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Bleeding Symptoms and von Willebrand Factor Levels: 30-Year Experience in a Tertiary Care Center

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Abstract

Correlations between bleeding symptoms and von Willebrand factor (VWF) levels may help to predict hemorrhagic severity in the Westerners with von Willebrand disease (VWD), but data in Asians are lacking. In this study, Thai patients with VWF levels <50 IU/dL without any secondary causes were enrolled from 1988 to 2018 to determine the relationship between VWF levels and hemorrhagic manifestations. According to the current concept, we reclassified VWD and low VWF by VWF levels $\leq$30 and 30 to 50 IU/dL, respectively. Type 2 VWD was diagnosed if VWF activity to antigen ratio was $\leq$0.6. Bleeding severity was determined by the condensed MCMDM-1VWD bleeding score (BS). Among 83 patients, VWF activities showed negative correlations with BS ($P = .001$), which were higher in type 2 (median: 7, interquartile range [IQR]: 5-11) compared with type 1 VWD (median: 3, IQR: 2-4) and low VWF (median: 4, IQR: 2-8). Bleeding symptoms were indistinguishable between type 1 VWD and low VWF using the 30 IU/dL cutoff point. However, VWF ristocetin cofactor activity or gain-of-function mutant glycoprotein Ib binding activity <36.5 IU/dL and VWF collagen binding activity <34.5 IU/dL could predict increased bleeding risk (BS $\geq$3) by 92.3% specificity and 70.0% sensitivity ($P < .0001$).

Keywords

von Willebrand disease, von Willebrand factor, bleeding score

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Introduction

von Willebrand disease (VWD) is caused by congenital defects of von Willebrand factor (VWF)[1] impairing platelet adhesion via glycoprotein Ib (GpIb) and causing faster factor VIII (FVIII) degradation.[2] Congenital VWD is classified into 3 types: type 1 (partial quantitative defects), type 2 (qualitative defects), and type 3 (complete deficiency).[3] The prevalence of VWD in Western populations was as high as 0.6% to 1.3%,[4] but the number in Thailand was only 1.1 per 1 million.[5] The US Hemophilia Treatment Center Network reported 13 239 patients with VWD comprising 85% of type 1, 13% of type 2, and 2% of type 3 VWD.[6] However, the proportions of VWD types were diverse among studies,[7] which were all published before the concept of "low VWF"[8] was introduced.

Bleeding symptoms are generally mild in type 1 VWD.[9] As mucocutaneous bleeding might occur in healthy subjects, the calculated bleeding scores (BS) using validated tools were

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invented to select patients for further investigations.[10] The condensed Molecular and Clinical Markers for the Diagnosis and Management of Type 1 VWD (MCMDM-1VWD) bleeding questionnaire has been used for evaluation of type 1 VWD since 2000s.[11] The latter Bleeding Assessment Tool[12] has been validated in healthy children and adults,[13] but not particularly in patients with VWD. A large study in 5196 Thai adult volunteers showed the median condensed MCMDM-1VWD BS of 0 with the 95% confidence interval (95% CI) of −2 to +2.[14] Another study in 16 VWD children revealed the minimum BS of +3.[15] Therefore, the normal threshold of BS <3 can be applied for general Thai population.

The VWF level of 50 IU/dL had been previously used as a cutoff diagnostic value for VWD.[3] However, 2 large cohorts from Europe and the United States[16,17] revealed that *VWF* gene mutations were detectable in 50% to 69% of participants with VWF levels of 30 to 50 IU/dL, which is now termed "low VWF," and 74% to 96% of type 1 VWD, which was classified by VWF levels <30 IU/dL. Nonetheless, some patients with low VWF can suffer from spontaneous or postprocedural hemorrhages that need VWF replacement.[18] Consequently, the diagnosis of VWD based also on hemorrhagic symptoms is more clinically relevant than using solely *VWF* mutations.

von Willebrand factor levels and BS generally exhibit the inverse correlations among patients with VWD in the Western cohorts,[10,11] but data from Asian population are lacking. Determining VWF levels that are associated with more severe bleeding may be helpful in prognostication and decision for replacement therapy. We, therefore, aimed to investigate the relationship between VWF levels and bleeding symptoms in Thai patients with VWD and low VWF.

Materials and Methods

Study Participants

The study population was enrolled from the list of all VWF assays performed at King Chulalongkorn Memorial Hospital, Bangkok, Thailand, from January 1988 to October 2018. Both adults and children were included if they had (1) personal or family history of bleeding tendency and (2) VWF levels <50 IU/dL according to the diagnostic criteria of VWD by UK Haemophilia Centre Doctors' Organization (UKHCDO) in 2004.[3] The patients with insufficient clinical information, acquired von Willebrand syndrome (AVWS), or diagnosis as other bleeding disorders rather than VWD were excluded.

