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Chapter 7

The effect of changes in thyroxine and thyroid stimulating hormone levels on the coagulation system

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Abstract

Introduction: Thyroid dysfunction is known to affect levels of factor VIII, von Willebrand factor and fibrinogen. Although altered coagulation parameters are related to levels of thyroid hormone, this is possibly partially mediated by thyroid stimulating hormone (TSH). Our aim was to examine the effect of TSH and free thyroxine (FT4) on coagulation factors, with a focus on factor VIII (FVIII), von Willebrand factor (VWF) and fibrinogen.

Methods: In patients successfully treated for well-differentiated thyroid carcinoma, either levothyroxine was withdrawn for 4 weeks (n=11) or recombinant TSH was administered (n=17) to stimulate thyroglobulin production. We measured levels of FVIII, VWF and fibrinogen, besides prothrombin, factor VII, factor IX, antithrombin, protein C and S, prothrombin fragment 1+2 and thrombin-antithrombin complex on two different occasions.

Results: In patients who changed from hypothyroidism to a slightly hyperthyroid state, a rise in FVIII (+39.1 U/dL), VWF (+32.0 U/dL), and fibrinogen (+0.6 g/L) was found. In patients in whom stable FT4 levels accompanied rising levels of TSH, no effect on coagulation parameters was observed.

Conclusions: The results of our study suggest that increasing levels of free thyroxine are associated with a rise in FVIII, VWF, and fibrinogen levels. This shift is not mediated by TSH.

Introduction

Thyroid dysfunction is known to affect the coagulation system[1-6]. In patients with overt hypothyroidism, an increased bleeding time, prolonged prothrombin time and activated partial thromboplastin time, as well as decreased levels of factor VIII (FVIII), fibrinogen and von Willebrand factor (VWF) have been observed[2,5], occasionally leading to a bleeding tendency, similar to acquired von Willebrand’s disease[7,8]. After adequate suppletion with levothyroxine, these coagulation factors return to normal levels[3,6]. In hyperthyroidism, elevated levels of VWF and fibrinogen have consistently been observed[2]. These changes in coagulation parameters may have clinical implications, such as an increased risk of venous thrombosis in hyperthyroidism[2,4]. Although the altered coagulation parameters are related to levels of thyroid hormone, it is possible that the effect on coagulation is either completely or partially mediated by thyroid stimulating hormone (TSH, or thyrotropin). We set out to examine and disentangle the effect of changes in TSH and serum free thyroxine (FT4) levels on FVIII, VWF and fibrinogen, which are the coagulation factors for which an effect of thyroid hormones is best documented[1,2,5]. In addition, we studied other coagulation factors to explore whether they were influenced by FT4 and TSH levels. We had the opportunity to study the separate effects of these hormones in two series of patients successfully treated for well-differentiated thyroid carcinoma.

For follow-up, serum thyroglobulin (TG) level is used as a tumour marker. To assess serum thyroglobulin levels in a maximally stimulated condition, high levels of TSH are induced[11]. This can be achieved either by withdrawal of levothyroxine for several weeks, or by the administration of recombinant human TSH (rhTSH) while continuing levothyroxine treatment. Measuring coagulation factors at different stages in both TG stimulating protocols can provide clues whether changes in levels of FT4 or TSH affect the coagulation system.

Materials and Methods

Study population

Patients successfully treated for differentiated thyroid carcinoma by surgery and radioactive iodine ablation of the residual thyroid gland were derived from a larger study on the influence of thyroid hormone on metabolism and gene expression in relation to heart rate and blood pressure.

Prior to a TSH-stimulated diagnostic protocol, consisting of either thyroxine withdrawal or recombinant TSH injection, patients from the Department of Endocrinology of the Leiden University Medical Centre, Leiden, the Netherlands (a tertiary referral centre for differentiated thyroid carcinoma) were asked to participate in the study. In all patients, successful treatment was defined as the absence of measurable serum TG levels during TSH stimulation and negative total-body scintigraphy. Patients with a haemoglobin level below 7.1 mmol/L, recent blood donation, diabetes mellitus, a body mass index above 35 kg/m², pregnancy, or other endocrine disorders, as well as postmenopausal women were excluded, in addition to patients using any drugs known to influence coagulation. In total, 12 patients undergoing thyroxine withdrawal and 17 patients receiving rhTSH were eligible for the present analysis.
For both studies written informed consent was obtained from all patients and the Medical Ethics Committee of the Leiden University Medical Centre approved both protocols.

