Systemic causes of heavy menstrual bleeding
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Chapter 5: No increased systemic fibrinolysis in women with heavy menstrual bleeding

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Chapter 5

Summary

Background: bleeding disorders have been recognized as important etiologic or contributing factors in women with heavy menstrual bleeding. Fibrinolysis in the endometrium plays a role in heavy menstrual bleeding. It is unknown whether increased systemic fibrinolysis might also increase the risk for heavy menstrual bleeding.

Objective: to investigate fibrinolytic parameters, including clot lysis time, in women with heavy menstrual bleeding.

Methods: we included 102 patients referred for heavy menstrual bleeding (Pictorial Blood loss Assessment Chart score >100) in our cohort. Patients and controls (28 healthy volunteers without heavy menstrual bleeding) had haemostatic testing in the 1st week after menstruation. For 79 patients and all controls fibrinolytic parameters (thrombin activatable fibrinolysis inhibitor-activity, plasminogen activator inhibitor-1, tissue plasminogen activator, plasmin inhibitor) and clot lysis time were available.

Results: fibrinolytic parameters were similar between patients and controls, except for thrombin activatable fibrinolysis inhibitor (89.4% vs 82.5%) and plasmin inhibitor (106% vs 96%), which were significantly higher in patients. In women with menorrhagia without gynecological abnormalities we found lower thrombin activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 levels than in women with gynecological abnormalities (thrombin activatable fibrinolysis inhibitor: 85.4% vs 94.8%; plasminogen activator inhibitor-1: 16.0 ug/l vs 24.5 ug/l).

Conclusion: systemic fibrinolytic capacity is not increased in women with heavy menstrual bleeding. Overall, the fibrinolytic inhibitors thrombin activatable fibrinolysis inhibitor and plasmin inhibitor were even higher in patients than in controls. However, in a subgroup of women without gynecological abnormalities, relatively lower levels of inhibitors may contribute to the heavy menstrual bleeding.
Introduction

Heavy menstrual bleeding is a common problem. At least 5-10% of women in the reproductive age seek medical attention for heavy menstrual bleeding. Heavy menstrual bleeding is known to be associated with gynecological abnormalities like uterine fibroids, endometrial polyps and adenomyosis. Moreover, heavy menstrual bleeding can also be associated with a wide range of haemostatic disorders. Von Willebrand’s disease has been recognized as an important etiologic or contributory factor, but platelet dysfunction and low factor XI are also prevalent. We previously found an underlying bleeding disorder in 29% of the patients with heavy menstrual bleeding, of these women 6% had Von Willebrand’s disease, 4% had factor XI <70%, and 23% had platelet defects.

There is evidence that fibrinolysis in the endometrium plays an important role in menstruation. In women with heavy menstrual bleeding increased fibrinolytic activity was observed in the menstrual fluid which suggested that this might be a contributing factor in the etiology of heavy menstrual bleeding. Moreover, antifibrinolytic agents, such as tranexamic acid, are effective in reducing menstrual blood loss. Notably, the role of systemic fibrinolysis in women with heavy menstrual bleeding has not been studied. We hypothesized that increased systemic fibrinolysis might contribute to heavy menstrual bleeding. To clarify this, we investigated fibrinolytic parameters, including clot lysis time in women with heavy menstrual bleeding and compared them with controls.

Material and methods

Patients

We included 102 consecutive patients referred to the University Medical Center of Groningen between March 2007 and December 2010 with a history of heavy, regular (every 23-39 days) menstrual periods. Exclusion criteria were: Pictorial Blood Assessment Chart score <100 (see below), known bleeding disorders, use of any intrauterine device within 2 months prior to inclusion, and treatment with anticoagulants, antifibrinolytics, non-steroidal anti-inflammatory agents, combined...
oral contraceptives, or progestagens. Referred patients who were potentially eligible received a structured questionnaire by mail to obtain information about baseline characteristics: medical, obstetrical and gynecological history and previous treatment for heavy menstrual bleeding. After reviewing the completed questionnaire we excluded 139 women with intermenstrual, irregular and postcoital bleeding. Eligible women were invited to our clinic and had a gynecological examination and pelvic ultrasonography in the first week after their menstruation. Patients with submucous uterine fibroids more than 2 cm in diameter or uterine polyps were classified as heavy menstrual bleeding with gynecological abnormalities, or explained heavy menstrual bleeding.

