese, Italy, patients with subclinical hypothyroidism occurring during VKAs treatment, who were treated with L- thyroxine and achieved an euthyroid state still on VKAs, were included in the study. VKAs sensitivity was calculated as median weekly dosage in mg. VKAs stability was measured as time spent in the therapeutic range (TTR). Weekly dosage and TTR were calculated during 6 and 12 weeks before objective diagnosis of subclinical hypothyroidism, during the first 6 and 12 weeks after L-thyroxine treatment was began, and during the first 6 and 12 weeks after objective euthyroidism has been reached.

Results: Twenty-six patients (8 males; median age 72.1 years) became subclinical hypothyroid during VKAs treatment. Half of the patients had a Hashimoto's thyroiditis and half an iatrogenic cause of subclinical hypothyroidism. Main indication for anticoagulation was atrial fibrillation and two third of the patients had a target INR of 2.5. During a 6 weeks time interval, mean weekly dosage was 29.9 mg (± 12.1 standard deviation [SD]) in subclinical hypothyroidism and 26.8 mg (± 12.1 SD) in euthyroidism ($P < 0.05$); median TTR was 61% (33-91 interquartile range [IR]) in subclinical hypothyroidism and 81% (51-100 IR) in euthyroidism (median difference = 13.5%; 95.2 % confidence interval -8.5 to 30.5%). During a 12 weeks time interval, mean weekly dosage was 30.8 mg (± 12.4 SD) in hypothyroidism and 27.0 mg (± 12.7 SD) in euthyroidism ($P < 0.05$); median TTR was 54 % (36-82 IR) in subclinical hypothyroidism and 65 % (55-79 IR) in euthyroidism.

Conclusions: Data of our pilot study suggest that subclinical hypothyroidism may affect both VKAs stability and sensitivity. Given the high prevalence of thyroid disorders in the anticoagulated population, larger studies are urgently warranted.

INTRODUCTION

A large proportion of patients on vitamin K antagonists spend up to half of their time outside the therapeutic range, with resultant increased risk of either thromboembolic or bleeding complications^[1]. Age, concurrent medications, comorbidities, in particular heart failure, and patient's compliance affect the quality of control of anticoagulant therapy in a predictable way. However, intraindividual variability in the response to vitamin K antagonists remains often unexplained^[2,3].

Thyroid disorders are common in patients on warfarin therapy, in particular because atrial fibrillation may be secondary to hyperthyroidism or because patients may develop amiodarone-induced dysfunction, either hypothyroidism or thyrotoxicosis^[4]. An influence of overt thyroid dysfunction on oral vitamin K antagonist sensitivity is well known: the effect of vitamin K antagonists is potentiated in thyrotoxicosis and attenuated in hypothyroidism^[5]. Data on subclinical hyperthyroidism suggest no significant impact on anticoagulation, but data on subclinical hypothyroidism are lacking^[6].

The aim of this pilot study was to explore, for the first time, the effect of subclinical hypothyroidism on vitamin K antagonists' sensitivity and stability.

MATERIAL AND METHODS

Study design and population

All patients followed at the Anticoagulation Clinic of the Department of Clinical Medicine of the University Hospital of Varese, Italy, at December 2009 and treated with the two available oral vitamin K antagonists, i.e. warfarin and acenocoumarol, were potentially eligible. One investigator (LG) retrospectively reviewed electronic and medical records to identify a past or present history of thyroid disease. Patients were asked to provide additional documents to objectively prove their thyroid disease. If information was lacking, data were retrieved by the electronic database of the Nuclear Medicine Laboratory. Patients were included if they fulfilled all three of the following criteria: 1. subclinical hypothyroidism occurring during vitamin K antagonist treatment; 2. treated with levothyroxine while on vitamin K antagonist; and 3. achieving an euthyroid state while still on vitamin K antagonists. We considered both naïve subclinical hypothyroidism and patients who developed subclinical hypothyroidism due to an insufficient replacement therapy with levothyroxine. No restriction was applied for target international normalized ratio (INR) or for vitamin K antagonist indication. For the included patients, we collected data on laboratory assessment of thyroid function as described above, and on INR values with PARMA 5.7 (Instrumentation Laboratory, Italy), the electronic database and software for INR monitoring used in our centre.

Definition of subclinical hypothyroidism and laboratory assay

Subclinical hypothyroidism was biochemically defined by increased serum TSH concentration in the presence of normal serum FT4 and/or FT3 concentrations^[7]. Serum free thyroxine (FT4; FT4 RIA, Brahms, Germany), free triiodothyronine (FT3; FT3 SPART RIA, Brahms), thyrotropin (TSH; TSH-CTK-3, DiaSorin, Italy), were assayed by commercial methods. Reference values in

our laboratory are as follows: FT4, 0.75 – 1.90 ng/dl; FT3, 1.50 – 5.30 pg/ml; TSH, 0.31 – 4.50 mU/l. Thyroid autoantibodies and antiphospholipid antibodies were not routinely checked.

Statistical analysis

Results of categorical variables were expressed as number and percentage. Continuous variables were reported as means or medians (range), depending on the distribution of data. Normal distribution was explored with Shapiro-Wilk test. If normally distributed, the paired t-test for mean value was applied; if not, the non-parametric Wilcoxon signed ranks test was used. A *P*-value below .05 was considered statistically significant. Vitamin K antagonist sensitivity was calculated as mean weekly dosage in mg. To include also patients on acenocoumarol, given that a validated transformation coefficient is not available, we considered 1 mg of acenocoumarol as equivalent to 2 mg of warfarin. Vitamin K antagonist stability was measured as time spent in the therapeutic range, estimated by linear interpolation between successive INR measurements, calculating the proportion of time during each interval that was spent in-range, summing across all intervals, and then dividing by the total duration of therapy^[8]. Weekly dosage and time spent in the therapeutic range were calculated during 6 and 12 weeks before objective diagnosis of subclinical hypothyroidism or suboptimal replacement therapy (phase A), during the first 6 and 12 weeks after levothyroxine treatment was began or the levothyroxine dose was altered (phase B), and during the first 6 and 12 weeks after objective euthyroidism had been reached (phase C). A period of six and 12 weeks was arbitrary selected to identify a period at high probability to be in the defined thyroid state. In case of vitamin K interruption for a dental, invasive or surgical procedure, time spent in the therapeutic range was not calculated or an adjacent period was included. Analyses were performed with PARMA, and with StatsDirect 2.7.2 (StatsDirect Ltd, United Kingdom).

