CLINICAL TRIALS AND OBSERVATIONS

The bleeding score predicts clinical outcomes and replacement therapy in adults with von Willebrand disease

Augusto B. Federici,[1,2] Paolo Bucciarelli,[1] Giancarlo Castaman,[3] Maria G. Mazzucconi,[4] Massimo Morfini,[5] Angiola Rocino,[6] Mario Schiavoni,[7] Flora Peyvandi,[1] Francesco Rodeghiero,[3] and Pier Mannuccio Mannucci[8]

[1]Angelo Bianchi Bonomi Hemophilia Thrombosis Center, Istituto Ricerca e Cura di Carattere Scientifico (IRCCS) Ca' Granda, Foundation Maggiore Policlinico Hospital, Milan, Italy; [2]Hematology and Transfusion Medicine, L. Sacco University Hospital and Department of Clinical Sciences & Community Health, University of Milan, Milan, Italy; [3]Division of Hematology, San Bortolo Hospital, Vicenza, Italy; [4]Hemophilia Center, Department of Hematology, University of Rome, Rome, Italy; [5]Hemophilia Center, Careggi Hospital, Florence, Italy; [6]Hemophilia Center, Pellegrini Hospital, Naples, Italy; [7]Hemophilia Thrombosis Center, Ospedale Policlinico, Bari, Italy; and [8]Scientific Direction, IRCCS Cà Granda Foundation Maggiore Policlinico Hospital, Milan, Italy

Key Points

- The bleeding score helps to predict clinical outcomes in adult patients with von Willebrand disease.
- High bleeding scores correlate with intensive on-demand therapy and may identify cases requiring regular prophylaxis.

Analyses of the bleeding tendency by means of the bleeding score (BS) have been proposed until now to confirm diagnosis but not to predict clinical outcomes in patients with inherited von Willebrand disease (VWD). We prospectively followed up, for 1 year, 796 Italian patients with different types of VWD to determine whether the previous BS of European VWD1 is useful to predict the occurrence of spontaneous bleeds severe enough to require replacement therapy with desmopressin (DDAVP) and/or von Willebrand factor (VWF)/factor VIII concentrates. Among the 796 patients included, 75 (9.4%) needed treatment of 232 spontaneous bleeding events. BS >10 and VWF:ristocetin cofactor activity <10 U/dL were associated with the risk of bleeding, but only a BS >10 remained highly associated in a multivariable Cox proportional hazard model (adjusted hazard ratio: 7.27 [95% confidence interval, 3.83-13.83]). Although the bleeding event-free survival was different in VWD types, only a BS >10 could predict for each type which patient had bleeding events severe enough to require treatment with DDAVP and/or concentrates. Therefore, BS can be considered a simple predictor of clinical outcomes of VWD and may identify patients needing intensive therapeutic regimens. (*Blood*. 2014;123(26):4037-4044)

Introduction

von Willebrand disease (VWD) is considered the most common inherited bleeding disorder, even though its prevalence varies considerably according to the setting of diagnosis[1-3]: in population-based studies, prevalence was estimated to be as high as 0.6% to 1.3%,[4,5] about 2 orders of magnitude higher than in specialized centers (0.005%-0.01%) to which symptomatic patients with VWD are usually referred.[6-10] VWD is due to quantitative and/or qualitative defects of von Willebrand factor (VWF), a multimeric glycoprotein synthesized by endothelial cells and megakaryocytes that mediates platelet adhesion/aggregation and stabilizes factor VIII (FVIII) in the circulation.[1-3] In VWD, bleeding events are caused not only by impaired platelet-VWF interactions, usually evaluated in plasma by ristocetin cofactor activity (VWF:RCo), but also by reduced FVIII levels that often accompany the VWF defect.

Whereas in classical hemophilia there is an excellent relationship between plasma levels of FVIII, frequency, and severity of clinical bleeding, in VWD such a relationship is less clear and straightforward. A plasma VWF level of 30 IU/dL has been suggested as a threshold to distinguish patients with a bleeding tendency from healthy subjects with low-borderline plasma levels of VWF.[10-13] Usually, the bleeding history is an essential criterion for the diagnosis of inherited bleeding disorders, including VWD.[14-17] A bleeding score (BS) based upon bleeding symptoms and calculated using the questionnaire proposed by Tosetto et al[18] was used to confirm diagnosis in a large cohort of European families with type 1 VWD[19] and was subsequently applied with some modifications to other clinical studies on VWD.[20,21] More recently, in a limited number of patients with some VWD types (1, 2A, 2B, 2M), attempts were made to use the BS not only for diagnostic purposes but also to evaluate the patients' tendency to bleed.[22-24] With this background, we have prospectively analyzed number and types of spontaneous bleeding episodes and their treatments in a cohort of 796 Italian VWD patients with centrally obtained laboratory measurements to determine whether the BS calculated at the time of the baseline visit predicts the rates of bleeding events requiring treatment with DDAVP and/or VWF/FVIII concentrates.

Patients and methods

Design and aims of the study

Italian National Registry of von Willebrand Disease in its second part (RENAWI-2) is a prospective observational cohort study carried out in

Submitted February 18, 2014; accepted April 16, 2014. Prepublished online as *Blood* First Edition paper, April 30, 2014; DOI 10.1182/blood-2014-02-557264.

There is an Inside *Blood* Commentary on this article in this issue.

