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RESEARCH ARTICLE

Utility of thromboelastography for the diagnosis of von Willebrand disease

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Abstract

Von Willebrand disease (VWD) is an inherited bleeding disorder that is caused by a quantitative or qualitative deficiency of von Willebrand factor (VWF). The National Heart, Lung, and Blood Institute (NHLBI) guidelines for the diagnosis of VWD state that a VWF activity (VWF:RCo) of <30 IU/dL or <50 IU/dL with symptoms of clinical bleeding are consistent with the diagnosis of VWD. However, current gold-standard diagnostic testing takes days to have complete results. Thromboelastography (TEG) is a testing method that provides a graphical trace that represents the viscoelastic changes seen with fibrin polymerization in whole blood, therefore providing information on all phases of the coagulation process. This study describes the TEG characteristics in 160 patients who presented for workup of a bleeding disorder and a subset of those were subsequently diagnosed with VWD. The TEG parameters, K-time (representing the dynamics of clot formation) and the maximal rate of thrombus generation (MRTG), were found to be sensitive in detecting patients with VWF:RCo <30 IU/dL. The TEG, unlike VWF:RCo, can be done in real time, and results are available to the clinician within an hour. This will definitely be beneficial in acute situations such as evaluation of and management of acute bleeding in patients with acquired deficiencies of VWF and may play an important role in the surgical management of patients with VWD.

KEYWORDS

hemostasis, pediatric hematology/oncology, thromboelastography, von Willebrand disease

1 | INTRODUCTION

Von Willebrand disease (VWD) is an inherited disorder associated with clinical bleeding abnormalities. Many subtypes exist based on quantitative or qualitative defects in von Willebrand factor (VWF). The gold-standard diagnostic testing at the time of the study included VWF activity (VWF:RCo), VWF antigen (VWF:Ag), and factor VIII (FVIII) activity[1]; newer assays such as the collagen binding and GP1bM are relatively new and were not available at the time this study was conducted. Patients with VWD may have a prolonged partial thromboplastin time (PTT) and decreased levels of FVIII activity secondary to increased clearance of FVIII because the protein is no longer protected by the FVIII–VWF circulating protein complex. According to the National Heart, Lung, and Blood Institute (NHLBI) guidelines, those with the diagnosis of VWD have VWF:Ag levels <50 IU/dL and VWF:RCo levels <50 IU/dL and variable levels of FVIII activity compared with normal laboratory reference ranges.[1]

There are many difficulties associated with current diagnostic methods (such as different methodologies available for laboratory tests, enzyme-linked immunosorbent assay [ELISA] vs line immunoassay [LIA], and a high coefficient of variation with each of these tests) and the inability to measure physiologic function.

TEG is a testing method that provides a graphical trace that represents the viscoelastic changes seen with fibrin polymerization in whole blood, therefore providing information on all phases of the coagulation process.[2–4] Because it is a global assay that evaluates clot formation from initiation to fibrinolysis, it has the ability to potentially pinpoint abnormalities in any step along the process.

Abbreviations: A30, maximal lysis; ANOVA, analysis of variance; CI, clotting index; ELISA, enzyme-linked immunosorbent assay; FVIII, factor VIII; LIA, line immunoassay; MA, maximal strength amplitude; MRTG, maximal rate of thrombus generation; NHLBI, National Heart, Lung, and Blood Institute; PT, prothrombin time; PTT, partial thromboplastin time; ROTEM, rotational thromboelastography; R-time, reaction time; TEG, thromboelastography; TF, tissue factor; TMRTG, time to maximal rate of thrombus generation; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor activity.

The TEG can be done in real time, unlike the VWF:Ag and VWF:RCo. The standard TEG assay has not been thought to be of use in VWD because of the lack of shear stress, which is essential for the activation of VWF. The aims of this study were to evaluate the parameters of tissue factor (TF)-initiated TEG in pediatric patients with VWD, to determine if this assay is sensitive to dysfunctional/low levels of VWF, as this does not require any significant change in procedure other than the use of TF as the activator instead of Kaolin.

