The hormonal influence on the haemostatic system and the risk of thrombosis
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Summary

This thesis further explores the link between the hormonal and haemostatic system. It describes, in three parts, the effect of the thyroid hormone, cortisol and prolactin on haemostasis and the risk of thrombosis.

Part I: Cortisol

Chapter 2 is a retrospective multicentre cohort study, performed in all academic medical centres in the Netherlands, on the incidence of venous thromboembolism in patients with Cushing's syndrome. A total of 473 patients diagnosed with endogenous Cushing's syndrome of benign origin between January 1990 and June 2010 were included; 360 with adrenocorticotropic hormone (ACTH)-depending and 113 with ACTH-independent Cushing's syndrome. Patients surgically treated for non-functioning pituitary adenoma served as controls for the incidence of post-operative venous thromboembolism in ACTH dependent Cushing's syndrome. Thirty-seven patients experienced VTE during the study period, resulting in an incidence rate of 14.6 (95% CI 10.3-20.1) per 1000 person-years. The incidence rate for first-ever VTE prior to treatment was 12.9 (95% CI 7.5-12.6) per 1000 person-years (17 events). The risk of post-operative VTE, defined as risk within 3 months after surgery, was 0% for ACTH-independent and 3.4% (95% CI 2.0-5.9) for ACTH-dependent CS (12 events in 350 patients); most events occurred between 1 week and 2 months after surgery. Compared to the controls, the risk of post-operative VTE in patients undergoing transsphenoidal surgery was significantly greater (p=0.01). In conclusion, patients with Cushing's syndrome are at high risk of venous thromboembolism, not only during active disease, but also after surgery. Guidelines on the choice, intensity and duration of thromboprophylaxis are urgently needed.

Chapter 3 describes the results of a large population based case-control study (a Dutch pharmacy registry in which hospitalization data is linked to pharmacy records) on the association between use of glucocorticoids and risk of symptomatic pulmonary embolism. The study consisted of 4495 cases hospitalized for pulmonary embolism and 16802 gender- and age-matched subjects without a history of pulmonary embolism. We calculated “daily dose equivalents” of prednisolone. The risk of pulmonary embolism was highest in the first 30 days of glucocorticoid exposure with an adjusted OR of 5.9 (95% CI 2.3-3.9) and gradually decreased with increasing duration of use to an OR of 1.9 (95% CI 1.3-2.9) for chronic users (>1 year). Low dose glucocorticoid usage (defined daily dose equivalent < 5 mg prednisolone) carried a 2-fold higher risk of pulmonary embolism (OR 1.8, 95% CI 1.3-2.4), whereas a 10-fold increased risk was observed for the highest dose of glucocorticoids (ddd-eq>30 mg prednisolone) (OR 9.6, 95% CI 4.3-20.5). Stratification for both duration and dose of glucocorticoids showed the highest risk of pulmonary embolism in short-term users compared to long-term and chronic users, irrespective of the dose of glucocorticoids. These data suggest that patients treated...
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with oral glucocorticoids may be at increased risk of pulmonary embolism, especially during the first month of exposure. Future clinical studies are necessary to adequately assess the risks and benefits of glucocorticoid use in specific disorders so that the anti-inflammatory, and thus beneficial, effects of glucocorticoid treatment can be weighted against the risk of adverse effects such as venous thrombosis.

Part II: thyroid hormone

Chapter 4 is a systematic review on observational and experimental studies assessing the effect of thyroid hormone excess on the coagulation and fibrinolytic system in vivo. Records were identified by a computer-assisted search of the MEDLINE and EMBASE electronic databases (1980-2012). Studies were divided into categories of low, medium, and high quality. Random effects models were used to obtain pooled estimates. A total of 29 articles consisting of 51 studies were included as in several articles more than one study was described. We included 4 intervention (before and after treatment in hyperthyroid patients), 5 cross-sectional (hyperthyroid subjects and euthyroid controls), and 4 experimental (before and after use of thyroid hormone in euthyroid subjects) medium/high quality studies for meta-analysis. We found that thyrotoxicosis shifts the haemostatic balance towards a hypercoagulable and hypofibrinolytic state with a rise in factors VIII and IX, fibrinogen, von Willebrand factor, and plasminogen activator inhibitor-1. This was observed in endogenous and exogenous thyrotoxicosis, and in subclinical as well as overt hyperthyroidism. This meta-analysis showed consistent evidence of a prothrombotic state in thyrotoxicosis. Only when well-designed studies with clinical outcomes are performed, the clinical relevance of these findings, especially in terms of prevention and treatment of venous thrombosis in hyperthyroid patients, can be determined.

