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

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Overview

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

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

Hyperthyroidism

The relation between high levels of thyroid hormone or thyrotoxicosis and venous thrombosis was first described in case reports in 1913 by Kaliebe et al. [13] and in 1928 by Doyle et al. [94]. Other case reports appeared
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Table 1: summary of identified studies and their exposures and outcomes.

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

**High FT4 levels and venous thrombosis risk**

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

Subsequently, three larger studies on the relation between thyroid hormone and risk of venous thrombosis have been performed: the ACT-study [42], the MEGA-study [67] and the TROL study [48], all using different study designs. All three studies collected blood samples and data on venous thrombosis. The most obvious difference in the design of the studies was the timing of the blood draw: In the TROL
study blood was sampled before the thrombosis, in the ACT-study blood was drawn at the time of thrombosis and in the MEGA study blood was drawn with a minimum of 3 months after the thrombosis. In addition, the MEGA study has substantially more power than the other two studies due to a large number of patients with thrombosis.

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

In the ACT study [42] FT4 levels and triiodothyronine (T3) levels were measured at the time of thrombosis in 190 cases and 379 controls. The authors reported that higher levels of FT4 gave rise to an increased risk of venous thrombosis, also in a dose response relation. A 1.7 fold increased risk (CI95 1.0 to 2.9) of venous thrombosis was observed in patients with FT4 above the 90th percentile (>18 pmol/L) compared with patients with a FT4 level beneath the 90th percentile. At the 99th percentile (>22 pmol/L) this risk increased to 4.7 (CI95 1.2 to 18.6). This effect was also seen for levels of T3, although less pronounced.
In the MEGA study [67], 2177 cases with venous thrombosis and 2826 controls were included in the analysis. Blood was drawn several months after the venous thrombosis. Here also a dose response relation was found with an OR of 1.2 (CI95 0.9 to 1.6) at the 90th percentile, gradually rising to an OR of 2.2 (CI95 1.0 to 4.6) at FT4 levels >24.4 pmol/L compared with FT4 levels between 15.5 pmol/L and 18.9 pmol/L (reference category). In the MEGA study, blood could have been drawn at various time points from the thrombotic events (3 months to 36 months). When only participants with blood drawn within 6 months after the event were included, the OR was 2.6 (CI95 0.7 to 9.6) at FT4 levels >24.4 pmol/L compared with the reference category. When only subjects were included in whom hyperthyroidism was established, by clinical diagnosis or blood draw, less than one year before or shortly after the index date, an odds ratio of 17.0 (CI95 2.2 to 133.0) was found.

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

Clinical hyperthyroidism and venous thrombosis risk

According to our search, only three studies reported on the relationship between clinically diagnosed hyperthyroidism and venous thrombosis. In a population based cohort from Taiwan, only focusing on pulmonary embolism, Lin et al. [49] included participants with hyperthyroidism over a 5 year period. A total of 8903 patients were found. They were compared to 44515 participants without hyperthyroidism. Fourteen patients with hyperthyroidism developed a pulmonary embolism (0.16%) as compared with 27 euthyroid participants (0.06%). They found that patients with hyperthyroidism had a 2.3-fold increased risk for pulmonary embolism compared with euthyroid subjects (CI95 1.2 to 4.5). The study by Kootte et al. [93] estimated the incidence of venous thrombosis in hyperthyroid patients in three hospitals in the Netherlands and found an incidence rate for first venous thrombosis of 5.3 per 1,000 person-years (CI95 1.1 to 15.6), which was considered to be higher than the population incidence of 1 per 1000 person-years [93]. A study by Danescu [71] et al was performed in the National Hospital Discharge Survey USA from 1979 to 2005. They looked at the discharge codes for hypothyroidism, hyperthyroidism, pulmonary
embolism and deep venous thrombosis. No increased risk for venous thrombosis with a relative risk of 0.98 (CI95 0.96 to 1.01) was found in patients with a diagnosis of hyperthyroidism compared to patients without a diagnosis of thyroid dysfunction in the database.

Hypothyroidism

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

Low FT4 levels and haemorrhage

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

Clinical hypothyroidism and venous thrombosis

If the effect on bleeding of low FT4 levels is causal, low levels of FT4 could translate in a protective effect against venous thrombosis. However, both a pro- and an anti-thrombotic effect of low levels of FT4 have been described. Peralta et al. claimed a role for hypothyroidism in the aetiology of cerebral vein thrombosis, by describing two cases with hypothyroidism concomitantly diagnosed with cerebral venous thrombosis [96]. The study by Danescu et al. reported no risk for venous thrombosis
with the diagnosis of hyperthyroidism as mentioned before, but did report on an increased risk for venous thrombosis with the diagnosis of hypothyroidism. An increased risk of 1.60 (CI95 1.59 to 1.60) was reported for patients diagnosed with hypothyroidism compared with participants without thyroid dysfunction.

