Chapter 3: Factor XI levels and coagulation parameters in women with heavy menstrual bleeding

Sophie Wiewel-Verschueren
H. Marieke Knol
Ellen R. Klinkert
Hanneke C. Kluin-Nelemans
Ate G.J. van der Zee
André B. Mulder
Karina Meijer

Submitted
Abstract

Objective: to confirm that FXI plasma levels in patients with heavy menstrual bleeding (HMB) are lower than in controls. Furthermore, to investigate whether other coagulation parameters are also decreased in women with HMB without obvious deficiencies.

Study design: after informed consent patients with regular HMB (Pictorial Blood loss Assessment Chart-score >100) were included. In all women gynecological examination was performed, blood was drawn (blood count and haemostatic parameters) and a standardized questionnaire was filled out. Controls had a regular cycle and subjectively normal blood loss.

Results: We included 106 patients with HMB and 53 healthy controls; 95% of the women were Caucasians. Median age was 43 years (range 18-55) in patients and 33 years (range 20-51 years) in controls (p<.001). The median PBAC score was 307 in patients versus 75 in controls (p< .001). Twenty-eight percent of patients had a low hemoglobin (Hb<12 g/dL) and 28% of the patients had gynecological abnormalities. FXI levels were significantly lower in the patient group versus the control group (FXI 102 IU/dL vs 113 IU/dL; p=.002), also when adjusted for age. No differences in FVIII, FIX, FXII and VWF:Ag were seen between patients and controls. In patients with and without gynecological abnormalities all measured coagulation parameters, including FXI levels, were not significantly different.

Conclusion: we confirm that FXI levels in women with HMB are significantly lower compared to controls. The other coagulation parameters do not show a similar pattern. Whether this relatively low FXI in patients plays a causative role or is the consequence of HMB is not clear.
Introduction

Heavy menstrual bleeding (HMB) is a common problem among women in the reproductive age. At least 5-10% of women in reproductive age will seek medical attention for HMB. HMB can be caused by a wide range of disorders. In addition to the gynecological abnormalities underlying bleeding disorders have been recognized as an important etiologic and/or contributory factor in the past decade. However, in approximately 50% of cases of HMB no pathology is found at hysterectomy and in less than 20% of the women an underlying bleeding disorder is found. Therefore, in a large proportion of women, HMB is unexplained.

In a previous study focusing on underlying bleeding disorders, we had the unexpected finding that patients had significantly longer aPTT compared to controls (26.5 vs 25.0 sec; p=0.001). Subsequent unplanned factor analysis revealed lower levels of FXI (100 vs 124 IU/dL; p<.001). This left us with the questions whether this was a true finding, or just higher levels in the control group and 2. whether women with HMB with no obvious deficiencies, might also be low in the normal spectrum with other coagulation factors.

The aim of our study was to confirm that the overall FXI plasma levels in patients with HMB are lower than in controls. Furthermore we wanted to investigate whether other coagulation parameters are also decreased in women with HMB without obvious coagulation deficiencies.

Material and Methods

Patients & controls

This is a single center prospective cohort study at the University Medical Center Groningen, The Netherlands. We included consecutive patients who were referred to the gynecological outpatient clinic between December 2010 and May 2015. The patients had a history of heavy, regular (every 23-39 days) menstrual periods. Exclusion criteria were pictorial blood loss assessment chart (PBAC) score < 100, known bleeding disorders, use of any intrauterine device in the past 2 months, and treatment with anticoagulant therapy, antifibrinolytics, nonsteroidal anti-inflammatory
agents, combined oral contraceptives, or progestogens. Referred patients who were potentially eligible received a structured questionnaire by mail to obtain information about baseline characteristics: medical, obstetric, and gynecologic history. Women with intermenstrual, irregular and postcoital bleeding were excluded. Thereafter, the women had a gynecological examination and transvaginal pelvic ultrasonography in the first week after their menstruation. If we suspected a gynecological abnormality during vaginal ultrasonography (e.g., a polyp, endometrial hyperplasia or submucous fibroid), we performed an additional gel instillation sonohysterogram and/or (diagnostic/therapeutic) hysteroscopy. One interviewer (S.W.-V.) took the menstrual history, recorded the number of other bleeding symptoms (items of the Tosetto bleeding score)\(^8\) such as easy bruising; nose, gum, postoperative, and post partum bleeding, and bleeding after tooth extractions; the interviewer also asked about a family history of bleeding disorders. Women with submucous uterine fibroid tumors >2 cm in diameter, uterine polyps, endometrial hyperplasia, or endometritis were classified as HMB with gynecologic abnormalities\(^5,9\).

