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Von Willebrand Disease and Risk for Obstetric Bleeding: Analysis of the Female UDC Project

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Von Willebrand Disease and Risk for Obstetric Bleeding:

Analysis of the Female UDC Project

A thesis submitted in partial satisfaction

of the requirements for the degree Master of Science

in Clinical Research

by

Gavin Daniel Roach

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ABSTRACT OF THE THESIS

Von Willebrand Disease and Risk for Obstetric Bleeding:

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Gavin Daniel Roach

Master of Science in Clinical Research

University of California, Los Angeles, 2014

Professor Robert M. Elashoff, Chair

Von Willebrand disease (VWD) is the most common inherited bleeding disorder. Patients are subject to defective platelet adhesion and aggregation that characteristically presents as mucosal bleeding. Women with VWD face bleeding risks during pregnancy, delivery, and the postpartum period. The female module of the Universal Data Collection system provides an excellent resource for analyzing the characteristics of women with VWD who experienced pregnancy-related bleeding, including bleeding during miscarriage, antepartum bleeding, and postpartum hemorrhage. We hypothesized that there are characteristics that are common to women with VWD who are likely to experience pregnancy-related bleeding complications. We compared the characteristics of women with VWD who have experienced these complications to those of women with VWD who

have been pregnant but have not had these complications. We then built a multivariable regression model for the purpose of identifying women with VWD who are at increased risk for pregnancy-related bleeding. We found that there are two risk factors that are associated with pregnancy-related bleeding in this group, 1) a history of anemia (OR 4.94), and 2) a history of bleeding symptoms (OR 1.36). Our results indicate that more attention needs to be paid to the manner in which providers take a bleeding history, and the instruments used. We propose that the best care is provided for these women when obstetricians and hematologists work together in a proactive approach.

The thesis of Gavin Daniel Roach is approved.

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Chapter 1: Thesis Manuscript

Background and Rationale

Von Willebrand Disease

Von Willebrand Disease (VWD) is characterized by quantitative or qualitative defects in the multimeric glycoprotein Von Willebrand Factor (VWF), which is produced in megakaryocytes and vascular endothelial cells. This protein plays a major role in normal coagulation via several mechanisms. VWF binds to factor VIII in the circulation and prevents its degradation. It binds to exposed collagen on the vascular endothelial surface at sites of vascular injury, and binds to platelet glycoprotein Ib under conditions of high shear stress, thus initiating platelet adhesion. It also binds to platelet glycoprotein IIbIIIa and assists in platelet aggregation. In the absence of functional VWF, from either a quantitative or qualitative deficiency, patients are subject to defective platelet adhesion and aggregation that characteristically presents as mucosal bleeding. This takes the form of epistaxis, gingival bleeding, menorrhagia, and bleeding from minor abrasions. Some affected individuals may experience more serious bleeding symptoms such as gastrointestinal bleeding, joint bleeding, or intramuscular bleeding. Women with VWD face additional bleeding risks during pregnancy, delivery, and the postpartum period.

VWD Prevalence

VWD is the most common inherited bleeding disorder, with an estimated prevalence of 13,000-16,000 per million (1.3-1.6%) based on population studies.[1,2] The number of

symptomatic, recognized cases, however, is much lower. Worldwide estimates using the number of patients referred to specialized centers divided by the total population served by those centers gives a range from 23 to 113 cases per million.[3,4] This method presumably identifies those with serious enough bleeding symptoms to warrant referral to a physician, but it does not detect people with below-normal levels of VWF or dysfunctional VWF who have not yet had serious bleeding symptoms, or have not recognized such symptoms, and therefore have not been referred to a specialized center. All of these individuals, both recognized cases, as well as unrecognized, are at risk for future bleeding episodes. There is currently no universal screening for VWD.

VWD Phenotypes

There are three recognized types of VWD. Patients with type 1 disease have a partial quantitative deficiency of VWF, patients with type 2 have qualitative defects of VWF, rendering the protein dysfunctional, and patients with type 3 have a total quantitative deficiency. Type 2 is further divided into four variants. In type 2A there is a deficiency of large molecular weight VWF multimers, which results in poor platelet adhesion, but relatively spared levels of VWF and FVIII. In type 2B the VWF has an increased affinity for platelets, which results in the degradation of large VWF multimers and often results in thrombocytopenia. Type 2M VWD is characterized by a decreased affinity of VWF for platelets, but normal multimer levels. Type 2N can be mistaken for hemophilia A because of impaired binding of VWF to FVIII and subsequently low serum levels of FVIII with spared levels of VWF. The most common type of VWD is type 1, which comprises approximately 60-80% of cases.

VWD in Women

Although VWD is inherited in an autosomal fashion, women are more regularly affected by bleeding symptoms as compared to men.[3,5] This is most likely because of menstruation, and the monthly bleeding challenge that presents to all females of menstruating age. Menorrhagia is a common symptom in women with VWD, occurring more frequently than in women without VWD. Some have estimated that rates of menorrhagia may be as high as 74-95%.[6-9] Childbirth represents another bleeding challenge. For women with type 1 disease, levels of VWF will start below normal in the beginning of pregnancy, then increase during the pregnancy, along with FVIII, and often reach the normal range during the third trimester.[10-12] Type 3 VWD does not usually produce an increase in VWF due to the homozygous loss of the gene. In type 2 the increased levels of VWF and FVIII do not ameliorate the risk of bleeding because of the underlying dysfunctional nature of the protein. Despite the elevation of VWF and FVIII during pregnancy, women with VWD experience more pregnancy-related bleeding than controls.[13] While rates of miscarriage have not been shown to be higher among women with VWD, the rate of bleeding with miscarriage is more frequent.[12] Women with VWD are also at an increased risk for postpartum hemorrhage, likely due to the fact that levels of VWF and FVIII can drop precipitously after delivery. Rates of postpartum hemorrhage are estimated to be as high as 31-59% in women with VWD compared to 10-21% of controls.[7,9]

