2. Hassell KL. Population estimates of sickle cell disease in the U.S. *Am J Prev Med*. 2010;38:S512-S5521.
3. Dufu K, Patel M, Oksenberg D, Cabrales P. GBT440 improves red blood cell deformability and reduces viscosity of sickle cell blood under deoxygenated conditions. *Clin Hemorheol Microcirc*. 2018;70: 95-105.
4. Vichinsky E, Hoppe CC, Ataga KI, et al. A phase 3 randomized trial of voxelotor in sickle cell disease. *N Engl J Med*. 2019;381: 509-519.
5. Howard J, Hemmaway CJ, Telfer P, et al. A phase 1/2 ascending dose study and open-label extension study of voxelotor in patients with sickle cell disease. *Blood*. 2019;133:1865-1875.
6. Cacoub P, Musette P, Descamps V, et al. The DRESS syndrome: a literature review. *Am J Med*. 2011;124:588-597.
7. Shiohara T, Kano Y. Drug reaction with eosinophilia and systemic symptoms (DRESS): incidence, pathogenesis and management. *Expert Opin Drug Saf*. 2017;16:139-147.
8. Picard D, Vellar M, Janela B, Roussel A, Joly P, Musette P. Recurrence of drug-induced reactions in DRESS patients. *J Eur Acad Dermatol Venereol*. 2015;29:801-804.
9. Darbari DS, Fasano R, Minniti CP, et al. Severe vaso-occlusive episodes associated with use of systemic corticosteroids in patients with sickle cell disease. *J Natl Med Assoc*. 2008;100:948-951.
10. Herity LB, Vaughan DM, Rodriguez LR, Lowe DK. Voxelotor: a novel treatment for sickle cell disease. *Ann Pharmacother*. 2021;55: 240-245.

Received: 11 August 2021 | Revised: 26 August 2021 | Accepted: 29 August 2021

DOI: 10.1002/ajh.26343

Is ≥ 100% the magic number to rule out the laboratory diagnosis of von Willebrand disease based on initial testing?

To the Editor:

Diagnostic criteria for von Willebrand disease (VWD) differ between guidelines although all require laboratory evidence of low von Willebrand factor (VWF) levels, typically in the setting of a personal bleeding history. The laboratory diagnosis is complicated by interassay and intraassay variability. Further, there are many modifiers of VWF levels that temporarily increase levels from baseline, such as pregnancy, aging, exercise, and oral contraceptives. Iron deficiency anemia is a frequent complication of VWD, and recently has been hypothesized to be enough of a biologic stressor to possibly obscure VWD diagnosis by temporarily increasing VWF levels.[1]

In addition, pre-analytic variables such as variations with temperature and time to sample transportation, storage, and preparation with processing of samples can lead to inaccurate VWF assays results.[2] Our group recently reported significant differences seen between VWF assay results drawn and processed at separate phlebotomy and processing sites (off-site) compared to samples drawn and processed in one location (onsite).[3] Normalization of VWF Antigen (VWF: Ag), VWF ristocetin cofactor activity (VWF: RCo) and/or Factor VIII (FVIII) was seen in 40%–60% of patients with abnormal results when testing was repeated with on-site processing under the guidance of a consulting hematologist. Given these challenges, repeat testing at a center with on-site assay processing has been recommended in patients with normal VWF levels and a high index of suspicion.

A subset of patients may not require repeat testing. The utility of repeat testing in patients with initial elevated levels has been evaluated by two different groups. In a study of patients aged 0–18 years, Doshi et al., found that values > 100 IU/dL on VWF Antigen (VWF:Ag) or VWF ristocetin cofactor activity (VWF:RCo) yielded negative predictive values (NPV) of > 95%.[4] Further, they found that 70% of patients were diagnosed with VWD on their initial testing although all testing was performed at one large academic medical institution. The effect of anemia on VWF levels was not examined although the authors do state that one woman with heavy menstrual bleeding (HMB) in the study was concurrently anemic. Brown et al. performed a similar analysis on adolescent females presenting to the emergency room with acute HMB and found that a VWF:Ag > 100 IU/dL and VWF:RCo > 100 IU/dL had a NPV of 93.2% and 95% respectively.[5] So, VWF:Ag and VWF:RCo levels were significantly higher at presentation for HMB, but a specific analysis for anemia was not done.

Our group sought to evaluate whether this finding could be generalized to initial VWF assays performed at facilities with offsite processing using data from a retrospective study comparing offsite and onsite testing through 17 institutions across the United States. We also sought to examine the role of anemia in possibly obscuring a VWD diagnosis.