Controls (included between January - April 2010) were women with regular cycles without using hormones and who considered their menstrual blood loss as normal. We hung posters with information about the study and inclusion criteria in the staff areas of our hospital. Women interested in participation could directly contact one of the authors by telephone or email, to receive more information, including the goal of the study. In- and exclusion criteria were checked, and the women signed informed consent. Controls underwent no gynecological examination. Patients and controls (28 healthy volunteers without heavy menstrual bleeding) had haemostatic testing in the 1st week after menstruation.

The study was approved by the Institutional Review Boards of the University Medical Center of Groningen. Informed consent was obtained from all patients and controls.

**Pictorial Blood loss Assessment Chart score**

The patients were informed about the Pictorial Blood loss Assessment Chart before the first hospital visit by a letter that contained standard instructions; they completed the Pictorial Blood loss Assessment Chart in the menses before the first hospital visit. The chart consists of a series of diagrams representing lightly, moderately and heavily soiled pads and tampons. Subjects recorded each discarded item for the duration of an entire cycle. Scoring of pads and tampons was done with tampons assigned 1, 5 and 10 and pads scored 1, 5, 20 for lightly, moderately and heavily soiled, respectively. Heavy menstrual bleeding was defined as a Pictorial
Blood loss Assessment Chart score of >100 based on the scoring system of Higham et al. 12. The healthy volunteers completed the pictorial chart in the first menses after blood samples were taken.

**Laboratory measurements**

A venous citrated blood sample was taken from all patients and controls in the first week after menstruation. In patients the blood samples were taken before the gynecological examination. Blood samples were also obtained for complete blood cell counts and ferritin. Venous blood was collected from the antecubital vein with a vacuum system. Venous blood samples were anticoagulated with 1:10 volume of 0.109 mol/L trisodium citrate. Platelet-poor plasma was prepared by centrifugation at 2500 x g for 15 minutes at 4⁰C, aliquoted and immediately frozen at -80⁰C and analysed after rapidly thawing at 37⁰C. The measurements of the fibrinolytic parameters were performed batch-wise at the end of the study.

Plasmin inhibitor measurements were performed with Berichrom® reagents from Siemens. Plasma antigen levels of plasminogen activator inhibitor-1 (reference values 4 to 43 ng/mL) and tissue plasminogen activator (reference values 2 to 12 ng/mL) were measured by enzyme-linked immunosorbent assays (Asserachrom; Diagnostica Stago, Asnieres-sur-Seine, France). Plasma levels of thrombin activatable fibrinolysis inhibitor were measured by a chromogenic substrate assay (Pefakit® TAFI, Pentapharm, Basel, Switzerland, reference values 79 to 126 U/dL) using a Behring Coagulation System®(Siemens, Marburg, Germany), as described before 13. Lysis of a tissue factor-induced clot by exogenous tissue plasminogen activator was studied by monitoring changes in turbidity during clot formation and subsequent lysis. In short, a 50 μL mixture containing phospholipid vesicles, tissue plasminogen activator (final concentration 56 ng/mL), tissue factor (final dilution 1:1000), and CaCl₂ diluted in HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid) was added by the use of a multichannel pipette. In a kinetic microplate the optical density at 405 mm was monitored every 20 seconds, resulting in a clot-lysis turbidity profile. The clot lysis time was derived from this clot-lysis profile and defined as the time from the midpoint of the clear to maximum turbid transition, representing clot formation, to the midpoint
of the maximum turbid to clear transition, representing the lysis of the clot.

Statistical analysis

Continuous variables, expressed as medians (IQR: Q1-Q3) were used for age, Body Mass Index (BMI), Pictorial Blood loss Assessment Chart scoring, hemoglobin, ferritin and all fibrinolytic parameters. To analyse differences in medians in women with heavy menstrual bleeding and controls, Hodges Lehmann differences of the median tests were used.

For the linear regression, we logtransformed all haemostatic variables, BMI and age, because they were non-normally distributed. To compare the relative strength of the various determinants within the model, all variables (clot lysis time, other fibrinolytic parameters, BMI and age) were standardized by calculating Z-scores. The Z-score for an observation of a subject is calculated by subtracting the mean from the observed value and dividing the residual by the standard deviation. Simple and multiple linear regression analysis was performed with the standardized variables, clot lysis time was the dependent variable. The resulting standardized regression coefficient ($\beta$) for a factor indicates the increase in standard deviations of log-clot lysis time, when that particular factor increases with 1 standard deviation and all other variables in the model are unchanged.

