Bleeding disorders in adolescents with heavy menstrual bleeding in a multicenter prospective US cohort

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Online Supplement

I. Supplemental methods:

The study was endorsed and advertised by the International Society of Thrombosis and Haemostasis (ISTH) (https://www.isth.org/news/203790/SSC-Subcommittee-to-Study-Bleeding-Disorders-in-Adolescents-with-Heavy-Menstrual-Bleeding.htm). Sources of referral included emergency department (ED), primary paediatricians and community gynaecologists and obstetricians (OB/GYN). The referral criteria have been previously described. (Supplemental Table 1) (1) and were the same across both centres. Participants referred for abnormal coagulation profiles, including initial abnormal von Willebrand factor (VWF) analysis, were excluded.

Descriptive variables and predictors

Patient demographics. This included race, ethnicity, age at first bleeding event, age at menarche, age at evaluation, age at diagnosis, and time to development of HMB. Menstrual bleeding patterns, assessment of HMB in ED, hospitalization, iron deficiency anaemia and treatment with packed red blood cells (pRBCs), personal and family history of bleeding were ascertained. All variables, predictors, and work-up of BD were defined *a priori*.

Definitions

We further defined HMB, for the purposes of this study, as refractory, when: a) HMB required ≥ 3 types of hormones for continued vaginal bleeding; b) a combination of hormones and long-acting reversible contraceptives; and, c) any breakthrough HMB requiring ≥ 2 hormone tapers and/or ED visit/hospitalization after an initial evaluation. Family history of BD or bleeding was determined to be positive when any first degree relative carried an established diagnosis of a BD or experienced *any* of the following bleeding symptoms: epistaxis or bleeding from minor

wounds, > 5 episodes/year or lasting more than 10 minutes; bruising (5 or more (> 1 cm) in exposed areas; oral cavity bleeding; GI bleeding (not associated with ulcer, portal hypertension or haemorrhoids); macroscopic haematuria, muscle hematomas, hemarthrosis, central nervous system bleeding, other bleeding such as umbilical stump bleeding, cephalohematoma, excessive bleeding following circumcision, venepuncture, dental extractions/surgery, surgeries or childbirth. The first bleeding event was any bleeding event, also judged by the criteria above. We used a composite of established BD or bleeding symptoms to capture family members with potentially undiagnosed BD. A gynaecologic or obstetric family history was considered positive when a female first degree relative self-reported menstrual bleeding as lasting ≥ 7 days or requiring change of pads more frequently than every 2 hours during heavy days, or causing iron deficiency with/without anaemia, requiring hormonal therapy or hospital admission and treatment to emergently control HMB, including dilatation and curettage, endometrial ablation or hysterectomy, irrespective of uterine structural abnormalities, or experienced post-partum haemorrhage after childbirth. Time until HMB was defined as the time from onset of menses or menarche until HMB, as defined above, or that resulting in an ED visit or hospitalization.

Laboratory testing

The rationale for testing FXI and FXIII activity assays upfront included the potential to miss these diagnoses with a normal initial aPTT. This was also based on evidence of abnormal bleeding in FXI deficiency not confined to severely deficient patients (2) and a previously reported 26% prevalence of HMB in FXIII deficient women with F XIII levels < 70 IU/dL -1 (3). A systematic approach to testing was undertaken, as previously described(1).

VWF analysis. Blood for VWF analysis was obtained irrespective of the menstrual cycle phase. As VWF analysis on oral contraceptives (OCs) containing 30-35 µg of oestrogen is unlikely to affect the laboratory diagnosis of VWD, none of the subjects were taken off OCs for testing (4,

5). For those on OCs, all were tested on 30-35 µg of oestrogen, monophasic, once-a-day pill, after at least 12 weeks of initiation or following an OC taper. VWF analysis included one-stage FVIII activity (Instrumentation Laboratories, Bedford, MA), VWF antigen (Instrumentation Laboratories, Bedford, MA), VWF activity by ristocetin cofactor activity (Siemens Inc. Marburg, Germany) and VWF: collagen binding assay (Hyphen-Biomed, Neuville-Sur-Oise, France) in all participants.

Qualitative platelet dysfunction (QPD). For platelet aggregation testing, all subjects were asked to withhold non-steroidal anti-inflammatory agents, or other platelet impairing medications, and any other over the counter herbal supplements for at least 7 days before testing. Confirmation of abnormal testing was performed in fasting state. Platelet aggregation (ADP, collagen, arachidonic acid, and ristocetin) and ATP secretion (thrombin, collagen and arachidonic acid) were performed using whole blood impedance and chemiluminescence (Chronolog, Haverton, PA) as the first tier, using same day healthy control.

Coagulation factor deficiencies. Factors XI assay (Instrumentation Laboratories, Bedford, MA) and FXIII chromogenic assay (Siemens Inc, Marburg, Germany) were performed in all participants. Select patients were also tested for FIX and FVII deficiencies when indicated by a prolonged aPTT and PT, respectively. We elected to study systemic fibrinolytic activity using ROTEM (ROTEM delta, Instrumentation Laboratories, Bedford, MA) rather than the measurement of individual fibrinolytic components.

