Prothrombotic changes due to an increase in thyroid hormone levels

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Abstract

Objective: With increasing free thyroxine levels, a gradually rising risk of venous thromboembolism has been described in case-control studies. However, reports on the influence of thyroid hormones on haemostasis, while suggesting a hypercoagulable state in thyrotoxicosis, have often been inconclusive. This study evaluates multiple markers of haemostasis and fibrinolysis in a paired design, making it more sensitive to changes in thyroid hormone levels.

Design: We analysed multiple variables in patients who shifted from severe hypothyroidism to mild hyperthyroidism during thyroid cancer treatment. Those with possible residual disease were excluded.

Methods: Ninety patients following total thyroidectomy were tested on two occasions: i) before radioiodine remnant ablation and ii) 6 weeks later, on levothyroxine (LT₄) suppression treatment, and the results were compared using the Wilcoxon's test for paired data.

Results: During LT₄ treatment, significant increases (all $P < 0.001$) in fibrinogen (from median 3.4 to 3.8 g/l), von Willebrand factor (from 85 to 127%), factor VIII (from 111 to 148%) and plasminogen activator inhibitor 1 (from 6.5 to 13.9 µg/l) were observed. In addition, the activation times of platelet adhesion and aggregation stimulated with collagen and epinephrine (EPI)/ADP, i.e. closure times in platelet function analyser (PFA-100), were significantly shortened ($P < 0.001$): for EPI from median 148 to 117 s and for ADP from 95 to 80 s. Changes in other tests were less prominent or insignificant.

Conclusions: An increase in thyroid hormone levels shifts the haemostatic balance towards a hypercoagulable, hypofibrinolytic state. This may contribute to the increased cardiovascular morbidity and mortality observed even in mild thyrotoxicosis.

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Introduction

With increasing free thyroxine (FT₄) levels, a gradually rising risk of venous thromboembolism has been described in case-control studies (1, 2). However, reports on the influence of thyroid hormones on coagulation and fibrinolysis, while mostly suggesting a hypercoagulable state in thyrotoxicosis, have often been of low methodological quality (3, 4, 5). Indeed, no high-quality study was identified in a systematic review in 2007 (3). Since then only two studies (6, 7) were assessed as high-quality in a recent meta-analysis (5). Demir *et al.* (6) report a significant increase in plasminogen activator inhibitor 1

(PAI1), von Willebrand factor (VWF) and fibrinogen following levothyroxine (LT₄) suppression treatment for thyroid nodules. In their randomised crossover study in healthy volunteers, van Zaane *et al.* (7) describe a dose-dependent effect of LT₄ exposure on several variables of coagulation and fibrinolysis, the most prominent being an increase in PAI1 (by 116%) and VWF activity and antigen (by 24 and 26% respectively).

In this study, we employed a large cohort of patients treated with radioactive iodine (RAI) ¹³¹I for differentiated thyroid cancer. In a short time, they shifted from severe

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hypothyroidism (before thyroid remnant RAI ablation) to mild hyperthyroidism (due to LT_4 suppression therapy), allowing analysis of their coagulation and fibrinolysis markers in a paired design. In addition, their primary haemostasis (i.e. platelet adhesion and aggregation) was assessed using the platelet function analyser (PFA-100) (8), and compared in the same design.

Subjects and methods

Patients and study design

Differentiated thyroid cancer patients ($n=112$), severely hypothyroid 4–5 weeks following total thyroidectomy without LT_4 replacement, were admitted for RAI thyroid remnant ablation. Before discharge, thyroid hormone suppression therapy (LT_4 , 150 $\mu\text{g/day}$) was initiated and 6–8 weeks later they were examined in the outpatient follow-up clinic. Blood samples were taken on two occasions: i) in severe hypothyroidism on admission and ii) in mild (subclinical) hyperthyroidism on LT_4 treatment. As cancer itself influences coagulation system, we excluded 22 patients with possible residual disease, i.e. with measurable thyroglobulin (TG) level and/or positive anti-TG antibodies (TG-Ab) on follow up. This made our sample more homogenous and hopefully not influenced by the underlying cancer diagnosis. Accordingly, in 90 patients (13 men and 77 women, median age of 54.1 years, interquartile range 39.2–64.0 years, 80 with papillary and