RESULTS

Overall, 996 patients were on vitamin K antagonist, and 118 (11.8%) had thyroid disease. Sixty-seven had hypothyroidism (57%), 16 hyperthyroidism (14%) and 35 (30%) a thyroid disease without abnormal hormone levels (e.g. multinodular goiter). Twenty-six patients (8 males; median age 72.1 years) had an episode of subclinical hypothyroidism during vitamin K antagonist treatment and objective data on thyroid disease available. Half of the patients had a Hashimoto's thyroiditis and the other half an iatrogenic cause of subclinical hypothyroidism (Table 1). Two third of the patients have never been treated with levothyroxine. Main indication for anticoagulation was atrial fibrillation and two third of the patients had a target INR of 2.5. Almost 70% of this population was on chronic therapy with 3 or more drugs other than vitamin K antagonists and levothyroxine. In some patients, co-medication was altered during one of the analyzed time intervals. Methimazole was stopped and prednisone was introduced in one patient during subclinical hypothyroidism. Two patients received a brief course of antibiotics during levothyroxine treatment. In the euthyroid phase, to the same patient whose therapy was modified in hypothyroidism lovastatin and glicazide were added, allopurinol was started in another patient and double antiplatelet therapy (clopidogrel plus aspirin) was introduced in a third patient. Other baseline characteristics were summarized in Table 1.

Table 1. Patient characteristics.

	Subclinical hypothyroidism (n=26)
Male, n (%)	8 (31)
Age, median (IR), years	72.1 (63.6-76.9)
VKA	
- Warfarin	21 (81)
- Acenocoumarol	5 (19)
INR target	
- 2.5	20 (77)
- 3.0	6 (23)
Primary indication for anticoagulation therapy ^a	
Atrial fibrillation, n (%)	13 (50)
Venous thromboembolism, n (%)	3 (11.5)
Heart valve prosthesis, n (%)	6 (23.1)
Antiphospholipid syndrome, n (%)	3 (13.9)
Severe dilatative cardiomyopathy, n (%)	1 (3.8)
On chronic therapy with 3 or more drugs other than VKA and levothyroxine	18 (69)
Thyroid disorder	
- Hashimoto's thyroiditis, n (%)	13 (50)
- Iatrogenic	13 (50)
- Amiodarone, n (%)	9 (35)
- Surgical, n (%)	2 (8)
- Radioiodine, n (%)	2 (8)

Type of hypothyroidism	
- Naïve patient, n (%)	18 (69)
- Insufficient levothyroxine treatment, n (%)	8 (31)
Time interval between VKA start and objective subclinical hypothyroidism diagnosis (median and IR, months)	14.2 (5.5-87.1)
Time interval between levothyroxine start and euthyroidism ^b (median and IR, months)	8.1 (2.9-15.4)

VKA indicates vitamin K antagonist; n, number; IR, interquartile range; and SD, standard deviation

^aThree patients had more than one disease requiring vitamin K antagonist

^bEuthyroidism was defined as the first period of 6 and 12 weeks in which thyroid hormones were within the normal ranges and treatment with VKA had not been interrupted

During a 6 weeks time interval, mean weekly dosage was 29.9 mg (± 12.1 standard deviation [SD]) in subclinical hypothyroidism and 26.8 mg (± 12.1 SD) in euthyroidism ($P < 0.05$); median time spent in the therapeutic range was 61% (33-91 interquartile range [IR]) in subclinical hypothyroidism and 81% (51-100 IR) in euthyroidism (median difference 13.5%; 95 % confidence interval for difference between population medians: - 8.5% to 30.5%). During a 12 weeks time interval, mean weekly dosage was 30.8 mg (± 12.4 SD) in subclinical hypothyroidism and 27.0 mg (± 12.7 SD) in euthyroidism ($P < 0.05$); median time spent in the therapeutic range was 54 % (36-82 IR) in subclinical hypothyroidism and 65 % (55-79 IR) in euthyroidism. A statistically significant difference in mean weekly dosage was shown also between the first period of replacement therapy with levothyroxine (phase B) and euthyroidism, but no difference was detected between subclinical hypothyroidism and the first period of replacement therapy with levothyroxine (Table 2 and 3).

Table 2. Vitamin K sensitivity (mean weekly dosage and standard deviation)

N	6 weeks	12 weeks
<i>Subclinical hypothyroidism (phase A)</i>		
25 ^a	29.9 mg $\pm$ 12.1 ^b	30.8 mg $\pm$ 12.4 ^c
<i>Levothyroxine treatment (phase B)</i>		
24 ^a	29.3 mg $\pm$ 13.0 ^d	28.1 mg $\pm$ 13.0 ^e
<i>Euthyroidism (phase C)</i>		
23	26.8 mg $\pm$ 12.2 ^{b,d}	27.0 mg $\pm$ 12.7 ^{c,e}

N indicates number of patient with available data

^aFor 6 weeks, data available for all 26 patients

^{b,c,d,e} $P < 0.05$

Table 3. Vitamin K stability (median time spent in the therapeutic range and interquartile range)