**Study design**

**Thyroxine withdrawal group**

A total of 12 patients undergoing thyroxine withdrawal were eligible for the present analysis. Blood was sampled at two points in time. The first blood sampling was performed after participants had stopped their levothyroxine treatment for 4 weeks. At this point, FT4 was expected to have decreased to very low levels and the accompanying TSH levels to have increased. Participants then restarted their levothyroxine treatment, and 8 weeks following the first blood sampling, when FT4 levels were expected to have returned to normal values and TSH levels to be suppressed, the second blood sample was drawn. Of note, the goal of substitution therapy in patients cured from differentiated thyroid carcinoma is a suppressed TSH, with an accompanying high normal or slightly elevated FT4. One patient did not return for the second blood sampling, leaving 11 patients (92%) within the thyroxine withdrawal group for final analysis.

**Recombinant human TSH group**

Seventeen patients received 0.9 mg rhTSH (Thyrogen) at 2 consecutive days. Blood samples were again drawn at two time points. At day 1, participants were at their regular substitution level and the first sample of blood was drawn. At day 4 and 5, 0.9 mg rhTSH was administered intramuscularly. At day 8, a second sample was drawn. At this point in time, TSH levels were expected to be high with unchanged FT4 levels.

**Biochemical analysis**

All plasma and serum samples were measured in one batch. Levels of FT4 and TSH were measured with an electrochemoluminescent immunoassay with a Modular Analytics E-170 system with an intra-assay CV of 1.6-2.2% and 1.3-5.0%, respectively (Roche Diagnostics, Almere, the Netherlands). Laboratory specific reference ranges were 10-24 pmol/L for FT4 and 0.3-4.8 mU/L for TSH. Coagulation levels were measured by activity assays for factor II, (STA Factor II), factor VII (STA Factor VII), factor VIII (STA deficient VIII), factor IX (STA factor IX) antithrombin (STA antithrombin III) and protein C (chromogenic method, STA protein C chromogen), and by antigen assays for von Willebrand factor (STA Liatest® VWF) and protein S (Liatest® Protein S and Liatest® free Protein S), all produced by Diagnostica Stago, Asnières, France. Furthermore, fibrinogen (method according to Claus, STA fibrinogen, Diagnostica Stago, Asnières, France), thrombin-antithrombin complex antigen (Enzygnost TAT) and prothrombin fragment 1+2 antigen (Enzygnost F1+2 micro; Dade Behring, Marburg, Germany) were measured. All tests were performed in duplicates by technicians who were unaware of the status of the sample (i.e. to which patient or patient group it belonged).

**Statistical analysis**

We assessed the effect of rises in FT4 levels (thyroxine withdrawal group) and TSH levels (Recombinant human TSH group) on several coagulation parameters. Median values and their ranges were determined for all measurements. In this research design, the participants served as their own controls and differences in coagulation factors were analysed with paired sample statistics. Mean differences of the pre-treatment and post-treatment scores were calculated with their 95% confidence intervals (95% CI). All statistical analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, IL)

**RESULTS**

**Thyroxine withdrawal group**

Patient characteristics

This study group consisted of 7 women and 4 men. Median age was 44 (range 29-56) years. Ten participants had been treated for papillary carcinoma while 1 participant was treated for a follicular carcinoma. The median time from surgery and radioactive iodine ablation therapy was 16 (range 8-64) months.

