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Laboratory Misdiagnosis of von Willebrand Disease in Post-Menarchal Females: A Multi-Center Study

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Abstract

Increased awareness of von Willebrand Disease (VWD) has led to more frequent diagnostic laboratory testing, which insurers often dictate be performed at a facility with off-site laboratory processing instead of a coagulation facility with onsite processing. Off-site processing is more prone to preanalytical variables causing falsely low levels of von Willebrand Factor (VWF) due to the additional transport required. Our aim was to determine the percentage of discordance between off-site and onsite specimen processing for VWD in this multicenter, retrospective study. We enrolled females aged 12 to 50 years who had off-site specimen processing for VWF assays, and repeat testing performed at a consulting institution with onsite coagulation phlebotomy and processing. A total of 263 females from 17 institutions were included in the analysis. There were 251 subjects with both off-site and onsite VWF antigen (VWF:Ag) processing with 96 (38%) being low off-site and 56 (22%) low onsite; 223 subjects had VWF ristocetin co-factor (VWF:RCo), 122 (55%) were low off-site and 71 (32%) were low onsite; similarly, 229 subjects had a Factor VIII (FVIII) assay, and 67 (29%) were low off-site with less than half, 29 (13%) confirmed low with onsite processing. Higher proportions of patients demonstrated low VWF:Ag, VWF:RCo, and/or FVIII with off-site processing compared to onsite (McNemar's test p-value < 0.0005, for all

assays). These results emphasize the need to decrease delays from sample procurement to processing for VWF assays. VWF assays should ideally be collected and processed at the same site under the guidance of a hematologist.

Introduction

Von Willebrand Disease (VWD) is the most common inherited bleeding disorder, estimated to affect up to 1% of the population [1,2]. Mucocutaneous bleeding is the hallmark of VWD, and is typified by epistaxis, bruising, gum bleeding, gastrointestinal bleeding, bleeding with trauma or surgery, as well as heavy menstrual bleeding (HMB). In post-menarchal females, HMB may be the only presenting symptom of VWD [3], and up to one-quarter of women and adolescents with HMB have VWD [4-6]. Unfortunately, many females with VWD have a delay in diagnosis on average of 16 years despite extensive bleeding [3,7]. To prevent the delays in diagnosis and subsequent complications, The American College of Obstetricians and Gynecologists (ACOG) advised that all patients with HMB, including adolescents, be screened initially by history and then by laboratory testing for an underlying disorder of hemostasis such as VWD, which has resulted in an increased evaluation by non-hematology specialists [8,9].

The laboratory diagnosis of VWD is based on three main assays, a quantitative measure of von Willebrand Factor (VWF) in the plasma, the activity of VWF and

specifically its ability to bind platelets and the activity of Factor VIII (FVIII) [10]. Multiple clinical variables, such as physiologic increases in VWF due to stress, illness, and cyclical estrogen levels in post-menarchal females, as well as physiologic decreases in VWF due to blood type, make correctly diagnosing VWD challenging [3,11]. Additionally, pre-analytical and laboratory variables such as inter-assay variability, poor assay sensitivity, sample collection technique, transportation, and storage contribute to the complexity of making an accurate diagnosis [12-15].

Insurers often dictate VWF assays to be performed at a laboratory within network (with off-site specimen processing), which is often not a hospital or academic based coagulation laboratory with onsite specimen processing. Onsite coagulation processing laboratories can collect and process samples in a timely manner, this is in contrast to a laboratory where sample collection and processing often take place at different locations leading to inevitable delays. Such a delay results in artificially low VWF or FVIII levels due to preanalytical variables, outside the control of the laboratories analyzing the samples [15,16]. Patients may then be mislabeled as having the laboratory diagnosis of VWD, leading to inappropriate interventions or inadequate therapy with attendant risks, lack of evaluation for alternate bleeding

conditions and increased health care costs due to repetitive testing, consultation and treatment [17,18].

To determine the consequences of collecting samples for VWF testing and sending them off-site for processing compared to onsite processing, a multi-site study was conducted. This study, to our knowledge is the first multi-institutional evaluation of VWF testing in post-menarchal females to determine the percentage with discordant testing results when comparing off-site to onsite coagulation processing.

Methods

Study Design

We present a retrospective study conducted at 17 institutions throughout the United States from July 2013 to October 2017. All participating institutions were part of The Foundation for Women and Girls with Blood Disorders, Learning Action Network (FWGBD LAN). This network was created to provide collaboration, education and resources for clinicians who care for women and girls with bleeding and other blood disorders. The study was approved by the institutional review board or given an exemption status at each institution.