N	6 weeks	12 weeks
<i>Subclinical hypothyroidism (phase A)</i>		
25 ^a	61 % (33-91) ^b	54 % (36-82)
<i>L-thyroxine treatment (phase B)</i>		
24 ^a	67 % (46-89)	62 % (52-84)
<i>Euthyroidism (phase C)</i>		
23	81 % (51-100)	65 % (55-79)

N indicates number of patient with available data

^aFor 6 weeks, data available for all 26 patients

^bP=0.27 compared to euthyroidism

DISCUSSION

Our pilot study suggests that subclinical hypothyroidism has an effect both on vitamin K antagonist stability and sensitivity. During subclinical hypothyroidism patients had a diminished sensitivity to vitamin K antagonists in comparison with the euthyroid state, both considering a 6 and 12 weeks time interval. This is in line with previous literature on overt hypothyroidism^[6]. We also noted a clear trend for decreased vitamin K antagonists' stability in subclinical hypothyroidism relative to the euthyroid state.

The effect of overt thyroid dysfunction on vitamin K antagonists sensitivity has been studied in the past^[4]. This disease-drug interaction has been described in a number of case reports and in animal studies in which a change in thyroid function was associated with a change in warfarin sensitivity, irrespective of the nature of the underlying thyroid disease: overt hyperthyroidism potentiated and overt hypothyroidism diminished the effect of vitamin K antagonists^[4;5]. Practically, it means that overt hypothyroid patients require higher dosages to reach target INR, and vice versa for overt hyperthyroidism. As the pharmacokinetic parameters – plasma clearance, volume of distribution, plasma protein binding – were unaffected by thyrotoxicosis, warfarin potentiation was thought to reflect an enhanced pharmacodynamic effect^[4].

Data on subclinical thyroid dysfunction and anticoagulation are sparse. To the best of our knowledge, only one study was published on patients with subclinical hyperthyroidism and our study is the first to investigate the effect of subclinical hypothyroidism on vitamin K antagonists' sensitivity and stability^[9]. Bucerius and colleagues studied 178 patients with subclinical hyperthyroidism undergoing iodine-131 therapy for a benign thyroid disease

that were on vitamin K antagonists with an INR target range of 2 to 3^[9]. The patients with subclinical hyperthyroidism were compared with two small control groups (overt hyperthyroidism, 15 patients; euthyroidism, 40 patients). The main outcome variable was an INR value within or out of target range during hospital stay for iodine-131. In a subgroup of 40 patients with subclinical hyperthyroidism, in which also INR values during euthyroidism were available, these INR values were compared. Apparently, they did not find any influence of subclinical state on the stability of vitamin K antagonist therapy. However, they did not measure time spent in the therapeutic range and no data on vitamin K antagonists' sensitivity were provided.

The clinical impact of our findings is potentially relevant. In our population of anticoagulated patients, more than 10% had a thyroid disease. Overt and subclinical hyperthyroidism may cause atrial fibrillation and their treatment may cause a temporary hypothyroid state. Thyroid blocking agents, such as methimazole, and antiarrhythmic drugs for atrial fibrillation modify oral vitamin K antagonist sensitivity. In particular, amiodarone causes an increased sensitivity to warfarin which causes an increased INR at the beginning of therapy^[4]. Beyond the predictable pharmacokinetic drug-drug interaction requiring a significant warfarin dose reduction, the iodine-rich amiodarone affects the thyroid gland, causing overt or subclinical hypothyroidism or thyrotoxicosis in more than 10% of cases^[4]. Many physicians are not familiar with these complex drug-drug-disease interactions.

Besides these relationships, subclinical hypothyroidism characteristics make our findings potentially of great impact. The prevalence of subclinical hypothyroidism in the adult population is about 4% to 8.5% in those without known thyroid disease^[7]. The prevalence increases with age, and in women older than 60 years, subclinical hypothyroidism is present in up to 20%^[7]. Subclinical hypothyroidism has no or few mild symptoms, and it usually cannot be recognised without thyroid hormone measurement^[7]. Therefore, patients may be at risk of INR fluctuations for several months. Moreover, when diagnosed, not every patient is routinely treated with Levothyroxine^[7]. Finally, patients already on levothyroxine often have periods of subclinical hypothyroidism due to an insufficient dosage. If our data will be confirmed by other studies, vitamin K antagonists could be a new criterion for the decision to start thyroid replacement therapy and to shorten time intervals between routinely thyroid hormones controls in patients on levothyroxine and vitamin K antagonists.

This study has some limitations. The included population is certainly underpowered to draw any definitive conclusions. We regard these data as hypothesis generating and useful for sample size calculations for future studies. Drug administration or withdrawal are well-known factors that may influence the quality of control of anticoagulant therapy and vitamin K antagonists sensitivity, and were not taken into account in the analysis. However, these factors were more prevalent in the euthyroid state. Moreover, as warfarin was self-administered, drug compliance was not specifically taken into account. Given the retrospective design of the study, it is probable that each patient had the same compliance in both hypothyroid and euthyroid state, and there is no reason to believe that the beginning of levothyroxine may increase patients compliance to vitamin K antagonists.

In conclusion, our study suggests a modest influence of subclinical hypothyroidism on vitamin K antagonists' stability and sensitivity. Even if future studies are needed to definitely conclude on this topic, physicians should remind to measure INR more frequently in patients with overt hyperthyroidism and hypothyroidism, and probably also in the situation of subclinical hypothyroidism. Moreover, it is possible that some of their unstable patients on vitamin K antagonists have a (subclinical) thyroid hormone excess or deficiency.

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Chapter 14

The effect of thyroid autoantibodies on warfarin stability

Francesco Dentali, Elena Rancan,
Bregje van Zaane, Victor EA Gerdes,
Water Ageno, Achille Venco,
Alessandro Squizzato

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ABSTRACT

Introduction: An influence of overt thyroid dysfunction on the sensitivity to oral vitamin K antagonists has been described. Data on the effect of thyroid dysfunction on warfarin stability are currently sparse and not conclusive. To our knowledge, no data have ever been published on the interaction between thyroid antibodies and warfarin. The aim this study was therefore to test the hypothesis of an effect of thyroid autoantibodies on warfarin stability.