ten with follicular cancer, all without residual disease), thyroid function tests, multiple coagulation and fibrinolysis tests, as well as the assessment of primary haemostasis using the PFA-100, were performed and analysed. Each patient gave his/her written informed consent and the investigation was approved by the ethical committee of our University Hospital. None of the patients had any previously known coagulation disorder, and none was treated with anticoagulants, antiaggregants or other medication capable of affecting coagulability.

Thyroid-related tests

Thyrotropin (TSH) was measured by IRMA (Immunotech, Beckman Coulter, Prague, Czech Republic). FT_4 , free triiodothyronine (FT_3) and TG-Ab were determined by RIAs (Immunotech, Beckman Coulter, Prague, Czech Republic). TG was assayed by immunofluorescence analysis (Thermo Scientific BRAHMS KRYPTOR, Hennigsdorf, Germany). The reference range (RR) for TSH, FT_4 and FT_3 is given in Table 1. The detection limit for TG was 0.8 $\mu\text{g/L}$, and TG-Ab were considered positive if >100 IU/ml. The inter-assay coefficients of variation (CV) were 5.5% for TSH, 8.4% for FT_4 , 6.4% for FT_3 , 5.6% for TG and 10.4% for TG-Ab.

Coagulation- and fibrinolysis-related tests

Fibrinogen was measured by clot-based assay – Clauss method (DG-FIB L Human, Diagnostic Grifols, Parets del

Table 1 The changes in haemostatic tests in 90 severely hypothyroid patients treated with suppressive dose of levothyroxine.

Variable (ref. range)	Hypothyroid	Mildly hyperthyroid	Relative change (%)	P
Thyroid function tests:				
TSH (0.15–5 mIU/l)	109.6 (78.6; 141.9)	0.14 (0.04; 0.50)	–99.9 (–100; –99.6)	<0.001
FT_4 (11–25 pmol/l)	<2.4 (<2.4 ; <2.4)	23.9 (22.1; 25.9)	>895 (>801 ; >980)	<0.001
FT_3 (2.5–5.8 pmol/l)	1.38 (1.08; 1.91)	5.12 (4.55; 5.60)	263 (162; 404)	<0.001
Tests related to haemostasis and fibrinolysis (in order of relative change and significance):				
PAI1:Ag (1–25 $\mu\text{g/l}$)	6.5 (4.2; 9.2)	13.9 (8.3; 23.5)	100 (44; 169)	<0.001
Factor VIII (60–150%)	111 (78; 135)	148 (104; 203)	46 (7; 90)	<0.001
VWF:Ag (50–160%)	85 (70; 107)	127 (94; 153)	43 (23; 71)	<0.001
CEPI-CT (75–145 s)	148 (119; 195)	117 (99; 145)	–21 (–38; –6)	<0.001
CADP-CT (62–104 s)	95 (80; 121)	80 (69; 96)	–15 (–33; 0)	<0.001
Fibrinogen (2–4 g/l)	3.4 (2.7; 3.7)	3.8 (3.4; 4.2)	13 (6; 32)	<0.001
F1 + 2 (69–229 pmol/l)	222 (164; 294)	197 (149; 259)	–13 (–36; 21)	0.021
Antithrombin (80–120%)	100 (93; 110)	109 (100; 115)	5 (0; 11)	<0.001
t-PA:Ag (1–10 $\mu\text{g/l}$)	2.1 (1.5; 3.0)	2.0 (1.2; 3.1)	–5 (–29; 27)	0.38
D-dimer (0–0.5 mg/l)	0.32 (0.23; 0.48)	0.30 (0.24; 0.54)	0 (–21; 26)	0.463
TAT (2.0–4.2 $\mu\text{g/l}$)	3.0 (2.3; 5.0)	3.2 (2.7; 4.3)	8 (–39; 44)	0.941