Gynaecological evaluation. All participants were screened with free T4 and TSH, and for polycystic ovarian syndrome in those with suggestive history and/or clinical criteria (6). Participants underwent a pelvic ultrasound at the discretion of the adolescent medicine physician or the OB-GYN.

Evaluation for hypermobility syndromes.

Participants were screened for benign joint hypermobility (BJH) syndrome by Beighton score; a score of 5/9 was considered positive (7, 8).

Bleeding Assessment Tools

Pictorial Blood Assessment Chart (PBAC). For those already on hormonal suppression for HMB, this was completed for cycles before the initiation of hormones by recall or using data from personally maintained menstrual calendars or smartphone apps.

Outcomes

Low VWF. Low VWF was defined as VWF:Ag and/or VWF:RCo between 30-50 IU dL^{-1} on ≥ 2 occasions and VWD was diagnosed when VWF: Ag or VWF: RCo was < 30 IU dL^{-1} or (RCo/VWF: Ag ratio <0.6), on ≥ 2 occasions, at least 8 weeks apart, using NHLBI guidelines (9). Qualitative platelet dysfunction was diagnosed when there was impaired aggregation to one or more agonists and/or impaired ATP secretion, both with reproducible findings on ≥ 2 occasions (10). To ensure accuracy, two coagulation experts (R.S and N.DS) reviewed platelet aggregation tracings and reached a consensus in those diagnosed with QPD. Clotting factor deficiency was defined when a particular clotting factor level was below the age-based reference range. Hyperfibrinolysis was diagnosed when EXTEM maximum lysis on ROTEM was > 13%(normal < 3%) (11).

Statistical analysis

Categorical variables were summarized using percentages and ratios; continuous variables were summarized using appropriate measures of central tendency and dispersion, according to data distribution. Two main groups, according to the pattern of menstrual bleeding, were compared: anovulatory and ovulatory HMB groups.

Main Outcome

The association between BD and the following predictors was investigated using univariable logistic regression: ethnicity, race, ED presentation/evaluation for HMB, hospitalization for acute HMB, pRBCs for acute anaemia, surgical bleeding, dental bleeding, family history of bleeding symptoms or established BD and family history of gynaecologic or obstetric bleeding; continuous predictors included age at menarche, age at first bleeding event, time until HMB development, PBAC score, ISTH BAT score, haemoglobin and ferritin.

Multicollinearity among potential predictors and influential outliers in the multiple variable model were investigated. Model fit was assessed with the Hosmer-Lemeshow test and c-statistics. Different regression models were compared using the Akaike Information Criterion (AIC). The final model was selected according to the lowest AIC and the clinical significance of its predictors.

Significance level was set at 0.05. Data analysis was generated using SAS software, version 9.2 (SAS institute Inc., Cary, NC). STROBE statement is reported in Supplemental Table 2.

II. Supplemental tables

Supplemental Table 1. Referral Criteria for evaluation of heavy menstrual bleeding

Table 1. Eligibility/Referral criteria for HMB evaluation in the Young Women's Blood Disorders Clinical Program
• Any HMB- self perceived or as perceived by the referring provider • Any acute HMB that leads to an emergency department visit or hospitalization • Persistent and recurrent breakthrough menstrual bleeding on hormonal therapy • Any HMB with a positive family history of HMB • Any HMB with a family history of bleeding disorder

HMB, heavy menstrual bleeding

Supplemental Table 2. STROBE Statement

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	x
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	x
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	x
Objectives	3	State specific objectives, including any prespecified hypotheses	x
Methods			
Study design	4	Present key elements of study design early in the paper	x
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	x
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	x
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	x
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	x
Bias	9	Describe any efforts to address potential sources of bias	x
Study size	10	Explain how the study size was arrived at	x
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	x
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	x
		(*b*) Describe any methods used to examine subgroups and interactions	x
		(*c*) Explain how missing data were addressed	x
		(*d*) If applicable, explain how loss to follow-up was addressed	x
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	x
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	x
		(b) Indicate number of participants with missing data for each variable of interest	x
		(c) Summarise follow-up time (e.g., average and total amount)	x
Outcome data	15*	Report numbers of outcome events or summary measures over time	x

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	x
		(*b*) Report category boundaries when continuous variables were categorized	x
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	x
Discussion			
Key results	18	Summarise key results with reference to study objectives	x
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	x
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	x
Generalizability	21	Discuss the generalizability (external validity) of the study results	x
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	x

*Give information separately for exposed and unexposed groups.