The methods have previously been described in detail.[3] Briefly, eligible subjects were females 12–50 years of age who were referred to a hematologist due to concern for a bleeding disorder. All subjects had VWF testing with offsite processing prior to referral, followed by VWF testing with onsite sampling and processing under the supervision of the consulting hematologist. The following data elements were collected: age, referral reason, bleeding symptoms, VWF assays (VWF: Ag, VWF:RCo and factor VIII assay) coagulation and hematologic laboratory results, details of onsite and offsite testing facilities, estrogen use, and final diagnosis as ascertained by the consulting hematologist. Although the most recent guidelines recommend using GP1bM as the platelet binding assay in the diagnosis of VWD, VWF:RCo was used as GP1bM is not widely available in the United States. In the current analysis, we focused on subjects with elevated VWF:Ag and/or VWF: RCo, defined as levels ≥ 100% at the referring ("off-site") institution. This subset was further classified by the qualifying test: elevated

TABLE 1 Initial and follow-up VWF testing on individuals diagnosed with VWD

Subject	Location	Test #	VWF Ag IU/dL (normal)	VWF RCo IU/dL (normal)	Hemoglobin g/dL	Diagnosis
CHLA16	Referring	1	60 (>50)	132 (42–200)	13.2	VWD
	Referring	2	68 (>50)		11.5	
	Consultation	3	210 (50–217)	179 (50–150)		
OHSU16	Referring	1	109 (55–200)	75 (55–200)	13.5	VWD
	Consultation	2	72 (60–150)	51 (60–150)	13.1	
UMIC01	Referring	1	192 (60–189)	203 (50–184)	5.7	VWD
	Consultation	2	55 (50–150)	38 (50–150)	12.1	
	Consultation	3	58 (50–150)	41 (50–150)	13.7	
UMIC03	Referring	1	104 (63–189)	47 (60–195)	13.6	VWD
	Consultation	2	115 (50–150)	51 (50–150)	12.7	
	Consultation	3		107 (50–150)		
	Consultation	4	139 (50–150)	52 (50–150)		
UMIC04	Referring	1	100	45 (49–204)	4.7	VWD
	Consultation	2	58 (50–150)	31 (50–150)	12.6	
	Consultation	3	66 (50–150)	36 (50–150)	13.1	

VWF:Ag only, elevated VWF:RCo only, or elevated VWF:Ag and VWF:RCo. Results from the referring institutions were then compared to the initial results from the consulting ("on-site") institutions. Results from the consulting institutions were classified as low (< 50%), normal (50%–100%), or elevated (≥ 100%). Anemia was defined as a hemoglobin of < 12 g/dL.

A total of 47 subjects, from a cohort of 263 subjects, were identified with elevated VWF:Ag and/or VWF:RCo from the referring institutions. Most of these subjects, 25 (53%), had elevated results for both VWF:Ag and VWF:RCo, while 18 (38%) had elevated VWF:Ag only. Four subjects (9%) had elevated VWF:RCo only. A majority of these subjects (n = 32, 68%) continued to have elevated VWF assays results when repeated at the consulting institution. A third of the subjects had normal laboratory values when repeated at the consulting institution. Five (11%) patients with elevated VWF antigen and/or ristocetin co-factor at the referring institution were eventually diagnosed with VWD, resulting in an negative predictive value (NPV) of 89%. For those patients with elevated VWF: Ag as well as VWF:RCo, the NPV increased to 96%. In those with isolated VWF:RCo or VWF:Ag elevation, NPV was 75% and 83% respectively. Laboratory results collected for the study for these five patients are shown in Table 1.

About half (n = 25, 53%) of those patients with elevated VWF:Ag and/or VWF:RCo levels (at consultation or prior to consultation) were anemic, compared to 18% (n = 39) of those with normal or low levels (chi-square $p < 0.001$). Three of the five patients (60%) with elevated levels and eventual diagnosis of low VWF or VWD had anemia. Two were severely anemic (hemoglobin < 6) at initial laboratory testing with correction of anemia prior to consultation. Eleven (23%) patients with elevated levels were ultimately diagnosed with a different bleeding disorder.

This study demonstrates a similar, though slightly lower, NPV for VWF:Ag or VWF:RCo levels > 100 IU/dL in the diagnosis of VWD compared to prior reports. The role of off-site processing in these findings is difficult to ascertain as typically pre-analytical variables will result in falsely low VWF levels. One limitation of the data arises from the VWF assays given a wide coefficient of variation, as well as the fact that they were not performed using identical instrumentation and reagents. Interestingly, the proportion of patients with anemia was higher in those patients with elevated VWF levels, adding support to the hypothesis that anemia is a sufficient stressor to increase VWF levels. The lowest NPV was seen in those patients with isolated elevation of VWF:Ag which might be expected given this finding can be seen in patients with Type 2 VWD.[6] However, this can also be seen with Type 1 VWD and of the five subjects with an eventual diagnosis of VWD, only one was diagnosed with Type 2 VWD. All diagnoses were made prior to the recent VWD diagnosis guidelines which suggest using an activity to antigen ratio of < 0.7 as the cut-off for diagnosis of Type 2 VWD.[7] Following these recommendations, some patients may have subsequently been reclassified. These findings reinforce prior reports that VWF levels above 100 IU/dL may be used to rule out VWD in a specific subset of patients. However, repeat testing should be considered in patients with a significant history of bleeding, concurrent anemia, or other biologic stressors even in the setting of elevated levels. Since there exists the possibility of other hemostatic disorders as we observed despite a VWF level exceeding 100%, referral to a hematologist for further specialized coagulation testing is warranted.