Guidelines for Management of VWD in Pregnancy

There is currently no consensus for how to monitor or treat women with VWD during pregnancy and the postpartum period, though many different guidelines exist.[14-21]

It is generally agreed that levels of factor VIII and VWF activity should be checked at least once during the third trimester, but the possibility of a miscarriage or antepartum bleeding in the first or second trimester necessitates earlier attention to these women. In addition to treatment for procedures such as an epidural, some women may benefit from prophylaxis during pregnancy. Within the population of women who have known VWD, there has not been an analysis of the risk factors that may lead some women to be at higher risk for obstetrical bleeding. While it is important for all women with VWD to have care provided by obstetricians and hematologists with knowledge of bleeding disorders in pregnancy, it would be of great value to be able to predict which women are at higher risk of bleeding than others.

Surveillance of Bleeding Disorders in Women

The Universal Data Collection (UDC) surveillance system was implemented by the Centers for Disease Control (CDC) in 1998 to collect information on persons with bleeding disorders at 130 Hemophilia Treatment Centers (HTCs) in the United States. Information about demographics, symptoms, treatments, and prophylaxis was collected to characterize the population being treated at HTCs and to study outcomes longitudinally. Over 24,000 people with bleeding disorders participated in the UDC. Due to the fact that hemophilia is predominantly a male disease, the majority of subjects enrolled in the UDC were men. The CDC added a female-specific module to the UDC in 2009 to assess the unique bleeding risks women face, and to monitor bleeding and reproductive complications over time.

The female UDC module was developed by a committee of experts from HTCs in collaboration with the CDC. The module was pilot-tested in 2008 at 17 HTCs and

implemented in 2009 at 30 HTCs, representing a minimum of 2 HTCs from each of 12 regions within the U.S. HTC network. The module collected information on diagnoses, gynecological and reproductive history, menstrual bleeding, obstetrical bleeding, treatments, and prophylaxis. A registration form was completed at enrollment and an annual form was completed at subsequent visits. The women enrolled in the female UDC module included females over the age of 2 years with a diagnosed bleeding disorder receiving care at an HTC.[22] The diagnoses included factor deficiencies, VWD, platelet disorders, and others. HTC care providers offered enrollment to all eligible females, and all participants or their legal guardians gave informed consent. The protocol was reviewed and approved by the institutional review boards at the CDC and the individual sites. Data collection occurred from September 2009 through September 2011 and included over 500 women with bleeding disorders, many of which had VWD. The female module of the UDC system provides an excellent resource for analyzing the characteristics of women with VWD who experienced pregnancy-related bleeding, including bleeding during miscarriage, antepartum bleeding, and postpartum hemorrhage.

Hypothesis and Specific Aims

Hypothesis

We hypothesize that there are characteristics that are common to women with VWD who are likely to experience pregnancy-related bleeding complications. These complications include bleeding during miscarriage, antepartum bleeding, and postpartum hemorrhage.

Specific Aims

The first aim is to use the information gathered in the female UDC module to compare the characteristics of women with VWD who have experienced pregnancy-related bleeding complications to those of women with VWD who have been pregnant but have not had pregnancy-related bleeding complications.

The second aim is to build a multivariable regression model using the information collected in the female UDC system for the purpose of identifying women with VWD who are at increased risk for pregnancy-related bleeding complications.

Methods

Study Population

We included women who were registered as part of the female module of the UDC beginning in September 2009 and continuing until the module was closed to registration in September 2011. Inclusion criteria were the diagnosis of VWD (any type or type unknown) and history of at least one reported pregnancy. The only exclusion criterion was a lack of any data for the primary outcome of interest.

Primary Outcome

The Registration form used by the female UDC module has 41 items organized into the following categories: Demographic/Referral Information, Bleeding Symptoms, Treatment, Reproductive/GYN History, and Menopause. Items 31, 32, and 33 ask if a

patient has ever had "a problem with bleeding during miscarriage," "any problems with bleeding during pregnancy," and "post-partum hemorrhage of concern to a healthcare provider," respectively. These three items are repeated on the Annual Visit form used at subsequent visits to the HTC. The responses to these three items comprised the primary outcome. We are interested in the risk factors associated with all pregnancy-related bleeding, and so a positive response to any of the three items constituted an unfavorable bleeding outcome.

A trained healthcare provider completed the UDC Registration form or the UDC Annual Visit form at the time of the patient visit. The recording of responses to these questions about pregnancy-related bleeding was at the discretion of the provider, informed by either discussion with the subject, or chart review. The glossary distributed to all providers defined postpartum hemorrhage as "excessive bleeding from the uterus after the delivery of a baby." It did not offer a quantitative definition of "problems with bleeding" during miscarriage or pregnancy. The information from the Registration form and the Annual Visit form were combined in our analysis in order to include women who may not have been pregnant or had a bleeding outcome at registration, but subsequently did at an annual visit.