Study objectives

Our primary objective was to determine the percentage of post-menarchal female patients with discordant VWF testing results when blood samples were drawn and processed at the same coagulation laboratory versus when phlebotomy and processing were performed at different sites. Secondary objectives were to determine how often the referring physician (1) performed partial versus complete testing for VWD and (2) referred a patient to a hematologist for VWD and they were diagnosed with an alternative bleeding disorder.

Study population

Eligible subjects were females of reproductive age, from 12 to 50 years who were referred to a hematologist due to the concern for a bleeding disorder. All eligible subjects had testing for VWD with off-site processing, which included at minimum a VWF antigen (VWF:Ag) and/or VWF activity/ristocetin co-factor (VWF:RCo) assay. Study subjects were then required to have VWF testing repeated at a consulting institution under hematology supervision with onsite coagulation test phlebotomy and processing. Subjects were excluded from the study if their hematology consultation was for reasons other than a suspected bleeding disorder

or if subjects were referred from another center with onsite coagulation laboratory processing and analysis.

Study procedures

Participating institutions identified and consecutively enrolled eligible subjects who had at least one visit with the consulting hematologist between July 2013 to October 2017. The subjects' medical records were reviewed retrospectively, including any medical history and laboratory work performed prior to the hematology consultation. Onsite processing was defined as using a hospital where phlebotomy and specimen processing were performed at the same laboratory, which did not include testing performed at satellite laboratories. Off-site processing was defined as using a clinic, community hospital or private facility where the phlebotomy and specimen processing were not performed at the same location. For onsite processing, plasma for the VWF assays was either analyzed onsite immediately after processing or frozen and later analyzed onsite or analyzed at a different location. A comparison was not made between evaluating processing and analyzing of VWF assays onsite to processing onsite and sending frozen samples off-site for analysis. Analyzing procedures were not collected for assays performed after off-site processing.

Low VWF was defined as a VWF:RCo or VWF:Ag of 30-50% (or 30-60% depending laboratory reference range) while type 1 VWD was defined as VWF:Ag or VWF:RCo <30% on at least one occasion. Prolongation of the prothrombin time (PT), activated partial thromboplastin time (aPTT) and specific factor deficiencies were determined based on individual laboratory reference ranges. The only coagulation factor activities routinely collected were FVIII and VWF. Hypofibrinogenemia was defined as a fibrinogen activity less than 150 mg/dL. The consulting hematologist at each institution defined pertinent bleeding symptoms (such as HMB) and determined the final diagnosis as documented in the medical record.

Data characteristics

Standardized case report forms through Research Electronic Data Capture (REDCap), a free, secure, web based, HIPPA compliant application were used to collect subject data. Information collected prior to hematology consultation included: subject age, specialty of referring physician, referral reason, results of VWF assays (VWF:Ag, VWF:RCo, FVIII activity and/or VWF multimer distribution), type of off-site laboratory that performed VWF assays, distance from phlebotomy site to processing site that performed VWF assays and value for other hematologic and coagulation testing performed (hgb, platelet count, PT, aPTT and fibrinogen).

Data collected at the hematology consultation included: subject age, bleeding symptoms, estrogen use, results of VWF assays, institutional procedure for VWF assay processing and analysis, value for other hematologic and coagulation testing performed identical to above, including platelet function assay (PFA-100™) and platelet aggregometry (whole blood impedance and light transmission aggregometry) results. Final diagnosis was collected and determined by the consulting hematologist after clinical and laboratory evaluation.

Statistical methods

The primary objective of the statistical analysis was to estimate the proportion of subjects with low VWF assays based on off-site processing laboratory values and were confirmed (i.e. concordant) when these laboratory assays were repeated at the consulting hospital with onsite phlebotomy and processing. VWD or low VWF diagnosis was classified first based only on the off-site values, and then based only on the onsite values, and concordance between these classifications was calculated. Basic summary statistics on patient age and data collected prior to and at consultation were provided.

McNemar's test was utilized on paired test results to examine the proportions of patients with low- or high-test results pertaining to VWF status, hematology or

coagulation function. The exact p-value from the McNemar's test was provided whenever frequency counts fell at or below 5. A two-sided p-value < 0.05 was deemed significant. All computations were completed using the statistical software Stata version 11[19]. Missing data results for VWF assays were excluded and analysis was performed only on subjects with paired VWD assay results. Loss to follow up was relevant for final diagnosis only and reported as unknown.