**Thyroid function and coagulation parameters (Table 1)**

At measurement 1 (4 weeks after levothyroxine withdrawal), levels of TSH were high with a median of 133.4 mU/L while levels of FT4 were extremely low: 1.5 pmol/L. At measurement 2 (8 weeks after restart of levothyroxine), TSH levels had decreased to 0.7 mU/L with FT4 levels of 24.2 pmol/L. Both measurement 1 and 2 showed levels of coagulation factors more or less within normal ranges. For FVIII (+39.1 U/dL, 95% CI 6.8 to 71.4), VWF (+12.0 U/dL, 95% CI 13.1 to 50.8) and fibrinogen (+0.6 g/L, 95% CI 0.2 to 1.0) a clear difference was observed between measurement 1 (deep hypothyroid state) and measurement 2 (slightly hyperthyroid state). In addition, levels of factor II (II) (+8.5 U/dL, 95% CI 2.1 to 14.9), factor IX (IX) (+30.8 U/dL, 95% CI 20.8 to 40.9), antithrombin (AT) (+13.4 U/dL, 95% CI 5.0 to 22.0), and total protein S (+70.0 U/dL, 95% CI 0 to 14.2) were all higher at measurement 2, while levels of factor VII (VII) (-29.9 U/dL, 95% CI -46.4 to -13.4) and protein C (+13.6 U/dL, 95% CI -22.8 to -4.3) had decreased. No clear changes were observed for prothrombin fragment 1+2 (F1+2) and the thrombin-antithrombin complex (TAT).

**Recombinant Thyroid Stimulating Hormone group**

Patient characteristics

The recombinant TSH group consisted of 1 male and 16 females. Median age of these patients was 49 (range 25-86) years. Fifteen patients had a diagnosis of papillary carcinoma and 2 were diagnosed with follicular carcinoma. The median time from surgery and radioactive iodine ablation therapy was 37 (range 13-303) months.

**Thyroid function and coagulation parameters (Table 2)**

A strong increase in median TSH levels was seen from measurement 1 (baseline situation) to measurement 2 (after administration of rhTSH): 0.1 mU/L versus 23.9 mU/L. Median levels of FT4 did not materially differ between the two measurements: 21.3 pmol/L versus 22.1 pmol/L. In this group, no clear differences in levels of FVIII, VWF or fibrinogen were observed between the two measurements, nor in any of the other coagulation factors. Only levels of FVII slightly differed between the first and the second measurement (mean change -8.8 U/dL, 95% CI -15.3 to -2.3).
Table 1. Effect on coagulation parameters of increasing levels of FT4 and decreasing levels of TSH (thyroxine withdrawal group).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Median (range)</th>
<th>Measurement</th>
<th>Median (range)</th>
<th>Mean difference</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT4 (pmol/L)</td>
<td>1.5 (0.0-2.4)</td>
<td>24.2 (17.4-31.2)</td>
<td>23.4</td>
<td>20.4 to 26.3</td>
<td></td>
</tr>
<tr>
<td>TSH (mU/L)</td>
<td>133.4 (99.1-191.9)</td>
<td>0.7 (0.0-3.0)</td>
<td>-141.7</td>
<td>-164.7 to -118.6</td>
<td></td>
</tr>
<tr>
<td>F1+2 (pmol/L)</td>
<td>125 (100-284)</td>
<td>158 (91-1888)</td>
<td>7.7</td>
<td>-47.0 to 62.5</td>
<td></td>
</tr>
<tr>
<td>TAT (ug/L)</td>
<td>2.3 (1.9-18.2)</td>
<td>2.7 (2.1-72.5)</td>
<td>-0.4</td>
<td>-6.5 to 4.6</td>
<td></td>
</tr>
<tr>
<td>AT (U/dL)</td>
<td>104 (82-141)</td>
<td>116 (90-152)</td>
<td>7.7</td>
<td>5.0 to 22.0</td>
<td></td>
</tr>
<tr>
<td>Protein C (U/dL)</td>
<td>132 (99-200)</td>
<td>113 (91-163)</td>
<td>-17.7</td>
<td>-22.8 to -4.3</td>
<td></td>
</tr>
<tr>
<td>Total Protein S (U/dL)</td>
<td>107 (88-143)</td>
<td>106 (93-160)</td>
<td>1.0</td>
<td>-0.1 to 14.2</td>
<td></td>
</tr>
<tr>
<td>FII (U/dL)</td>
<td>114 (83-137)</td>
<td>122 (92-150)</td>
<td>7.4</td>
<td>2.1 to 14.9</td>
<td></td>
</tr>
<tr>
<td>FVII (U/dL)</td>
<td>136 (106-213)</td>
<td>105 (88-157)</td>
<td>-30.7</td>
<td>-46.4 to -13.4</td>
<td></td>
</tr>
<tr>
<td>FVIII (U/dL)</td>
<td>95 (55-155)</td>
<td>133 (84-219)</td>
<td>38.1</td>
<td>6.8 to 71.4</td>
<td></td>
</tr>
<tr>
<td>FIX (U/dL)</td>
<td>96 (70-134)</td>
<td>141 (101-171)</td>
<td>45.0</td>
<td>20.8 to 40.9</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.1 (2.0-3.9)</td>
<td>3.7 (2.5-4.6)</td>
<td>0.6</td>
<td>0.2 to 1.0</td>
<td></td>
</tr>
<tr>
<td>VWF (U/dL)</td>
<td>79 (51-120)</td>
<td>102 (67-186)</td>
<td>23.2</td>
<td>13.1 to 50.8</td>
<td></td>
</tr>
</tbody>
</table>