In this study, the participants with VWF levels, activities and/or antigen, of <30 IU/dL were diagnosed as VWD.[4] The patients with VWF levels of 30 to 50 IU/dL would be reclassified into low VWF according to the current concept.[8] All of patients with low VWF were previously diagnosed as type 1 VWD by UKHCDO criteria.[3] Type 2 VWD was defined by ratios of GpIb binding activity or collagen binding activity (VWF:CB) to VWF antigen (VWF:Ag) ≤ 0.6.[8]

Laboratory Tests

The in-house enzyme-linked immunosorbent assays were applied for VWF:Ag and VWF:CB as previously described.[19] The in-house ristocetin cofactor (VWF:RCo) assay[20] was performed to measure GpIb binding activity. In brief, 200 μL washed formalinized platelet suspension[21] yielding 350×10^3 platelets/μL mixed with 50 μL diluted platelet-poor plasma (PPP) was assayed in a standard light transmission aggregometry (LTA) (Chrono-log 560VS; Chrono-log Corp, Havertown, Pennsylvania) with 1 mg/mL ristocetin (ristomycin monosulfate, R7752; Sigma-Aldrich, St Louis, Missouri). Each specimen was tested in duplicate using 3 different concentrations and diluted standard human plasma (SHP) calibration (Siemens Healthineers, Marburg, Germany). The interassay coefficients of variation (CVs) for VWF:RCo were 9.2% and 8.3% for commercial lyophilized normal (control plasma N [CPN]) and pathological plasmas (control plasma P [CPP]; Siemens Healthineers), respectively. Since 2016, VWF:RCo has been replaced by gain-of-function mutant GpIb binding (VWF:GPIbM) assay (INNOVANCE VWF Ac; Siemens Healthineers)[22,23] due to its less complexity, independence of the ristocetin-binding site, and excellent correlations with VWF:RCo.[24,25] The VWF:GPIbM assay was conducted as described by Patzke et al[22] in an automated coagulation system (CA-1500; Sysmex UK Ltd, Milton Keynes, United Kingdom) with the interassay CVs of 5.2% and 8.1% for CPN and CPP, respectively.

von Willebrand factor multimer analysis[26] was performed per physician decision. Low-dose ristocetin-induced platelet agglutination (RIPA) test,[27] FVIII coagulant activity (FVIII:C) assay,[28] LTA[29] with or without lumi-aggregometer release assay,[30] and modified Ivy bleeding time[31] were conducted when indicated.[4,32] A FVIII:C assay utilized a single-stage clot-based method[28] measuring activated partial thromboplastin time of PPP mixed with FVIII-deficient plasma (Siemens Healthineers) in an automated analyzer (CS-2400; Sysmex UK Ltd). The FVIII:C level of assayed PPP was calibrated by the standard curve of diluted SHP. The interassay CVs of FVIII:C for CPN and CPP were 8.1% and 9.2%, respectively. von Willebrand factor multimer analysis showed reduced high-molecular-weight (HMW) multimers in type 2A and 2B VWD. Low-dose (0.5 mg/mL) RIPA revealed positive agglutination in type 2B VWD.[27] Type 3 VWD was diagnosed when both VWF antigen and activities were <5 IU/dL without visualized bands on VWF multimer analysis.[32]

Study Design and Data Collection

The data were retrieved retrospectively from medical records for the patients before August 2015 and prospectively after that. The extracted information included symptoms and signs of bleeding, family history of bleeding disorders, and other pertinent medical history. Bleeding score was calculated by the condensed MCMDM-1VWD bleeding questionnaire.[11] For the previously diagnosed patients, the bleeding history was

Table 1. Clinical and Laboratory Characteristics of 83 Patients Classified as von Willebrand Disease (VWD) or Low von Willebrand Factor (Low VWF).