TSH, thyroid-stimulating hormone; FT_4 , free thyroxine; FT_3 , free triiodothyronine; PAI1:Ag, plasminogen activator inhibitor 1 antigen; VWF:Ag, von Willebrand factor antigen; CEPI-CT, closure time with collagen/epinephrine in platelet function analyser (PFA-100, see Subjects and methods section); CADP-CT, closure time with collagen/ADP in PFA-100; F1 + 2, prothrombin fragment 1 + 2; t-PA:Ag, tissue-type plasminogen activator antigen; TAT, thrombin-antithrombin complex. The values are expressed as medians with 1st and 3rd quartile in parentheses. Wilcoxon's rank sum test for paired data was used to assess the significance.

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Vallès, Spain); RR in Table 1. The limit of detection was 0.70 g/l. The intra-assay CV were 3.20% in the normal values and 4.60% in the pathological values. The inter-assay CV were 4.30% in the normal values and 6.50% in the pathological values.

Antitrombin was measured by chromogenic assay (BIOPHEN AT (LRT), Hyphen BioMed, Neuville-Sur-Oise, France); RR in Table 1. The limit of detection was $\leq 10\%$. The intra-assay CV were 0.99% in the normal values and 1.38% in the pathological values. The inter-assay CV were 2.73% in the normal values and 0.57% in the pathological values.

D-dimer was measured by immuno-turbidimetric method (STA – Liatest D-Di, Diagnostica Stago, Asnières sur Seine, France); RR in Table 1. The limit of detection was 0.27 mg/l (fibrinogen equivalent unit (FEU)). The intra-assay and inter-assay CV were 2.23 and 3.86% (pathological values) respectively.

Factor VIII was measured by one-stage method based on the Activated Partial Thromboplastin Time with deficient plasma (DG-FVIII, Diagnostic Grifols; C. K. Prest, Diagnostica Stago); RR in Table 1. The limit of detection was 1.3%. The intra-assay CV were 6.40% in the normal values and 6.90% in the pathological values. The inter-assay CV were 8.00% in the normal values and 8.40% in the pathological values.

VWF was measured by immuno-turbidimetric method (STA – Liatest VWF:Ag, Diagnostica Stago); RR in Table 1. The limit of detection was 3%. The intra-assay CV were 1.90% in the normal values and 2.70% in the pathological values. The inter-assay CV were 2.90% in the normal values and 4.40% in the pathological values.

PAI1 antigen was measured by ELISA (ZYMUTEST PAI1 Antigen, Hyphen BioMed); RR in Table 1. The limit of detection was ≤ 0.5 ng/ml. The intra-assay and inter-assay CV were 3–8 and 5–10% respectively.

Tissue type plasminogen activator antigen (t-PA) was measured by ELISA (ZYMUTEST t-PA Antigen, Hyphen BioMed); RR in Table 1. The limit of detection was ≤ 0.5 ng/ml. The intra-assay and inter-assay CV were 3–8 and 5–10% respectively.

Prothrombin fragment F 1+2 (F1+2) was measured by ELISA (Enzygnost F 1+2 (monoclonal), Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany); RR in Table 1. The measuring range was 20–1200 pmol/l. The intra-assay and inter-assay CV for F1+2 were 3.6–5.5 and 4.4–11.2% respectively.

Thrombin–antitrombin complex (TAT) was measured by ELISA (Enzygnost TAT micro, Siemens Healthcare Diagnostics Products GmbH); RR in Table 1. The measuring

range was 2.0–60.0 μ g/l. The intra-assay and inter-assay CV were 4.0–6.0 and 6.0–9.0% respectively.

PFA-100 test

The process of primary haemostasis was measured by the PFA-100 System (Dade Behring, Vienna, Austria) using collagen (COL)/ADP and COL/EPI cartridges. The principle of the test is flow of citrated whole blood through the cartridge containing a membrane coated with inductors (COL/ADP or COL/EPI); the output is closure time (CT) of the cartridge, reflecting velocity of platelet plug formation (8). The RR for PFA-100 System is given in Table 1, measuring range is 0–300 s, and sensitivity and specificity are 96.1 and 88.6%, respectively, and coefficient of accuracy is 0.979. The intra-assay CV are 8.95% for COL/ADP and 10.51% for COL/EPI and the inter-assay CV are 0.6% for COL/ADP and 4.0% for COL/EPI.