The study was approved by the institutional review board of the University Medical Center of Groningen (Registration number: NL 42716.042.12). Informed consent was obtained from all patients and controls.

The 53 healthy controls were recruited through posters in the employee wings of the hospital. These were women with a subjectively normal menstruation, regular cycle and no use of hormonal treatment or intrauterine devices; their haemostatic test results in the first week after menstruation were used for comparison. Exclusion criteria were treatment with anticoagulant therapy, nonsteroidal anti-inflammatory agents, or known bleeding disorders. Controls completed the same questionnaire as patients but had no gynecological examination. We did not exclude controls on the basis of their PBAC-score.

**Pictorial Bleeding Assessment Chart-score**

Before the first hospital visit, the patients were informed about the PBAC\(^10,11\) by a letter that contained standard instructions; they completed the PBAC in the menses before the first hospital visit. HMB was defined as a PBAC-score of ≥100 based on
the scoring system of Higham et al. After informed consent was given, controls completed the PBAC in the first menses, thereafter blood samples were taken.

Laboratory measurements

A venous citrated blood sample was taken from all patients and controls in the first week after the menstruation. In patients, the blood samples were taken before the gynecological examination. In patients, beside haemostatic measurements, blood samples were obtained for ABO blood group typing, complete blood cell counts and ferritin measurements. In controls, beside haemostatic measurements, hemoglobin, mean platelet volume, platelets and ferritin were measured.

Reagents that were used for activated partial thromboplastin time (aPTT; Dade Actin, FS Reagent), prothrombin time (PT, Dade Innovin Reagent) and fibrinogen (Dade Thrombin Reagent) were obtained from Siemens (Marburg, Germany). The 1-stage factor VIII, IX, XI and XII assays were performed with aPTT reagent and factor deficient plasmas from Siemens. Von Willebrand factor antigen (VWF:Ag) was measured by enzyme linked immunosorbent assay with polyclonal antiserum from DakoCytomation (Glostrup, Denmark), with von Willebrand factor ristocetin cofactor activity (vWF:Rco), and with von Willebrand reagent (lyophilized stabilized platelets and ristocetin) from Siemens in an optical aggregometer from Chrono-Log Corp (Haverton, PA).

For the haemostatic parameters, the normal ranges in our laboratory were as follows: aPTT, 23-33 seconds; PT, 9-12 seconds; fibrinogen, 1.7-4.0 g/L; factor VIII:C, 50-150 IU/dL; factor IX, 50-150 IU/dL; factor XI, 65-130 IU/dL; factor XII, 65-150 IU/dL; vWF:Ag, 50-150 IU/dL; and vWF:RCo, 50-150 IU/dL.

Values below the lower limit of normal reference range were confirmed in a second independent sample. A diagnosis of VWD was made if the vWF:Ag or vWF:RCo was <50 IU/dL in two independent measurements.

Data analysis

In our previous study we found a FXI plasma level of 100 IU/dL (SD: 17 IU/dL) in women with HMB versus 124 IU/dL (SD: 23 IU/dL) in controls. As this was an
unexpected finding, we assumed an overestimation of the true difference. Assuming a true difference of 10 IU/dL, a confidence interval of 95%, a power of 80% and a ratio of 2 patients versus 1 control, we needed to include 106 patients and 53 controls. Differences in the results of the coagulation tests between the patients and the control subjects were evaluated by Student t-test or Mann Whitney U test, depending on the normality of data for continuous data, and by Chi-square test or Fisher exact test for categoric data. Simple linear regression was performed to examine univariate associations between age (divided in tertiles) and coagulation parameters. A two tailed P value ≤.05 indicated statistical significance.