2 | MATERIALS AND METHODS

At Children's Hospital of Michigan, all patients who present for workup of a bleeding disorder have TEG and VWD studies as part of the initial evaluation, and these data were obtained from the patient medical records. Those patients with a diagnosis of VWD had confirmatory VWF multimer analysis completed, and were further classified based on their specific VWD type. D1472H, VWF:Gp1bm, and collagen binding testing were not widely available at the time of study and thus were not completed. All other patients were not identified to have any underlying bleeding disorder.

A retrospective chart review of patients who presented for a bleeding disorder workup that had TF-initiated TEG analysis and von Willebrand studies completed between January 2007 and December 2015 was performed at Children's Hospital of Michigan. Institutional review board approval was obtained, and current diagnostic tests for VWD (complete blood count [CBC] with platelet count, VWF:RCo and VWF:Ag, FVIII, ABO blood type; PT, PTT, fibrinogen) and TF-initiated TEG parameters, including K-time and MRTG, were compared. Patient demographics including age, gender, and ethnic background were also reviewed.

2.1 | TEG analysis

In all subjects, the dynamics of blood clot formation was recorded by the TEG 5000 (Version 4.2) using the following method. Each sample was allowed to rest for 30 minutes as recommended by Sorensen et al[3] following which the thromboelastographic analysis was performed. To perform the TEG analysis, 20 μL of 1:10 000 dilution of recombinant human TF (Innovin, Dade Behring) and 20 μL of $CaCl_2$ were added to the TEG cup, followed by 340 μL whole blood. All samples were run for 90 minutes.

The TEG analysis has five parameters. The first parameter is the reaction time (R-time), which is the period from initiation of the test until the clot initiated reaches 2 mm amplitude and represents time to fibrin initiation. The K-time refers to the time it takes the amplitude of the curve to go from 2 to 20 mm and represents the dynamics of clot formation. The third parameter is the α angle, which is the slope between *R* and *K*, which represents the rate of clot formation. The fourth parameter is the MA, which measures the strength of the clot formed. Finally, A30 is the difference between MA and the amplitude of the curve after 30 minutes.[2,3,5,6]

MRTG and TMRTG: A velocity curve, as noted above, creates a graph relating clot strength over time, which correlates with the rate and amount of clot formation or fibrinogen polymerization.[7]

2.2 | Von Willebrand studies

VWF:Ag was analyzed by the STA-Liatest VWF kit (Diagnostica Stago Inc., Parsippany, NJ, USA), which is an immunoturbidimetric method that uses microlatex particles coated with rabbit anti-human vWF and measures the absorbance as the particles agglutinate in proportion to the antigen level present.

VWF:RCo is determined by a turbidimetric method that measures the change in optical density based on the sample's VWF, which initiates aggregation of the platelet reagent in the presence of ristocetin (BC von Willebrand Reagent; Siemens Healthcare Diagnostics Inc., Newark, DE, USA).

2.3 | Fibrinogen

Quantitative determination of fibrinogen in plasma was performed by the Clauss clotting method. The fibrinogen quantitation and clotting factor assays were performed on the STA-R Evolution (Diagnostica Stago, Inc., Parsippany, NJ, USA), at the Special Coagulation Laboratory at Children's Hospital of Michigan.

2.4 | Blood counts

CBC results were obtained as a part of the routine clinical workup for the patient.

2.5 | Coagulation factor assays

The one-stage PTT based clotting assay was used to determine FVIII activity and was performed on the STA-R Evolution (Diagnostica Stago, Inc., Parsippany, NJ, USA) using Kaolin PTT reagent (CK Prest; Diagnostica Stago, Inc., Parsippany, NJ, USA) and George King Biomedical congenital FVIII-deficient plasma (Overland Park, KS, USA).

2.6 | Statistical analysis

Comparison data by VWD types, VWD percentage groups, and controls are reported using means and standard deviations. Mean differences between groups were conducted using a parametric analysis of variance procedure (ANOVA), with a Tukey test to balance type 1 error and power. If assumptions of homogeneity of variance were violated, a nonparametric Kruskal–Wallis test would be substituted, followed by pair-wise comparisons using a nonparametric Mann–Whitney *U* test. An independent receiver operating characteristic (ROC) curve was performed to compare K-time on TEG to VWF:RCo in patients with VWF:RCo < 30 IU/dL. A curvilinear procedure was performed to examine best fit for changes in MRTG related to changes in K-time. Statistical significance was considered achieved at a P value < 0.05, two-tailed. All statistical procedures were performed using SPSS Version 25.0 IBM Inc., Chicago, IL.