Statistical analyses

For statistical analysis, SigmaStat Software (Point Richmond, CA, USA), version 3.1, was used. As data were mostly non-normally distributed, their summary values were expressed as median and interquartile range and comparisons were performed using non-parametric Wilcoxon's test for paired data. For the assessment of possible influence of age and BMI on the observed changes, the whole sample was divided into tertiles and non-parametric analysis of variance (Kruskal–Wallis test) was used.

Results

The effects of LT_4 treatment on coagulation and fibrinolytic parameters are summarised in Table 1. Clearly, significant increases ($P < 0.001$) were observed in procoagulation factors fibrinogen (median relative change 13%), VWF:Ag (43%) and factor VIII (46%). The most prominent change observed was an increase by 100% in the antifibrinolytic factor PAI1:Ag. Although significant, only a small increase in antithrombin (by 5%) and a less significant ($P = 0.021$) decrease in F1+2 (13%) were found. The prevailing response to LT_4 exposure thus was a shift favouring prothrombotic events over fibrinolytic events in the coagulation balance.

The activation times of primary haemostasis (i.e. platelet adhesion and aggregation), evaluated by the PFA-100 CTs after stimulation with collagen and EPI or ADP, were significantly shortened, median relative change being –15% for EPI and –21% for ADP. This also suggests a prothrombotic change.

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There was no significant influence of age and BMI on the observed significant changes (data not presented). We did not confirm a significant decrease in t-PA or an increase in D-dimer. Also TAT was not affected.

Discussion

Alterations in coagulation and fibrinolysis associated with abnormal thyroid hormone levels have recently been reviewed (4, 5). While many studies suggest a trend towards a hypercoagulable state in thyrotoxicosis and a hypocoagulable state in hypothyroidism, many reports are inconclusive and most of them were assessed as low-to-medium methodological quality (3, 5). Two high-quality studies on the effect of thyroid hormone excess (6, 7) have supported the prothrombotic shift in coagulation/fibrinolysis balance. Demir *et al.* (6) used lT_4 suppression treatment (0.05–0.15 mg/day for 1 year) in 30 women with nodular goitre and found a significant increase in fibrinogen (ca. 16%), D-dimer (ca. 23%), VWF (ca. 40%), tissue factor (ca. 43%) and PAI1 (ca. 22%) levels; when the data were controlled for age and BMI, only fibrinogen, VWF and PAI1 remained significant. In the other study (7), healthy volunteers were given lT_4 in a controlled randomised crossover pattern, and with the higher dose (0.45 or 0.6 mg/day for 14 days, $n=12$) there was a significant increase in fibrinogen (ca. 17%), VWF activity (ca. 24%), VWF antigen (ca. 26%), factor VIII (ca. 19%), factor IX (ca. 14%), factor X (ca. 7%), PAI1 (ca. 116%) and clot-lysis time (ca. 14%), while activated partial thromboplastin time was decreased by 3%. With the lower dose (0.3 mg/day for 14 days, $n=16$), only the increase in VWF activity (7%) and VWF antigen (10%) remained significant.

Our study had several important differences from the above mentioned studies, while also addressing the question of lT_4 effect on coagulation and fibrinolysis in a paired design. First, the sample was larger ($n=90$), which may have increased sensitivity. Second, our patients were treated for thyroid cancer, which may have affected some of the tests; we believe to have avoided this by excluding those with possible residual disease (using TG and TG-Ab). Third, our patients started the study in severe hypothyroidism (median TSH 109.6 mIU/l and $\text{FT}_4 < 2.4$ pmol/l) and finished in mild hyperthyroidism (median TSH 0.14 mIU/l and FT_4 23.9 pmol/l) whereas the patients in the first (6) and the volunteers in second study (7) started both euthyroid and finished either with values similar to ours (mean TSH 0.17 mIU/l and FT_4 23.0 pmol/l), which also reflects a similar dose of lT_4 (6), or in overt

hyperthyroidism (median TSH 0.02 mIU/l and FT_4 40.0 pmol/l) with the higher dose (6). Fourth, the duration of our study was 6–8 weeks, rather reflecting rapid changes, which was similar to the second study (2 weeks) (7) but clearly different from the first one (1 year) (6).