Chapter 5 describes a young woman who attempted suicide by auto-intoxication with 25 mg of levothyroxine, which presented us with a unique situation of assessing the haemostatic effects during extreme thyroid hormone excess. 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. We found a marked increase in coagulation factors VIII, IX and X, von Willebrand factor, and plasminogen activator inhibitor-1 resulting in a rise in endogenous thrombin potential indicating an enhanced thrombin generation in thyrotoxicosis. Levels tended to normalize once euthyroidism was restored. Moreover, levels of FVIII, FIX and VWF even further decreased during subclinical hypothyroidism. These findings are in line with chapter 4, in that thyroid hormone excess shifts the haemostatic balance towards a hypercoagulable and hypofibrinolytic state, which is likely to reflect an increased risk of venous thrombosis.
Chapter 6 studies the effect of hyperthyroidism and hypothyroidism on thrombin activatable fibrinolysis inhibitor (TAFI), the link between the coagulation and fibrinolytic system. The effect of hypothyroidism on TAFI was studied in a multicenter observational cohort study described in chapter 8. The effect of hyperthyroidism on TAFI was studied in a single blinded, cross-over randomized controlled trial in which healthy volunteers were treated with levothyroxine for 2 weeks. Hyperthyroidism resulted in a hypofibrinolytic condition and in enhanced TAFIa-dependent prolongation of clot-lysis, despite slightly decreased plasma TAFI levels. Hypothyroidism resulted in hyperfibrinlysis, a reduced contribution of TAFIa during fibrinolysis and a trend towards increased plasma TAFI levels. The results of this study might further explain the thrombotic tendency in hyperthyroid patients, and the bleeding tendency in hypothyroid patients.

In Chapter 7 we investigate fibrin clot formation and fibrinolysis in hypothyroidism and hyperthyroidism using observational and interventional studies. We demonstrated that hyperthyroidism is associated with the formation of compact fibrin networks ex vivo, which are more resistant to fibrinolysis providing one mechanism for increased thrombosis risk in this condition. Interestingly, less compact fibrin networks with enhanced fibrinolysis during hypothyroidism was found, consistent with a bleeding tendency in this condition. In both disorders these changes are reversible upon restoration of euthyroidism. Altered clot structure/lysis may be one mechanism for increased thrombotic risk in hyperthyroidism and a bleeding tendency in hypothyroidism.

Chapter 8 studies the prevalence of acquired von Willebrand syndrome (aVWS) and bleeding symptoms in patients with hypothyroidism in an observational cohort study. Ninety consecutive hypothyroid patients before or within the first 48 hours of replacement therapy were enrolled. Repeat-samples were obtained after restoration of euthyroidism. The prevalence of aVWS, defined as von Willebrand factor antigen (VWF:Ag) ≤ 50% and/or VWF ristocetin activity (VWF:RiCo) ≤ 50%, was 33%. No patients had VWF levels below 10%, 9% had VWF levels between 10 and 30%, 23% had VWF levels between 30 and 50%. Bleeding score was negatively correlated to both VWF:Ag (β -0.32, p=0.03) and VWF:RiCo (β -0.32, p=0.02). After restoration of euthyroidism, VWF:Ag had significantly increased by 44%, VWF:RiCo by 36%, factor VIII by 39%, and endogenous thrombin potential by 10%. We conclude that acquired von Willebrand syndrome has a high prevalence in hypothyroid patients. Physicians should be aware of this association, especially since it may easily go unnoticed owing to the fact that most patients with aVWS do not bleed until they are exposed to trauma or surgery and further diagnostic tests are performed.
In Chapter 9 we explore the effect of subclinical hypothyroidism on vitamin K antagonists' sensitivity and stability. Among 996 patients followed at the Anticoagulation Clinic, 26 patients who became subclinical hypothyroid during vitamin K antagonists treatment and achieved an euthyroid state while still on vitamin K antagonists were included. 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 begun, 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 therefore suggest that subclinical hypothyroidism may affect both stability and sensitivity of vitamin K antagonist. Physicians should measure INR more frequently in patients with thyroid disorders and measure thyroid levels in patients that are, despite all efforts, unstable on vitamin K antagonists.