3 | RESULTS

A total of 160 patients (ages ranging 2 weeks to 18 years) who had a workup for a bleeding disorder that included von Willebrand studies and TEG were included in the study. Patient demographics, including age, sex, race, and presenting symptoms were compared and showed

no significant difference in patients with diagnosis of VWD compared with control patients. Patient demographics based on VWF:RCo levels are presented in Supporting Information Table S1. As previously described, we did see a significantly higher FVIII activity in patients of African American ethnicity in both our control patients and VWD patients.[8] Our control group consisted of 82 patients (34 males and 48 females) with the majority of patients being of Caucasian (43%) and African American (22%) descent. Males most commonly presented with epistaxis (41%) and bruising (26%), and females with menorrhagia (25%), epistaxis (21%), and bruising (19%). None of the female patients were on hormonal therapy at the time of diagnostic workup.

Seventy-eight patients were diagnosed with VWD based on laboratory evaluation and presence of bleeding symptoms. There were 36 males and 42 females, who were further categorized into type 1 VWD (67 patients) and type 2A VWD (11 patients). The most prominent ethnicity was Caucasian (58%) followed by African American (19%), Hispanic (12%), and other (11%). There were no patients diagnosed with type 2 B, 2 N, 2 M, 3, or platelet-type VWD.

There were no significant differences in the clinical presentation of patients with type 1 VWD versus type 2A VWD. Presenting symptoms in patients aged 10 and younger ($N = 41$; 23 males and 18 females) and those aged 11 and older ($N = 37$; 13 males and 24 females) were compared. The most common presenting symptom in male patients was epistaxis (51%) regardless of age. However, in female patients, the age of presentation impacted their presenting bleeding symptoms. Females younger than 10 years of age presented most frequently with epistaxis (44%); and in patients aged 11 years and older, the most frequent symptoms were epistaxis (27%) and menorrhagia (27%). The general laboratory characteristics as well as the TEG data showed no significant differences between type 1 and type 2A VWD patients (Supporting Information Tables S2 and S3). TEG data were reported in medians due to one outlier in the type 2A VWD group, which skewed the means and did not represent overall finding in type 2A VWD patients.

Of the 160 patients evaluated in this study, 85 of 160 patients had VWF:RCo > 50 IU/dL; 14 of 85 of these patients were diagnosed with VWD due to significant bleeding symptoms and had levels between 50 and 60 IU/dL (normal range for VWF:RCo is 60 IU/dL to 120 IU/dL). There were 45 of 160 patients with VWF:RCo 30–50 IU/dL; 34 of 45 of these patients were diagnosed with VWD, and 11 of 45 were not diagnosed with any bleeding disorder. Of the 11 patients not diagnosed as a bleeding disorder, 9 of 11 were blood group O and 2 of 11 were blood group A and had normal levels on repeat testing. There were 30 of 160 patients with VWF:RCo < 30 IU/dL, and all were diagnosed with VWD. All 75 patients with VWF:RCo < 50 IU/dL were divided into two groups based on VWF:RCo levels: 30–50 IU/dL and <30 IU/dL. The TEG parameter, K-time (time for increase in amplitude from 2 to 20 mm representing the dynamics of clot formation, normal <2.2 minutes) was determined abnormal for values ≥2.2 minutes. An example of a normal TEG in a healthy control in comparison with patients with VWD with varying VWF:RCo is reflected in Figure 1.

Lab characteristics of these patients are presented, including FVIII, platelet count, and fibrinogen levels (Supporting Information Table S4).

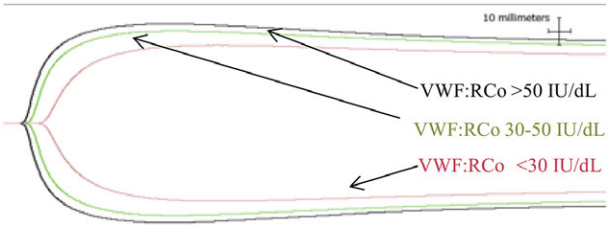

FIGURE 1 Sample TEG graphical trace comparing K-time with varying VWF:RCo levels

Patients with VWF:RCo < 30 IU/dL had significantly longer K-times than those with VWF:RCo >50 IU/dL. PTT assays on four patients with VWF:RCo < 50 IU/dL and three patients with VWF:RCo > 50 IU/dL were not available, and are not included in the means.