Methods: A retrospective inception cohort study was carried out in a population of 100 consecutive adult outpatients with provoked or unprovoked deep venous thrombosis followed at the Anticoagulation Clinic of the Department of Clinical Medicine of the University Hospital of Varese, Italy, and treated with warfarin to achieve therapeutic range of the international normalized ratio (INR) between 2.0 and 3.0. Thyroid autoantibodies, i.e. anti-thyroid peroxidase (AbTPO), anti-thyroglobulin (AbTg), and anti-TSH receptor antibodies (AbTR), were measured in all patients. We compared warfarin stability in patients with elevated antibodies levels to those with antibodies within the normal range. Stability of oral anticoagulation was evaluated during a period of three months, before or after the date of antibody measurement, and presented as the percentage of time in range and the standard deviation (SD) of the mean INR values.

Results: Overall, 36 patients, 12 with elevated antibody levels (5 women; mean age 71.0 years) and 24 with normal antibody levels (10 women; mean age 66.5 years) with available INR values were analysed. Eight patients had elevated AbTPO levels (one also elevated TrAb), four elevated AbTg, and none isolated elevated TrAb. Percentage of time in range was slightly lower, although not significantly, in patients with elevated antibodies (61.9% vs 70.1%, $p=0.16$), whereas the SD of the INR values was higher in patients with elevated antibodies (0.83 vs 0.65, $p=0.05$), suggesting that thyroid autoantibodies may be responsible for the instability of the INR control.

Conclusions: The presence of thyroid autoantibodies may be associated with an increased instability of the INR control and, therefore, deserves further investigation in prospective clinical trials.

INTRODUCTION

A large proportion of patients on warfarin treatment spend up to half of their time outside the therapeutic range, with resultant increased risk of either thromboembolic or bleeding complications^[1]. While changes in concurrent medications, comorbidities, and patient's compliance affect the quality of control of anticoagulant therapy in a predictable way, intraindividual variability in the response to warfarin treatment remains often unexplained^[2]. Thyroid disorders are not uncommon in patients on warfarin therapy, in particular because atrial fibrillation may be secondary to hyperthyroidism or because patients develop amiodarone-induced hypothyroidism. An influence of overt thyroid dysfunction on the sensitivity to oral vitamin K antagonists has been described^[3]. The effect of vitamin K antagonists is potentiated in thyrotoxicosis and is attenuated in hypothyroidism. This disease-drug interaction has been described in a number of case reports and in animal studies, in particular for overt hyperthyroidism, where a change in thyroid function appears to be associated with a change in warfarin sensitivity, irrespectively of the nature of the underlying thyroid disease^[4]. However, data on the effect of thyroid dysfunction on warfarin stability are currently sparse and not conclusive^[5]. To our knowledge, no data have ever been published on the interaction between thyroid antibodies and warfarin.

METHODS

The aim of our retrospective inception cohort study was therefore to test the hypothesis of an effect of thyroid autoantibodies on warfarin stability. The study was carried out in a population of 100 consecutive adult outpatients with provoked or unprovoked deep venous thrombosis who were enrolled in an etiological cross-sectional study^[6]. Subjects and methods have been previously described^[6]. Briefly, all patients were followed at the Anticoagulation Clinic of the Department of Clinical Medicine of the University Hospital of Varese, Italy, and treated with warfarin to achieve therapeutic range of the international normalized ratio (INR) between 2.0 and 3.0. Thyroid autoantibodies, i.e. anti-thyroid peroxidase (AbTPO), anti-thyroglobulin (AbTg), and anti-TSH receptor antibodies (AbTR), were measured in all patients. The following commercial test were used: AbTPO, DYNtest anti-TPO_n, Brahms, Germany; AbTg, Anti-Tgn RIA, Brahms; TRAb, TRAK human RIA, Brahms. Reference values in our laboratory are as follows: AbTPO_{b60} U/mL; AbTg_{b60} U/mL; TRAb, b1 IU/L. Both cases and controls signed a written informed consent for the original study. For the purpose of this study, we compared warfarin stability in patients with elevated antibodies levels to those with antibodies within the normal range. For each case, we randomly selected two controls matched for age, gender and cause of deep venous thrombosis (provoked/unprovoked). Stability of oral anticoagulation was evaluated during a period of three months, before or after the date of antibody measurement, and presented as the percentage of time in range and the standard deviation of the mean INR values. INR values within the first month of treatment following the acute thrombotic event were not included in the analysis. A period of three months was arbitrary selected, based on the mean available anticoagulation period. The difference in measures of variability of anticoagulation (percentage time in range and standard deviation

of INR) between cases and controls were calculated. Percentage of time in range was estimated by linear interpolation between successive INR measurements, calculating the proportion of time during each interval that was spent in-range, summing across all intervals, and then dividing by the total duration of therapy^[7]. Unpaired t tests were used to calculate the mean difference in measures of stability of anticoagulation (percentage time in range and standard deviation of INR during the study period) between the two groups. Results are presented as means unless stated otherwise. A P-value below .05 was considered statistically significant.

