Hormones, haemostasis, and the risk of thrombosis

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Chapter 15

Prolactin and venous thrombosis: indications for a novel risk factor?

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Submitted for publication
**Introduction**

Prolactin is a neuro-endocrine stress hormone synthesised and secreted by the pituitary gland. A unique feature of prolactin-control is its inhibitory character, by dopamine mediated suppression. Any disease or drug that interferes with the secretion of dopamine or its delivery to the hypothalamus can therefore cause hyperprolactinaemia. The most common cause of pathophysiological prolactin levels are pituitary adenomas (prolactinomas). Prolactin levels also increase substantially during pregnancy and the first six weeks after delivery, and to a lesser degree in response to any kind of physical or psychological stress [1].

Several conditions that are characterised by high levels of prolactin, such as pregnancy, puerperium, and the use of oral contraceptive agents (OCP) or hormone replacement therapy (HRT), are associated with an increased risk of venous thrombosis (VT) [2;3]. Although this increased risk is to a large extent explained by the simultaneous increase in circulating oestrogen levels, prolactin itself may play an additional role. In prolactinoma patients, an increased incidence of venous thrombosis has been reported, and there are indications that higher levels of prolactin may contribute to a hypercoagulable state [4;5]. Conversely, higher levels of prolactin were found in patients with venous thrombosis, in whom no congenital or acquired risk factors could be identified, compared to those with congenital risk factors or healthy control subjects [5]. In addition, venous thrombosis has been related to the use of anti-psychotic drugs, which are known to induce hyperprolactinaemia by blocking dopamine receptors [6-8].

These considerations highlight that elevated prolactin levels may be a possible cause of venous thrombosis. Therefore, in this case-control study, we attempted to determine the association between different levels of prolactin and the risk of venous thrombosis.

**Material and methods**

**Study population**

Consecutive outpatients with suspected deep venous thrombosis were potentially eligible for this study (n=944). Patients with objectively confirmed deep venous thrombosis (DVT), calf vein thrombosis, or superficial thrombophlebitis of the lower extremities (cases) and subjects in whom leg vein thrombosis was objectively ruled out (controls) were derived from a larger study, designed to investigate new risk factors for venous thrombosis. Patients were excluded if they were under the age of 18 (n=3), if they had experienced a previous deep venous thrombosis or pulmonary embolism (n=119), if they were already receiving vitamin K antagonists or heparin for more than 24 hours (n=3), if they were admitted to the hospital (n=58), or if they were unwilling to participate or unable to give consent (n=7). A total of 754 patients were included in the study (Figure 1).

**Setting and location**

Patients were recruited at the Academic Medical Centre, Amsterdam, the Netherlands between September 1999 and August 2006. The study was approved by the institutional medical ethical review board, and all patients provided written informed consent.
Data collection and diagnosis of venous thrombosis

At presentation, i.e. prior to diagnostic testing for venous thrombosis, participants were subjected to a standardised questionnaire including items on medical history, family history, concomitant medication, and the presence of known risk factors for venous thrombosis. Participants subsequently provided a non-fasting venous blood sample. Blood was collected in 0.109 mol/L trisodium citrated tubes. Within 1 hour, platelet-poor plasma was obtained by centrifugation, and re-centrifugation of the supernatant, for 20 minutes at 1600 x g at 4°C. Plasma was stored at -80°C until further use.

All participants underwent diagnostic testing for deep vein thrombosis according to an algorithmic management strategy combining clinical pretest probability and D-dimer assay (Tinaquant, Roche Diagnostics, Basel, Switzerland), followed by compression ultrasound (CUS) of the lower extremities, if indicated. For those who had been judged likely to have deep vein thrombosis, a second ultrasound examination was performed one week later if the first test was negative. The diagnosis of venous thrombosis, including deep venous thrombosis (i.e. proximal thrombosis of the iliac or superficial femoral vein, or thrombosis involving at least the upper third part of the deep calf veins), calf vein thrombosis, and superficial thrombophlebitis, was confirmed by failure to fully collapse the lumen of the deep or superficial veins during compression testing. Thrombosis was considered provoked if at least one of the following acquired risk factors were present: use of oestrogen- or progestosterone 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 high clinical pretest probability and negative first ultrasound failed to return for the second ultrasound examination. Diagnosis of venous thrombosis was confirmed in 211 participants who were therefore included as cases, whereas in 537 participants the diagnosis of venous thrombosis was objectively ruled out using the above-mentioned diagnostic management strategy. These 537 participants were included as controls (Figure 1).

Laboratory assay of prolactin levels

Citrated plasma of 187 cases and 486 controls was available for laboratory assay of prolactin levels. To obtain a control to case ratio of 2:1, we randomly selected 374 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).