Results

Baseline characteristics

We included 106 patients with HMB and 53 healthy controls. Patients were older than controls (43 years (range 18-55) vs 33 years (range 20-51), p<.001), 93% of patients and 98% of controls were Caucasian. Patients had higher BMI than controls (24.9 (range 15.2-41.0) vs 22.2 (range 17.1-33.5), p<.001). The median PBAC score was 307 (range 105-1063) in patients versus 75 (range 15-393) in controls (p< .001). Twenty-eight percent of patients had a low hemoglobin (Hb<12 g/dL), with lower mean cell volume and ferritin levels, compared to 4% in controls. Twenty-eight percent of the patients had gynecological abnormalities (6% uterine polyps and 22% submucous uterine fibroid tumors) (table 1). The median cycle day of the laboratory analysis was 9 (range, 4-14 days) for patients and controls.

Factor XI and other haemostatic variables

FXI level was significantly lower in the patient group versus the control group (102 IU/dL vs 113 IU/dL, p=.002). One patient had a mild factor XI deficiency (FXI: 61 IU/dL). FXI levels were correlated with age: levels were higher with increasing age. The unadjusted FXI level was 11.6 IU/dL lower in patients vs controls, when adjusted for age this difference increased to 12.8 IU/dL.

There was no significant difference in the other coagulation factors (table 2), the FVIII and VWF:Ag levels were even slightly higher in patients than in controls.
If patients with gynecological abnormalities were compared with patients with no gynecological abnormalities (table 3), FXI levels were not different. Also for the other coagulation parameters no relevant or statistical significant differences were seen. Women with gynecological abnormalities were older though than women without gynecological abnormalities.

**Bleeding disorders**

Five patients (4.7%) had a bleeding disorder; four had von Willebrands disease and one had the abovementioned factor XI deficiency (FXI: 61 IU/dL). There were no combinations of bleeding disorders in patients. In the control group there were also two women with a low VWF:Ag level. These women were not classified as von Willebrands disease, because they did not meet the predefined criteria (including low von Willebrand factor measured twice). Excluding the women with bleeding disorders, the FXI levels in patients were still significantly lower than in controls (FXI: 102 IU/dL vs 113 IU/dL, respectively; p=.002).

**Bleeding symptoms**

Overall, 18% of the patients with HMB who had previous surgeries, deliveries or tooth extractions reported bleeding complications, versus 8% of controls with these exposures. Median Tosetto score in patients was 0 (-2 – 9) and in controls -1 (-3 – 3). The patients in the lowest quartile of the FXI levels had a higher overall score of 1 (-2 – 7) on the Tosetto bleedingscore than patients who had a higher FXI level who had a Tosetto score of 0 (-2 – 9). As the PBAC score was not different between these two groups, this indicates that women with lower levels had more bleeding symptoms.

**Comment**

In this study we confirm that the FXI level in women with HMB is significantly lower compared with controls. The overall coagulation parameters were not significantly different in women with HMB compared with the controls.

BMI was significantly different between patients and controls, but there was no
relation between FXI and BMI (data not shown). In our study BMI only correlated with FIX. This correlation has been previously described by Luxembourg et al.\textsuperscript{12}. These authors checked blood donors at different (coagulation) parameters and saw the same effect: in their study an increase in BMI also correlated with an increase in the FIX level. In our study BMI had no significant effect on the FXI levels; therefore we did not adjust for BMI.

In our study we only found a bleeding disorder in 5\% of the women. About 5-20\% of the patients with HMB are described to have von Willebrand disease (VWD) as an underlying bleeding disorder\textsuperscript{6,13}. FXI deficiencies are found in approximately 1-10\% of women with HMB\textsuperscript{7,14,15}. The lower percentage of bleeding disorders in our cohort could be explained by the strict criteria for a bleeding disorder. Additionally, we repeated low values in a separate sample, further reducing false positives. Some well-established platelet disorders are clinically relevant, but the value of an unconfirmed single disturbed platelet light transmission aggregometry is uncertain. Our group\textsuperscript{7} previously showed that 11\% of healthy controls had one or more platelet aggregation abnormalities at single testing, according to laboratory cut-offs. Laboratory tests for platelet function disorders are not well standardized because of test complexity and the need to rapidly process freshly collected blood samples\textsuperscript{16}. We therefore chose not to include abnormal platelet light transmission aggregometry in our study.