RESULTS

Overall, 36 patients, 12 with elevated antibody levels (5 women; mean age 71.0 years) and 24 with normal antibody levels (10 women; mean age 66.5 years) with available INR values were analysed. Eight patients had elevated AbTPO levels (one also elevated TrAb), four elevated AbTg, and none isolated elevated TrAb. Five patients with elevated antibody levels were excluded because blood sample was obtained far distant from anticoagulation period. All other patients had antibodies within the normal range. One patient with elevated antibodies and two controls temporarily discontinued warfarin treatment to undergo an invasive procedure. New medications were administered in none of the patients with elevated antibodies and in 5 (20.8%) of the controls. In particular, one patient received long-term atorvastatin, and a brief course of levofloxacin and N-acetylcysteine, one long-term chlorthalidone, one amoxicilline for few days, and the last two a brief course of an unspecified non-steroidal antiinflammatory drug and an antibiotic, respectively. Two patients with elevated antibodies had a subclinical hypothyroidism; one of the controls had a euthyroid goiter. Percentage of time in range was slightly lower, although not significantly, in patients with elevated antibodies (61.9% vs 70.1%, $p=0.16$), whereas the SD of the INR values was higher in patients with elevated antibodies (0.83 vs 0.65, $p=0.05$), suggesting that thyroid autoantibodies may be responsible for the instability of the INR control.

DISCUSSION

Several mechanisms can be advocated to explain the potential interference of thyroid autoantibodies on warfarin stability. Thyroid autoantibodies may interfere with warfarin metabolism, clearance, absorption, or transportation; another possibility is that thyroid autoantibodies exert an in vitro effect, thus altering the quality of laboratory control. This mechanism could be similar to that observed in patients with antiphospholipid antibodies, which largely depends on the type of thromboplastin used^[8]. Our study results are limited by some methodological drawbacks. First, we did not routinely measure lupus anticoagulant in each patient, therefore an influence cannot be excluded. Second, there is limited sample size. Third, well-known factors that influence the quality of control of anticoagulant therapy – such as drugs and interruption for surgical procedure – were not taken into account in the analysis. However, these factors were more prevalent in the control group. Moreover, as warfarin was self-administered, drug compliance could not be analysed as additional confounding factor.

Fourth, the lack of a power calculation limited our study quality, but it was not possible to perform given that it is the first study investigating a possible association. Finally, an antibody-specific effect could not be explored given the low sample size.

In conclusion, although these findings are hampered by the limited sample size of the study and are not statistically significant, our hypothesis - that the presence of thyroid autoantibodies may be associated with an increased instability of the INR control - deserves further investigation in prospective clinical trials.

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Chapter 15

Recurrent deep venous
thrombosis during optimal
anticoagulation and overt
hyperthyroidism
A case report

Alessandro Squizzato, Josè Vitale,
Victor EA Gerdes, Erica Romualdi,
Harry R Büller, Walter Ageno

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ABSTRACT

Recurrent deep venous thrombosis despite well-conducted anticoagulant treatment is an uncommon, but possible, event. It has been hypothesized that overt hyperthyroidism may increase thromboembolic risk. We present the case of an elderly man with a recurrent episode of deep venous thrombosis during optimal oral vitamin K antagonist treatment, associated with a new diagnosis of overt hyperthyroidism, with no evidence of occult cancer and normal levels of antiphospholipid antibodies.

INTRODUCTION

Recurrent venous thromboembolism (VTE) despite well conducted anticoagulant treatment is an uncommon, but possible, event^[1]. Overt or occult cancer and antiphospholipid syndrome are the main underlying disorders^[2]. Overt hyperthyroidism is hypothesized to increase the risk of VTE: the occurrence of sinus and cerebral vein thrombosis during severe hyperthyroidism, and a number of coagulation abnormalities, such as increased activity of plasma factors VIII and IX, have been reported^[3-5]. We present the case of an elderly man with a recurrent episode of deep venous thrombosis (DVT) during optimal vitamin K antagonist treatment, associated with a new diagnosis of overt hyperthyroidism, with no evidence of occult cancer after extensive screening and normal levels of antiphospholipid antibodies.

CASE REPORT

A 74-year-old Caucasian man, on chronic oral anticoagulation with warfarin because of a permanent atrial fibrillation, referred with an important oedema of the right leg during a follow-up visit at the Anticoagulation Clinic of the University of Insubria, in Varese, on 31 May 2006. After compression ultrasound, a femoral-popliteal DVT of the right leg was diagnosed. On the same day, the International Normalized Ratio was 3.42. No International Normalized Ratios below 2.00 were detected during regular monitoring at the Anticoagulation Clinic for more than 1 year before DVT. A full therapeutic dose of low molecular weight heparin (enoxaparin 10 000 IU twice daily for 10 days) was started, and the target International Normalized Ratio was elevated to 3.0. Neither temporary nor permanent thromboembolic risk factors were identified, but previous contralateral unprovoked proximal DVT was objectively diagnosed with a compression ultrasound on 4 June 2002. At that time, neither temporary nor permanent thromboembolic risk factors were identified. Thrombophilia screening had not been performed given the age of the patient. Until May 2006, neither thrombotic nor major bleeding complications had occurred. Other relevant concomitant diseases in the patient history included chronic obstructive pulmonary disease, obstructive sleeping apnoea syndrome, obesity, prostatic adenoma, and a surgically repaired aortic abdominal aneurysm.

The patient was also on treatment with digoxin, alfuzosine, dutasteride, amiloride, and hydrochlorothiazide. At the time of recurrent DVT, anticardiolipin antibodies, antiβ₂-glycoprotein I antibodies, and lupus anticoagulant were normal. In the suspicion of an occult cancer, chest/abdominal computed tomography was performed: an enlarged thyroid gland with a nodule in the right lobe, and a bilateral surrenalic adenoma were identified. Thyroid ultrasound confirmed a multinodular gland with a larger nodule of 43mm diameter in the right lobe; thyroid scintigraphy with 99mTc showed that the larger right lobe nodule was functionally autonomous. Thyrotropin (thyroid-stimulating hormone) was below 0.05mIU/ml (reference range 0.31–4.50mIU/ml), free triiodothyronine was 6.67 pg/ml (reference range 1.50–5.30 pg/ml), and serum free thyroxine was 1.72 ng/dl (reference range 0.75–1.90 ng/dl); antithyroid peroxidase, antithyroglobulin, and antithyroid-stimulating hormone receptor antibodies were not elevated. The patient was temporarily started on thiamazole before