Another well-designed study by Debeij *et al.* (9), though not mentioned in the comprehensive review by Stuijver *et al.* (5), addressed similar questions. Although the study had been smaller and primarily focused on the role of TSH in the observed coagulation abnormalities, it had important similarities, both in design and results. In 11 patients treated with a suppression dose of lT_4 following thyroid ablation for cancer (ten papillary and one follicular), lT_4 was withdrawn for 4 weeks, leading to increased TSH (median 133.3 mIU/l) and decreased FT_4 (median 1.5 pmol/l), and the first sample was taken for coagulation tests. After lT_4 treatment was restarted for 8 weeks, leading to suppressed TSH (median 0.7 mIU/l) and elevated FT_4 (median 24.2 pmol/l), and the second sample was drawn. With the rapid shift from severe hypothyroidism to mild hyperthyroidism, a significant rise was observed in the following: antithrombin (ca. 13%), factor II (ca. 7%), factor VIII (ca. 41%), factor IX (ca. 32%), fibrinogen (ca. 19%) and VWF (ca. 41%) as well as a significant decrease in protein C (ca. 10%) and factor VII (ca. 22%).

In spite of some methodological differences, the main results of the four studies were in reasonably good accord. In all of them, a significant increase in fibrinogen and VWF was found in lT_4 -treated persons. The increase in PAI1 was clearly more prominent (and quantitatively greatest among the variables tested, ca. 100%) in our study and in that by van Zaane *et al.* (7) than in Demir's study (6). This may reflect either shorter duration (weeks) or greater shift in thyroid hormone levels in the first two studies. In a similarly short study by Debeij *et al.* (9), PAI1 was not assessed. In our study, also, factor VIII was clearly increased as in that by van Zaane *et al.* (7) and by Debeij *et al.* (9), while it was not assessed by Demir *et al.* (6). Taken together, these results suggest that an increase in thyroid hormone levels favours coagulation and suppresses fibrinolytic processes.

The increased levels of VWF and fibrinogen have been the most consistently found changes in hyperthyroidism (4, 5). As these factors are closely related to platelet plug formation, it may suggest an important role of platelet-vascular wall interaction in the lT_4 effect. The assessment of primary haemostasis using PFA-100 thus seems to be a suitable method to address this question. Our finding that lT_4 treatment shortened CT in the PFA-100, i.e. lT_4 activated platelet plug formation, lends further support

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to this hypothesis. While the PFA-100 is widely used as a 'global' test system for primary haemostasis in cardiology and haematology (8), we have only found a single report on its use in abnormal thyroid function (10). Homoncik *et al.* (10) described a prolonged CT in hypothyroidism and shortened CT in hyperthyroidism. They also reported a decrease in CT associated with an increase in T₄ and VWF in the patients treated with lT₄ for hypothyroidism (mostly due to Hashimoto's thyroiditis). Though their design was different the general pattern of the PFA-100 response to lT₄ was similar in our larger study.

These results, together with a 100% increase in PAI1, suggest an important role of endothelium activation in thyroid hormone excess because both VWF and PAI1 are synthesised in and secreted from endothelium. Indeed, in a small ($n=14$) study, Burggraaf *et al.* (11) report an influence of hyperthyroidism on some (e.g. VWF, t-PA, PAI1 and thrombomodulin), but not all (e.g. E-selectin) endothelium-associated proteins. As human endothelial cells express thyroid hormone receptors alpha and beta 1 (12), endothelium seems to be an important target for thyroid hormone action.