Results

Baseline characteristics

We included 102 patients with heavy menstrual bleeding. Fibrinolytic parameters were available for 79 patients with heavy menstrual bleeding and 28 healthy volunteers (see table 1). Median age was 46 years (IQR: 41-49) in patients and 42 years (IQR: 32-47) in controls. BMI was similar between patients and controls (respectively 24.2 kg/m$^2$ vs 23.6 kg/m$^2$). Median hemoglobin levels in patients and controls were respectively 7.6 mmol/L (IQR: 6.9-8.1) and 8.4 mmol/L (IQR: 8.0-8.7), for ferritin respectively 13.5 ug/L (IQR: 6-22.3) and 34.7 ug/L (IQR: 18.6-41.8). Gynecological abnormalities were present in 33% of the patients. There were no baseline differences between the 79 patients with measurement of the fibrinolytic parameters and the 23 patients in whom no
fibrinolytic parameters were measured.

**Overall fibrinolytic parameters**

Fibrinolytic parameters were similar between patients and controls, except for thrombin activatable fibrinolysis inhibitor and plasmin inhibitor, which were higher in patients (see also table 2).

Linear regression was performed to investigate the association between factors of fibrinolysis and clot lysis time by use of the log transformed clot lysis time as dependent variable. In simple linear regression analyses all fibrinolytic factors were associated with clot lysis time (see table 3). The strongest association was found between plasminogen activator inhibitor-1 and clot lysis time with a regression coefficient of 0.74 (95% CI 0.59-0.90). Including all fibrinolytic factors in a multiple regression model increased the explained variance to 69%. In controls we also observed an association between clot lysis time and all fibrinolytic factors in the simple model, especially for plasminogen activator inhibitor-1, tissue plasminogen activator and BMI (see table 4). The multiple regression model increased the explained variance to 82% in the control group. In the multiple linear model only plasminogen activator inhibitor-1, plasmin inhibitor and BMI remained significantly associated with logtransformed clot lysis time in patients (table 3), while in controls only BMI kept a significant association (table 4).

**Fibrinolytic parameters and gynecological abnormalities**

In women with heavy menstrual bleeding without gynecological abnormalities we found lower thrombin activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 levels than in women with gynecological abnormalities (thrombin activatable fibrinolysis inhibitor: 85.4 vs 94.8%; plasminogen activator inhibitor-1: 16.0 vs 24.5 ug/l). The clot lysis time seemed shorter in patients without gynecological abnormalities (66.5 vs 73.6 minutes).

**Fibrinolytic parameters and BMI**

Overall, in patients with a BMI >25 kg/m² (n=32; 43%) all fibrinolytic parameters,
except for thrombin activatable fibrinolysis inhibitor, were longer/higher than in women with a BMI ≤25 kg/m\(^2\) (respectively clot lysis time: 82.0 vs 63.2 minutes; plasminogen activator inhibitor-1: 32.5 vs 12.0 ug/l; tissue plasminogen activator: 12.0 vs 6.8 ug/l; plasmin inhibitor: 109.5 vs 105.0%). Thus, in women with a BMI >25 kg/m\(^2\), all values except for tissue plasminogen activator indicated decreased fibrinolytic capacity.

**Discussion**

In this single-centre cross-sectional study we found that, on average, the fibrinolytic capacity is not increased in women with heavy menstrual bleeding. Inhibitors of fibrinolysis (thrombin activatable fibrinolysis inhibitor and plasmin inhibitor) were even higher in patients with heavy menstrual bleeding. However, in a subgroup of patients with unexplained heavy menstrual bleeding we found lower thrombin activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 levels than in women with gynecological abnormalities.

In our study we investigated the role of systemic fibrinolysis. Heavy menstrual bleeding is associated with an increase in local fibrinolysis due to elevated levels of endometrium derived plasmin and plasminogen activators \(^1\). The fibrinolytic system in women with heavy menstrual bleeding and the effects of levenorgestrel-intrauterine system after 6 months was monitored in a study by Koh et al. \(^2\). They did not find any effect of the levenorgestrel-intrauterine system on the systemic fibrinolytic parameters. On the other hand, the local fibrinolytic inhibitor plasminogen activator inhibitor 1 and 2 were highly expressed in the endometrium after 6 months of levenorgestrel-intrauterine system, which means that these levels inhibited the fibrinolytic activity in the endometrium and the women did not experience heavy menstrual bleeding anymore. This might indicate that the systemic fibrinolysis is not relevant in heavy menstrual bleeding, only the local fibrinolysis.