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patients diagnosed and followed up at 6 comprehensive hemophilia centers, members of the Italian Association of Hemophilia Centers (AICE). This study is the continuation of the previously described Italian National Registry of von Willebrand Disease (RENAWI-1).[25] The aim of RENAWI-2 is to evaluate the incidence, type, and severity of spontaneous bleeding episodes requiring treatment with DDAVP and/or VWF/FVIII concentrates in a large cohort of patients in whom phenotypic and genotypic diagnosis was centrally confirmed. Patients were informed about the anonymous use of their data and the purpose of this study and gave informed consent in accordance with the Declaration of Helsinki. This study was approved by the institutional review board of the participating hospitals after the endorsement as an independent study by the AICE.

Inclusion and exclusion criteria

Patients were included in RENAWI-2 between June and December 2008 during a baseline visit, and prospectively followed from January 1, 2009 for 12 months onward. Moreover, those patients who bled during this period were further observed beyond the RENAWI-2 follow-up until 2013 to evaluate any shift from on-demand treatment to prophylaxis. The BS was calculated by the investigators on the basis of symptoms that had occurred before inclusion in RENAWI-2 using a standardized questionnaire[18] administered to patients when blood samples were withdrawn. For the purpose of RENAWI-2, all cases were included without distinguishing between propositi and affected family members. Patients unable to participate in the follow-up visits or give informed consent were considered ineligible (n = 39) as well as those who did not meet criteria for VWD diagnosis after centralized laboratory reevaluation (n = 36). We also excluded individuals with concomitant hepatic or renal insufficiency (n = 43), those using antiplatelet or anticoagulant drugs (n = 9), pregnant women (n = 5), or those with active malignancy (n = 6) at the time of the first study visit. Patients with incomplete follow-up (n = 10), and the only VWD2N case, were not included in the final analyses. At the end, 796 VWD patients were included in RENAWI-2.

Laboratory methods for VWD diagnosis

At each participating center, venous blood was withdrawn at the time of the baseline visit. Platelet-poor plasma was obtained and kept frozen at –40°C by each center. The following tests were performed at the central laboratory in Milan. FVIII was measured by a 1-stage coagulation assay, VWF:antigen (Ag) by commercial kits or homemade enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies against VWF.[25] VWF:RCo was measured by aggregometry using formalin-fixed platelets from normal donors; however, when plasma VWF:RCo was <15 U/dL, patients were reinvestigated using the more sensitive ELISA-VWF:RCo assay.[26] According to predefined inclusion criteria, patients were included in this study if their plasma VWF:RCo was below the lower normal limit of 56 U/dL of the central laboratory, corresponding to 2 standard deviations (SDs) below the plasma levels of normal donors with non-O blood groups. The plasma VWF multimeric pattern was evaluated in patients' plasma by low- and intermediate-resolution gel electrophoresis.[25] For molecular diagnosis, genomic DNA was extracted from peripheral blood using standard methods. The oligonucleotides used and the detailed testing procedures are available on request.[27] The final classification according to VWD types was confirmed by the coordinating center in Milan by also using multimeric analysis and mutations of the VWF gene.[28] DNA was available in all affected patients with VWD2A, VWD2B, VWD2M, and in most VWD3 (82%) and VWD1 (63%). The majority of the identified mutations had been previously recorded in the International Society on Thrombosis and Haemostasis–Scientific Standardization Committee on VWF database (www.vwf.group.shef.ac.uk). To analyze the association of the bleeding phenotypes with VWF and FVIII functional activities, the 796 patients were arbitrarily grouped as follows: severe (VWF:RCo <10 and FVIII < 20 U/dL), moderate (VWF:RCo = 10-30 and FVIII = 20-40 U/dL), and mild (VWF:RCo = 31-56 and FVIII >40 U/dL).

Type and intensity of on-demand treatments

According to its licensing in Italy, desmopressin (DDAVP) can be given IV (Minirin-DDAVP [Ferring] and Emosint [Kedrion]) or subcutaneously (Emosint; Kedrion) at a dosage of 0.3 μg/kg. An infusion trial of DDAVP has been performed according to published criteria in at least 1 family member for all VWD types except for VWD2B and VWD3.[16,29] Three plasma-derived concentrates containing both VWF and FVIII were licensed in Italy for VWD at the time of the study[30-32]: Alphanate (Grifols), Fahndi (Grifols), and Haemate-P (CSL-Behring). Patients were treated on demand at each participating center with varying doses of VWF (25-80 IU VWF:RCo/kg) chosen according to sites and severity of bleeds. For the purpose of this RENAWI-2 study, treatments with DDAVP and/or VWF/FVIII concentrates were analyzed only in case of spontaneous bleeding events (on-demand treatment). Treatments used to prevent bleeding at the time of surgery or invasive procedures in 72 patients as well as those used for secondary long-term prophylaxis in 11 cases were not analyzed in this study, which was specifically designed to evaluate only the phenotypic determinants of spontaneous bleeds. However, those patients treated on demand with VWF/FVIII concentrates were also observed beyond the RENAWI-2 follow-up until 2013 to evaluate their inclusion in a prophylaxis program because of the high frequency of spontaneous bleeds.