Independent Predictor Variables

The female UDC module collected demographic and health services information as well as clinical data regarding bleeding symptoms and treatments. Many of these characteristics and data could conceivably be associated with bleeding outcomes in pregnancy. The investigators chose items from the Registration and Annual Visit forms

that could reasonably be considered as possible predictors of risk for pregnancy-related bleeding using clinical acumen. The demographic variables that were examined include: race/ethnicity, age at diagnosis, age at menarche, and family history of a bleeding disorder. The health services variables included: frequency of HTC utilization, lost time from school, work, or recreation since last visit because of periods, and prenatal visit to a health care provider before most recent pregnancy to plan for a healthy pregnancy. The clinical variables included: VWD phenotype, baseline VWF:Ristocetin Cofactor activity level, ABO blood type, history of anemia, history of menorrhagia, , and history of other gynecologic abnormalities. Other gynecologic abnormalities included: bleeding ovarian cysts, fibroids or fibroid tumors, endometriosis, irregular cycles, breakthrough spotting, abdominal pain mid-cycle ("Mittlesmerz"), pain during menses (dysmenorrhea), uterine or cervical polyps, and uterine or cervical cancer. These other abnormalities were added together to produce a cumulative score ranging from 0 to 9.

There is an item (number 20 on the Registration form, 32 on the Annual Visit form) that asks about twelve bleeding symptoms and whether provider intervention was required for these symptoms. The data from this item was analyzed as follows: each "yes" response to a bleeding symptom was added cumulatively to obtain a total bleeding symptom score, ranging from a possible 0 to 12. Each "yes" response to a provider intervention was added cumulatively to obtain a total intervention score ranging from 0 to 12. These were included in the analysis as two distinct variables. The bleeding symptoms included the following: more than one nosebleed per year lasting 10 minutes or longer, oral mucosal bleeding lasting 10 minutes or longer, bleeding after dental procedures of concern to a health care provider, bleeding from minor cuts lasting 5 minutes or longer, bruises

larger than a quarter size occurring at least once a month without trauma, bleeding after surgery of concern to health care provider, menstrual bleeding that required protection change at least every 2 hours on heaviest day, bleeding with pregnancy/post-partum of concern to health care provider, joint bleeding, muscle bleeding, CNS bleeding, and GI bleeding. Because pregnancy and postpartum bleeding are included in the primary outcome, this item was discarded from the total bleeding symptom score and the total intervention score, making the possible range of responses from 0 to 11.

There is an item (number 23 on the Registration form, 35 on the Annual Visit form) that asks about specific treatments for bleeding problems and menorrhagia. These are broken down into categories: Medications/Devices, Surgeries, and Blood/Factor Products. The Medications/Devices category includes subcategories: antifibrinolytics, desmopressin, oral contraceptives, levonorgestrel IUD, and other hormonal contraceptives. Surgeries include: dilation and curettage, endometrial ablation, uterine artery embolization, hysterectomy, other gynecological surgery, and nasal cauterization. Blood/Factor products include: clotting factor products, blood or plasma products, and platelet transfusion. And answer of "yes" to treatment usage for any bleeding problems or menorrhagia was counted as one point for that subcategory, and points were added cumulatively to obtain a total score for each category, with a range of 0 to 5 for Medications/Devices, 0-6 for Surgeries, and 0-3 for Blood/Factor Products. These three categories of treatments were included in the analysis as three distinct variables. See Figure 1 for a complete list of the independent variables.

Fig. 1 Independent Variables

Demographic Variables	Clinical Variables
Race/Ethnicity	VWD phenotype
Age at diagnosis	Baseline VWF:RCof activity level
Age at menarche	ABO blood type
Family history of bleeding disorder	History of anemia
	History of menorrhagia
	History of other gynecologic abnormalities
Health Services Variables	Bleeding symptom score
Frequency of HTC utilization	Intervention score
Lost time from school, work, or recreation because of periods	Medication/Device treatment score
Prenatal visit to health care provider to plan for healthy pregnancy	Surgery treatment score
	Blood/Factor Product treatment score

Sample Size and Power Calculation

The sample size is fixed because the UDC system is no longer enrolling subjects. To estimate the magnitude of the relationships that will be detectable with this sample size we simplified the analysis plan assuming a dichotomization of the bleeding symptom score using a median split into high versus low symptom score. Based on a preliminary report of the UDC data published by Byams et al in 2011, the overall proportion of VWD subjects with pregnancy-related bleeding was reported to be approximately 48% (76.7% for miscarriage, 31.1% for pregnancy, and 37.6% for postpartum.[22]) If we assume the proportion with bleeding in the low symptom group is 35% and the proportion with bleeding in the high symptom group is 60%, a Fisher's exact test with a 0.05 two-sided significance level will have 80% power to detect the difference in bleeding proportion

between the groups when the sample size is 62 subjects per group, or 124 total subjects. The actual power to detect differences should be somewhat higher since using the continuous bleeding scores should result in an increased ability to detect relationships.

Univariate Analysis

Each of the independent variables was examined for distribution of responses, normalcy, potential outliers, and missing data. Variables with a large proportion of missing data were considered for exclusion. These included lost time from school, work, or recreation because of periods, and prenatal visit to health care provider to plan for a healthy pregnancy. Knowing that the results would be suspect, we included both these variables in the univariate analysis for the sake of completeness. Neither variable was significant.

Independent variables were examined between outcome groups using the Fisher's exact test or Pearson's chi-squared test for categorical variables. Student's *t*-test or logistic regression was used for continuous variables, including the bleeding score, intervention score, and treatment subcategory scores. Results are reported as means with standard deviations or medians with interquartile ranges. *p* values are reported and considered significant if $p < 0.05$.

