PBAC score: an easy-to-use tool to predict coagulation disorders in women with idiopathic heavy menstrual bleeding

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Women with inherited bleeding disorders suffer significant morbidity and impaired quality-of-life associated with heavy menstrual-related bleeding [1]. Heavy menstrual bleeding (HMB), also referred to as menorrhagia, is defined as uterine bleeding that lasts for > 7 days or blood loss > 80 mL per menstrual cycle [2]. The wide variation in what constitutes ‘normal’ bleeding makes identifying patients with HMB difficult for both clinicians and patients [1]. Indeed some women, especially those from families with hereditary bleeding disorders, do not realize they have HMB so do not seek medical advice for their condition.

HMB is often a presenting symptom of coagulation disorders, and may be the only bleeding symptom [3]. An increased prevalence of von Willebrand disease (VWD) has been observed in women with HMB (13% [95% CI 11–16%] compared with 0.8–1.3% in the general population) [4] and it has been proposed that mild platelet function defects are even more common, with ~50% diagnosed with platelet aggregation defects compared with a control frequency of 17.3% [5].

A semi-objective method to quantify menstrual blood loss is the alkaline haematin technique, which requires the collection of all tampons or sanitary towels for laboratory analysis [6]. Although the most accurate method, it is too time-consuming and expensive for routine clinical practice. Alternatives include collecting and weighing all products used or the use of vaginal cups for menstrual collection; however, these methods can encounter problems with fluid evaporation and/or leakage. Another method is the pictorial blood assessment chart (PBAC) Score [7]. This method records the number of tampons or towels used and the degree to which they are stained with blood. A validation study found that the PBAC Score was superior to a woman’s subjective assessment of menstrual blood loss, with a positive predictive value of 85.9% [8]. Additionally, a comparison of PBAC Score with the alkaline haematin method found a significant correlation between the two [9]. PBAC Scores > 100 have been confirmed in the majority of women diagnosed with coagulopathic disorders [10].

We performed a retrospective analysis to assess whether the amount of menstrual blood loss, determined using the PBAC Score, can be used as a predictor for the presence of coagulation disorders in women with idiopathic HMB. All patients with a history of HMB referred to our coagulation centre between September 2011 and October 2013 were included. Known causes of gynaecological and endocrinological HMB had been ruled out by the patients’ gynaecologists, and patients were not receiving any treatment that may have affected the PBAC Score, such as tranexamic acid, desmopressin or factor concentrate. Women without a history of HMB were recruited from among the patients’ family, friends and associates as a control group; patients were not age-matched, but were similar in age. None of the participants were on oral contraceptives. Informed consent...
for participation was obtained, and the study was approved by the Ethics Committee Nordrhein. A full blood count was carried out, and the following coagulation tests were performed within 4 h of blood collection: von Willebrand factor antigen (VWF:Ag) and ristocetin cofactor (VWF:RCo), VWF-multimers, fibrinogen (by Clauss assay), and residual activities of Factor II (FII), FV, FVII, FX, FVIII (one-stage and chromogenic assay), FIX, FXI, FXII and FXIII. Sampling was not performed in relation to the menstrual cycle. A diagnosis of VWD or factor deficiency was made if the results of the relevant coagulation tests were below the reference range values used in our laboratory. All participants were instructed how to complete the PBAC Score throughout their next menstrual cycle. The chart consists of images representing lightly, moderately and heavily stained sanitary towels (scored as 1, 5 and 20 respectively) and tampons (scored as 1, 5 or 10 respectively); passage of clots (assigned ascending scores from 1–5) and episodes of flooding are also recorded. HMB was defined as PBAC Score >100 [7].

Overall, 199 women with a history of HMB and 106 women in the control group were included. Age range between groups was comparable, with a median (range) age of 22 (11–56) years in the HMB group and 29 (13–54) years in the control group. The number of women with a coagulation disorder was noticeably higher in the HMB group (151/199, 76%) than the control group (4/106, 4%). Among the 151 HMB women with bleeding disorders, the most common diagnosis was VWD in 118 (78%). Other disorders included FXIII deficiency (15/151, 10%), FVII deficiency (8/151, 5%), and prolonged platelet function assay (PFA), thrombocytopenia, or other mild factor deficiencies (33/51, 22%). Nineteen women (13%) were diagnosed with two coagulation disorders, and two women were diagnosed with three. For women from the control group, diagnosis was made on the basis of one blood test; In the HMB group, 18% (36) were also tested only once, but the majority (163/199, 82%) were tested twice or more.

The median (range) PBAC Score was higher in the HMB group (266 [31–4212]) than the control group (60 [4–100]) (Fig. 1a). Menstrual duration was also longer in women with HMB (7 [3–19] days) than the control group (5 [1–9] days) (Fig. 1b). PBAC Score was 258 (70–4212) in women with VWD, 297 (173–1080) in FXIII deficiency, 254 (140–1080) in FVII deficiency, 266 (70–2670) in women with prolonged PFA and 320 (110–985) in women with thrombocytopenia.

Using the Mann–Whitney test for independent samples, there was a highly significant difference in PBAC Score and the duration of menstruation between the control and HMB groups, both with and without a coagulation disorder as an underlying cause for HMB (P < 0.001). We also compared results between women with and without a coagulation disorder within each group, but found no significant difference in terms of PBAC Score or duration of menstruation (Table 1).