Statistical analysis

Continuous variables were expressed as medians with minimum (min) and maximum (max) values, and categorical variables as counts and percentages. Comparison between groups was made by the Mann-Whitney *U* test for continuous variables and the χ^2 test for categorical variables. The relationship between VWF:RCo plasma levels and BS (both taken as continuous) was assessed by means of linear regression analysis, using a restricted cubic spline function to check for possible deviations from linearity, and adjusting for age and sex. For different risk categories formed according to values and levels of BS, VWF:RCo, and FVIII, the incidence rate of bleeding (any type, mucosal and nonmucosal) was calculated as the number of episodes during the follow-up period divided by the total number of patient-years for that period, and 95% confidence intervals (CIs) were calculated under the Poisson distribution assumption. For each patient, the follow-up started on January 1, 2009 and ended at the date of the first bleeding event or on January 1, 2010 (administrative censoring). An additional observation until 2013 in the group of bleeders treated on demand with VWF/FVIII concentrates was carried out, with the only purpose to describe their inclusion in a prophylaxis program. At the beginning of follow-up, all VWD patients were free from bleeding for at least 1 month. A multivariable Cox proportional hazard model was used to calculate the risk of bleeding, expressed as hazard ratio (HR) with 95% CI, according to different predictors' categories (BS [<5, 5-10, >10], VWF:RCo [>30, 10-30, <10 IU/dL], FVIII [>40, 20-40, <20 IU/dL]) and in which the effect of each predictor was adjusted for the confounding effect of the others and for those of age and sex. The reference group was the highest category for VWF:RCo and FVIII and the lowest for BS. Bleeding-free survival was computed with the Kaplan-Meier method, and the log-rank test was used for comparison between groups. $P < .05$ was taken as cutoff for statistical significance. All analyses were performed with the statistical software SPSS (release 19.0), except for the linear regression analysis using the spline function, for which the statistical software R was used (release 2.9.1; R Project for Statistical Computing).

Results

BS as predictor of bleeding in VWD

The main demographics and clinical-laboratory features of the whole cohort are shown in Table 1. This cohort was mainly composed of adult VWD: only 16 of 796 (2%) patients had <18 years at enrollment. Among the 796 cases, 75 (9.4%) had at least 1 spontaneous bleeding event requiring treatment during the 1-year follow-up: therefore, these patients can be defined as bleeders. Bleeders had median values of BS and baseline VWF:RCo/FVIII levels, respectively, higher and lower than nonbleeders ($P < .001$ for all comparisons). Considering

Table 1. Demographic and clinical-laboratory features at baseline of the entire cohort (n = 796) vs bleeders (n = 75) of the VWD patients according to types

	VWD1		VWD2A		VWD2B		VWD2M		VWD3		All VWD types	
	All cases, n = 457	Bleeders, n = 23	All cases, n = 65	Bleeders, n = 10	All cases, n = 56	Bleeders, n = 5	All cases, n = 169	Bleeders, n = 12	All cases, n = 49	Bleeders, n = 25	All cases, n = 796	Bleeders, n = 75
Gender, M/F	183/274	10/13	31/34	7/3	23/33	3/2	75/94	6/6	21/28	11/14	333/463	37/38
Age, median (range), y	39 (6-99)	39 (13-67)	39 (7-81)	59 (11-85)	37 (8-84)	45 (16-61)	39 (10-89)	37 (18-73)	29 (5-60)	33 (10-61)	38 (5-99)	37 (10-85)
BS, median (range)	4 (−3-28)	6 (2-28)	8 (1-19)	9 (3-19)	6 (0-24)	7 (1-24)	5 (0-28)	4 (0-28)	14 (3-35)	16 (5-35)	5 (−3-35)	9 (0-35)
VWF:RCo, median (range), IU/dL	31 (3-56)	25 (6-56)	6 (2-53)	6 (2-22)	20 (6-79)	12 (6-23)	10 (4-50)	10 (6-37)	<2 (—)	<2 (—)	17 (<2-79)	6 (<2-56)
<10 IU/dL, case no. (%)	128 (28)	8 (35)	45 (69)	6 (60)	8 (14)	2 (40)	77 (46)	5 (42)	49 (100)	25 (100)	307 (38)	46 (61)
10-30 IU/dL, case no. (%)	94 (21)	12 (52)	18 (28)	4 (40)	38 (68)	3 (60)	71 (42)	4 (33)	0	0	221 (28)	16 (21)
>30 IU/dL, case no. (%)	235 (51)	3 (13)	2 (3)	0	10 (18)	0	21 (12)	3 (25)	0	0	268 (34)	13 (17)
FVIII:C, median (range), IU/dL	47 (2-187)	36 (13-129)	48 (17-140)	43 (21-110)	46 (16-140)	47 (35-57)	43 (7-144)	33 (22-48)	3 (1-26)	3 (1-19)	44 (1-187)	29 (1-129)
Previous DDAVP, case no. (%)	204 (45)	23 (100)	11 (17)	9 (90)	5 (9)	1 (20)	54 (44)	9 (75)	0	0	274 (34)	42 (56)
Previous conc., case no. (%)	146 (32)	11 (48)	55 (85)	10 (100)	35 (62)	5 (100)	106 (63)	10 (83)	46 (94)	25 (100)	388 (49)	61 (81)

Bleeders were defined as those VWD patients who had at least 1 spontaneous bleeding event requiring treatment during the 1-y follow-up.
conc., concentrates; F, female; M, male.

Figure 1. Restricted cubic spline curve. Curve showing the age- and sex-adjusted relationship between VWF:RCo plasma levels and BS in all RENAWI-2 patients with VWD. Dotted lines represent 95% CIs. A plateau was found at a mean BS value of 3.5 (dashed horizontal line), that was reached for VWF:RCo levels above 30 IU/dL.

the baseline levels of VWF:RCo, the proportion of mild (31-56 IU/dL), moderate (10-30 IU/dL), and severe (<10 IU/dL) cases was 34%, 28% and 38%, respectively, with differences within the VWD types. Only 13 of the 268 mild VWD cases (5%) had an episode of bleeding, and accounted for the 17% of the total bleeders (13 of 75). The BS measured at the time of inclusion was inversely related to baseline levels of VWF:RCo, reaching a plateau at a mean value of 3.5, corresponding to VWF:RCo levels above 30 U/dL (Figure 1).