Results

Characteristics of subjects

The 17 institutions identified 278 subjects between July 2013 and October 2017. Fifteen subjects were under 12 years of age at the time of diagnostic testing and excluded from the analysis. The main analytic cohort included a total of 263 females. The median subject age when VWF assays were drawn by the referring physician visit was 15 years (range 12-50), and the majority of subjects, 72% (189), had HMB as their referring complaint (Table 1). Primary care physicians were most often the referring physician (63%, n=167) followed by obstetricians/gynecologists (24%, n=64).

The median age at the hematology consultation was 15 years (range 12-50) and 83% (217) reported HMB, followed by easy bruising 37% (96) and epistaxis 30%

(80) stated in their initial hematology consult note. Testing was conducted while on hormonal contraception in 99 (38%) subjects at the initial hematology consultation.

Von Willebrand factor testing

A total of 251 (95%) subjects had both off-site and onsite sample processing assays for VWF:Ag, 223 (84%) subjects for VWF:RCo, and 229 (87%) subjects for FVIII. Of the 251 subjects who had VWF:Ag testing, 96 (38%) had low VWF:Ag with off-site processing, but only 56 (22%) had low VWF:Ag with onsite processing (Figure 1). Among 223 subjects with VWF:RCo results, 122 (55%) had low VWF:RCo with off-site processing, while only 71 (32%) were confirmed with onsite processing. Similarly, among 229 subjects with paired FVIII assay results, 67 (29%) were low with off-site processing and less than half, 29 (13%), were confirmed with onsite processing. Higher proportions of subjects demonstrated low VWF:Ag, VWF:RCo, and/or FVIII with off-site processing compared to onsite (McNemar's test p-value < 0.0005, for all assays) (Figure 1). Eighty-six (33%) subjects had normal or elevated VWF:Ag and/or VWF:RCo prior to their hematology consultation.

All three VWF assays (VWF:Ag, VWF:RCo and FVIII) were sent in 210 (80%) subjects by referring physicians. Referring physicians sent two assays, VWF:Ag

and VWF:RCo in 8 (3%) subjects, and only one assay (either VWF:Ag or VWF:RCo) in 44 (17%) subjects. At the onsite consulting institutions, VWF assays were collected, processed and frozen for future analysis in 68%-73% of subjects depending on the assay. Assays were analyzed immediately after processing in 10-18% of subjects at the onsite institutions. Consulting hematologists repeated VWF testing more than once for 74 (28%) subjects. Subjects with subnormal VWF assays at onsite institutions were routinely re-tested again onsite.

Among the 56 subjects with low VWF:Ag onsite, 31 had repeat testing onsite and 23 (74%) continued to be low. Twenty-nine of the 71 subjects had repeat VWF:RCo onsite after being initially low onsite and 24 (83%) continued to be low; 11 of the 29 subjects with initial low FVIII onsite had repeat testing and 8 subjects (73%) continued to have low levels onsite. In comparison, among subjects with low VWF:Ag with off-site processing, 58% (56/96) were low when repeated onsite. Among the subjects with low VWF:RCo after off-site processing, 58% (71/122) were low when repeated onsite, and among subjects with low FVIII with off-site processing, 43% (29/67) were low when repeated on-site.

Other hematologic testing

There were 89 subjects (34%) who had a PT performed with both off-site and onsite processing, with 15 subjects initially having a prolonged PT and 10 (66%) normalized when retested with onsite processing (p=0.09) (Table 2). Only 117 subjects (44%) had an aPTT performed with off-site processing and then repeated onsite processing. A prolonged aPTT was noted in 26 subjects with off-site processing and 17 (65%) normalized when retested with onsite processing during the hematology consultation (p=0.041). A fibrinogen level was evaluated in 26 subjects by the referring physician and in 158 subjects by the consulting physician. Hypofibrinogenemia was identified in only one subject by the consulting hematologist, and final diagnosis was not made due to lack of follow up.

PFA-100™ was performed in 121 subjects by the consulting hematologist, and 73 (60%) subjects had a normal result. Of the 48 subjects with abnormal results, 13 subjects had isolated prolongation of the collagen/epinephrine cartridge, 12 subjects had isolated prolongation of the collagen/adenosine diphosphate (ADP) cartridge and 23 subjects had prolongation of both closure times. The majority of subjects (n=33, 69%) with abnormal PFA-100™ results were given the diagnosis of VWD or low VWF. Other identified bleeding disorders in subjects with abnormal PFA-100™ results included immune thrombocytopenia purpura, platelet dysfunction and factor deficiency.

