Hormones, haemostasis, and the risk of thrombosis
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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 Buller, 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[5]. 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 our assay, all samples were also measured in citrated plasma using a similar assay (Tinaquant, Roche Diagnostics, Basel, Switzerland). The intra- and interassay coefficients of variations (CVs) were below or equal to 4.8% and 5.1% for FT4, 3.6% and 0.9% for T3, 9.2% and 3.2% for TSH, and 4.3% and 7.4% for antiTPO, respectively. The percentage agreement between the two methods was 97.1% for FT4, 97.2% for T3, 97.7% for TSH and 97.8% for antiTPO. In one plasma sample, thyroid hormones could not be assessed due to insufficient volume.

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.

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 already available 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, triiodothyronine; 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.

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

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

### Table 2. Thyroid function.

<table>
<thead>
<tr>
<th></th>
<th>All cases (n=190)</th>
<th>DVT only (n=155)</th>
<th>Controls (n=379)</th>
<th>p-value (2-sided) A vs C</th>
<th>p-value (2-sided) B vs C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FT4, pmol/L, mean (95% CI)</strong></td>
<td>16.0 (15.6-16.4)</td>
<td>16.2 (15.8-16.7)</td>
<td>15.4 (15.1-15.6)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>T3, nmol/L, mean (95% CI)</strong></td>
<td>1.94 (1.87-2.00)</td>
<td>1.90 (1.83-1.97)</td>
<td>1.79 (1.75-1.82)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>TSH, mU/L, median (95% CI)</strong></td>
<td>1.41 (1.22-1.51)</td>
<td>1.37 (1.17-1.51)</td>
<td>1.21 (1.11-1.30)</td>
<td>0.03</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>AntiTPO, U/mL, median (95% CI)</strong></td>
<td>3.0 (2.73-3.08)</td>
<td>2.89 (2.72-3.07)</td>
<td>2.98 (2.90-3.07)</td>
<td>0.48</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Hypothyroidism, n (%)</strong></td>
<td>3 (1.6)</td>
<td>3 (1.9)</td>
<td>0 (0)</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>- Sub. Hypothyroidism, n (%)</td>
<td>0 (4.7)</td>
<td>3 (4.5)</td>
<td>18 (4.7)</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Euthyroidism, n (%)</strong></td>
<td>170 (89.5)</td>
<td>138 (89.0)</td>
<td>348 (91.8)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>- Sub. Hypothyroidism, n (%)</td>
<td>8 (4.2)</td>
<td>7 (4.5)</td>
<td>12 (3.2)</td>
<td>0.50</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Hyperthyroidism, n (%)</strong></td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (0.3)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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.

### 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).

<table>
<thead>
<tr>
<th>FT4a (pmol/L)</th>
<th>Percentileb</th>
<th>n</th>
<th>VTc (n=190)</th>
<th>DVT (n=155)</th>
<th>Unprovoked VTc (n=69)</th>
<th>Unprovoked DVT (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 LoRef</td>
<td>2</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>&lt;11</td>
<td>10</td>
<td>16</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&lt;12</td>
<td>36</td>
<td>5</td>
<td>50.0 (14.5-16.2)</td>
<td>61</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&lt;13</td>
<td>10</td>
<td>10</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&lt;14</td>
<td>50</td>
<td>36</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&gt;16</td>
<td>30</td>
<td>85</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&gt;17</td>
<td>80</td>
<td>68</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&gt;18</td>
<td>90</td>
<td>35</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&gt;19</td>
<td>95</td>
<td>12</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>99</td>
<td>6</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&gt;21</td>
<td>99</td>
<td>3</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
<tr>
<td>&gt;22</td>
<td>99</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>1</td>
<td>0.1 (0.0-0.4)</td>
<td>0.4 (0.2-0.7)</td>
</tr>
</tbody>
</table>

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, and the 90th percentile with the 95th percentile, as we compared subjects below and above each cut-off level.

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[25-26]. 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]. 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.

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 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[25-28]. 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[25-28]. Other hypotheses include indirect effects mediated through beta-adrenergic receptor function[25-28]. 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.
is known to affect thyroid function, and multiple alterations in thyroid hormone levels have been observed in patients with this non-thyroidal illness syndrome\[34,35\]. 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\[34,35\]. 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\[34,35\]. 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 incidence. 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 disabilitating 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), a man of age 83, was 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.