Of the patients with VWF:RCo < 30 IU/dL, 23 of 30 (77%) had an abnormal K-time of ≥2.2 minutes ($P \leq 0.001$), whereas of the patients with low VWF:RCo 30–50 IU/dL, only 13 of 45 (29%) had an abnormal K-time of ≥2.2 minutes, which was not statistically significant. Regression analysis of K-time and MRTG in comparison with VWF:RCo shows that for every 1 IU/dL decrease in VWF:RCo, there is an equal increase in both K-time and MRTG levels (Table 1). An ROC curve for patients with VWF:RCo < 30 IU/dL and abnormal K-time showed an area under the curve of 0.67 ($P = 0.003$) (Figure 2). An analysis of the MRTG showed a mean of 9.45 in patients with VWF:RCo < 30 IU/dL, a mean of 10.9 in patients with VWF:RCo 30–50 IU/dL, and a mean of 11.26 in patients with normal VWF:RCo. A strong correlation (0.86) was seen between patients with abnormal K-time and MRTG. When analyzing regression data, the regression accounts for 74% (linear) and 84% (exponential/logistic) of the explained variance in abnormal K-time (Figure 3). A moderate correlation (0.34) was seen between patients with abnormal K-time and FVIII; however, when analyzing regression data, the correlation only accounts for 11% of the explained variance in abnormal K-time values. Patients with abnormal K-time and abnormal fibrinogen levels and platelet counts were also compared and showed no significant correlation, indicating that these were not the determinants that influenced the K-time.

In patients diagnosed with VWD, 32 patients had previously qualified for a DDAVP trial and VWF:Ag, VWF:RCo, and TEG parameters were compared at 1 hour, 2 hours, and 4 hours after treatment with DDAVP. There were 13 males and 19 females, with a mean age of 10.91 years. Of the 32 patients who underwent a DDAVP trial, 29 patients had normalization of VWF:RCo at 1 hour after DDAVP and 25 patients had normalization of K-time at 1 hour after DDAVP treatment. Three of 32 patients were found to be nonresponders to DDAVP treatment in regard to serial VWF:RCo levels at 1 hour, 2 hours, and 4 hours after DDAVP; however 2 of 3 of the patients did have normalization of their K-times. In reviewing K-time normalization, 2 of 7 patients who were abnormal at 1 hour after DDAVP had normalization at 2 hours after DDAVP, suggesting that some patients may be slow responders to treatment. Mean MRTG of patients was compared before and after administration of DDAVP using the test of sphericity within subject effects and was found to be significant; $P \leq 0.001$ in all paired samples test (Table 2).

TABLE 1 Regression for method comparison against the reference method, VWF:RCo

Variable	Intercept	95% CI	Slope	95% CI	*P*
K-Time	2.1922	2.0149 to 2.3694	−1.0014	−1.0036 to −0.9992	<0.0001
MRTG	10.1912	9.2173 to 11.1650	−0.9907	−1.0027 to −0.9788	<0.0001

Abbreviations: CI, confidence interval; MRTG, maximal rate of thrombin generation; VWF:RCo: von Willebrand factor activity.