definitive treatment with surgery or radiometabolic therapy could be performed. Hormonal dosages for detecting functional active surrenalic adenomas showed only a mild decrease in the bloodadrenocorticotrophic hormone level at 0800 h (7 pg/ml, reference range 9–55 pg/ml), a mild increase in blood cortisol level at 1600 h (103 ng/ml, reference range 10–90 ng/ml), with a normal free cortisol level in 24-h urine volume. Routine blood tests revealed a mild increase in erythrocyte sedimentation rate and in reactive C protein. Prostatic soluble antigen was 1.3 ng/ml (reference range <4ng/ml). The patient is still regularly followed at the Anticoagulation Clinic and no cancer has been diagnosed until now, April 2007.

DISCUSSION

VTE is a multicausal disease^[6]. More than one acquired or environmental risk factors, as well as a genetic predisposition, often coincide to produce a vein thrombosis. Minor risk factors, therefore, may be relevant if other risk factors coexist: VTE occurs if a combination of these risk factors leads to crossing the thrombosis threshold^[6]. At the time of the first DVT, three known minor risk factors were present: age, male sex, and obesity. No other potentially relevant conditions were identified. The same three risk factors still coexisted at the time of the second DVT, but this time the patient was on vitamin K antagonist treatment. For this reason, we believe that a new determinant event must have occurred. Cancer and antiphospholipid syndrome are the main reasons for VTE recurrence during adequate anticoagulation, but have been excluded^[2]. Even the risk of cancer is increased for many months (even years) after the thrombotic event, our patient is apparently cancer-free after 9 months. No other relevant clinical events concomitantly occurred.

Even if inherited thrombophilia tests could be theoretically advocated as necessary, natural coagulation factor inhibitor deficiency (antithrombin, protein C, and protein S) is improbable, given the old age of the patient^[7]. Moreover, thrombophilias associated with increased levels of function of the coagulation factors (e.g. factor V Leiden, prothrombin gene mutation) are probably not relevant as they may not be risk factors for recurrent thrombosis, particularly under anticoagulant treatment^[7]. Finally, if any congenital thrombophilic defects were present, these risk factors would have been present at the time of both the first and the second DVT, and therefore would have contributed the thrombotic risk in both episodes. Apparently a new risk factor increased the VTE risk at the time of the second DVT, given the optimal warfarin treatment. Overt hyperthyroidism may therefore have contributed to DVT recurrence on the top of the existing risk factors. While recognizing that the association of hyperthyroidism and venous thromboembolism may be purely coincidental, thyroid dysfunction was newly diagnosed and an extensive literature supports its potential role of this unusual VTE risk factor.

The link between the haemostatic system and thyroid disease has been investigated since the beginning of the last century^[5]. In particular, in-vitro studies showed that the effects of thyroid hormones on the coagulation system are mainly mediated by hormone/receptors interaction^[5,8]. In-vivo studies on the effect of overt thyroid dysfunction on plasma coagulation

and fibrinolytic factors suggested a procoagulant state in overt hyperthyroid patients. Even if several methodological drawbacks obscure the real in-vivo effects of hyperthyroidism on the haemostatic system, these findings are further supported by two studies in which oral thyroid hormones were administered to healthy volunteers^[9;10]. A few coagulation tests were performed, but a clear increase of factor VIII, von Willebrand antigen and activity, and tissue plasminogen activator antigen was observed (no statistically significant effect was shown on antithrombin activity). No clinical studies evaluating the relationship between thyroid dysfunction and VTE events have been published, but case reports confirmed the hypothesis that hyperthyroidism is associated with a hypercoagulable state^[5]. Sinus and cerebral vein thrombosis have been reported in overt hyperthyroidism, in particular during thyrotoxic crisis: this association is relevant, since sinus and cerebral vein thrombosis is a rare disease (four per million inhabitants per year)^[5]. To our knowledge, the present case is the first report of an association of overt hyperthyroidism with a recurrent episode of VTE. Only one case report^[11] has described a DVT occurring in a patient with Graves' disease after a long-haul flight.

While recognizing that the association may be coincidental rather than causal, our case report reinforces the available evidence on the possible role of overt hyperthyroidism as VTE risk factor. Given that a definitive conclusion cannot be drawn, it seems worthwhile to prospectively measure thyroid hormones in aetiological clinical studies to definitively assess their causal role in VTE. Serum TSH measurement would be sufficient as a first screening test in order to avoid useless laboratory tests.

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AUTHOR'S AFFILIATIONS

Walter Ageno

Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Varese, Italy

Luigi Bartalena

Endocrinology Unit, Department of Clinical Medicine, University of Insubria, Varese, Italy

Sara Biere-Rafi

Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Anton de Boer

Division of Pharmacoepidemiology and Pharmacotherapy, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, the Netherlands

Dees PM Brandjes

Department of Internal Medicine, Slotervaart Hospital, Amsterdam, the Netherlands

Harry R Büller

Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Suzanne C Cannegieter

Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

Jan Debeij

Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

Olaf M Dekkers

Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, the Netherlands

Francesco Dentali

Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Varese, Italy

Eric Fliers

Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Luca Galli

Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Varese, Italy

Victor EA Gerdes

Department of Internal Medicine, Slotervaart Hospital, Amsterdam, the Netherlands
Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Roeland Huijgen

Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Elisa Manfredi

Department of Internal Medicine, S. Antonio Abate Hospital, Gallarate, Italy

Joost CM Meijers

Department of Experimental Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Hans-Martin Otten

Department of Internal Medicine, Slotervaart Hospital, Amsterdam, the Netherlands