Characteristics	Low VWF (N = 34)	Type 1 VWD (N = 8)	Type 2 VWD (N = 40)	Type 3 VWD (N = 1)
Age at the diagnosis (years)	29 ± 14	24 ± 12	28 ± 18	35
Gender (female, %)	27/34 (79.4)	5/8 (62.5)	22/40 (55.0)	0/1 (0)
ABO blood group O (%)	20/20 (100)	5/5 (100)	17/25 (68.0)	1/1 (100)
Prolonged aPTT (N, %)	10/22 (54.5)	4/6 (66.7)	18/30 (60)	1/1 (100)
Prolonged bleeding time (N, %)	1/8 (12.5)	N/A	15/17 (88.2)	N/A
Iron deficiency anemia (N, %)	2/21 (9.5)	0/4 (0)	7/30 (23.3)	1/1 (100)
VWF:Ag (IU/dL, range)	46.6 (32.0-66.0)	30.0[a] (26.0-45.0)	34.8 (8.0-85.0)	<1.0
VWF:RCo or VWF:GPIbM (IU/dL, range)	44.0 (30.0-59.0)	27.5[b] (18.0-49.0)	6.0[c] (3.0-35.0)	4.7
VWF:CB (IU/dL, range)	42.7 (34.0-69.0)	30.5[a] (20.0-35.0)	6.5[c] (1.8-31.0)	<1.0
FVIII:C (IU/dL, range)	55.5 (30.0-85.0)	50.0 (25.0-71.5)	42.4 (6.0-94.0)	<1.0
Ratio of VWF:RCo or VWF:GPIbM to VWF:Ag (range)	0.92 (0.62-1.49)	0.93 (0.66-1.53)	0.21[c] (0.06-0.67)	NE
Ratio of VWF:CB to VWF:Ag (range)	0.95 (0.63-1.20)	1.01 (0.64-1.25)	0.19[c] (0.04-0.86)	NE
Ratio of FVIII:C to VWF:Ag (range)	1.21 (0.73-2.24)	1.65 (0.96-2.54)	1.29 (0.46-2.61)	NE
Bleeding score ≥3 (N, %)	17/26 (65.4)	4/7 (57.1)	31/33 (93.9)[d]	1/1 (100)
Bleeding score ≥4 (N, %)	14/26 (53.8)	2/7 (28.6)	30/33 (90.9)[e]	1/1 (100)

Abbreviations: aPTT, activated partial thromboplastin time; FVIII:C, factor VIII coagulant activity; IQR, interquartile range; N/A, no data available; NE, not estimable; VWD, von Willebrand disease; VWF:Ag, von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding activity; VWF:GPIbM, gain-of-function mutant GpIb binding activity; VWF:RCo, von Willebrand factor ristocetin cofactor activity.
[a] $P < .001$ for Mann Whitney *U* test comparing type 1 VWD to low VWF.
[b] $P < .05$ for Mann Whitney *U* test comparing type 1 VWD to low VWF.
[c] $P < .001$ for Mann Whitney *U* test comparing type 2 to type 1 VWD.
[d] $P < .05$ for Fisher exact test comparing type 2 to type 1 VWD.
[e] $P < .01$ for Fisher exact test comparing type 2 to type 1 VWD.

obtained from either medical records or prospective interviews when they came for follow-ups.

Statistical Analysis

For continuous data with normal distribution, means and standard deviations were used for description and *t* test or analysis of variance for comparison among VWD types. For skewed data by the Shapiro-Wilk test, medians with ranges or interquartile ranges (IQRs) and nonparametric tests were applied. Linear regression and rank correlation and receiver operating characteristic (ROC) curve analyses were performed to estimate the correlations between factors and accuracy of diagnostic parameters, respectively.

SPSS Statistics 22.0 (IBM Corporation, Armonk, New York) and GraphPad Prism 8.0 (GraphPad Software Inc, San Diego, California) were the softwares for analyses, considering the level of significance at $P < .05$.

Results

Baseline Characteristics

Among 94 patients who had VWF levels <50 IU/dL, 83 participants from 65 families were diagnosed as VWD or low VWF comprising 12.2% of all requested VWF assays. Eleven patients were excluded due to a case of liver cirrhosis (with borderline VWF:CB) and 10 cases of AVWS.

There were 34 patients classified as low VWF (41%), 8 as type 1 (9.6%), 40 as type 2 (48.2%), and 1 as type 3 VWD (1.2%). Among patients with type 2 VWD, there were 26 patients subclassified as type 2A (31.3%) and 1 as type 2M (1.2%) VWD. None of the patient was diagnosed as type 2B VWD. Thirteen (15.7%) were type 2 VWD of an unspecified subtype because VWF multimer analysis and low-dose RIPA were not performed.

The mean age at diagnosis was 28 ± 16, ranging from 1 to 67 years old. Sixteen (19.3%) patients were diagnosed before 15 years old. Although the patient with type 3 VWD was diagnosed at the age of 35, he was previously misdiagnosed as hemophilia A at the age of 6 months. The majority of patients were females (65.1%) and had blood group O (84.3%) that included all patients in low VWF and type 1 VWD groups. Baseline characteristics of each VWD type are shown in Table 1.