ACKNOWLEDGMENTS

The authors would like to thank The Foundation for Women and Girls with Blood Disorders, Learning Action Network (FWGBD LAN) members who contributed data to this study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Angela C. Weyand[1], Peter Kouides[2,3], Jemily Malvar[4], Julie Jaffray[4]

[1]Department of Pediatrics, Division of Hematology/Oncology, University of Michigan Medical School, Ann Arbor, Michigan, USA
[2]Department of Medicine, Division of Hematology/Oncology, Mary M. Gooley Hemophilia Center, Rochester, New York, USA
[3]Department of Medicine, Division of Hematology/Oncology, University of Rochester, Rochester, New York, USA
[4]Department of Pediatrics, Division of Hematology/Oncology/Bone Marrow Transplant, Children's Hospital of Los Angeles, Los Angeles, California, USA

Correspondence
Julie Jaffray, Department of Pediatrics, Division of Hematology/Oncology/Bone Marrow Transplant, Children's Hospital of Los Angeles, 4650 Sunset Blvd, Mailstop #54, Los Angeles, CA 90027, USA.
Email: jjaffray@chla.usc.edu

ORCID

Angela C. Weyand https://orcid.org/0000-0003-2595-8541
Julie Jaffray https://orcid.org/0000-0002-1175-7266

REFERENCES

1. Moyer G, Huguelet P. Iron deficient anemia may obscure a diagnosis of low VWF, VWD or mild hemophilia a in biological females with heavy menstrual bleeding. *Blood*. 2020;136:32-33. https://doi.org/10.1182/blood-2020-138697
2. Preston FE, Lippi G, Favaloro EJ, Jayandharan GR, Edison ES, Srivastava A. Quality issues in laboratory haemostasis. *Haemophilia*. 2010;16(suppl 5):93-99. https://doi.org/10.1111/j.1365-2516.2010.02305.x
3. Jaffray J, Staber JM, Malvar J, et al. Laboratory misdiagnosis of von Willebrand disease in post-menarchal females: a multi-center study. *Am J Hematol*. 2020;95(9):1022-1029. https://doi.org/10.1002/ajh.25869
4. Doshi BS, Rogers RS, Whitworth HB, et al. Utility of repeat testing in the evaluation for von Willebrand disease in pediatric patients. *J Thromb Haemost*. 2019;17(11):1838-1847. https://doi.org/10.1111/jth.14591
5. Brown MC, White MH, Friedberg R, et al. Elevated von Willebrand factor levels during heavy menstrual bleeding episodes limit the diagnostic utility for von Willebrand disease. *Res Pract Thromb Haemost*. 2021;5(4):e12513. https://doi.org/10.1002/rth2.12513
6. Ledford MR, Rabinowitz I, Sadler JE, Kent JW, Civantos F. New variant of von Willebrand disease type II with markedly increased levels of von Willebrand factor antigen and dominant mode of inheritance: von Willebrand disease type IIC Miami. *Blood*. 1993;82(1):169-175.
7. James PD, Connell NT, Ameer B, et al. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. *Blood Adv*. 2021;5(1):280-300.

Received: 4 August 2021 | Revised: 28 August 2021 | Accepted: 29 August 2021

DOI: 10.1002/ajh.26344

Red blood cell lifespan is reduced in severe aplastic anemia and improves with response to immunosuppressive treatment

To The Editor:

Aplastic anemia (AA) is a rare and heterogeneous disease, characterized by bone marrow failure leading to pancytopenia and empty marrow. Immune-mediated damage of hematopoietic stem cells is the major modality leading to bone marrow aplasia in acquired AA. Our previous cross-sectional study found that the life span of RBCs in severe (SAA) and very severe (VSAA) patients was shortened, but it was close to normal in patients exhibiting a hematologic response (HR) after immunosuppressive therapy (IST).[1] We report here a prospective trial by carbon monoxide (CO) breath test to quantify RBC lifespan in VSAA/SAA, and its changes according to response to treatment.

A total of 22 patients were included in this study: 14 SAA and 8 VSAA (14 males, eight females) with median age 25 (6–46), between November 2016 and November 2017. Pretreatment blood cell counts of the 22 patients were as follows: neutrophils 0.27×10^9/L ($0.04–0.9 \times 10^9$/L), Hb 71 g/L (42–118 g/L), absolute reticulocytes 12.9×10^9/L ($2.5–26.5 \times 10^9$/L), and platelets 10×10^9/L ($0–16 \times 10^9$/L). All the patients had no evidence of hemolysis with indirect bilirubin (IBIL), haptoglobin (HP), lactate dehydrogenase (LDH) in the normal range. Erythrocyte osmotic fragility (EOF) test and Coombs test were also negative. As a control, 13 healthy people (three males, 10 females) were included with a median age of 36 (28–47).

The CO breath tests were obtained with no blood transfusion or 2 weeks after transfusion, no smoking within 24 h, on an empty stomach. An automatic instrument (ELS TESTER, Seekya Biotec Co. Ltd, Shenzhen, China) was used.

The IST treatment and the response criteria were in on-line supplementary material.