Multivariable Analysis

All independent variables with a *p* value less than 0.15 in the univariate analysis were considered for inclusion in the multivariable logistic regression model. Mulicollinearity of any two variables was assessed by constructing a correlation matrix.

Spearman's rank correlation coefficient was used, with 0.25 or higher signifying a moderate correlation. When multicollinearity was detected, only one of the highly correlated variables was used in the multivariable logistic regression.

The exploratory multivariable logistic regression model was constructed in both the forward and backward stepwise fashion. Potential interactions were included in the regression model. The exploratory modeling used the minimum BIC criterion in order to avoid model overfitting. The results of the multivariable logistic regression model are reported with the parameter estimates, standard errors, odds ratios, and the 95% confidence intervals for the variables included in the final model.

Results

Study Population Characteristics

562 women were registered in the female UDC module. Of these women, 479 (85%) were identified having von Willebrand disease, with phenotypes as follows: 345 (72%) type 1, 52 (11%) type 2 (23 type 2A, 12 type 2B, 5 type 2M, 12 type 2N), 19 (4%) type 3, and 63 (13%) type unknown. 174 women of the 479 with VWD (36%) also reported having at least one pregnancy, and were eligible for inclusion in our study. There were 507 total current and historical pregnancies reported by these women. The outcomes of these pregnancies were reported as follows: 307 (67%) full term deliveries, 35 (8%) preterm deliveries, 73 (16%) first trimester miscarriages, 26 (6%) second trimester miscarriages, 12 (3%) elective terminations, 7 (2%) ectopic, tubal, or molar pregnancies, 1 (0.2%) stillbirth, and 46 (10%) uncategorized. Table 1 summarizes the study population.

Table 1. Study Population Characteristics (n= 174)

	n*	n (%)
Mean Age at Registration, years (range)	173	42 (17-83)
Race/Ethnicity	173	
White (non-Hispanic)		146 (84)
Other		27 (16)
Family History of Bleeding Disorder	122	
Yes		109 (89)
No		13 (11)
VWD Phenotype	174	
Type 1		126 (72)
Type 2		15 (9)
Type 3		10 (6)
Unknown		23 (13)
Mean Age at Diagnosis, years (range)	163	25.8 (0-76)
Mean VWF:RCof Baseline Activity, % (range)	83	51.6 (1-210)
Mean VWF: Ag Baseline Activity, % (range)	82	57.5 (0-140)
Mean F VIII Baseline Activity, % (range)	84	70.8 (9-192)

n* indicates the total number of women with available data for each characteristic. A number less than 174 indicates that there was missing data.

Primary Outcome

The 174 women who were included in this study all had at least one response (positive or negative) reported for one or more of the three pregnancy-related bleeding questions. Only positive responses ("yes" to bleeding) were counted as positive outcomes, thus items that had missing data were assumed to be negative. Among the 174 pregnant women with VWD there were a total of 62 (36%) who had miscarriages, and 52 (84%) of these women reported problems with bleeding during miscarriage. Of the 174 women who

reported pregnancy, 54 (31%) of these reported a problem with antepartum bleeding. Of 146 women who had a term or preterm delivery, 70 (48%) reported postpartum hemorrhage of concern to a health care provider. Outcomes for bleeding with miscarriage, antepartum bleeding and postpartum hemorrhage are reported in Table 2. The total number of women reporting bleeding with miscarriage, antepartum bleeding, and/or postpartum hemorrhage is 107 out of 174, or 61%.

Table 2. Primary Outcome: Bleeding in Pregnant Women with VWD

	Bleeding Incidence*
Bleeding with Miscarriage n = 62	52 (84%)
Antepartum Bleeding n = 174	54 (31%)
Postpartum Hemorrhage n = 146	70 (48%)
Total women reporting bleeding with miscarriage, antepartum, and/or PPH, n = 174	107 (61%)

** Bleeding with miscarriage calculated out of 62 total women who reported a miscarriage. Antepartum bleeding calculated out of 174 total women who reported a pregnancy. Postpartum hemorrhage calculated out of 146 total women who reported a full-term or pre-term delivery.*

Univariate Analysis Results

The results of the univariate analysis are reported in Table 3. Women who were more likely to have an unfavorable bleeding outcome were: those with a history of anemia (p = 0.0351), those who reported a higher number of gynecologic abnormalities (p = 0.0003), those with a higher bleeding symptom score (p <0.0001), those with a higher

intervention score (p = 0.0005), and those who had higher scores on all three subcategories of treatment for bleeding or menorrhagia: medication/device (p <0.0001), surgery (p = 0.0002), and blood/factor products (p = 0.0195). In addition to these variables which were significant at a level of p <0.05, age at diagnosis (p = 0.0505) was included in the multivariable analysis because it met the criterion of p <0.15 as specified in the analysis plan.