The overall incidence rate of bleeding was 10.02 per 100 patient-years (95% CI, 7.75-12.29). The bleeding incidence rates (any, mucosal and/or nonmucosal) according to different BS, VWF:RCo, and FVIII levels are shown in Figure 2. BS > 10 was associated with the highest incidence of both mucosal (20.63 per 100 patient-years [95% CI, 12.20-29.06]) and nonmucosal bleeding (12.54 per 100 patient-years [95% CI, 6.90-19.95]). The incidence of bleeding was inversely related to VWF:RCo levels and, when VWF:RCo or FVIII were very low (<10 and <20 IU/dL, respectively), the incidence of bleeding was very high. The detailed calculations of the risk of bleeding according to BS, VWF:RCo, and FVIII as predictors are shown in Table 2. In the multivariable Cox proportional hazard model, only BS remained highly associated with the risk of bleeding (adjusted HR: 7.27 [95% CI, 3.83-13.83] for BS > 10 compared with BS <5), whereas VWF:RCo lost its effect. This is likely due to the high inverse relationship between VWF:RCo and BS for VWF:RCo levels <30 IU/dL (as shown in Figure 1), as well as to the high correlation between VWF:RCo and FVIII, particularly in VWD1 (in our case: $\rho = 0.70$ for the whole VWD group, and $\rho = 0.74$ for VWD1; $P < .001$ for both).

The cumulative probability of being free from bleeding during the 1-year follow-up, as calculated by the Kaplan-Meier method, is shown in Figure 3 according to the 5 VWD types (Figure 3A), BS and VWF:RCo in all VWD types (Figure 3B), BS and VWF:RCo in VWD1 only (Figure 3C), BS and FVIII in VWD3 only (Figure 3D). The event bleeding-free survival at 6 months was clearly shorter in VWD1 with BS >10 and VWF:RCo <10 U/dL (0.81 [95% CI, 0.62-0.99]) or in VWD3 patients with BS >10 and FVIII levels <5 U/dL (0.39 [95% CI:0.16-0.62]). In patients with VWD2A,

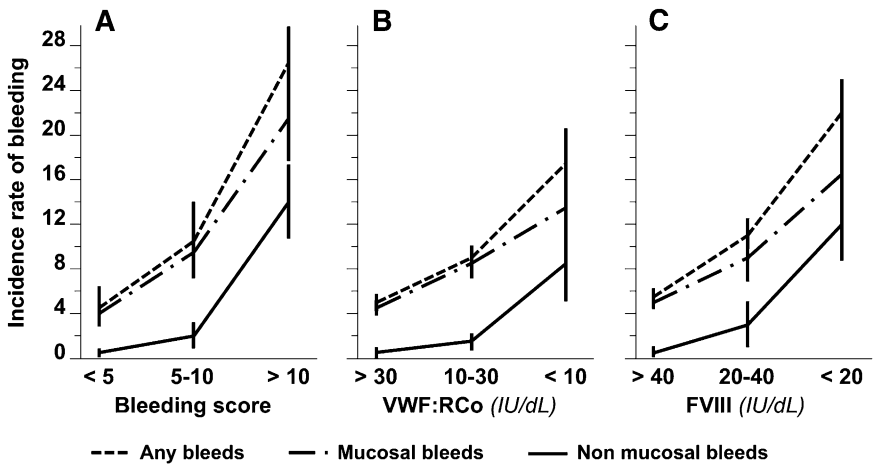

Figure 2. Bleeding incidence rate. Incidence rate of bleeding (per 100 patient-years; any type [dashed line], mucosal [dash-dotted line], nonmucosal [solid line]) according to BS (A), VWF:RCo (B), and FVIII (C). Vertical bars represent 95% CIs.

VWD2B, and VWD2M analyzed both separately and together, there was a nonstatistically significant difference in event bleeding-free survival according to BS and VWF:RCo levels (data not shown).

Primary outcome: annual bleeding events with replacement therapy

Among the 75 VWD bleeders, a total of 232 spontaneous bleeding events was recorded during the 1-year follow-up. The number of annual events per patient progressively increased according to VWD types (Table 3) but, when patients were analyzed according to BS, the proportion observed in all bleeders with BS < 5 was significantly lower (18 of 232 [8%]) than that observed in those with BS 5 to 10 (58 of 232 [25%]) and with BS > 10 (156 of 232 [67%]; $P < .0001$ for comparison between the 3 groups). The sites of mucosal (epistaxis, menorrhagia, gums, and gastrointestinal [GI] bleeds) and nonmucosal (hematoma and joint bleeding) bleeding events according to different VWD types are shown in Figure 4A. Overall, mucosal bleeds were more frequent than hematoma and joint bleeds: epistaxis and menorrhagia occurred in all VWD types, whereas GI bleeding occurred mainly in VWD3 and VWD2A, characterized by partial or complete loss of intermediate- and high-molecular-weight VWF multimers. On the other hand, hematomas and joint bleeds occurred mainly in VWD3 and in those patients with more severe VWD1 characterized by lower FVIII levels.