Platelet aggregation testing was performed in 60 (23%) subjects at the consulting institution and 10 subjects had an abnormality with at least one agonist. The majority of abnormalities were with epinephrine or ADP secretion. One subject was given the diagnosis of VWD and had decreased aggregation to both ristocetin and epinephrine. Other identified bleeding disorders consisted of platelet dysfunction, immune thrombocytopenia purpura and factor deficiency.

Final diagnosis

Less than 40% (100) of the subjects were ultimately diagnosed with VWD by the consulting hematologist. Of the 100 subjects, 94 (36%) were diagnosed with type 1 VWD, 5 (2%) with type 2 and 1 subject was diagnosed with type 3 VWD. Less than 20% (47) of the subjects were diagnosed with an alternative bleeding disorder such as low VWF (7%, n=19), factor deficiency (2%, n=6), platelet dysfunction (2%, n=5), and other unclassified bleeding disorder (6%, n=17). However, approximately 40% of the subjects referred to a hematologist for the concern of a bleeding disorder had a normal hemostatic evaluation (Figure 2).

Discussion

Increased awareness that HMB may be the only bleeding symptom in young women has led to primary care physicians and obstetricians/gynecologists to conduct initial hemostatic evaluation for VWD [8,9]. Primarily regulated by hospital and insurance contracts, these physicians typically send the VWF assays to coagulation laboratories with offsite processing that are generally more cost efficient due to the high volumes of testing compared to hospital-based laboratories with onsite processing. In many cases these companies with off-site processing have multiple locations for phlebotomy that can be more convenient for patients compared to academic-based coagulation laboratories that may require patients to travel far distances. In this large, multi-institutional study, significant differences were seen between assays drawn and processed off-site where phlebotomy and processing are conducted in separate locations compared to samples drawn and processed in one location (onsite). Abnormal VWF:Ag, VWF:RCo, and/or FVIII results identified with off-site testing normalized in 40-60% of the subjects when retested with onsite processing under the guidance of a consulting hematologist.

We do acknowledge the possibility of VWF variation within an individual. Subjects who initially had low VWF assay results at laboratories with off-site processing may have had a stress induced response when tested at laboratories with onsite

processing causing a normalization of the results or they may have a rapid clearance of their VWF such as in type 1C VWD [20]. This may also reflect the inherent variability of VWD where 16 different patterns of VWF:RCo, VWF:Ag, FVIII and bleeding time have been reported in a well described cohort of 50 individuals in 25 families [21]. However, it appears the main discordance between assay results found in our study is most likely secondary to delayed or inappropriate processing of the specimen prior to analyzing. Concerns have been raised that mislabeling of VWD is increasing due to these pre-analytical variables such as delay in centrifuging the sample or heat inactivation or cold activation of the sample that are outside of the scope of the analyzing laboratory [22]. Due to the concern for misdiagnosis, many patients inevitably require VWF testing to be performed multiple times to establish a diagnosis or confirm normal VWF assay results, especially when they were previously low with off-site processing [17]. Our study found one-quarter of the subjects had VWF assays repeated up to 3 times at the consulting institution in order to confirm the final laboratory diagnosis of VWD. Depending on the assay, 6-21% of subjects who initially had normal results with off-site processing had low levels with onsite processing, which may be due to stress induced elevation at the first blood draw with the referring physician. These variations underscore the need for repeat testing in patients with normal

testing results and significant bleeding or to confirm VWD in patients with low VWF assay results.

Our results are consistent with a previous, smaller, single institution study, which found normalization of VWF assays in 73-100% of subjects when the off-site testing was repeated at their onsite facility [23]. Our large, multi-institutional study provides additional verification that the use of laboratories with off-site processing with potential multiple pre-analytical variables affects VWF assays, causing falsely low levels and misdiagnosis of VWD.

Although primary care physicians and non-hematology specialists are ordering VWF testing, our study found referring physicians sent a partial work-up for VWD in 20% of subjects. Thus, although referring physicians are considering the diagnosis of VWD and bleeding disorders in females with HMB, attention to improved education regarding the required laboratory investigations for VWD testing is necessary. In addition, due to the challenges of interpreting VWF assays as well as the inherent variability of VWF levels, consultation with hematologists for final VWD diagnosis is imperative. Although laboratory assay results are crucial a detailed personal history of bleeding and family history of bleeding or VWD are important components of this diagnosis as well as newer assays such as collagen

binding and GPIbM assays which are now becoming standard to assist with establishing an accurate diagnosis. For patients with negative or inconclusive VWF testing, additional evaluation by a hematologist is also necessary to evaluate for other bleeding disorders when the clinical context warrants.