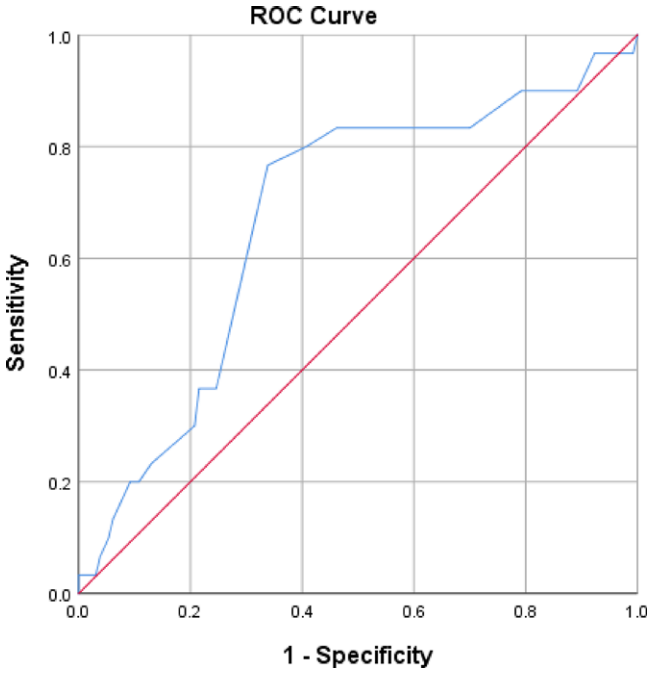

FIGURE 2 ROC curve comparing K-time on TEG with VWF:RCo in patients with VWF:RCo < 30 IU/dL; $R^2 = 0.067$, $P = 0.003$

Eleven patients in our study have now had the collagen binding assay completed to complement their diagnostic evaluations. These results were compared with their TEG parameters and VWF:Ag and VWF:RCo levels. We did not find any correlation when comparing this assay to these parameters; however, this may be the result of the extremely small sample size.

4 | DISCUSSION

VWD is the most common bleeding disorder and has been estimated to have a prevalence of up to 1.3%, and affecting males and females equally.[9,10] Typically, patients present with skin and mucosal bleeding, including easy bruising, epistaxis, bleeding from the gums, and heavy menstrual bleeding. Management of acute bleeding episodes in these patients primarily consists of replacement of the deficient coagulation protein. Treatment is often empiric as monitoring of the VWF levels in real time is not possible in most clinical centers. Significant bleeding is often associated with VWF:RCo levels < 30 IU/dL, although patients with levels between 30 and 50 IU/dL are also described to have bleeding symptomatology.

In many situations, prothrombin time (PT) and PTT may be sufficient for monitoring patients with coagulation disorders and their response to medications, including those with VWD, but these tests are neither specific nor sensitive. In critically ill patients or those with acute bleeding, it is imperative that quick and reliable information be available to assist in physician decision-making. The aim of our study was to evaluate the use of TF-activated TEG and its role in diagnosing VWD. We were able to show that patients with VWF < 30 IU/dL had significant changes of the TEG parameters: K-time, MRTG, and TMRTG. With this knowledge, it may be beneficial to clinicians to use TEG as one of the monitoring tools in patients with this diagnosis.

The lack of shear stress in the TEG assay was thought to make it insensitive to VWF activity. A recent prospective study aimed to differentiate adult patients with VWD from healthy controls using TEG and rotational thromboelastography (ROTEM)[11] and were able to find differences in R-time and the clotting index (CI) in those with VWD using TEG; however, no differences were seen using ROTEM. Modified

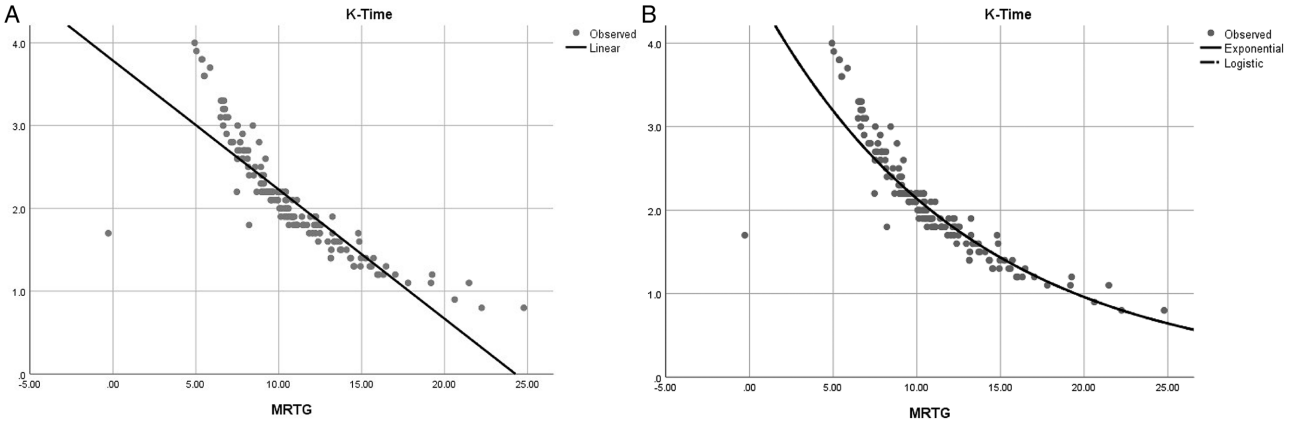

FIGURE 3 A, Linear curve fit for changes in MRTG related to K-time, $R^2 = 0.74$, $P \leq 0.001$; B, exponential/logistic curve fit for changes in MRTG related to K-time, $R^2 = 0.84$, $P \leq 0.001$