Table 2. Risk of bleeding in the 796 VWD patients according to clinical and laboratory predictors

	Crude HRs (95% CI)	Adjusted HRs (95% CI)*
BS		
<5	1†	1†
5-10	2.10 (1.10-3.90)	2.05 (1.07-3.91)
>10	6.80 (3.80-12.30)	7.27 (3.83-13.83)
VWF:RCo, IU/dL		
>30	1†	1†
10-30	1.51 (0.72-3.14)	1.16 (0.54-2.47)
<10	3.27 (1.77-6.06)	1.12 (0.50-2.51)
FVIII:C, IU/dL		
>40	1†	1†
20-40	2.07 (1.16-3.69)	1.52 (0.80-2.90)
<20	4.20 (2.43-7.26)	2.20 (1.05-4.62)

*The effect of each predictor on the bleeding risk was adjusted for that of the other ones, and for age and sex, in a multivariable Cox proportional hazard model.
†Reference group.

Other outcomes: intensity of replacement therapy and indications for prophylaxis

In general, a total of 292 (7.7 per patient per year) DDAVP injections and 795 (15 per patient per year) infusions of VWF/FVIII concentrates were used to treat spontaneous bleeds during the 1-year observation period. Pertaining to DDAVP, 38 of 75 patients (51%) with VWD1 (n = 18), VWD2A (n = 8), and VWD2M (n = 12) were treated with varying median annual doses per patient depending on types (Table 3). The most intensive on-demand therapy was used for VWD2A and VWD1. DDAVP was the only treatment of the majority of relatively mild bleeding events (epistaxis, menorrhagia, gum bleeds) although it was used together with VWF/FVIII concentrates in cases of more severe events such as GI bleeding, hematomas, or joint bleeds.

Pertaining to VWF/FVIII concentrates, 53 of 75 (71%) patients were treated on demand with varying annual doses per patient, not only in VWD3 (n = 25), VWD2A (n = 10), VWD2B (n = 5) but also in VWD2M (n = 7) and VWD1 (n = 6); median doses were particularly high in VWD3, VWD2A, and in a few patients with more severe forms of VWD1 (Table 3). GI or joint bleeds did usually require the most intensive treatment with VWF/FVIII concentrates. Treatment intensity was also analyzed according to the BS of bleeders (Table 3); for both DDAVP (Figure 4B) and VWF/FVIII concentrates (Figure 4C), cumulative dosages progressively increased with increasing BS values. Pertaining to VWF/FVIII concentrates, a few patients with severe forms of VWD1 were treated with median annual dosages similar to those used for VWD3 patients. Because of the intensive on-demand treatment needed for recurrent spontaneous bleeds, 12 of 53 patients (23%) were shifted by the investigators at the end of 12-month follow-up to long-term prophylaxis, owing to GI bleeds (5 of 12 cases) in VWD1 (n = 2) and VWD3 (n = 3), to hemarthroses (5 of 12 cases) in VWD3, and menorrhagia (2 of 12 cases) in VWD3 and VWD1. All 12 of these VWD patients had very high BS (median, 22; range, 11-33).

Discussion

The main goal of this prospective observational cohort study of 796 Italian patients with VWD was to use the BS not only for diagnostic purposes as hitherto but also to evaluate clinical outcomes by assessing the annual incidence rate of spontaneous bleeding and the