Table 3. Univariate Analysis Results

	Women without bleeding	Women with Bleeding	*p* value
Race/Ethnicity (n=173)			0.9472
White	57	89	
White Hispanic	3	6	
Black	5	8	
Black Hispanic	0	0	
Asian and Pacific Islander	2	1	
American Indian and Alaskan Native	0	1	
Other	0	1	
Von Willebrand Disease Phenotype (n=151)			0.5838
Type 1	43	83	
Type 2	5	10	
Type 3	5	5	
Baseline VWF:RCof Activity Level (median and IQ range) (n=83)	40.5 (30.25–59)	43 (27-64)	0.7266
ABO Blood Type (n=71)			0.4757
O	16	42	
A	4	6	
B	0	3	
AB	0	0	
Age at Diagnosis (median and IQ range) (n=163)	22 years (10.5-34)	28.5 years (17.75-38)	0.0505
Age at Menarche (median and IQ range) (n=172)	12 years (11-13)	12 years (11-13)	0.9208
HTC Utilization (n=107)			0.3893
Frequent (once per year)	27	48	
Infrequent (every 2-3 years)	10	12	
Rare (every 4 or more years)	1	1	
First Visit	1	7	
Family History of Bleeding Disorder (n=122)			0.5471
Yes	39	70	
No	6	7	
History of Anemia (n=124)			0.0351
Yes	32	81	
No	7	4	
History of Perceived Heavy Periods (n=156)			0.2839
Yes	53	100	
No	2	1	
Lost Time from School, Work, or Recreation (n=37)			0.4908
Yes	4	9	
No	11	13	

Prenatal Visit to Health Care Provider to Plan for Healthy Pregnancy (n=11)			0.4909
Yes	4	0	
No	5	2	
History of Other Gynecologic Abnormalities (Median and IQ range) (n=173)	2 (0-4)	3 (2-5)	0.0003
Bleeding Symptom Score (Median and IQ Range) (n=170)	3 (2-4)	5 (3-7)	<0.0001
Intervention Score (Median and IQ Range) (n=170)	1 (0-2)	2 (1-4)	0.0005
History of Medication/Device Use for Bleeding or Menorrhagia (Median and IQ Range) (n=163)	1 (1-2)	2 (1-3)	<0.0001
History of Surgery for Bleeding or Menorrhagia (Median and IQ Range) (n=163)	0 (0-1)	1 (0-2)	0.0002
History of Blood or Factor Products for Bleeding or Menorrhagia (Median and IQ Range) (n=163)	0 (0-1)	1 (0-2)	0.0195

Multicollinearity

Before attempting the multivariable model, Spearman's rank correlation coefficients were calculated and a matrix was constructed to assess for correlation between the independent variables identified in the univariate analysis. Two variables that are moderately or strongly correlated will essentially convey the same effect on the dependent variable. If two or more correlated variables are included in a multivariable model, the overall reliability of the model may be preserved, but the information about each individual variable may not be valid. Correlated variables tend to increase the standard errors of the coefficients, leading to misinformation about the significance of each independent variable in the model. The correlation matrix is reported in Table 4. To identify moderate or strong

correlation, we used a coefficient > 0.25 as the cutoff. (See Appendix for additional discussion.)

Table 4. Correlation Matrix with Spearman's Rank Correlation Coefficients

	Dx Age	Anemia	Other Gyn	Bleeding	Interven	Med/Dev	Surgery	Blood/Fac
Dx Age	1	0.0780	0.0649	0.0808	-0.1275	-0.0899	0.1859	-0.0232
Anemia	0.0780	1	0.0837	0.0336	0.1709	0.0236	0.0988	0.0764
Other Gyn	0.0649	0.0837	1	0.3774	0.2660	0.2780	0.2784	0.1033
Bleeding	0.0808	0.0336	0.3774	1	0.5888	0.4134	0.3660	0.2700
Interven	-0.1275	0.1709	0.2660	0.5888	1	0.2786	0.2891	0.3058
Med/Dev	-0.0899	0.0236	0.2780	0.4134	0.2786	1	0.3144	0.1376
Surgery	0.1859	0.0988	0.2784	0.3660	0.2891	0.3144	1	0.4447
Blood/Fac	-0.0232	0.0764	0.1033	0.2700	0.3058	0.1376	0.4447	1

The correlation matrix identifies two variables that are not correlated with any others: age at diagnosis, and history of anemia. The remaining variables all have correlation with the bleeding symptom score, with the strongest correlation between bleeding score and intervention score (0.5888). This is not a surprising finding given that a higher bleeding score would conceivably be associated with a higher number of interventions for bleeding. One can easily understand how the intervention score and the treatment subcategories would also be correlated, after all, each of the treatments is itself an intervention. We removed all the variables that were correlated with the bleeding score, leaving only three variables for our multivariable model: age at diagnosis, history of anemia, and bleeding symptom score.

Multivariable Analysis Results

The three independent variables were analyzed along with their interaction terms in a stepwise regression model. Forward and backward stepwise regression was performed using the minimum BIC (Bayesian Information Criterion) to select the best model and avoid overfitting. The final model included two independent variables: history of anemia, and bleeding symptom score. The parameter estimates, their standard errors, the odds ratios for bleeding versus no bleeding, and the 95% confidence intervals for the odds ratios are reported in Table 5. The odds of having pregnancy-related bleeding for women with a history of anemia compared to women without a history of anemia is 4.94 (95% CI 1.30-21.63). For the bleeding symptom score, each additional point in the cumulative score increases the odds of having pregnancy-related bleeding by 1.36 (95% CI 1.14-1.66), with an odds ratio of 21.75 over the entire range of scores.