Supplemental Table 3. Types of VWD and Clotting factor abnormalities in the cohort

Total Number with VWD		**17**
Type 1		13
	Type 1 Severe	1
	Type 1 C	1
Type 2		4
	Type 2B	1
	Type 2M	3
Total Numbers with Clotting Factor deficiencies		**3**
	Mild FXIII deficiency*	1
	Mild haemophilia A*	1
	Symptomatic haemophilia carrier[#]	1

VWD indicates von Willebrand Disease
*FXIII of 40%; FVIII:C 29%
[#] FVIII:C: 142%; + for intron 22 inversion mutation

Supplemental Table 4. Types of Qualitative Platelet Disorders

Participant	**Type of abnormality**	**Degree**	**Comments**
1	ADP aggregation defect	Severe	Repeated thrice
2	Global aggregation defect	Moderate	History of (resolved) ITP*
3	ADP aggregation defect	Moderate	Repeated thrice
4	COX-1 defect	Severe	Repeated thrice
5	COX-1 defect	Severe	Repeated twice
6	ADP and COX-1 aggregation defect	Mild-Mod	Repeated thrice
7	COX-1 defect	Moderate	Repeated twice
8	ADP and COX-1 aggregation defect	Mild	Repeated twice
9	COX-1 aggregation defect	Moderate	Familial thrombocytopenia*

ADP indicates adenine diphosphate; ITP, immune thrombocytopenia; COX, cyclooxygenase
*Participant 2 and 9 only had platelet aggregation studies performed once. The global aggregation defect in the participant 2 with a history of ITP is likely from autoantibodies against GP1b and GPIIb-IIIa

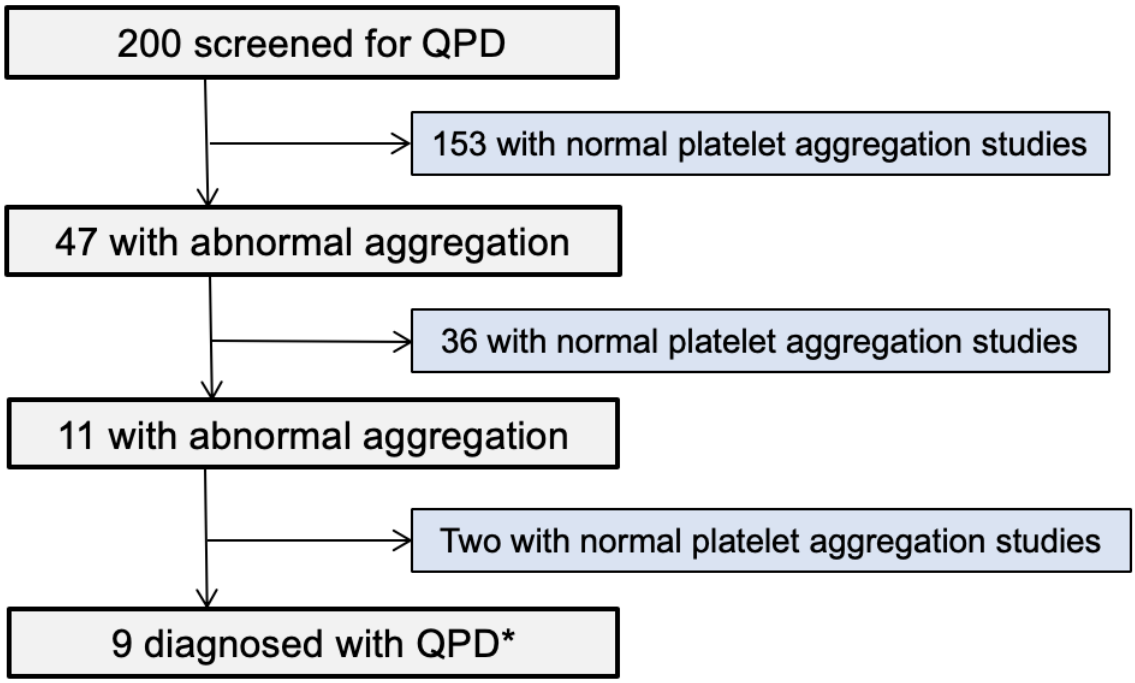
Supplemental Table 5. Frequency of additional bleeding symptoms in the cohort

	BD (n=67)	No BD (n=133)
Epistaxis	25 (37%)	9 (6.7%)
Post-surgical bleeding	8 (12%) *	5 (3.75%) $
Cutaneous	16 (24%)	3 (1.8%)
Oral cavity bleeding	8 (12%)	3 (1.8%)
Dental extraction bleeding	6 (9%) *	2 (1.2%) $
Minor wound bleeding	3 (4.5%)	1 (0.6%)
Other bleeding#	1 (1.5%)	0 (0%)

*Of those with bleeding disorders, 20 participants had surgery but only eight experienced bleeding and 26 had dental procedures but only 6 experienced bleeding.
$No Of those without bleeding disorders, 42 had surgery but only five participants experienced bleeding and 29 had dental procedures but only two experienced bleeding.
The surgeries and dental procedures in the entire cohort were pre-diagnosis in the entire cohort. The surgeries that occurred after diagnosis are not included.
#This includes bruising with immunization

III. Supplemental Figure

Supplemental Figure 1. Platelet Aggregation Testing Flow



QPD denotes Qualitative Platelet Dysfunction
*One participant with familial thrombocytopenia and one participant with history of immune thrombocytopenia (resolved) were not re-tested

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