Differences were not as dramatic between PT and aPTT assays when tested with off-site and onsite processing as expected. Since it was not a requirement of the study to have a PT and aPTT obtained by the referring or consulting physician, this was only performed in 60-65% of the subjects. The decreased sample size may have added to a lack of significance seen between these assay results.

Overall, less than half of the subjects were ultimately diagnosed with VWD or low VWF, and 39% had a normal hemostatic evaluation. Many patients with significant bleeding and normal coagulation testing are given the diagnosis of unknown cause (BUC). Prevalence of BUC may be as high as 40% in patients undergoing investigation for a bleeding disorder [24]. These patients still suffer from typical mucocutaneous bleeding, such as HMB, post-surgical bleeding and bleeding after dental extractions [25]. With almost 40% of our subjects with significant bleeding, but a normal hemostatic work-up, improved specialized coagulation testing and further investigation of bleeding causes in this patient group are needed. Hopefully, next

generation gene sequencing will uncover underlying disorders of hemostasis in such patients [26].

This study does have several limitations besides its retrospective design. The first is the inability to determine the specific duration of time or transit details between sample collection to off-site processing. The actual duration may have been minutes or hours and the transportation of the blood samples may have been in a temperature cooler or stored only in collection bags. Details regarding hospital policies for VWF assay collection, processing and analyzing were collected for consulting institutions with onsite processing, but were not available for laboratories with off-site processing. A second limitation is that not all potential subjects were included into the study due to missing laboratory information (typically from the referring physicians). Data from missing subjects would not have affected the main results of the study but may have provided a larger sample size to identify statistical differences. A third limitation arises from the VWF assays: the wide coefficient of variation of the VWF assays and VWF assays in the 20% range and the fact that the assays were not done with identical instrumentation and reagents [27]. To address these limitations, a study could be designed to simultaneously draw two samples: one to be processed onsite and one to processed off-site with both locations following a specific protocol.

In conclusion, HMB is increasingly recognized as a symptom of an underlying bleeding disorder, leading to increased evaluation, diagnosis, and treatment of bleeding disorders in women. Significant differences were seen between assays drawn and processed off-site where phlebotomy and processing are in separate locations compared to samples drawn, prepared and processed in one location. These results highlight the need for VWF testing to ideally be both drawn and processed with little delay at laboratories with onsite processing under the guidance of a hematologist.

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References

1. Werner EJ, Broxson EH, Tucker EL, Giroux DS, Shults J, Abshire TC. Prevalence of von Willebrand disease in children: a multiethnic study. *J Pediatr*. 1993;123(6):893-898.
2. Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. *Blood*. 1987;69(2):454-459.