Table 5. Multivariable Analysis Results

Term	Estimate	Std Error	Odds Ratio	Lower 95% CI	Upper 95% CI
Intercept	-1.2986	0.5724			
Anemia	0.7987	0.3508	4.9406	1.2989	21.6339
Bleeding	0.3079	0.0962	1.3606	1.1359	1.6611

Discussion

Women with VWD face a significant risk of bleeding during pregnancy and after delivery. In our study population the rates of bleeding with miscarriage, antepartum

bleeding, and postpartum bleeding were 84%, 31%, and 48%, respectively. In our effort to determine predictors of bleeding risk in this population we used the female module of the UDC and found that there are two risk factors that are associated with pregnancy-related bleeding in this group, 1) a history of anemia, and 2) a history of bleeding symptoms. Our results indicate that the odds of having pregnancy-related bleeding increase significantly if the patient has had anemia, as well as for each cumulative symptom recorded in the bleeding score. The final multivariable model is parsimonious and has an Area Under the Curve (AUC) of 0.74299 (see ROC curve in Appendix).

Significance

These results contribute to the growing body of literature that seeks to better understand the clinical and public health burdens faced by females with genetic bleeding disorders. This population, and the gynecologic and obstetric bleeding complications that they face have been largely overlooked due to the male sex-predilection of the second most common inherited bleeding disorder, hemophilia. In recent years there has been more attention paid to the unique bleeding challenges women face. As more women are enrolled in surveillance programs for inherited bleeding disorders, we can begin to better quantify the incidence, prevalence, and predictors for favorable and unfavorable bleeding-related outcomes.

Our results support the idea that a woman VWD who has a history of more severe bleeding symptoms is likely to have bleeding in the future, and underscores the need for an accurate bleeding history. Tosetto and colleagues found similar results when they looked at the mucocutaneous bleeding score and found it could significantly predict the risk for

surgical bleeding and bleeding after tooth extraction in men and women with type 1 VWD.[23] To the extent that a subject's recollection of bleeding symptoms is a reliable measure, it appears to be an important aspect of the history. Our results suggest that clinicians should pay special attention to the bleeding history and specifically to the number of different bleeding symptoms a woman reports when evaluating a woman with VWD, as it is likely to inform her risk for later bleeding, including during pregnancy.

Limitations

The female module of the UDC was designed as a targeted surveillance system to characterize the population of women with bleeding disorders, describe the severity and scope of those disorders, and monitor symptoms and outcomes. It was designed to be a prospective cohort, but most of the data was collected retrospectively. Women were asked to recall historical information about bleeding symptoms at the time of registration, and the data was updated on an annual basis. Since the data collection only spanned two years, the UDC was completed before the benefits of a longitudinal design could be fully realized for the female module. The retrospective nature of the data collection limits our ability to track outcomes over time and determine incidence of pregnancy-related bleeding. There is little information about the timing of risk factors compared to outcomes, which significantly restricts our ability to draw causal inferences between them. As such, our study and its results can serve only as an exploration of the possible risks that lead some women with von Willebrand disease to have pregnancy-related bleeding. These risks will need to be validated in prospective studies before they can gain widespread use in clinical practice.

There are additional limitations in this study design. Recall bias may interfere with accurate reporting by subjects. Provider subjectivity in answering the questionnaires may occur across the participating HTCs. To reduce this subjectivity, a glossary containing clarifications was distributed to the providers, but they may still have encountered ambiguity in questions like, "Did she have a problem with bleeding during miscarriage?" Presumably all miscarriages involve bleeding, and determining if it is significant enough to present a problem involves a subjective determination. Our inability to account for individual subjectivity has the potential to artificially inflate or deflate the rates of bleeding reported in this study.

Even for variables that should be free from subjectivity, such as baseline VWF activity and antigen levels, there appeared to be inaccurate reporting. Among 10 women with type 3 disease, who by definition should have an undetectable baseline level of VWF:RCof, the reported VWF:RCof levels for four of these subjects were 43%, 53%, 56%, and 94%. VWF:Ag levels for these women were reported as 60%, 71%, 79%, 105%, and 118%. These cannot be accurate baseline levels if these women truly have type 3 disease. For women with type 1 disease, who by definition should have a baseline VWF:RCof level less than 50%, the reported range of baseline levels was 1% to 207%, with a mean of 50%. Based on these observations, it is not surprising that there was no association of baseline VWF:RCof levels with bleeding outcome in our study. These baseline levels must be viewed with skepticism. With accurate measurement of VWF:RCof, VWF:Ag and FVIII, others have found a significant inverse relationship to the bleeding score and to bleeding outcomes.[23] Future prospective trials, in which study personnel have real-time communication with care providers, could alleviate this ambiguity.

Another limitation of our study is that it only captures information for women who have already been referred to a federally supported HTC. This group of women may not be representative of the broader population of females with VWD. While VWD is very common, it is also very heterogeneous, and many women with the disease may never come to the attention of a hematologist because they do not recognize symptoms of bleeding. The sample of women included in the UDC have come to medical attention, been referred to a specialty center, and therefore are likely to have more severe disease. The results of our analysis may only apply to women with VWD who have more severe phenotypes.

Lastly, we observed a fair amount of variability in the number of responses to each item on the questionnaires. Of the 174 women included in this study, some variables (age at first period, age at diagnosis) had almost complete response rates, while others (baseline VWF:RCof activity level) had very low response rates. Some of the low response rates are explained by the fact that some items were only on the Annual Visit form, and not all subjects had a follow-up visit within the two-year enrollment of the female UDC module. The variables included in our final multivariable model, history of anemia and bleeding symptom score, had 124 and 170 responses each, respectively.