Unfortunately, our patients were significantly older than our controls, although the controls met the same inclusion criteria as the patients. Fortunately, this did not influence the results. We did adjust for age at the FXI levels, this even increased the difference between patients and controls. As the other coagulation parameters increase with ageing, the difference between patients and controls would even be smaller, when patients and controls were of the same age. As the other coagulation parameters at this time are not different between patients and controls, the age difference does not influence our results.

In our study there was also a significant age difference between women with and without gynecological abnormalities. Because fibroid tumors are hormonally dependent and develop primarily in premenopausal women, the cumulative incidence estimates for the older women in reproductive age will approximate the lifetime risk
of the development of fibroid tumors 17.

The volunteers have been enrolled on the basis of a subjectively normal amount of blood loss during their menstruation. Remarkably, a number of controls had a PBAC-score over 100 points. This has not influenced our results: when the controls were divided into a PBAC <100 or ≥100, there were no differences in coagulation parameters (including FXI) between those two groups (data not shown). This illustrates that a PBAC-score of >100 might be very sensitive, but the score is not specific in this Western European setting. Other studies 18, 19 confirm that a PBAC score of ≥150 is a more accurate cut-off level in clinical practice. If we had used the PBAC cut-off level of 150 in patients, we would lose 7 out of 106 patients. This would not influence our results in coagulation parameters (data not shown), the significant difference in FXI levels still exists.

Two controls had low von Willebrand antigen levels (VWF:Ag 48 IU/dL and 42 IU/dL). As the prevalence of von Willebrand disease is estimated to be 1-2% in the population 20, this is not an unexpected finding. The levels in our controls were only measured once, so these women do not officially meet the criteria for von Willebrand disease.

A weakness of our study is that at the start of our study only the Tosetto bleeding score was available as a screening tool for von Willebrand disease. The Tosetto bleeding score was developed to quantify the bleeding tendency of patients by 12 questions on different parts of the body 21. A few years later, the ISTH-BAT (International Society on Thrombosis and Haemostasis - Bleeding Assessment Tool) was published 22, 23. This tool was developed to create uniformity in the field of bleeding disorders and to compare and analyze different studies 24. Unfortunately, this tool was not available at the start of our study. The differences between these tools are two extra items (‘hematuria’ and ‘other bleeding symptoms’), and no negative scores when patients have had, for example, surgical procedures without bleeding complications in the ISTH-BAT. Also for some items the definitions used and number of point attributed were changed. For example in the ISTH-BAT at the post-partum hemorrhage item dilatation & curettage is deleted from the 2 points column and an extra item is added at the 3 points column, which is ‘requiring examination under anesthesia and/or the
use of uterin balloon/package to tamponade the uterus’. Because of this, we could not convert our scores to the ISTH-BAT scores.