As for proteins of hepatic synthesis, in their above mentioned study (11) Burggraaf *et al.* found an increase in α 2-antiplasmin and fibronectin, and a decrease in plasminogen, while there was no significant change in fibrinogen. Still increased fibrinogen levels similar to ours have rather consistently been reported in other studies (4, 5), probably reflecting an increased hepatic synthesis due to thyroid hormone receptor-dependent transcriptional regulation demonstrated in hepatoma cell line (13). The increase in FVIII, observed in our study as well as in others (5), may have been due to the same process though not addressed in the hepatoma cell line study (13).

We conclude that the increase in thyroid hormone levels shifted the haemostatic balance towards a hypercoagulable and hypofibrinolytic state, namely due to an increased capacity of primary haemostasis (endothelium-platelet interaction) and a decreased capacity of fibrinolysis. However, none of our patients actually developed any clinical thrombotic event in the subacute setting. In addition, as there was no laboratory sign of actually occurring increase in coagulation (assessed by F1+2 and TAT), also no change in fibrinolysis (assessed by D-dimer) was observed.

The clinical importance of prothrombotic changes related to thyroid hormone excess has been a matter of controversy (4, 5). However, the updated meta-analyses of population cohort studies demonstrated a clear association of hyperthyroidism (even subclinical) with increased

cardiovascular morbidity and mortality (14, 15). From combined community-based studies, Yang *et al.* (14) concluded that the general population with subclinical hyperthyroidism was at a 31% increased risk of cardiovascular disease. As nearly all the studies were adjusted for conventional cardiovascular risk factors (age, BMI, blood pressure, diabetes, cholesterol and smoking), the results suggested that subclinical hyperthyroidism could be an independent risk factor for cardiovascular disease. Thrombotic events (both arterial and venous) related to thyroid hormone excess may partly explain this increased risk. Subclinical hyperthyroidism was associated with a 21% increased risk of coronary heart disease and a 68% increased risk of atrial fibrillation (15), which may be further complicated by stroke. Indeed, Schultz *et al.* (16) reported an increased incidence of stroke among subjects with subclinical hyperthyroidism (hazard ratio 3.39; 95% CI 1.15–10.00, $P=0.027$), even after adjusting for sex, age and atrial fibrillation. Also, an increased morbidity due to venous thromboembolism was observed (1, 2, 17). Though no controlled intervention studies confirming benefit of subclinical hyperthyroidism treatment are available (18), the recent guidelines advocate treatment, particularly in older patients (≥ 65 years) and those with clearly suppressed TSH (<0.1 mIU/l) (19).

In addition, the prothrombotic risk related to exogenous (even subclinical) thyroid hormone excess should be taken into consideration. Namely, lT₄ overtreatment in hypothyroid patients that may occur rather frequently (20) should be avoided. Also for lT₄ suppression treatment in thyroid nodules and differentiated thyroid cancer (especially, low risk cases following surgery and RAI ablation), the risk/benefit considerations should include prothrombotic changes.

As a potential limitation of the study, its short duration may be considered. The study design was focused on the impact of a relatively rapid and pronounced change in thyroid hormone concentrations (within 6–8 weeks) on the observed variables, not allowing for extrapolation into further 6 months or later, which would be better related to clinical events. In fact, most published interventional studies on this topic, reviewed in (5), used a similar time pattern of weeks. However, chronic changes related to subclinical hyperthyroidism were observed by others. Demir *et al.* (6) report changes in fibrinogen, VWF and PAI1 similar to ours after 1 year of lT₄ suppression treatment for benign thyroid nodules. We therefore believe that at least some of the observed changes may be longer-lasting. Despite this limitation, our study possesses several strengths. The major strength is that

the comparison is made within the patients themselves, so it is unlikely that other factors can explain the findings. Together with sample size ($n=90$, i.e. much greater than studies reviewed in reference (5)) and homogeneity, this makes the results more reliable. In addition, the spectrum of tests was relatively wide, including those of primary haemostasis (PFA-100), which allowed us to suggest an important role of platelet–endothelium interaction in the observed effects. We believe that participation of thyroid hormones in this interaction deserves further research.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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