Future Directions

We have demonstrated that pregnancy-related bleeding outcomes are common in the cohort of women enrolled in the female module of the UDC. These unfavorable outcomes represent a significant burden not only to the women themselves, but also to providers caring for these at-risk women. Our results indicate that a bleeding history and a

history of anemia may be helpful in identifying women who are at the greatest risk for bleeding, but there is more work that needs to be done. Specifically, more attention needs to be paid to the manner in which providers take a bleeding history, and the instruments used.

There are several bleeding score questionnaires that have been used in patients with bleeding disorders, most notably the Vicenza and its iterations, which have been previously studied in von Willebrand's disease.[23-25] This instrument was the basis for the bleeding symptom assessment in the female UDC, although the Vincenza Score takes the severity of symptoms into account whereas the UDC does not. In order to validate using a bleeding assessment tool for the monitoring and risk-stratification in women with bleeding disorders, a prospective study would need to be undertaken. Such a study, if continued for a sufficient length of time, would give invaluable insight into the precise role that bleeding symptoms play in the prediction of future bleeding episodes, including those that occur during pregnancy. Thus far the usefulness of bleeding scores has been mostly in their negative predictive value for screening for possible bleeding disorders, i.e. they have high sensitivity and low specificity. It remains to be seen if they can be used prospectively to accurately risk-stratify bleeding disorder patients. They are also time consuming and require provider administration—they have not been validated for self-reporting.[26-28]

Analysis of the female UDC module allows us to reflect on the characteristics of this surveillance system that were effective, and to consider what will be important for the next generation of surveillance tools. Explicitly worded questions, focused on the age-related presentation of symptoms and their resulting management will help to not only characterize the population being served by HTCs, but also to drive hypothesis-driven

research that will improve outcomes over time. The next phase of surveillance is already underway at HTCs in collaboration with the CDC and the American Thrombosis & Hemostasis Network (ATHN). The CDC Registry for Bleeding Disorders Surveillance Project aims to learn more about the bleeding and clotting disorders community, understand how patients use the health services offered at HTCs, and monitor the health of these patients.

In has become increasingly evident that women with bleeding disorders face challenges in hemostasis during pregnancy. Some of these women receive care from an obstetrician, some from a hematologist, but we propose that the best care is provided when these two disciplines work together in a proactive approach. Of note, only 11 of the 174 women had data available for the question, "Before patient became pregnant with most recent pregnancy, did she see a health care provider to plan for a healthy pregnancy?" but of the 4 women who responded "yes," none of them experienced any pregnancy-related bleeding.

Chapter 2: Statistical Appendix

Statistical Approach to Univariate Analysis

As per the planned analysis, the independent variables were compared to the outcome variable using the statistical test that suited the data. The outcome was binary in all cases, women either had a bleeding complication or they did not. The statistical software package, JMP 11 Pro (SAS Institute, 2013), was used for all analyses. JMP will automatically choose which statistical test to use dependent upon how the independent variables are categorized by the user as continuous, ordinal, or nominal. For the continuous variables (VWF baseline activity, Age at diagnosis, Age at first period, Other gynecologic abnormalities, Bleeding symptom score, Intervention score, Medication/Device use for bleeding or menorrhagia, Surgery for bleeding or menorrhagia, and Blood/Factor products for bleeding or menorrhagia), a logistic regression was used to compare the two outcome groups. For the nominal and categorical independent variables (Race/ethnicity, Disease phenotype, ABO bloodtype, HTC utilization, Family history of bleeding disorder, History of anemia, History of perceived heavy periods, Visited healthcare provider to plan for a healthy pregnancy, and Lost time from school, work, or recreational activities), the Chi-squared test was used to compare the frequencies in the outcome groups. In this study, every independent variable had at least one cell in the contingency table with less than 10 subjects, and almost all of them had at least one cell with less than 5, therefore in order to avoid relying on the approximation of the Chi-squared distribution, we used the Fisher's exact test.

There were 18 independent variables examined in this study. We know that Type I error increases dramatically as the number of comparisons increases, leading to falsely positive significance in multiple hypothesis testing. In order to avoid this pitfall, we chose only the variables that we believed could be plausible predictors of the outcome. In addition we assessed the variables for multicollinearity to make sure they were not redundant, and then we fit the significant variables into a multivariable logistic regression in order to identify as few variables as necessary to achieve a well-fit model.

Statistical Approach to Multivariable Analysis

To assess the degree of association between variables we constructed a correlation matrix. Spearman's rank correlation coefficient was examined for each pair of independent variables and plotted in the matrix. The scatterplots for each coefficient were also generated. We chose to use the Spearman's rank correlation coefficient, a non-parametric equivalent of the Pearson's correlation coefficient, because our variables were not all continuous and not all normally distributed. We pre-specified 0.3 as the magnitude of the correlation that we considered to be significant, although this is admittedly an arbitrary cutoff. We sought to identify moderate and strong correlations, knowing that several of our independent variables were already suspect to be correlated. For example, we could reasonably expect that those women with a higher bleeding score would also have a higher intervention score, because intervention is a direct result of having bleeding. In addition, it was considered likely that the three subcategories of treatment for bleeding or menorrhagia were a representation of the all-encompassing Intervention score, and thus might be correlated. We chose to err on the side of detecting lower levels of correlation,

and when we evaluated the *p* values for our correlation coefficients, we noted that even using a cutoff of 0.25 for the correlation coefficient resulted in significant *p* values < 0.05, and thus we lowered our threshold to 0.25.