Our data support the previously found difference in FXI levels between women with HMB and controls with normal amount of blood loss. Whether this relatively low FXI in patients is underlying the HMB or whether it is a consequence of HMB is not clear. In a previous study by Kadir et al. FXI levels showed no cyclic relationship over the monthly period in women with a regular cycle and no heavy menstrual bleeding was described. One would need to investigate the cyclic variation in FXI levels in women with HMB in comparison to cycle-controlled controls. The low levels of FXI levels could not be explained by our population, which were almost all Caucasians and no Ashkenazi Jews, where the prevalence of FXI deficiency is around 9%. At this moment the difference in FXI levels will not result in changes in the treatment of women with HMB. Perhaps there is a relationship between the cyclic variation in systemic fibrinolysis and FXI levels in women with HMB, which can result in a better understanding of the effectiveness of tranexamic acid in certain patients. Ultimately it is debatable whether this really will lead to changes in the clinic, as tranexamic acid is inexpensive, and an easily available treatment to try against heavy menstrual bleeding.
### Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=106)</th>
<th>Controls (n=53)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y*</td>
<td>43 (18-55)</td>
<td>33 (20-51)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age of menarche, y*</td>
<td>13 (9-17)</td>
<td>13 (11-16)</td>
<td>.207</td>
</tr>
<tr>
<td>Duration of period, d*</td>
<td>7 (4-14)</td>
<td>5 (3-8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Length of cycle, d*</td>
<td>28 (24-35)</td>
<td>28 (25-35)</td>
<td>.064</td>
</tr>
<tr>
<td>Body Mass Index*</td>
<td>24.9 (15.2-41.0)</td>
<td>22.2 (17.1-33.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pictorial blood loss assessment chart score*</td>
<td>307 (105-1063)</td>
<td>75 (15-393)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hemoglobin, g/dL**</td>
<td>12.5 (±1.3)</td>
<td>13.3 (±1.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mean cell volume, fL **</td>
<td>85.8 (±6.4)</td>
<td>89.5 (±4.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ferritin, ng/mL **</td>
<td>25.6 (±27.1)</td>
<td>45.2 (±30.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gynecologic abnormalities, n (%)</td>
<td>30 (28)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Data are given as median (range); **Data are given as mean (±SD).
### Table 2: Univariate analysis in haemostatic variables between patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=106)</th>
<th>Controls (n=53)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXI, IU/dL**</td>
<td>101.6 (±30.3)</td>
<td>113.2 (±21.3)</td>
<td>.002</td>
</tr>
<tr>
<td>aPTT, sec**</td>
<td>26.5 (±1.8)</td>
<td>26.8 (±1.8)</td>
<td>.26</td>
</tr>
<tr>
<td>PT, sec**</td>
<td>10.8 (±0.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FVIII, IU/dL**</td>
<td>152.1 (±34.8)</td>
<td>145.6 (±42.5)</td>
<td>.30</td>
</tr>
<tr>
<td>VWF:Ag, IU/dL**</td>
<td>96.4 (±29.2)</td>
<td>88.4 (±27.7)</td>
<td>.09</td>
</tr>
<tr>
<td>FIX, IU/dL**</td>
<td>109.3 (±20.1)</td>
<td>104.3 (±20.0)</td>
<td>.14</td>
</tr>
<tr>
<td>FXII, IU/dL*</td>
<td>106 (33-174)</td>
<td>103 (37-147)</td>
<td>.16</td>
</tr>
<tr>
<td>Fibrinogen, g/L**</td>
<td>2.9 (±0.6)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Data are given as median (range); **Data are given as mean (±SD).

### Table 3: Haemostatic variables in patients with no gynecological and gynecological heavy menstrual bleeding

<table>
<thead>
<tr>
<th></th>
<th>No gynecological abnormalities (n=76)</th>
<th>Gynecological abnormalities (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXI, IU/dL**</td>
<td>102.0 (±20.0)</td>
<td>100.6 (±23.8)</td>
<td>.77</td>
</tr>
<tr>
<td>aPTT, sec**</td>
<td>26.3 (±1.8)</td>
<td>26.8 (±1.74)</td>
<td>.26</td>
</tr>
<tr>
<td>PT, sec*</td>
<td>10.9 (9.5-12.1)</td>
<td>10.7 (9.8-11.7)</td>
<td>.10</td>
</tr>
<tr>
<td>FVIII, IU/dL**</td>
<td>148.7 (±34.3)</td>
<td>160.8 (±35.1)</td>
<td>.11</td>
</tr>
<tr>
<td>VWF:Ag, IU/dL**</td>
<td>98.5 (±30.9)</td>
<td>91.2 (±24.0)</td>
<td>.24</td>
</tr>
<tr>
<td>FIX, IU/dL**</td>
<td>107.8 (±20.0)</td>
<td>112.9 (±20.1)</td>
<td>.24</td>
</tr>
<tr>
<td>FXII, IU/dL**</td>
<td>107.9 (±29.5)</td>
<td>107.8 (±32.6)</td>
<td>.99</td>
</tr>
<tr>
<td>Fibrinogen, g/L*</td>
<td>2.9 (1.2-4.4)</td>
<td>2.9 (2.0-4.5)</td>
<td>.62</td>
</tr>
<tr>
<td>Age, y*</td>
<td>41 (18-54)</td>
<td>47 (36-55)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body Mass Index*</td>
<td>24.9 (15.2-41.0)</td>
<td>24.8 (19.3-37.1)</td>
<td>.93</td>
</tr>
</tbody>
</table>

*Data are given as median (range); **Data are given as mean (±SD).
References