ROC analysis demonstrated that the PBAC Score (AUC = 0.980, 95% CI: 0.967–0.993) was superior to menstrual duration (AUC = 0.858, 95% CI: 0.814–0.902) in identifying patients with HMB and a coagulation disorder. The optimal cut-off for the PBAC Score was 100, with a sensitivity of 91%, a specificity of 100%, positive predictive value (PPV) of 100% and negative predictive value (NPV) of 85.5%. A more adequate cut-off for the duration of HMB is 5 days, with a sensitivity of 91.5%, specificity of 34.9%, PPV of 83.1% and NPV of 80.2%.

In this study, 75% of women with HMB suffered from a coagulation disorder, compared to 4% of the control group, confirming earlier findings that HMB is
an indicator of coagulopathy [5, 10–12]. A similar study was described by Miller et al. [5] in which 232 women with a PBAC Score >100 were examined for coagulation disorders; a laboratory abnormality was found in 170 patients (73.3%) compared to 75% in our study. Both our study and that described by Miller et al. included patients where the coagulation factor levels were only slightly below the reference range, indicating a mild deficiency which may have limited clinical significance. The incidence of VWD observed in our study (59% of HMB patients) is notably higher than the rate of 6% observed by Miller et al. [5], and the rate of 13% reported in a systematic review [4]. Differences in study methods may account for these variations, as it was noted that studies recruiting women from the general population report lower prevalence rates [4]. Additionally, previous studies may include women self-diagnosed with HMB who may not have met the criteria used in our study, which could have diluted the reported prevalence rates, while our study may have included a selection bias due to the patients’ awareness that they were being evaluated for HMB. The ethnic mix of the population tested may be an influencing factor, as a lower incidence of VWD has been reported in black women, and ethnic variations in VWF levels can influence the diagnosis of VWD [4,5].

Mild platelet function defects are frequently observed in women with HMB [5,13]. We did not evaluate platelet aggregation, but observed thrombocytopenia in 4% and prolonged PFA in 8% of HMB patients, comparable with the reported incidence of abnormal PFA alone (6.5%) [5]. We also observed low levels of mild coagulation factor deficiencies in our HMB population, confirming similar findings by earlier studies [5,12,14].

The observation that HMB can indicate an underlying coagulation disorder further emphasizes the need for a reliable method to measure blood loss during menstruation. We have demonstrated that a PBAC Score >100 and/or a menstrual duration >5 days is linked to the presence of a coagulation disorder. We did not use standardized towels or tampons, which may have affected the variability of PBAC Scores in our study. However, our results demonstrated that PBAC Score had greater diagnostic accuracy than menstrual duration. PBAC Scores >100 have been confirmed in 74%, 57% and 59% of women with VWD, haemophilia and FXI deficiency respectively [10]. In addition, we observed high interindividual, but low intra-individual variation in PBAC Scores; supporting earlier published data [15].

In conclusion, the results of this study indicate that a high PBAC Score is a strong indicator of an underlying coagulation disorder, and may be suitable for use in defining treatment endpoints for clinical use or in trials.

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Reduced immunogenic response to residual CHO cell protein in recombinant factor IX (IB1001) drug product in normal healthy rabbits

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IXINIITY® [coagulation factor IX (recombinant)] (IB1001) is a human recombinant factor IX (rFIX) developed for the management of haemorrhagic episodes in individuals with haemophilia B. IB1001 is manufactured in Chinese hamster ovary (CHO) cells using a state-of-the-art process that incorporates three validated viral reduction steps and selectively purifies active forms of FIX. The purification process results in IB1001 drug product that is consistently greater than 96% purity with a low level of CHO cell protein (CHOP) as impurities. Host cell proteins (HCP) in general carry the risk of functioning as an adjuvant and thus triggering an immunologic reaction to the active ingredient [1,2]. During clinical development, immunogenicity to residual CHOP was noted in 30% of patients without any clinical significance. The anti-CHOP response was confirmed by the western blot analysis with positive patient sera [3]. This finding was not unexpected as similar reactivity to HCP was also observed in patients after treatment with other recombinant factor products such as FVIII [4]. As prolonged exposure with rFIX is required for the treatment of haemophilia B, removal of any potential antigens or material that could act as an adjuvant in IB1001 was of paramount importance [5]. Although there were no apparent anti-CHOP antibody-associated adverse events identified in IB1001 trial subjects, an additional hydrophobic interaction chromatography (HIC) step designed to further remove CHOP residue was implemented and validated for the manufacture of IB1001.

An ELISA developed using a process-specific polyclonal antibody (sheep derived) and CHOP reference standard was used to monitor CHOP levels in the drug substance. The IB1001 produced using the newly modified process (MP) with the HIC step exhibited significant reduction (~2000 fold less) in the residual CHOP levels (<26 ng CHOP per mg of IB1001) compared to the levels (58 500 ng CHOP per mg of IB1001) in the IB1001 produced using the former process (FP). The addition of HIC step did not alter IB1001 physicochemical characterization, or its pharmacokinetic profile in animals [3]. The specific aim of this study was to compare the immunogenic potential of residual CHOP in IB1001 product produced with and without the HIC step, in rabbits. A total of 48 rabbits (24 males and 24 females) were randomly assigned into two groups (FP and MP). IB1001 was administrated intravenously at 0.5 mg kg$^{-1}$ twice weekly for a total of 64 days. Serum samples for anti-CHOP reactivity testing were collected prior to dosing on days 1 (baseline), 29 and 57. To measure the anti-