CI indicates confidence interval; FT4, free thyroxine; TSH, thyroid stimulating hormone; AT, antithrombin; F1+2, prothrombin fragment 1 and 2; FII, factor II; FVII, factor VII; FVIII, factor VIII; FIX, factor IX; TAT, thrombin-antithrombin complex; and VWF, von Willebrand factor.

Table 2. Effect on coagulation parameters of increasing levels of TSH with stable levels of FT4 (Recombinant human TSH group).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Median (range)</th>
<th>Measurement</th>
<th>Median (range)</th>
<th>Mean difference</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT4 (pmol/L)</td>
<td>21.3 (15.2-27.0)</td>
<td>22.1 (17.8-27.2)</td>
<td>1.0</td>
<td>0.1 to 1.9</td>
<td></td>
</tr>
<tr>
<td>TSH (mU/L)</td>
<td>0.1 (0.0-3.0)</td>
<td>23.9 (5.5-46.7)</td>
<td>-23.2</td>
<td>17.4 to 29.1</td>
<td></td>
</tr>
<tr>
<td>F1+2 (pmol/L)</td>
<td>258 (124-392)</td>
<td>204 (108-343)</td>
<td>-30.5</td>
<td>-69.0 to 7.9</td>
<td></td>
</tr>
<tr>
<td>TAT (ug/L)</td>
<td>3.9 (2.3-21.9)</td>
<td>3.4 (2.0-23.8)</td>
<td>3.3</td>
<td>-3.2 to 5.8</td>
<td></td>
</tr>
<tr>
<td>AT (U/dL)</td>
<td>126 (107-145)</td>
<td>118 (101-140)</td>
<td>-1.7</td>
<td>-6.4 to 3.0</td>
<td></td>
</tr>
<tr>
<td>Protein C (U/dL)</td>
<td>125 (90-150)</td>
<td>122 (80-140)</td>
<td>6.3</td>
<td>-8.0 to 0.2</td>
<td></td>
</tr>
<tr>
<td>Total Protein S (U/dL)</td>
<td>126 (94-159)</td>
<td>124 (88-169)</td>
<td>-2.1</td>
<td>-5.6 to 1.4</td>
<td></td>
</tr>
<tr>
<td>FII (U/dL)</td>
<td>113 (95-130)</td>
<td>111 (99-126)</td>
<td>-7.7</td>
<td>-7.1 to 1.1</td>
<td></td>
</tr>
<tr>
<td>FVII (U/dL)</td>
<td>118 (90-172)</td>
<td>112 (95-153)</td>
<td>-6.8</td>
<td>-15.3 to 2.3</td>
<td></td>
</tr>
<tr>
<td>FVIII (U/dL)</td>
<td>123 (71-324)</td>
<td>138 (59-258)</td>
<td>-15.0</td>
<td>-54.1 to 33.4</td>
<td></td>
</tr>
<tr>
<td>FIX (U/dL)</td>
<td>160 (114-234)</td>
<td>160 (97-214)</td>
<td>-12.7</td>
<td>-25.7 to 0.3</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.7 (2.4-4.6)</td>
<td>3.8 (2.3-5.6)</td>
<td>0.1</td>
<td>-0.2 to 0.4</td>
<td></td>
</tr>
<tr>
<td>VWF (U/dL)</td>
<td>105 (61-316)</td>
<td>150 (44-293)</td>
<td>7.3</td>
<td>-16.8 to 31.4</td>
<td></td>
</tr>
</tbody>
</table>