Another explanation why we found no increased systemic fibrinolysis could be the moment of testing in the menstrual cycle. We measured the fibrinolytic parameters one week after the menstruation. This is the week in which the body has used many of its reserves to compensate for the blood loss during the menstruation. Therefore,
we may only see the increased production of fibrinolytic parameters and not the continuous low levels during the menstrual cycle. Probably there is cyclic variation in menstruating women. In a systematic review \(^16\), we previously reported that in most of the studies there was no cyclic variation in fibrinolytic factors (plasminogen activator inhibitor, tissue plasminogen activator, urokinase-type plasminogen activator and plasmin inhibitor). However, this systematic review did not focus on patients with heavy menstrual bleeding, as the present study.

We see that all fibrinolytic parameters, except for thrombin activatable fibrinolysis inhibitor and tissue plasminogen activator, indicate a significantly decreased fibrinolytic capacity in the overweight group of patients (BMI >25 kg/m\(^2\)). This is in line with previous studies: in the study of Carter \(^17\) patients with metabolic syndrome had prolonged clot lysis time, partly due to increased circulating levels of plasminogen activator inhibitor-1. Koh et al. \(^18\) found that in overweight women (BMI >25 kg/m\(^2\)) tissue plasminogen activator antigen and plasminogen activator inhibitor-1 levels were systemically elevated in the menstrual cycle compared with levels in women with normal weight. The fact that in our study, patients and controls had similar BMI, might indicate that decreased fibrinolysis in obesity does not protect from heavy menstrual bleeding.

We used the clot lysis assay, which is a test that reflects the overall fibrinolytic activity of plasma. Our study showed in patients and controls that all fibrinolytic factors were associated with clot lysis time, plasminogen activator inhibitor-1 having the strongest influence on the clot lysis time. The clot lysis time is calculated from the turbidity profile of the clot formation and clot lysis is thought to represent overall plasma fibrinolytic capacity \(^19\). Meltzer et al. \(^19\) reported in healthy volunteers that the variation in clot lysis time could be explained by plasminogen activator inhibitor-1, thrombin activatable fibrinolysis inhibitor, plasmin inhibitor and plasminogen plasma levels. We also found the same influence of the fibrinolytic parameters on the clot lysis time in a hemorrhagic group.

Our study had some limitations. Firstly, we performed our measurements of the fibrinolytic capacity in only 79 of the 102 patients with heavy menstrual bleeding, because material was not available in all. This depended on the amount of blood that
was collected from the venapunction. Sometimes this was not enough, in a random fashion.

Secondly, the number of controls is relatively low, although we still had a power of 80% to detect 20% difference in clot lysis time. Also, the controls had longer clot lysis time, higher thrombin activatable fibrinolysis inhibitor and higher plasmin inhibitors than patients, making it unlikely that we missed true increased values.

As third, our study patients were significantly older than controls. Studies report discordant effects of age: the clot lysis time was slightly longer in older patients 20 but another study showed an up-regulation of the fibrinolytic response of tissue plasminogen activator by increasing age 21. In our cohort we observed the same effects on clot lysis time and tissue plasminogen activator (see table 2), although not at a significant level.

At present, the clinical relevance of our study has to be investigated. Further investigation will be needed to analyze systematic fibrinolysis in the different phases of the menstrual cycle in women with heavy menstrual bleeding. Also, the relation between success of antifibrinolytic therapy and fibrinolytic parameters should be evaluated.

In conclusion, we showed that the systemic fibrinolytic capacity is not increased in women with heavy menstrual bleeding. However, inhibitors of fibrinolysis are lower in a subgroup of women without gynecological abnormalities and heavy menstrual bleeding.
Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=79)</th>
<th>Controls (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, yrs (Q1-Q3)</td>
<td>46 (41-49)</td>
<td>42 (32-47)</td>
</tr>
<tr>
<td>Body Mass Index, kg/m² (Q1-Q3)</td>
<td>24.2 (21.8-28.9)</td>
<td>23.6 (21.9-25.3)</td>
</tr>
<tr>
<td>PBAC score, median (Q1-Q3)</td>
<td>279 (225-398)</td>
<td>126 (78-165)</td>
</tr>
<tr>
<td>Hemoglobin &lt;7.5 mmol/L, n (%)</td>
<td>31 (39.2)</td>
<td>2 (7.1)</td>
</tr>
<tr>
<td>Ferritin &lt;15 ug/L, n (%)</td>
<td>43 (55.1)</td>
<td>4 (14.3)</td>
</tr>
<tr>
<td>Gynecological abnormalities, n (%)</td>
<td>26 (33)</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