Prolactin was measured using direct chemiluminometric technology (ADVIA Centaur® immunoassay system, Siemens Healthcare Diagnostics, Marburg, Germany) and corrected for the 10% dilution with citrate. The intra- and interassay coefficients of variations (CVs) were below or equal to 4.4% and 4.9%, respectively. The local reference range was below 22 µg/L for women and below 15 µg/L for men. Considering that prolactin is a stress-hormone, which may increase during an acute phase response induced by the thrombotic event itself, we analysed high sensitivity C-reactive protein (hsCRP) in 158 randomly selected subjects (79 cases and 79 controls) as described elsewhere[11].

Statistical analysis

Results of categorical variables were expressed as number and percent. Prolactin levels were presented as medians (95% confidence interval), and the difference between groups was calculated using the Mann-Whitney U-test. We subsequently calculated odds ratios (OR) and 95% confidence intervals (95% CI) for the risk of venous thrombosis at different levels of prolactin using binary logistic regression. Cut-off levels for prolactin were set according to the different percentiles of the values observed in controls. Prolactin levels below the 50th percentile were used as reference and compared with levels above the 75th, 80th, 90th, 95th, and the 97.5th percentile. Multivariate analysis was performed to adjust for gender to take the frequency matching on gender into account. The influence of potential confounding factors was analysed using the univariate model, and if a significant contribution (change in crude OR of more than 5%) was found for any of the independent factors, these variables were added to the multivariate logistic regression model. An exception was made for pregnancy and puerperium, since there was only a limited number of women in these conditions included in the study. However, pregnancy and puerperium were considered strong confounders in the relation between prolactin and venous thrombosis, and we therefore felt it was more accurate to exclude these women from the analysis than to incorporate these variables in the multivariate model.
Prolactin and venous thrombosis

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>All cases(^a) (n=187)</th>
<th>DVT only(^b) (n=152)</th>
<th>Controls(^c) (n=374)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>79 (42.2)</td>
<td>66 (43.4)</td>
<td>158 (42.2)</td>
</tr>
<tr>
<td>Age, median (range), years</td>
<td>57 (19-90)</td>
<td>58 (19-90)</td>
<td>57 (18-90)</td>
</tr>
<tr>
<td>Unprovoked VT, n (%)</td>
<td>64 (34.2)</td>
<td>47 (30.9)</td>
<td>-</td>
</tr>
<tr>
<td>Provoked VT, n (%)</td>
<td>123 (65.8)</td>
<td>105 (69.1)</td>
<td>-</td>
</tr>
<tr>
<td>- Oral contraceptive pill</td>
<td>36 (19.3)</td>
<td>26 (17.1)</td>
<td>30 (8.0)</td>
</tr>
<tr>
<td>- Hormone replacement therapy</td>
<td>3 (1.6)</td>
<td>3 (2.0)</td>
<td>5 (1.3)</td>
</tr>
<tr>
<td>- Recent trauma</td>
<td>26 (13.9)</td>
<td>22 (14.5)</td>
<td>53 (14.2)</td>
</tr>
<tr>
<td>- Malignancy</td>
<td>26 (13.9)</td>
<td>24 (15.8)</td>
<td>24 (6.4)</td>
</tr>
<tr>
<td>- Pregnancy</td>
<td>1 (0.5)</td>
<td>0 (0.0)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>- Puerperium</td>
<td>1 (0.5)</td>
<td>1 (0.7)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>- Surgery/bedridden</td>
<td>30 (16.0)</td>
<td>29 (19.1)</td>
<td>30 (8.0)</td>
</tr>
<tr>
<td>- Paralysis of the symptomatic leg</td>
<td>16 (8.6)</td>
<td>15 (9.9)</td>
<td>30 (8.0)</td>
</tr>
<tr>
<td>- Hospitalisation</td>
<td>35 (18.7)</td>
<td>33 (21.7)</td>
<td>46 (12.3)</td>
</tr>
<tr>
<td>- Long distance travel</td>
<td>30 (16.0)</td>
<td>23 (15.1)</td>
<td>43 (11.5)</td>
</tr>
<tr>
<td>Prolactin level (μg/L), median (95% CI)</td>
<td>6.7 (5.6-7.8)(^d)</td>
<td>6.7 (5.6-6.7)</td>
<td>5.6 (5.6-6.7)</td>
</tr>
<tr>
<td>- Men</td>
<td>5.6 (5.6-6.7)</td>
<td>5.6 (4.4-6.7)</td>
<td>5.6 (5.6-5.6)</td>
</tr>
<tr>
<td>- Women</td>
<td>7.8 (6.7-8.9)(^e)</td>
<td>7.8 (6.7-8.9)(^e)</td>
<td>6.7 (5.6-6.7)</td>
</tr>
<tr>
<td>Use of PRL-increasing agents, n (%)</td>
<td>8 (4.3)</td>
<td>8 (5.3)</td>
<td>21 (5.6)</td>
</tr>
<tr>
<td>- Calcium channel blockers (verapamil)</td>
<td>1 (0.5)</td>
<td>1 (0.7)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>- Dopamine receptor antagonist</td>
<td>1 (0.5)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>- Dopamine synthesis inhibitors</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>- Oestrogen influencing drugs (tamoxifen)</td>
<td>3 (1.6)</td>
<td>3 (2.0)</td>
<td>5 (1.3)</td>
</tr>
<tr>
<td>- Opiates</td>
<td>1 (0.5)</td>
<td>1 (0.7)</td>
<td>9 (2.4)</td>
</tr>
<tr>
<td>- Serotonin-reuptake inhibitors</td>
<td>2 (1.1)</td>
<td>2 (1.3)</td>
<td>4 (1.1)</td>
</tr>
</tbody>
</table>