3. Ragni MV, Bontempo FA, Hassett AC. von Willebrand disease and bleeding in women. *Haemophilia.* 1999;5(5):313-317.
4. Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Frequency of inherited bleeding disorders in women with menorrhagia. *Lancet.* 1998;351(9101):485-489.
5. Dilley A, Drews C, Miller C, et al. von Willebrand disease and other inherited bleeding disorders in women with diagnosed menorrhagia. *Obstet Gynecol.* 2001;97(4):630-636.
6. Shankar M, Lee CA, Sabin CA, Economides DL, Kadir RA. von Willebrand disease in women with menorrhagia: a systematic review. *BJOG.* 2004;111(7):734-740.
7. Kirtava A, Crudder S, Dilley A, Lally C, Evatt B. Trends in clinical management of women with von Willebrand disease: a survey of 75 women enrolled in haemophilia treatment centres in the United States. *Haemophilia.* 2004;10(2):158-161.
8. Committee on Adolescent Health C, Committee on Gynecologic P. Committee Opinion No.580: von Willebrand disease in women. *Obstet Gynecol.* 2013;122(6):1368-1373.
9. Screening and Management of Bleeding Disorders in Adolescents With Heavy Menstrual Bleeding: ACOG COMMITTEE OPINION SUMMARY, Number 785. *Obstet Gynecol.* 2019;134(3):658-659.
10. Branchford BR, Di Paola J. Making a diagnosis of VWD. *Hematology Am Soc Hematol Educ Program.* 2012;2012:161-167.
11. Kadir RA, Economides DL, Sabin CA, Owens D, Lee CA. Variations in coagulation factors in women: effects of age, ethnicity, menstrual cycle and combined oral contraceptive. *Thromb Haemost.* 1999;82(5):1456-1461.
12. Lippi G, Franchini M, Montagnana M, Salvagno GL, Poli G, Guidi GC. Quality and reliability of routine coagulation testing: can we trust that sample? *Blood Coagul Fibrinolysis.* 2006;17(7):513-519.
13. Favaloro EJ, Lippi G, Adcock DM. Preanalytical and postanalytical variables: the leading causes of diagnostic error in hemostasis? *Semin Thromb Hemost.* 2008;34(7):612-634.
14. Favaloro EJ, Bonar R, Marsden K. Lower limit of assay sensitivity: an under-recognised and significant problem in von Willebrand disease identification and classification. *Clin Lab Sci.* 2008;21(3):178-183.
15. Kim YA, Lewandrowski KB, Lucien FA, Van Cott EM. The effects of transport temperature and time on routine and specialized coagulation assays. *Blood Coagul Fibrinolysis.* 2018;29(2):184-188.
16. Favaloro EJ, Soltani S, McDonald J. Potential laboratory misdiagnosis of hemophilia and von Willebrand disorder owing to cold activation of blood samples for testing. *Am J Clin Pathol.* 2004;122(5):686-692.

17. Doshi B, Rogers, R., Whitworth H., Stabnick, E., Britton, J., Butler, R. Utility of Repeat Testing in the Evaluation for Von Willebrand Disease in Pediatric Patients. *Blood.* 2018;132:981.
18. Sidonio RF, Jr., Smith KJ, Ragni MV. Cost-utility analysis of von Willebrand disease screening in adolescents with menorrhagia. *J Pediatr.* 2010;157(3):456-460, 460 e451.
19. *Stata Statistical Software* [computer program]. College Station, TX2009.
20. Sharma RaH, S.L. New Advances in the diagnosis of von Willebrand disease. American Society of Hematology 2019; Orlando, FL.
21. Abildgaard CF, Suzuki Z, Harrison J, Jefcoat K, Zimmerman TS. Serial studies in von Willebrand's disease: variability versus "variants". *Blood.* 1980;56(4):712-716.
22. Preston FE, Lippi G, Favaloro EJ, Jayandharan GR, Edison ES, Srivastava A. Quality issues in laboratory haemostasis. *Haemophilia.* 2010;16 Suppl 5:93-99.
23. Coyle T, Gopaluni, S., Newman, N., Hansen, J. Milk Box Misdiagnosis of Von Willebrand Disease Is Common. American Society of Hematology; 2008.
24. Quiroga T, Goycoolea M, Panes O, et al. High prevalence of bleeders of unknown cause among patients with inherited mucocutaneous bleeding. A prospective study of 280 patients and 299 controls. *Haematologica.* 2007;92(3):357-365.
25. Obaji S, Alikhan R, Rayment R, Carter P, Macartney N, Collins P. Unclassified bleeding disorders: outcome of haemostatic challenges following tranexamic acid and/or desmopressin. *Haemophilia.* 2016;22(2):285-291.
26. Downes K, Megy K, Duarte D, et al. Diagnostic high-throughput sequencing of 2396 patients with bleeding, thrombotic, and platelet disorders. *Blood.* 2019;134(23):2082-2091.
27. de Maat MP, van Schie M, Kluft C, Leebeek FW, Meijer P. Biological Variation of Hemostasis Variables in Thrombosis and Bleeding: Consequences for Performance Specifications. *Clin Chem.* 2016;62(12):1639-1646.

Figure Legends

Figure 1. Graphical representation comparing von Willebrand factor antigen, ristocetin co-factor and Factor VIII activity results in patients who had both off-site and onsite testing.

*Represents statistically significant discordance between off-site versus onsite testing.

Figure 2. Final diagnosis of subjects by the consulting hematologist after clinical and laboratory evaluation.

Table 1. Subject and referring physician characteristics.