Eliana Piantanida

Endocrinology Unit, Department of Clinical Medicine, University of Insubria, Varese, Italy

Elena Rancan

Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Varese, Italy

Erica Romualdi

Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Varese, Italy

Patrick C Souverein

Division of Pharmacoepidemiology and Pharmacotherapy, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, the Netherlands

Alessandro Squizzato

Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Varese, Italy
Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Jan Stam

Department of Neurology, Academic Medical Center, Amsterdam, the Netherlands

Danka JF Stuijver

Department of Internal Medicine, Slotervaart Hospital, Amsterdam, the Netherlands

Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Maria Tanda

Endocrinology Unit, Department of Clinical Medicine, University of Insubria, Varese, Italy

Achille Venco

Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Varese, Italy

Chantal JN Verkleij

Department of Experimental Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Josè Vitale

Research Center on Thromboembolic Disorders and Antithrombotic Therapies, Department of Clinical Medicine, University of Insubria, Varese, Italy

Bregje van Zaane

Department of Internal Medicine, Slotervaart Hospital, Amsterdam, the Netherlands

Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands

Anton van Zanten

Department of Clinical Biochemistry, Slotervaart Hospital, Amsterdam, the Netherlands

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SUMMARY

The interactions between dysfunctional thyroid diseases and haemostasis are the main focus of the thesis. After the introduction and outline, Chapter 2 gives an overview on the complex interactions of hormones with the coagulation and fibrinolytic system.

The thesis is divided into two parts. The first part (Chapters 3-11) is focused on the effect of thyroid hormone on laboratory markers of coagulation and fibrinolysis, and venous thromboembolism; the second part (Chapters 12-15) addresses the influence of thyroid disorders on vitamin K antagonists.

Part I: Thyroid, coagulation and fibrinolysis, and venous thromboembolism

In **Chapter 3, 4 and 5** we review the available evidence on the effects of hyperthyroidism and hypothyroidism on these systems, on the association between hypothyroidism and acquired von Willebrand's syndrome, and the relationship between thyroid diseases and cerebrovascular diseases. In **Chapter 3**, we systematically summarise and analyse all published case-control or interventional cohort studies that evaluated the effects of hyperthyroidism and hypothyroidism on the coagulation-fibrinolytic system *in vivo* that were identified by a computer-assisted search of the MEDLINE and EMBASE electronic databases. A total of 36 papers were included. No high quality study was identified. A total of 19 tests were investigated in the medium-quality studies. These tests indicate a hypocoagulable state for overt hypothyroidism and a hypercoagulable state for overt hyperthyroidism.

In **Chapter 4**, we systematically describe all published clinical epidemiological and interventional studies, case reports and *in vitro* studies that investigated the association between hypothyroidism and acquired von Willebrand's syndrome that were identified by a computer-assisted search of the MEDLINE and EMBASE electronic databases. A total of 41 papers were included. No high quality *in vivo* study was identified. Almost all bleeding episodes described in the case reports were mucocutaneous bleedings, and we describe the range of the observed low levels of von Willebrand factor antigen VWF activity and factor VIII activity. Acquired von Willebrand's syndrome may be the main factor responsible for bleeding diathesis in overt hypothyroid patients.

Chapter 5 summarises certain and possible cerebrovascular complications of thyroid disorders. In overt hyperthyroidism, cardioembolic stroke is clearly associated to thyrotoxic atrial fibrillation, and in subclinical hyperthyroidism with serum thyroid-stimulating hormone levels <0.1 mU/L, the incidence of atrial fibrillation is increased. *In vitro* and *in vivo* studies indicate a hypercoagulability state in hyperthyroidism and case reports suggest an increased risk of acute cerebral venous thrombosis. Possible associations between hyperthyroidism and Moyamoya or Giant cell arteritis have only been described in case reports. There is enough evidence that overt hypothyroidism is associated with several traditional and newer atherosclerotic risk factors, especially hypertension, hyperlipidemia, and hyperhomocysteinemia. For subclinical hypothyroidism, these associations are less certain.

Chapter 6 is the first published study that evaluated the relationship between thyroid dysfunction and a deep venous thrombosis (DVT). Fifty consecutive adult outpatients with a previous diagnosis of provoked DVT, 50 consecutive adult outpatients with a previous diagnosis

of unprovoked DVT, both of the lower legs, and 50 control subjects were enrolled. Previously unrecognized subclinical hypothyroidism was diagnosed in seven (14.0%) unprovoked DVT patients, one (2%) provoked DVT patient, and one (2%) control. The results of this pilot study suggest an increased prevalence of subclinical hypothyroidism in patients with unprovoked DVT.

In **Chapter 7**, parameters of thyroid function were assessed in 190 venous thrombosis cases on the day of the diagnosis of thrombosis and 379 sex-matched controls. We found the risk of venous thrombosis to gradually rise with increasing levels of free thyroxine. In the absence of traditional acquired risk factors, free thyroxine levels above 17 pmol/L yielded a sex- and age-adjusted odds ratio (OR) of 2.2 (95% confidence interval [CI] 1.2-4.2) for deep venous thrombosis, which further increased up to an OR of 13.0 (95% CI 1.1-154.1) for free thyroxine levels above reference range. These data suggest increasing levels of free thyroxine to be a risk factor for venous thrombosis.

The risk of pulmonary embolism (PE) during thyroid dysfunction were tested in **Chapter 8** with a nested case-control study using the PHARMO Record Linkage System, a Dutch population-based registry that links medication histories to hospital admission records. New use of antithyroid or thyreomimetic agents, and thyroid-related hospitalizations, were also used as indicators for diagnosis of thyroid disease. The study population consisted of 3479 cases admitted to the hospital for PE and 11830 controls. Diagnosis of hypothyroidism prior to the index date, i.e. treated hypothyroidism, was significantly associated with PE (OR 2.05, 95% CI 1.11-3.78), especially within the first three months after diagnosis (OR 4.98; 95% CI 1.39-17.82). No association was found for diagnosis of hypothyroidism after the index date. Our findings suggest that patients with hypothyroidism are at increased risk of pulmonary embolism and it is probably related to treatment with thyreomimetic agents rather than hypothyroidism itself. There were only few patients who used antithyroid agents. As a result we were not able to draw strong conclusions about hyperthyroidism before or within the first months after PE although the data suggest an association within the first months after diagnosis.