PBAC score: Pictorial Bleeding Assessment Chart score

Table 2: Fibrinolytic variables in patients vs controls

<table>
<thead>
<tr>
<th>Median (Q1-Q3)</th>
<th>Patients (n=79)</th>
<th>Controls (n=28)</th>
<th>Median differences (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotlysis, min.</td>
<td>67.9 (62.0-81.4)</td>
<td>63.4 (57.3-73.3)</td>
<td>5.0 (-0.7, 11.3)</td>
</tr>
<tr>
<td>TAFI, %</td>
<td>89.4 (79.2-102.3)</td>
<td>82.5 (78.3-91.0)</td>
<td>6.1 (0.3, 12.6)</td>
</tr>
<tr>
<td>PAI-1, μg/l</td>
<td>18.0 (9.3-36.0)</td>
<td>19.0 (10.0-22.8)</td>
<td>1.0 (-4.3, 8.0)</td>
</tr>
<tr>
<td>tPa, μg/l</td>
<td>8.3 (6.3-12.0)</td>
<td>7.2 (5.3-12.0)</td>
<td>0.9 (-0.8, 2.5)</td>
</tr>
<tr>
<td>PI, %</td>
<td>106.0 (98.0-111.3)</td>
<td>96.0 (92.0-101.8)</td>
<td>8.0 (4.0, 12.0)</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; TAFI: thrombin activatable fibrinolysis inhibitor; PAI-1: plasminogen activator inhibitor-1; tPA: tissue plasminogen activator; PI: plasmin inhibitor
**Table 3**: Mean change in clot lysis time* with 1 SD increase in fibrinolytic factor in patients

<table>
<thead>
<tr>
<th>Simple model</th>
<th>Controls (n=53)</th>
<th>Multiple model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>R²</td>
</tr>
<tr>
<td>TAFI*</td>
<td>0.53 (0.34, 0.73)</td>
<td>0.28</td>
</tr>
<tr>
<td>PAI-1*</td>
<td>0.74 (0.59, 0.90)</td>
<td>0.55</td>
</tr>
<tr>
<td>tPA*</td>
<td>0.52 (0.32, 0.71)</td>
<td>0.27</td>
</tr>
<tr>
<td>PI*</td>
<td>0.40 (0.19, 0.61)</td>
<td>0.16</td>
</tr>
<tr>
<td>BMI*</td>
<td>0.68 (0.50, 0.84)</td>
<td>0.45</td>
</tr>
<tr>
<td>Age*</td>
<td>0.04 (-0.18, 0.28)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

SD: standard deviation; CI indicates confidence interval; TAFI: thrombin activatable fibrinolysis inhibitor; PAI-1: plasminogen activator inhibitor; tPA: tissue plasminogen activator; PI: plasmin inhibitor; BMI: Body Mass Index; * Clot lysis time, TAFI, PAI-1, tPA, PI, BMI and age were log-transformed

**Table 4**: Mean change in clot lysis time* with 1 SD increase in fibrinolytic factor in controls

<table>
<thead>
<tr>
<th>Simple model</th>
<th>Controls (n=53)</th>
<th>Multiple model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>R²</td>
</tr>
<tr>
<td>TAFI*</td>
<td>0.51 (0.17, 0.86)</td>
<td>0.26</td>
</tr>
<tr>
<td>PAI-1*</td>
<td>0.79 (0.54, 1.04)</td>
<td>0.62</td>
</tr>
<tr>
<td>tPA*</td>
<td>0.77 (0.52, 1.03)</td>
<td>0.60</td>
</tr>
<tr>
<td>PI*</td>
<td>0.54 (0.20, 0.88)</td>
<td>0.29</td>
</tr>
<tr>
<td>BMI*</td>
<td>0.81 (0.58, 1.05)</td>
<td>0.66</td>
</tr>
<tr>
<td>Age*</td>
<td>0.43 (0.06, 0.79)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

SD: standard deviation; CI indicates confidence interval; TAFI: thrombin activatable fibrinolysis inhibitor; PAI-1: plasminogen activator inhibitor; tPA: tissue plasminogen activator; PI: plasmin inhibitor; BMI: Body Mass Index; * Clot lysis time, TAFI, PAI-1, tPA, PI, BMI and age were log-transformed
References

with age in healthy humans, while endothelium-dependent vasodilation is unaffected. Thromb Haemost. 2003 Feb;89:374-81