	Total N=263
Variable	**Prior to consultation**
Median age (range), y	15 (12-50)
Bleeding history/referral reason, n (%) *	
Heavy menstrual bleeding	196 (75)
Epistaxis	35 (13)
Easy bruising	35 (13)
Gum bleeding	10 (4)
Post-partum bleeding	3 (1)
Post-surgical bleeding	9 (3)
Other gastrointestinal bleeding	0
Prolonged bleeding with lacerations	--
Trauma	--
Family history of VWD	27 (10)
Unspecified/other bleeding	3 (1)
Unknown	1 (0)
Type of referring physician, n (%) *	
General practioner	168 (64)
Obstetrics/gynecology	64 (24)
Private hematologist/oncologist	10 (4)
Surgeon	4 (1.5)
Adolescent medicine	2 (1)
Other specialist	4 (1.5)

Unknown	10 (4)

*Some subjects had >1 bleeding symptom or referral reason

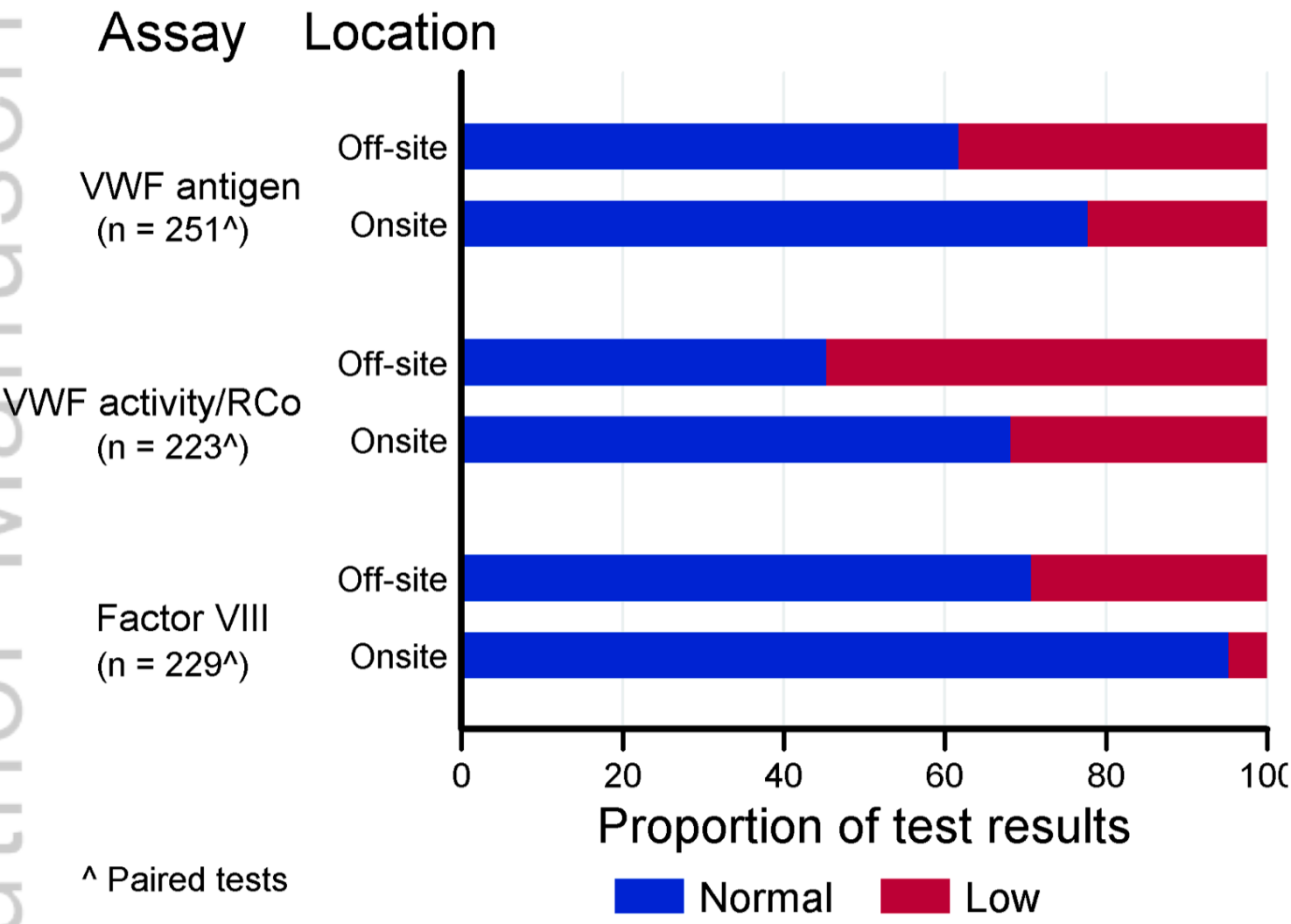
VWD, von Willebrand disease

Table 2. Comparing off-site to onsite testing of other hematologic laboratory assays.