N indicates number; DVT, deep venous thrombosis; VT, venous thrombosis; CI, confidence interval; and PRL, prolactin.
\(^a\)All thrombosis patients combined (DVT, calf vein thrombosis, and thrombophlebitis).
\(^b\)P<0.05 patients with deep venous thrombosis vs controls.
\(^c\)P=0.05 patients with deep venous thrombosis vs controls.
\(^d\)P=0.01 patients with deep venous thrombosis vs controls.

Separate analysis was performed for patients with deep venous thrombosis, excluding those with calf vein thrombosis or superficial thrombophlebitis. Considering gender-based differences in basal prolactin levels, subgroup analysis was performed for men and women separately. To evaluate the possible influence of circulating oestrogen levels, we performed additional analyses in subgroups of pre-menopausal and post-menopausal women applying the same cut-off levels as those used in the analysis of all women. Women were considered pre-menopausal if aged 55 years or below and not using hormone replacement therapy, whereas post-menopausal women were defined as those aged above 55 years or those using hormone replacement therapy. Last, to assess the relation between levels of prolactin and C-reactive protein, linear regression was performed using log-transformed values of both variables. Statistical analysis was performed with the use of SPSS 15.0 software package (SPSS Inc, Chicago, IL, USA).

Results

Patient characteristics

A total of 187 cases and 374 controls were included. Mean age (57 years) and female gender (58%) were similar in cases and controls. Of all cases with venous thrombosis, 152 (81%) had a deep venous thrombosis, 12 (6%) an isolated calf vein thrombosis and 23 (12%) a superficial thrombophlebitis of the lower extremities. Overall, in 123 (66%) patients with venous thrombosis, and in 105 (69%) of the patients with deep venous thrombosis, at least one acquired risk factor for venous thrombosis was present at the time of event, and thrombosis was therefore considered provoked.

In total, 45 (24%) patients with venous thrombosis and 33 (14%) controls were using drugs known to increase circulating prolactin levels: 36 patients with venous thrombosis and 30 controls were using oral contraceptive agents; 3 patients with venous thrombosis and 5 controls were on hormone replacement therapy; and 8 patients with venous thrombosis and 21 controls were using prolactin-increasing drugs other than oral contraceptive agents or hormone replacement therapy. Of these, 2 patients with venous thrombosis and 3 controls used more than one prolactin-increasing agent. (Table 1).

Prolactin levels

The median prolactin level in cases with venous thrombosis (6.7 µg/L, 95% CI 5.6-7.8) was higher than in control subjects (5.6 µg/L, 95% CI 5.6-6.7) (p=0.02) (Table 1). In both groups, median prolactin levels were higher for women than for men. Nine patients with venous thrombosis (6 women) and 7 control subjects (3 women) had prolactin levels above reference range. We did not find an association between prolactin levels and C-reactive protein among the 158 subjects in whom levels of C-reactive protein were analysed, neither in cases (regression coefficient \(\beta\) -0.05; 95% CI -0.17 to 0.07; \(p=0.43\)) nor in controls (regression coefficient \(\beta\) 0.03; 95% CI -0.06 to 0.12; \(p=0.52\)).
Prolactin and the risk of venous thrombosis

We found that for each percentile level, the odds ratios increased with the percentile used (adjusted for gender) (Table 2). For example, for prolactin levels above the 75th percentile (8 µg/L), we found an odds ratio of 1.7 (95% CI 1.1-2.7) compared to levels below the 50th percentile (6 µg/L). This further increased up to an odds ratio of 4.7 (95% CI 1.9-11.7) for prolactin levels above the 97.5th percentile (16 µg/L). Adjustment for age, use of oestrogen-containing agents, surgery, and malignancy slightly decreased the odds ratios. Similar results were observed in women (Table 3 and Figure 2), with a particular high relative risk of venous thrombosis in the subgroup of pre-menopausal women (Figure 2). Adjustment for age, use of oestrogen-containing agents, surgery, and malignancy did not materially alter the crude odds ratios (data not shown). No clear association between higher prolactin levels and the risk of venous thrombosis was observed for men (Table 3) or for post-menopausal women (Figure 2).