TABLE 2 Mean MRTG and K-time values in patients with diagnosis of VWD who participated in the DDAVP trial

	Pre-DDAVP ($N = 32$)	1 Hour post-DDAVP ($N = 32$)	2 hours post-DDAVP ($N = 32$)	4 Hours post-DDAVP ($N = 31$)
Mean MRTG	9.91[a]	12.34	12.9	12.6
Mean K-time	2.17	1.75	1.65	1.71

Abbreviations: VWD, von Willebrand disease; MRTG, maximal rate of thrombin generation.
[a]$P \leq 0.001$.

TEG using ristocetin activation has been found to be useful in the diagnosis of VWD. To date, there have not been any published studies looking at TEG and VWD in children and also no studies that have looked at using a tissue factor activator in patients with VWD.

VWF is an important component of fibrinogen polymerization. Previously, it has been shown that fibrinogen binding to the glycoprotein IIb–IIIa complex induces platelet aggregation under low shear stress environments, and the aggregates formed under these conditions are unstable, making the effects reversible. In high shear stress conditions, VWF interacts with both platelet binding sites, glycoprotein IIb–IIIa and glycoprotein Ib–IX, to induce platelet aggregation without the influence of fibrinogen.[12] Although no published data exist, we hypothesize the prolonged K-time seen in patients with VWD despite normal fibrinogen levels may be due to their low levels of VWD:Ag and/or VWD:RCo, which, in turn, interferes with the clot kinetics in these patients.

Our study shows that the TF-activated TEG demonstrates impaired clot formation in patients with VWF:RCo < 30 IU/dL as reflected by the prolongation in the K-time and the low MRTG and can be helpful in identifying patients with VWF:RCo levels in this range who are at higher risk of major bleeding. This is of benefit in non-life-threatening acute bleeding (e.g., epistaxis) where often replacement therapy is still empiric as results of the evaluation often may take several hours to days. It also reflects response to DDAVP treatment as reflected by the correction in K-time and MRTG. This can be extremely helpful in preparation for surgery, where post DDAVP levels are often not available prior to surgery and correction is assumed based on previous testing results. Because fibrinogen is a major determinant of the K-time, when patients present with acute bleeding and have normal fibrinogen activity/levels, but abnormal K-time on TF-activated TEG, substitution with VWF containing factor products would be considered instead of nonspecific substitution with fresh frozen plasma.

The TEG, unlike VWF:RCo, can be done in real time, and results are available to the clinician within an hour. It may also help physicians monitor response to treatment, frequency of treatment, and the need for prophylactic dosing in patients with VWD undergoing surgical interventions or those at high risk for bleeding episodes. Therefore, obtaining a baseline TF-initiated TEG evaluation in patients with VWD may be of benefit for diagnosis as well as monitoring of therapy.

Given that our study is retrospective, newer diagnostic tests were unable to be completed, including D1472H heterozygote testing for those with low VWF levels and collagen binding assays for those with type 2 disease. In addition, we were unable to access bleeding scores in patients prior to 2012, and thus we could not analyze these data to see if those with higher scores had a higher likelihood of derangements in their TEG.

The next step will be to evaluate if this difference is also seen with Kaolin, which is the standard TEG assay. Kaolin activates the intrinsic coagulation pathway in addition to activating platelets by releasing platelet factor 3, and thus may allow for normalization of the TEG despite deficient VWF leading to lack of sensitivity.[13,14] We anticipate that Kaolin being a stronger agonist may make the test less sensitive to VWF-related changes on TEG (K-time) and velocity curve (MRTG).

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CONFLICTS OF INTEREST

KR, SK, RT, and WH have no disclosures to acknowledge. MC has received honoraria for attendance at advisory boards from Novo Nordisk, Genentech, Baxalta/Shire, CSL Behring, HemaBiologics, and Bayer Pharmaceuticals Inc.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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