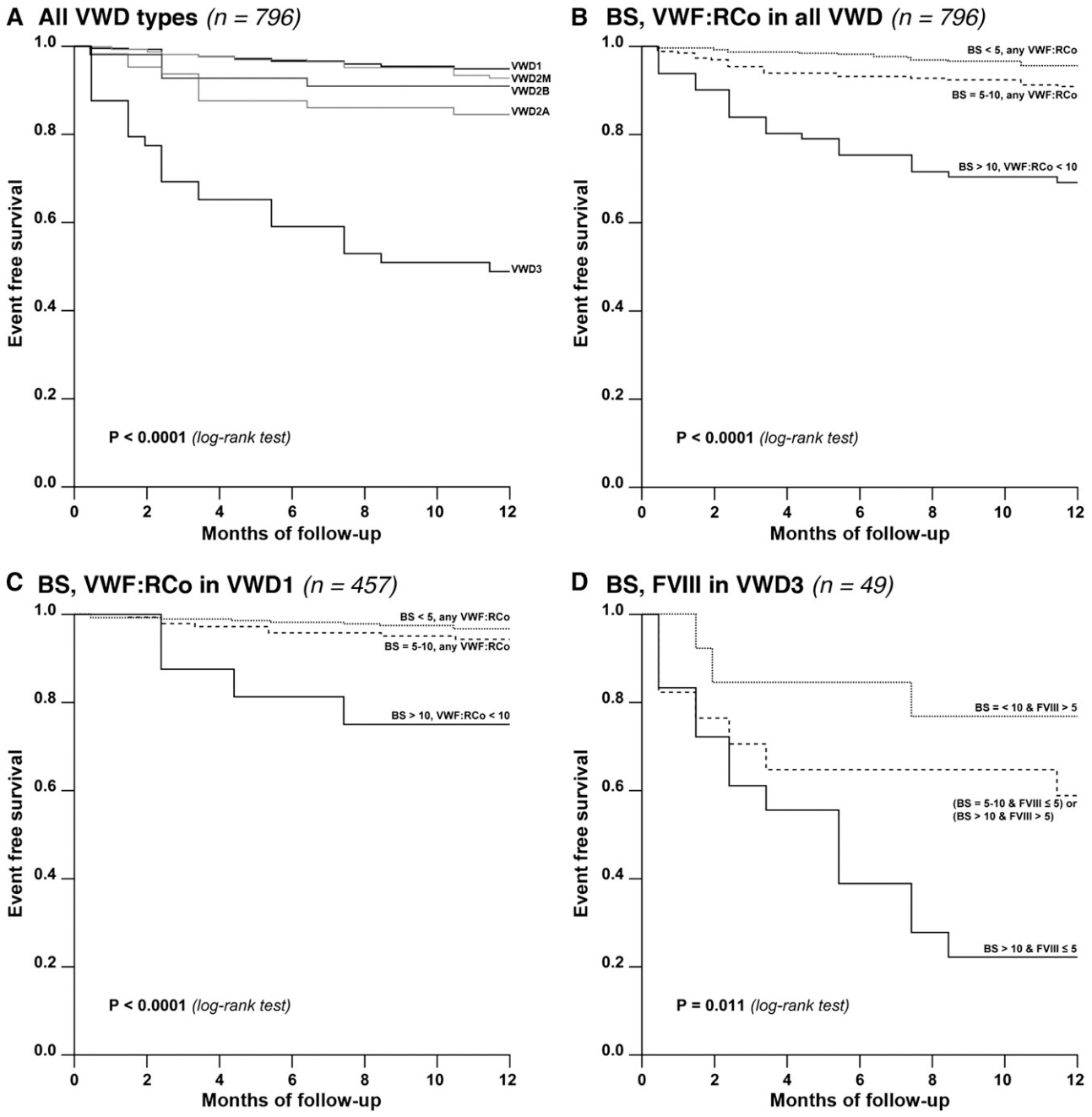

Figure 3. Bleeding event-free survival (Kaplan-Meier method). According to: (A) VWD types; (B) BS and VWF:RCo levels in all VWD; (C) BS and VWF:RCo levels in VWD1; (D) BS and FVIII levels in VWD3.

actual number of bleeding events requiring on-demand therapy with DDAVP and/or VWF/FVIII concentrates. The diagnosis of VWD types was confirmed by central reevaluation of the laboratory measurements as reported previously[25]; in RENAWI-2, patients were also evaluated using the BS calculated at baseline visit according to the questionnaire originally used by Tosetto and colleagues in the frame of the European study on VWD type 1.[18] This BS was subsequently proposed together with VWF levels and family history to obtain evidence-based diagnosis of VWD type 1.[33] More recently, BS was also considered as a determinant of clinical outcomes in a few VWD types.[34] With this background, we thought that the prospective approach of RENAWI-2 was appropriate to assess whether there was a more extensive role for the use of BS in all VWD types.

At variance with other studies that limited the evaluation of VWD patients to those with plasma levels of VWF:RCo <30 IU/dL, we chose to include in RENAWI-2 the largest possible number of VWD patients with mild (34%), moderate (28%), and severe (38%) laboratory phenotypes according to the European and Italian recommendations.[3,6,35] Thus, a relatively large proportion (34%) of mildly affected VWD patients with low-borderline levels of VWF activity was also included, and only 5% of them had a spontaneous bleeding event. Pertaining to VWD types, the most frequent was VWD1 (58%), followed by VWD2M (21%), VWD2A (8%), and VWD2B (7%), whereas the rarest VWD3 patients accounted for 6% of the total cohort. These proportions of VWD types in RENAWI-2 are similar to those previously reported.[1-3,6-10]

Table 3. Amount of DDAVP and VWF/FVIII concentrates used to treat 232 spontaneous bleeding events in the 75 VWD patients who bled during the 1-y follow-up, according to VWD types and BS

Bleeders at follow-up	VWD1, n = 23	VWD2A, n = 10	VWD2B, n = 5	VWD2M, n = 12	VWD3, n = 25	All VWD, n = 75
Total no. of bleeding events (events/pt)	49 (2.13)	35 (3.50)	13 (2.60)	36 (3.00)	99 (3.96)	232 (3.09)
No. of bleeds/y in VWD with BS >10 (% by VWD type)	28 (57)	19 (54)	7 (54)	18 (50)	84 (85)	156 (67)
Cases treated with DDAVP, no. (%)	18 (78)	8 (80)	0 (0)	12 (100)	0 (0)	38* (51)
DDAVP dose,† μg/pt/y, median (range)	105 (42-252)	189 (63-305)	0 (0)	63 (21-345)	0 (0)	105 (21-345)
DDAVP dose in VWD with BS >10, median (range)	210 (189-252)	235 (189-305)	0 (0)	302 (251-345)	0 (0)	241 (189-345)
Cases treated with VWF/FVIII concentrates, no. (%)	6 (26)	10 (100)	5 (100)	7 (58)	25 (100)	53* (71)
Concentrate dose‡ of VWF/RCo 1000 U/pt/y, median (range)	61 (16-256)	77 (5-126)	45 (7-93)	27 (5-75)	105 (18-235)	75 (5-256)
Concentrate dose in VWD with BS >10, median (range)	157 (48-256)	82 (75-126)	93	44 (27-75)	156 (63-235)	138 (27-256)

pt, patient.

*Some patients were treated with both DDAVP and VWF/FVIII concentrates.

†Each patient was treated with the same dosage of DDAVP equal to 0.3 μg/kg. The median doses of DDAVP calculated in microgram per patient per year are shown also according to BS <5, 5 to 10, >10 by 3 VWD types (see also Figure 4A-C).

‡Patients have been treated with different dosages (30-80 VWF:RCo U/kg) according to the different sites and severity of bleedings based on the judgment of the investigators. The median doses of VWF/FVIII concentrates expressed in total VWF:RCo IU × 1000/patient per year are shown also according to BS >10 by the 5 VWD types (see also Figure 4A-C).