Chapter 10 describes the results of a retrospective multicentre cohort study in which we aimed to determine the incidence of venous thromboembolism in a cohort of biochemically confirmed overt hyperthyroid patients between 6 months before and 6 months after the diagnosis was made. This was performed by studying all handwritten and electronic available data of patients selected from the hospitals’ electronic systems with diagnostic codes. A total of 587 patients (23%) was included out of 2578 patients selected purely on diagnostic codes. Five patients experienced a VTE during the study period, resulting in an incidence rate of 8.7 (95% CI 2.8-20.2) per 1000 person-years. Three of these five patients had a first VTE (incidence rate for first VTE was 5.3 (95% CI 1.1-15.6) per 1,000 person-years). Incidence rates of VTE in the general population are between 0.6 and 1.6 per 1,000 person-years for first VTE and 0.7 and 1.8 per 1,000 person-years for all VTE. In conclusion, these findings support the hypothesis that hyperthyroidism leads to an increased risk of venous thromboembolism. Prospective studies are necessary to further define the relationship between hyperthyroidism and venous thromboembolism. These finding also show that diagnostic codes are primarily used for registration purposes, which is probably not performed with the precision needed for research.

In Chapter 11 we performed a nested case control study with data from a large population based study of patients with a first venous thrombosis (MEGA study), in which blood samples were taken in the months after first venous thrombosis. Information on recurrent venous thrombosis was retrieved. Patients with a recurrence (381 cases) were matched (1:2) with
patients without a recurrence (761 controls) on time from first VT to blood sampling. The risk of venous thrombosis was not affected by different levels of FT4 when compared with the reference category (FT4 levels of 15.5 to 18.9 pmol/l). The VT risk for FT4 levels < 15.5 pmol/l was 0.8 (CI95 0.6-1.1); the VT risk for FT4 > 24.4 pmol/l was 0.6 (CI95 0.1-3.8). Also for TSH, the ORs did not essentially vary. In a restricted analysis in which only cases were included in whom the recurrent VT occurred within one year after blood sampling, the odds ratios remained the same. We hypothesize that hyperthyroidism present at a first venous thrombotic event is not a risk factor for recurrence, when properly diagnosed and treated. This emphasizes the need for awareness of possible hyperthyroidism when a patient with unprovoked venous thrombosis is examined, so that the patient will not only benefit from recovery of thyroid disease but also from a reduced risk of recurrent venous thrombosis.

In Chapter 12 we describe two cases with hypothyroidism-induced dyslipidemia in whom the lipid abnormalities after thyroid hormone substitution therapy completely resolved. Decreased thyroid function increases both the number of LDL particles in the blood, but also promotes LDL oxidability thereby increasing the atherogenic effect. Statins are less effective in hypothyroid patients whose HMG-CoA reductase (the rate limiting enzyme in cholesterol biosynthesis) activity is already reduced by the hypothyroid state. Hypothyroidism is by itself a risk factor for myopathie en renal insufficiency, however it also increases the risk of statin-induced rhabdomyolysis. Besides the insidious clinical presentations and diagnostic challenges of hypothyroidism, these cases illustrate that clinical and biochemical screening for thyroid dysfunction is of paramount importance in all dyslipidemic patients before start of lipid-lowering medication both in primary and secondary care.

Chapter 13 describes the results of a double-blind, multicenter trial in which we assessed whether treatment with the thyroid hormone metabolite 3,5-diiodothyronamine (T2) leads to activation of coagulation and inhibition of fibrinolysis. In total, 40 male patients with the metabolic syndrome were randomised to receive either the thyroid hormone metabolite TRC150094 (at a dose of 50mg per day) or placebo for 28 days. For the present analysis, parameters of thyroid function and haemostasis were assessed before and after treatment. We found that treatment with TRC150094 did not affect parameters of coagulation or fibrinolysis, possibly explained by the suggested mainly nongenomic effects of T2 due to a direct interaction with the mitochondria, while the effects of triiodothyronine (T3) and thyroxine (T4) are initiated via the nucleus. In this 4 week trial, treatment with T2 did not lead to activation of coagulation, however; it does appear to affect thyroid hormone metabolism: levels of thyroid hormone free thyroxine (FT4) increased by 11% and T3 by 21%. However the rise might still be to small to detect any differences in coagulation parameters as compared to studies in which synthetic T4 is given.
Summary

Given the idea of a thyroid hormone-mediated upregulation of coagulation factors at the hepatic level, TRβ such as ‘eprotirome’ that bind and activate the β1 isoform of the nuclear thyroid hormone receptor and have hardly any affinity for the α1 isoform expressed in the cardiac system selective agonist, might be able to affect the regulation of liver-synthesized clotting factors. Even though thyroid hormone derivates deserve further study as potential agents in the treatment of dyslipidemia and other risk factors for atherosclerosis, we would urge future prospective clinical trials of to assess the effect on coagulation and fibrinolysis of this class of agents.