**Low FT4 levels and venous thrombosis**
The first controlled study on risk of venous thrombosis was done by Squizzato et al[20]. In patients with unprovoked deep venous thrombosis, a 5.5-fold increased risk (CI95 0.6 to 52.6) for subclinical hypothyroidism was found. In provoked DVT patients a 6.8-fold higher chance to find subclinical hypothyroidism was found compared with controls. However, this study was small, so that chance findings were plausible.

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

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

There is not much known on the pathophysiological mechanisms underlying the effect of thyroid hormones on the coagulation system. Thyroid hormone affects gene transcription, which may form the basis of changes in pro- and anticoagulant proteins. Since thyroid hormone influences the basal rate of the carbohydrate, lipid but also the protein metabolism, it is likely that thyroid hormone also affects the production and clearance of coagulation proteins. In vitro studies have shown a direct effect of tri-iodothyronine (T3) on hepatocytes and endothelial cells. T3 causes an up-regulation of fibrinogen, factor II (prothrombin), factor X, von Willebrand factor and plasminogen [36,66,69]. Also in vivo, a quick response of the coagulation system was seen with increased production of nearly all coagulation factors, but most notably VWF and FVIII, on increasing levels of FT4 caused by restarting levothyroxine substitution after withdrawal [46,47]. Additional proof comes from a clinical study [67], where we found a positive association between FVIII and VWF with levels of FT4. Also the risk for venous thrombosis attenuated upon adjustment for FVIII and VWF, which suggests that VWF and FVIII are in the causal pathway between thyroid hormone and venous thrombosis. Although not much is known about the relation between thyroid specific auto-immune antibodies and the effect on the coagulation system, some claims of an effect via this pathway have been made. In this thesis, we have looked in 3 different studies whether there was an effect of anti-thyroid peroxidase antibodies on the risk of thrombosis. None of the studies were able to find a relation between these antibodies and venous thrombosis. A third mechanism was recently proposed by Hooper et al. [81] who studied the influence of thyroxine on fibrin formation. A cross-over design was applied with two arms: Blood was sampled from 19 patients with hyperthyroidism en from 19 euthyroid matched controls. Another blood sample was gathered after treatment of the hyperthyroid patients, i.e., when they returned to a euthyroid state. Levothyroxine was added to the blood samples of the euthyroid participants. In hyperthyroidism, a dense clot structure with impaired fibrinolysis was found. The clot structure partly returned to normal when a euthyroid state was restored. Short term exogenous hyperthyroidism was not associated with alterations in clot structure. Inflammation may also play a
role because complement C3 levels were also associated with clot formation in hyperthyroid patients. The denser clot structure and higher resistance to fibrinolysis might, in itself or in combination with faster clot formation as hypothesized earlier, also be a plausible explanation for the clinical effects found.

The last pathophysiological explanation is the association between hypothyroidism and cardiovascular risk factors. These cardiovascular risk factors include obesity, LDL cholesterol, mild increases of homocysteinemia and endothelial dysfunction; hypothyroidism also increases diastolic blood pressure and induces a mild increase in C-reactive protein [59,60,62,63]. Since recent reports state that arterial cardiovascular risk factors also tend to increase the risk for venous thrombosis to some extent [87-89], it might be possible that these cardiovascular parameters are somewhere in the pathway between hypothyroidism and venous thrombosis.

The effect of low levels of free thyroxine via cardiovascular risk factor would result in a pro-thrombotic effect, while the direct effect of hypothyroidism on the coagulation system is anti-coagulant.

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

In conclusion, in the studies in this thesis we found strong evidence that higher than average levels of FT4 increase the risk of venous thrombosis. Weak evidence is
provided that lower than average levels of FT4 give an increased risk of bleeding in patients using vitamin K antagonists. A hypothyroid state possesses probably both pro- and anti-coagulant properties. The effect of FT4 on venous thrombosis is mediated at least by coagulation factors factor VIII and von Willebrand factor.

**Clinical implications**

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

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

For physicians treating patients with thrombosis, there are several clinical implications. In patients with venous thrombosis, it is advisable to look for signs of thyroid disease, such as goiter, sweating, heat or cold intolerance etc. Based on our and the above mentioned previous findings in three other studies [42,48,49], routine testing for hyperthyroidism of patients with an unprovoked thrombosis may be considered, as the diagnosis is not always obvious clinically and testing is easily done and relatively cheap. A positive result will have a strong impact for the patient:
not only will an undetected condition be treated, but also can the patient receive anticoagulant treatment for a shorter period (3 instead of 6 months (ACCP guidelines [97])), with hence a lower bleeding risk.
Summary