Because the patient population was mainly composed of adults, the use of a pediatric BS[36,37] was not deemed necessary in the few included children. There were no significant difference in the BS pertaining to sex and age within VWD types. In RENAWI-2, the BS calculated at baseline was inversely and linearly correlated with plasma levels of VWF activity up to VWF:RCo levels of 30 IU/dL. Above this level, the relationship reached a plateau at a BS mean value of 3.5. These data are consistent with previous suggestions of Sadler and colleagues, who proposed a threshold level of VWF activity of 30 IU/dL to identify clinically relevant VWD patients.[10-13] Over a 1-year observation, only 75 patients (9.4%) had at least 1 spontaneous bleeding. The clinical (BS) and laboratory (VWF:RCo and FVIII) parameters were all associated with a high risk of bleeding. However, only a BS >10, a measurement that can be obtained easily during medical visits, remained the most significant determinant of the risk of any bleeding at multivariable analysis. The cumulative probability of being free from bleeding events differed among the VWD types, confirming a progressively increased degree of severity from the milder VWD1, VWD2M, and VWD2B to more severe VWD2A and VWD3. To evaluate the role of the BS together with that of VWF:RCo, that is, the measurement of VWF activity chosen for this study, the bleeding-free survival was calculated in the whole cohort of patients and in different VWD types, comparing BS <5 or BS 5 to 10 and any level of VWF activity with BS >10 and VWF:RCo <10. In the whole patient cohort, 3 different curves did result, with the shortest bleeding-free survival in those with BS >10 plus VWF:RCo <10. Furthermore, considering that levels of VWF activity may affect the BS and the bleeding risk, patients with partial (VWD1 = 457) or complete (VWD3 = 49) VWF deficiency were analyzed separately. In both groups, the bleeding-free survival was heterogeneous and resulted in 3 different curves, being shorter in patients characterized by BS >10 plus VWF:RCo <10 (VWD1) or plus FVIII <5 (VWD3). The heterogeneous bleeding tendency in VWD3 patients, considered by definition homogeneous because of the complete absence of VWF in plasma and cellular compartments, is intriguing. Indeed, there were cases with severe (ie, bleeds every week), moderate (bleeds every month), or mild (1-2 events per year) phenotypes. The possible role played in this heterogeneity by tiny amounts of VWF present in patient plasma or platelets, as well as the role of varying plasma levels of FVIII, is currently under investigation in a large prospective study on VWD3 [see http://www.vwd-3winters-ips.com/]. Conversely, the probability of being free from bleeding was not significantly different according to BS and VWF levels in VWD2A, VWD2B, and VWD2M. Perhaps other hemostasis-related parameters play a role in these VWD types as additional determinants of clinical severity, such as thrombocytopenia in VWD2B [22] and the more marked defects of the high molecular weight multimers in VWD2A than in VWD2M.[24]

The BS of the 75 bleeders (who had a total number of 232 annual events) was significantly higher than that of nonbleeders and the total annual rate of bleeds was significantly higher in patients with BS >10, irrespective of the VWD type. All of the other data on the type and frequency of bleeds were as expected from previous retrospective studies.[1-10,25] DDAVP was used not only in VWD1 but also in more than half of the bleeders with VWD2A and VWD2M, the most intensive regimens being used in VWD2A and VWD1. DDAVP was the only treatment of the majority of minor bleeding events (epistaxis, menorrhagia, gum bleeds), although it had to often be used together with VWF/FVIII concentrates to handle GI bleeding or hematomas. More than two-thirds of the bleeders needed at least 1 infusion of VWF/FVIII concentrates, with varying annual doses not only in VWD3, VWD2A, VWD2B but also in VWD2M and VWD1: the doses were higher in VWD3, VWD2A, and in a few severe bleeders with VWD1. However, not all of these bleeders required the same treatment intensity with DDAVP and/or VWF/FVIII concentrates because dosages did progressively increase from patients with BS of <5 to those with scores of 5 to 10 and >10. A few patients with clinically severe forms of VWD1 received median annual dosages of VWF/FVIII concentrates similar to those used in VWD3, suggesting that the intensity of replacement therapy does not depend on the VWD types but is mainly related to the BS, VWF, or FVIII levels.

Approximately one-fourth of VWD patients treated on demand with VWF/FVIII concentrates during the 12-month observation of RENAWI-2 were shifted to continuous prophylaxis because of their frequent and/or severe bleeding episodes. Information about correct