Multivariable Model

After excluding the variables that were found to be correlated in the assessment of multicollinearity, we were left with only three independent variables from the univariate analysis to include in the multivariable model. Age at diagnosis, History of anemia, Bleeding symptom score, and their interaction terms were included in the forward and backward stepwise regression, and the model with the minimum BIC was selected. We used the minimum Bayesian Information Criterion (BIC) because it penalizes the model more strongly than the Akaike Information Criterion (AIC) for free parameters. Either criterion would have been acceptable. The final model included only the intercept, History of anemia, and Bleeding symptoms score. The resulting equation for our multivariable model became:

$$\text{Logit (bleeding outcome)} = -1.30 + 0.80(\text{Anemia}) + 0.31(\text{Bleeding})$$

The standard error of the estimates and the odds ratios and 95% confidence intervals are reported in the results.

Alternatively, since only three independent variables were considered for the multivariable model, we could have done away with forward and backward stepwise

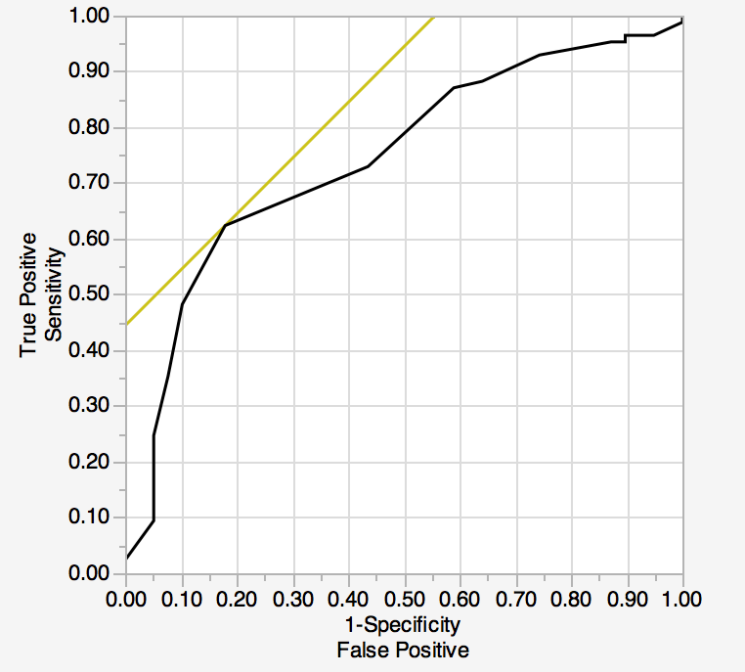
regression and just fit the model with all three variables. The results would have looked like:

Logit (bleeding outcome) = -1.73 + 0.023(Dx age) + 0.74(Anemia) + 0.28(Bleeding)

In this model the BIC was slightly higher and the estimate for Dx age was not strictly significant (p = 0.1316). This model would be an acceptable alternative, but since we pre-specified our analysis plan using stepwise regression and the minimum BIC, we chose to select the first model.

Adequacy Tests of the Model

An ROC curve was plotted for the selected multivariable model using History of anemia and Bleeding symptom score:



The Area Under the Curve (AUC) for this model is 0.74299. We would expect the AUC representing chance alone to be 0.50, and the AUC for a perfect model to be 1.00, therefore our model is only moderately predictive of the outcome. A better preforming model would have a higher AUC closer to 1.

To further assess the adequacy of our model we can look at the whole model - LogLikelihood Chi-square, which compares the whole model fit to the model that omits the explanatory variables except for the intercept. It essentially tests the hypothesis that all explanatory parameters are zero. The *p* value in this case is the probability of obtaining a greater Chi-square value by chance alone if the specified model fits no better than the model that includes only the intercept. The *p* value for the whole model Chi-square is 0.0002, suggesting that our model does fit the data better than a model that includes only the intercept.

Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	8.526527	2	17.05305	0.0002*
Full	68.684126			
Reduced	77.210653			

RSquare (U)	0.1104
AICc	143.568
BIC	151.829
Observations (or Sum Wgts)	124

We can also assess the predictive efficacy of our model, in effect the ability of our model to correctly discriminate between those who have the outcome (bleeding) versus those who do not have the outcome (no bleeding). To do this, we can construct a 2x2 table. On one axis we plot the predicted outcomes using the model and on the other axis, the

actual outcomes. To obtain the predicted outcome, we calculate the predictive probability of each subject using the regression equation, and assign an outcome of 1 if the probability is >0.5 and an outcome of 0 if the probability is <0.5. The 2x2 table is shown below:

Predicted Outcome	Count / Total % / Col % / Row %	Bleeding Outcome 0	Bleeding Outcome 1	
0		8	6	14
		6.45	4.84	11.29
		20.51	7.06	
		57.14	42.86	
1		31	79	110
		25.00	63.71	88.71
		79.49	92.94	
		28.18	71.82	
		39	85	124
		31.45	68.55	

Our model correctly predicted the bleeding outcome of 8 out of 39 subjects without bleeding and 79 out of 85 subjects with bleeding. The Sensitivity is 79 / 85 = 0.93. The Specificity is 8 / 39 = 0.21. Our model does quite well predicting those with the bleeding outcome (it misses few who actually had bleeding), but has a high false positive rate. Though a higher degree of specificity would be ideal, this is an acceptable outcome for our model. We would rather be overly sensitive to predicting bleeding outcomes in pregnant women than the alternative, to be overly specific but risk missing those who are likely to have bleeding.

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