Part III: Prolactin

Chapter 14 studies the association between increasing levels of prolactin, coagulation parameters and venous thrombosis using a large population-based case-control study into etiology of first venous thrombosis. Prolactin levels were determined in 2068 patients with venous thrombosis and 2785 age- and sex matched control subjects and odds ratios and 95% confidence intervals were calculated. We found a rise in factor VIII and von Willebrand factor with increasing levels of prolactin in the controls. An increased risk of VT was observed when blood was sampled within 6 months after thrombosis (OR 2.9, 95% CI 1.1-8.1) for prolactin levels above the 99th percentile (42.6 umol/l) relative to levels between the 20th to 80th percentile. When blood was sampled more than 6 months after VT no clear association could be observed (OR 1.3, 95% CI 0.7-2.3). In conclusion, we found a modest association between prolactin and clinical venous thrombosis, particularly when blood was sampled close to the event. This may be explained by a causal relation or by prolactin being a marker of stress due to the thrombotic event.

In Chapter 15, microvascular homeostasis (sublingual and retina) was evaluated in ten prolactinoma patients and ten age- and sex-matched healthy controls using sidestream dark field (SDF) imaging. Lipids, coagulation and inflammatory markers were assessed using an extensive chemistry panel. SDF imaging revealed a marked perturbation of sublingual microcirculation in prolactinoma patients when compared to control subjects, as attested to by significant changes in microvascular flow index (2.74±0.12 versus 2.91±0.4, respectively; p=0.001), heterogeneity index (0.25±0.09 vs. 0.11±0.4, respectively; p=0.006) and lower proportion of perfused vessels (90±4.0% vs. 95±3.0%, respectively; p=0.02). In the retina, a trend towards dilatated perifoveal capillaries was seen. Systemically, prolactinoma patients displayed biochemical markers indicative of an increased cardiovascular risk, comprising decreased HDL cholesterol levels, increased interleukin-6 as well as higher endogenous thrombin potential and prothrombin levels, indicative of a pro-inflammatory and pro-coagulant state, respectively. These finding show that patients with elevated prolactin levels
are characterised by microvascular dysfunction as well as systemic markers indicative of a pro-atherothrombotic state. Further studies are required to evaluate if prolactin is a pro-atherogenic hormone in patients at increased cardiovascular risk.

For the detection of important unwanted outcomes of new interventions, physicians often rely on adverse event reporting. From previous studies we know that underreporting of adverse events is a major concern in clinical trials, although was generally assumed that the underreporting of serious adverse events, such as venous or arterial thrombosis is relatively limited. During this PhD course we hypothesized that there is a underreporting of venous and arterial thrombosis.

Chapter 16 describes a systematic review in which we aimed to quantify the reported incidence of venous thromboembolism and arterial thrombosis in randomized clinical trials (RCTs), and to evaluate the extent of underreporting. The EMBASE database was searched to identify all RCTs published in the 4 highest impact general medical journals (New England Journal of Medicine, Lancet, Journal of the American Medical Association, and Annals of Internal Medicine) between January 1, 2011 and July 1, 2011. The occurrence of VTE and AT, either as predefined outcome or as adverse event, was assessed per study and per population. A total of 131 RCTs were identified. VTE and AT were not reported in 89% and 70% of these studies, respectively. Under these conditions, meta-analytical calculation of incidences is problematic. When calculating the raw-unweighted reported incidence among the 3 studies that had predefined outcomes for VTE, the reported incidence for VTE was 8.4 (95% CI 7.8-9.1) per 1000 person-years. In the 128 studies without predefined outcomes for VTE (consisting of 322029 individuals, including many patients with cancer, inflammatory disease, cardiovascular disease or surgery, adding up to a follow up period of over 500,000 person-years) an incidence of VTE of 0.4 (95% CI 0.4-0.5) per 1000 person-years was found. The raw unweighted reported incidence of AT in the 18 studies in which AT was part of the predefined outcomes was 25.6 (95% 24.9-26.3) per 1000 person-years. In the 92 studies without predefined outcomes for AT (consisting of 231638 individuals adding up to a follow-up period of over 200,000), the raw unweighted incidence of AT was 2.5 (95% CI 2.3-2.7) per 1000 person-years. These data suggest that the reported incidence of both venous thromboembolism and arterial thrombosis is highly underreported. Uniform registration of adverse events in clinical trials, even when unlikely to be related to the intervention, is necessary to be able to inform physicians about the potential toxicities of new therapeutic strategies.