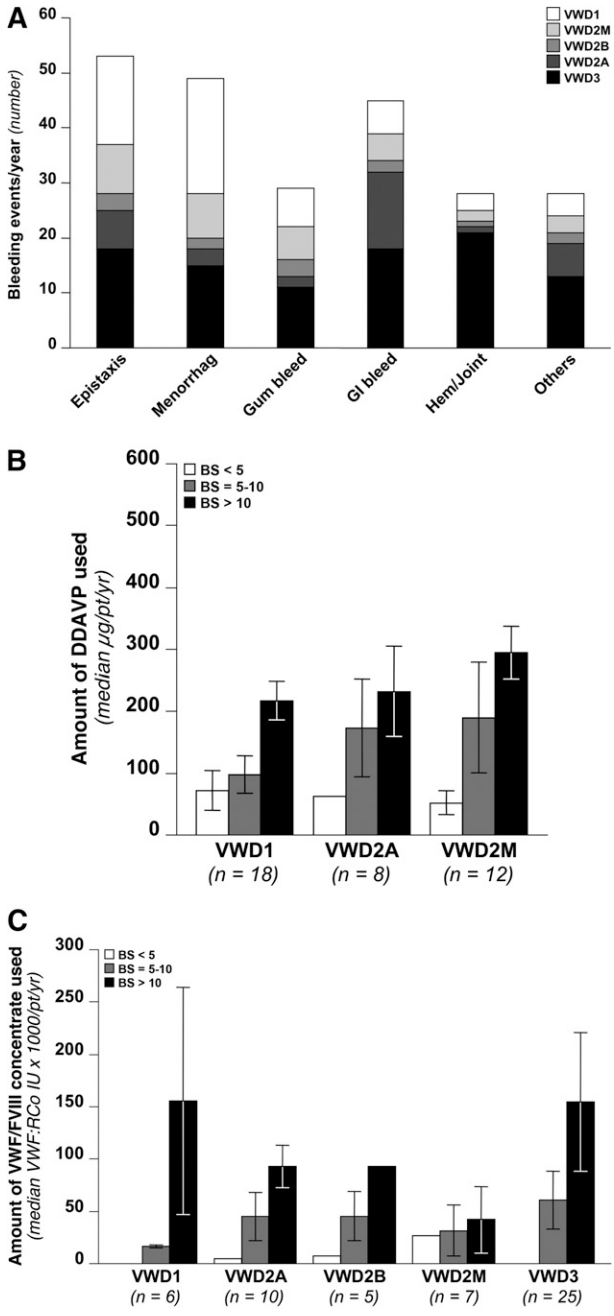

Figure 4. Frequency, types, and treatments of bleeding events. (A) The distribution of 232 spontaneous bleeding events is shown by bars according to different sites and, inside each bar, according to different VWD types. (B) The amount of DDAVP (expressed in median microgram per patient per year) used to treat spontaneous bleeding events in VWD1 (n = 18), VWD2A (n = 8), and VWD2M (n = 12) is shown according to different BS. (C) The amount of VWF/FVIII concentrates (expressed in median VWF:RCo IU per 1000 per patient per year) used to treat spontaneous bleeding events in VWD1 (n = 6), VWD2A (n = 10), VWD2B (n = 5), VWD2M (n = 7), and VWD3 (n = 25) is shown according to different BS.

indications and benefits of prophylaxis vs on-demand therapy in VWD is scanty and based mainly on retrospective analyses,[38-41] and the final results of the few ongoing studies are still unavailable.[39,41] Our data provide, for the first time, evidence that high BS may identify cases requiring prophylaxis.

The main limitation of this study is the relatively short period of observation (1-year follow-up). Although this time interval was probably sufficient to reflect the bleeding tendency of the more severe VWD3 and VWD2A types, a more prolonged observation would have been useful for the moderate or milder cases of the other VWD types.[22-24] Our cohort was mainly composed of adult VWD patients, with a relatively small number of pediatric VWD: hence, the potency of BS might have been different in children. An additional limitation is the absence of patients with VWD2N, who might represent a peculiar bleeding phenotype. Furthermore, because this project started before 2009, we could not adopt the bleeding assessment tool (International Society on Thrombosis and Haemostasis, Bleeding Assessment Tool [ISTH-BAT]) recommended by the International Society on Thrombosis and Hemostasis.[42] However, both ISTH-BAT and the BS used in the RENAWI-2 were derived from the first proposed BS.[15] On the other hand, strengths of this study are the prospective follow-up of a large cohort representative of the general distribution of VWD types and the centralized performance of the main laboratory assays.

Based upon these findings, we conclude that, at variance with hemophilia A, spontaneous bleedings occur in a relatively small proportion of patients with VWD from a cohort representative of the 5 different VWD types. Patients characterized by high BS and low VWF activity are more likely than others to have a high bleeding tendency and to use more therapeutic products for replacement therapy. Therefore, a simple and inexpensive parameter such as BS is proposed as a useful predictor of clinical outcomes of inherited VWD, and may also help to identify those cases at higher risk of frequent and severe bleeds requiring more intensive regimens, such as long-term prophylactic replacement therapy.

Acknowledgments

The authors thank all those who participated in the RENAWI-1 and RENAWI-2. The authors acknowledge the work of Luciano Baronciani and Maria Teresa Canciani at the Angelo Bianchi Bonomi Hemophilia Thrombosis Center in Milan who were involved in the genotype and phenotype assessments used to confirm VWD diagnosis in such a large cohort of patients. The authors greatly appreciate the comments and suggestions of Alberto Tosetto, and also thank Luigi Ghilardini who prepared figures and tables.

RENAWI-2 was partially supported by a grant of the Italian Ministry of Health (A.B.F. and P.M.M.).

Authorship

Contribution: A.B.F. designed the study, enrolled the patients, supervised the centralized confirmation of VWD phenotypic and molecular diagnoses, analyzed the data results, and wrote the manuscript; P.B. performed statistical analysis, analyzed the data results, and reviewed the manuscript; G.C. designed the study, enrolled patients, supervised the molecular diagnosis, analyzed the data results, and reviewed the manuscript; M.G.M., M.M., A.R., and M.S. enrolled the patients, analyzed the data results, and reviewed the manuscript; F.P. supervised the molecular diagnosis, analyzed the data results, and reviewed the manuscript; F.R. analyzed the data results and reviewed the manuscript; and P.M.M. discussed the original study, analyzed the data results, and extensively reviewed the final draft of the manuscript.

Conflict-of-interest disclosure: A.B.F. has been involved in advisory boards and had received honoraria as a speaker at educational meetings organized by Baxter, Csl-Behring, Grifols, Kedrion Biopharma, LFB, and Octapharma. P.M.M. had received honoraria as

a speaker at educational meetings organized by Bayer, Grifols, Kedrion Biopharma, and Novo Nordisk. The remaining authors declare no competing financial interests.

Correspondence: Augusto B. Federici, Department of Clinical Sciences and Community Health, University of Milan, Via Francesco Sforza 35, 20122 Milan, Italy; e-mail: augusto.federici@unimi.it.

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