Total N=263					
Laboratory Test	**Total off-site processing n (%)**	**Abnormal off-site n (%)**	**Total onsite processing n (%)**	**Normalized onsite n (%)**	**p-value**
PT	156 (59)	22 (14)	152 (58)	10 (45)	p=0.09
aPTT	171 (65)	30 (18)	172 (65)	17 (57)	p=0.04
Fibrinogen	26 (10)	0	158 (60)	†	†
PFA-100™	0	0	121 (46)	73	†

† refers to non-applicable or statistical testing was not conducted

PT, prothrombin thrombin time; aPTT activated partial thromboplastin time; PFA, platelet function assay



AJH_25869_Figure 1 VWD Revision TIFF.tiff

Table 1. Subject and referring physician characteristics.

	Total N=263
Variable	**Prior to consultation**
Median age (range), y	15 (12-50)
Bleeding history/referral reason, n (%) *	
Heavy menstrual bleeding	196 (75)
Epistaxis	35 (13)
Easy bruising	35 (13)
Gum bleeding	10 (4)
Post-partum bleeding	3 (1)
Post-surgical bleeding	9 (3)
Other gastrointestinal bleeding	0
Prolonged bleeding with lacerations	--
Trauma	--
Family history of VWD	27 (10)
Unspecified/other bleeding	3 (1)
Unknown	1 (0)
Type of referring physician, n (%) *	
General practioner	168 (64)
Obstetrics/gynecology	64 (24)
Private hematologist/oncologist	10 (4)
Surgeon	4 (1.5)
Adolescent medicine	2 (1)
Other specialist	4 (1.5)
Unknown	10 (4)

*Some subjects had >1 bleeding symptom or referral reason

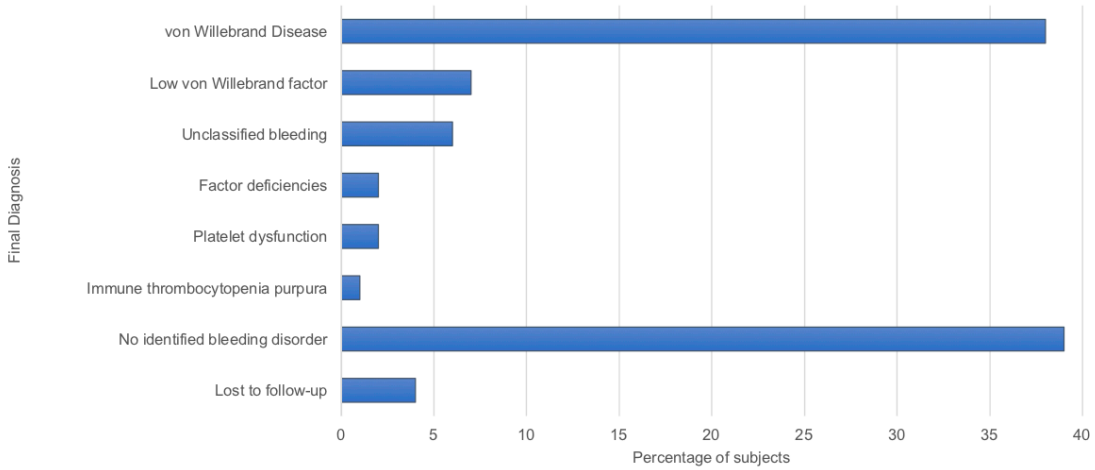
VWD, von Willebrand disease

Table 2. Comparing off-site to onsite testing of other hematologic laboratory assays.

Total N=263					
Laboratory Test	**Total off-site processing n (%)**	**Abnormal off-site n (%)**	**Total onsite processing n (%)**	**Normalized onsite n (%)**	**p-value**
PT	156 (59)	22 (14)	152 (58)	10 (45)	p=0.09
aPTT	171 (65)	30 (18)	172 (65)	17 (57)	p=0.04
Fibrinogen	26 (10)	0	158 (60)	†	†
PFA-100™	0	0	121 (46)	73	†

† refers to non-applicable or statistical testing was not conducted

PT, prothrombin thrombin time; aPTT activated partial thromboplastin time; PFA, platelet function assay



AJH_25869_VWD paper Figure 2 TIFF.tiff