In **Chapter 9**, the effect of supraphysiological doses of levothyroxine on coagulation and fibrinolysis was tested in healthy volunteers randomized to receive levothyroxine or no medication for 14 days in a crossover design. 16 participants received levothyroxine in a dose of 0.3 mg/day, and 12 received levothyroxine 0.45 or 0.6 mg/day depending on body weight. Levels of von Willebrand factor activity and antigen, factor VIII, factor IX, factor X, fibrinogen, PAI-1 and clot-lysis time higher compared to no medication and activated partial thromboplastin time were decreased. Overall data suggest that thyroid hormone excess increases coagulation factor levels, and inhibits fibrinolysis, in a dose-dependent fashion.

The effect on thrombin-activatable fibrinolysis inhibitor (TAFI) was specifically addressed in another study, described in **Chapter 10**. The effect of hyperthyroidism on TAFI was studied in the same patients of Chapter 9. The effect of hypothyroidism on TAFI was studied in a multicenter observational cohort study. Blood was drawn before treatment of patients with newly diagnosed hypothyroidism and when euthyroidism was achieved. Hyperthyroidism resulted in a hypofibrinolytic condition and in an enhanced TAFI-dependent prolongation of clot-lysis. Hypothyroidism resulted in hyperfibrinolysis

and a reduced TAFIa-dependent prolongation of clot-lysis.

In **Chapter 11**, the case of a young woman who attempted suicide by intoxication with 25 mg of levothyroxine was presented. Levels of thyroid hormones and parameters of coagulation and fibrinolysis were assessed in the days following the auto-intoxication. We found a marked increase in levels of coagulation factors VIII, IX and X, von Willebrand factor and plasminogen activator inhibitor-1, resulting in enhanced thrombin generation. These findings, similarly to the previous ones, suggest that thyroid hormone excess shifts the haemostatic balance towards a hypercoagulable and hypofibrinolytic state, and is therefore likely to reflect an increased risk of venous thrombosis.

Part II: Thyroid and vitamin K antagonist

Chapter 12 is a published editorial in which we commented the available evidence on the relation between thyroid dysfunction and vitamin K antagonist, and highlighted the lack of published studies, in particular for subclinical hypo- and hyperthyroidism, even if the old literature provided a solid pathophysiological rationale.

To fill the gap of the literature and to explore the role of thyroid autoantibodies, we tested in two pilot studies the effect of subclinical hypothyroidism on vitamin K stability and sensitivity and the effect of thyroid autoantibodies on warfarin stability.

In **Chapter 13**, 26 patients with subclinical hypothyroidism occurring during vitamin K antagonist treatment, who were treated with L-thyroxine and achieved an euthyroid state still on vitamin K antagonist treatment, were included in the study. Weekly dosage and time spent in the therapeutic range were calculated during 6 and 12 weeks before objective diagnosis of subclinical hypothyroidism, during the first 6 and 12 weeks after L-thyroxine treatment was began, and during the first 6 and 12 weeks after objective euthyroidism has been reached. During a 6 weeks time interval, mean weekly dosage was 29.9 mg during subclinical hypothyroidism and 26.8 mg during euthyroidism ($P < 0.05$); median time spent in the therapeutic range was 61% during subclinical hypothyroidism and 81% during euthyroidism (median difference = 13.5%; 95.2 % CI -8.5 to 30.5%). Data of our pilot study suggest that subclinical hypothyroidism may affect both VKAs stability and sensitivity.

In **Chapter 14**, 36 patients, 12 with elevated antibody levels and 24 with normal antibody levels with available INR values were analysed. Stability of oral anticoagulation was evaluated during a period of three months, before or after the date of antibody measurement, and presented as the percentage of time in range and the standard deviation of the mean INR values. Percentage of time in range was slightly lower in patients with elevated antibodies (61.9% vs 70.1%, $p = 0.16$), whereas the standard deviation of the INR values was higher in patients with elevated antibodies (0.83 vs 0.65, $p = 0.05$), suggesting that thyroid autoantibodies may be responsible for the instability of the INR control.

Finally, in **Chapter 15**, we present the case of an elderly man with a recurrent episode of deep venous thrombosis during optimal oral vitamin K antagonist treatment, associated with a new diagnosis of overt hyperthyroidism, with no evidence of occult cancer and normal levels of antiphospholipid antibodies.

DANKWOORD

Just a few words to say 'thanks' to you,
who has encouraged me,
who has trusted me,
who has worked with me,
who has lived with me,
who has simply spent some time with me,
who is enjoying this day with me.

Just a few words to say 'thanks so much' to you,
Victor, Bregje and Harry.

And just to say ...
Alessandra.

CURRICULUM VITAE

Alessandro Squizzato was born in 1975, 21st of August, in Tradate, Italy. Claudia and Giovanni are his mother and father, Maurizio his brother. He was christened in 1975, 14th of September. He married Alessandra Vecchi in 2005, 5th of February. They have two children, the 'angel' Pietro and the 'princess' Elisabetta.

He graduated in Medicine in 2000, 18th of October, and specialised in Internal Medicine in 2005, 24th of October. He co-founded the Research Center on Thromboembolic Disorders and Antithrombotic Therapies of the University of Insubria, Varese, Italy, in 2009. He is currently Assistant Professor in Internal Medicine at the University of Insubria, Varese, Italy.