CI indicates confidence interval; FT4, free thyroxine; TSH, thyroid stimulating hormone; AT, antithrombin; F1+2, prothrombin fragment 1 and 2; FII, factor II; FVII, factor VII; FVIII, factor VIII; FIX, factor IX; TAT, thrombin-antithrombin complex; and VWF, von Willebrand factor.

**Discussion**

The aim of the present study was to study the separate effects of thyroxine and TSH on coagulation factor levels, notably FVIII, VWF and fibrinogen, as these are best known to be influenced by thyroid function. In patients in whom stable FT4 levels accompanied increasing levels of TSH, no clear effect on coagulation parameters was observed. In patients who changed from a state of high TSH and low FT4 to low TSH and slightly elevated FT4 levels, a clear rise in FVIII, VWF and fibrinogen was demonstrated. These results suggest that changes in coagulation factors related to thyroid function are mainly mediated by FT4 rather than TSH.
Due to the setting of this study within patient care, there were some differences between the two study groups; notably, TSH was higher in the thyroxine withdrawal group (141.7 mU/L) than in the recombinant TSH group (23.2 mU/L). However, it is known that shortly after rhTSH administration levels of TSH rise to values above 100 mU/L\textsuperscript{2,3}, and gradually decrease towards values like the 23.2 mU/L that we have measured at day 4. These results can therefore be interpreted as both groups having been exposed to similar peak levels of TSH. Another limitation could be the difference in length of time between the blood sampling between the groups. While, in the thyroxine withdrawal group, there were 56 days between measurement 1 and 2, the time interval consisted of only 4 days of rhTSH stimulation in the recombinant TSH group. It can be debated whether changes occurring after 4 days can be observed within this short time interval. However, in several studies using a direct trigger for the activation of the coagulation system, one could see activation of coagulation within 5 to 8 hours from the initial trigger\textsuperscript{10-12}. Lastly, since all included participants were treated for well-differentiated thyroid cancer, the role of undetectable residual tumour must be taken into account. This is important because cancer is known to be a cause of prothrombotic changes in itself\textsuperscript{16,17}. There are two arguments for an unlikely role of residual cancer: Firstly, all patients were asserted to be cured at the time of the study, based on both the clinical picture as well as undetectable TG levels. Secondly, the subjects were their own controls and it is unlikely that, within one single patient, residual cancer will have a differential effect on coagulation factors within this short time period.

Despite these limitations, we observed clear changes in levels of coagulation parameters with rising levels of FT4, while this was not the case when only levels of TSH changed. The changes in coagulation parameters were not entirely in one direction: rising FT4 levels were associated with increasing levels of FVIII, VWF and fibrinogen as well as FII, FIX, AT and total protein S, whereas FVII and protein C decreased. Although most individual factors moved towards a more procoagulant state, as could have been expected from the literature\textsuperscript{18-20}, the change of some factors in anticoagulant direction can possibly be explained by the level of FVII which was only mildly elevated. This study precludes prediction of what will happen to coagulation parameters at higher levels of free thyroxine (as in clinical overt thyrotoxicosis), although the large increases in FVIII and FIX support a prothrombotic effect of hyperthyroidism.

How thyroxine influences the coagulation system is not well known. \textit{In vitro} studies showed a direct effect of tri-iodothyronine (T3), the active form of thyroid hormone, on hepatocytes and endothelial cells causing an upregulation of fibrinogen, FII, FIX, VWF and plasminogen\textsuperscript{19,20}. This would support the hypothesis of a direct effect on coagulation gene transcription by thyroid hormone.

In conclusion, the results of our study suggest that higher levels of free thyroxine are associated with higher levels of factor VII, von Willebrand factor and fibrinogen, and that this shift in coagulation parameters is not mediated by TSH levels. An overall shift in other coagulation factors, mostly towards a prothrombotic tendency was also observed.

**References**