Table 3. Risk of venous thrombosis for different levels of prolactin in men and in women.

<table>
<thead>
<tr>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Per-</strong>&lt;br&gt;pentile&lt;sup&gt;a&lt;/sup&gt;</td>
<td><strong>PRL</strong>&lt;sup&gt;b&lt;/sup&gt; (μg/L)</td>
</tr>
<tr>
<td>50</td>
<td>&lt;6</td>
</tr>
<tr>
<td>75</td>
<td>&gt;6</td>
</tr>
<tr>
<td>90</td>
<td>&gt;10</td>
</tr>
<tr>
<td>95</td>
<td>&gt;13</td>
</tr>
<tr>
<td>97.5</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

PRL indicates prolactin; n, number; OR, odds ratio; and CI, confidence interval.

Pregnant women and women within the first six weeks after delivery (puerperium) were excluded from the analysis.

Analysis was performed for the 75th, 80th, 90th, and 95th percentile. Risk applies for PRL levels above the cut-off point compared to reference (below the 50th percentile).

Venous thrombosis: deep venous thrombosis, calfvein thrombosis, and thrombophlebitis.

Adjusted for age, use of oestrogen-containing agents (in women), surgery, and malignancy.
Prolactin may be involved in the pathogenesis of venous thrombosis via several pathways. First, prolactin induces an inflammatory response characterised by infiltration of lymphocytes, macrophages, and neutrophils, as well as adhesion of circulating mononuclear cells to the endothelium, thereby indirectly altering coagulation. Second, in rats, prolactin was reported to enhance the synthesis of prothrombin in hepatic microsomes, and to increase levels of coagulation factor XII. This proposed direct effect on coagulation is compatible with recent clinical data showing high platelet count, increased levels of fibrinogen, antithrombin, and plasminogen activator inhibitor-1 (PAI-1), and decreased levels of tissue factor pathway inhibitor (TFPI) in prolactinoma patients compared to healthy age- and gender-matched controls. Third, prolactin has been suggested to enhance platelet activation, possibly by binding to a platelet-located prolactin receptor. However, two recent studies neither showed an effect of prolactin on platelet function, nor the presence of a platelet-located receptor confirmed, leaving the role of prolactin in platelet activation and aggregation still unclear.

Given that stress of any kind can cause an increase in prolactin secretion, the question arises whether our findings are not merely the result of an acute phase response induced by the thrombotic event itself. This may particularly be true since women show a greater prolactin response than men to almost all physiological stimuli, presumably due to the effect of higher oestrogen concentrations on the lactotroph cells. However, the lack of association between hsCRP and prolactin argues against the acute phase response as explanation for our findings. Also, control subjects were persons with a suspicion of venous thrombosis. Consequently, controls were not entirely free of (inflammatory) stress either, which makes it unlikely that our finding is an epiphenomenon. Future prospective studies are still needed to disentangle the complex relationship between prolactin, oestrogen, and the acute phase response in all details.

Theoretically, all factors that induce physical or psychological stress may increase levels of prolactin, which includes most of the acquired risk factors for venous thrombosis. Therefore, these risk factors may be considered confounders. To adequately identify possible confounding factors, the influence of each of the acquired risk factors was analysed using a univariate model. Apart from pregnancy, puerperium, and use of oestrogen-containing agents (oral contraceptives and hormone replacement therapy, only malignancy and surgery were found to influence the relation between prolactin and venous thrombosis. However, adjustment for these factors did not materially alter the study findings.

A possible limitation of our study is the fact that prolactin levels were measured at presentation rather than at a fixed time of day. The diurnal rhythm of prolactin, with peak levels occurring early in the morning, could have resulted in small physiological fluctuations between subjects. However, owing to the design of our study, this would have affected both cases and controls in a similar manner. Such random misclassification could at most have led to an underestimation of the association.
In conclusion, our data suggest that higher prolactin levels are associated with venous thrombosis. The relation is most pronounced in pre-menopausal women, suggesting that oestrogen-dependent pathways in crosstalk with prolactin levels may underlie this association. Future studies are needed to evaluate causality of the observed relation between prolactin levels and venous thrombosis.

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We would like to acknowledge all the work done by the "vasculisten", a group of medical students, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands, who had an essential role in the execution of this study, especially the recruitment and data collection. We thank Huib Bout, Department of Clinical Biochemistry, Slotervaart Hospital, Amsterdam, the Netherlands, for his laboratory efforts.

REFERENCES