Twelve (35.3%) patients with low VWF had a history of bleeding tendency in parents and/or siblings. Fourteen patients with low VWF underwent LTA and 6 (42.9%) cases revealed mild abnormalities: 3 cases with reduced responses to adenosine diphosphate (ADP) and epinephrine, 2 cases with reduced responses to ADP and collagen, and a case with only adenosine triphosphate release defect on lumi-aggregometry. Among these 6 cases, only 1 (16.7%) patient had family history of bleeding.

von Willebrand Factor Assays

von Willebrand factor: Ag and FVIII:C of type 1 were not significantly different from those of patients with type 2 VWD.

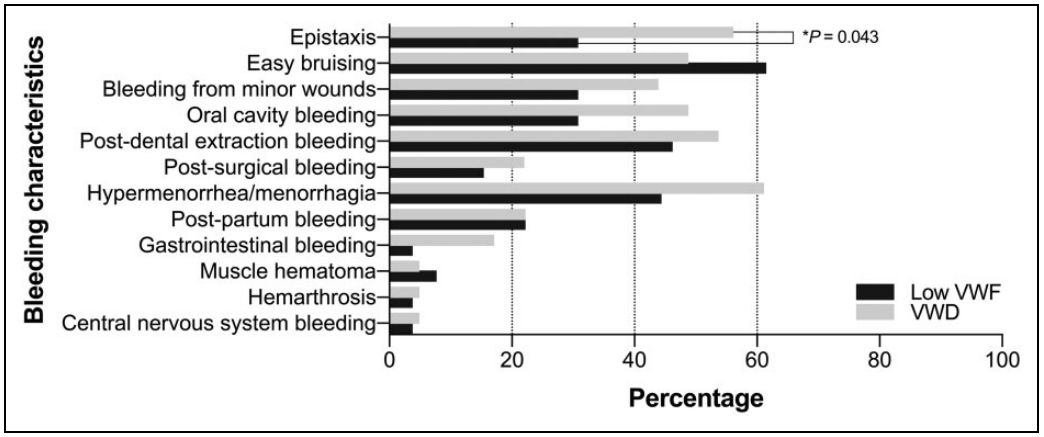


Figure 1. Frequencies of bleeding sites among 67 patients with VWD and low VWF. VWD indicates von Willebrand disease.

However, VWF activities and activity to antigen ratios were significantly lower in type 2 VWD. All VWF assays are displayed in Table 1. There was no difference in clinical characteristics and VWF levels between type 2A and unspecified type 2 VWD (data not shown).

von Willebrand factor: RCo was used to measure GpIb binding activities in 67 (80.7%) cases before the test was substituted by VWF:GPIbM. In all patients, GpIb binding activity and VWF:CB exhibited a strong correlation by the R^2 of 0.83 ($P < .001$). On the other hand, FVIII:C modestly related to VWF:Ag, GpIb binding activity, and VWF:CB exhibiting the R^2 of 0.17 ($P = .001$), 0.09 ($P = .025$), and 0.16 ($P = .002$), respectively.

Twenty-two patients (7 of low VWF, 2 of type 1, and 13 of type 2 VWD) had repeated assays at the median time of 31.5 months (IQR: 1.8-95.8) apart. The median difference in VWF:RCo or VWF:GPIbM levels was +3.40 IU/dL, IQR: −0.08 to +10.18 (Wilcoxon signed-rank $P < .001$). However, VWF:Ag and VWF:CB levels did not significantly change.

von Willebrand Factor Multimer Analysis and Low-Dose RIPA

von Willebrand factor multimer analysis was performed in 45 (54.2%) patients. Loss of HMW multimers was found in 29 of 30 patients with type 2 VWD in whom multimer analyses were done. The patient with type 2M and 14 patients with type 1 VWD or low VWF showed normal multimer distribution. There were 7 patients with type 2 VWD who were assigned subtypes according to the assays of their relatives. Low-dose RIPA was achieved in 23 patients of type 2 VWD. All cases did not show platelet agglutination induced by low-dose ristocetin.

Bleeding Characteristics

The complete history of bleeding could be obtained in 67 patients, including 26 cases of low VWF (38.8%), 7 of type 1 (10.5%), 24 of type 2A (35.8%), 9 of unspecified type 2 (13.4%), and 1 (1.5%) of type 3 VWD. Most bleeding symptoms were at mucocutaneous sites. Bleeding after invasive procedures (58.2%), either dental extraction (50.7%) or surgery (19.4%), was the most common presentation followed by easy bruising (53.7%), epistaxis (46.3%), bleeding in oral cavity (41.8%), and minor wound bleeding (38.8%), respectively. Bleeding patterns were similar among VWD types (Figure 1), except epistaxis which was more frequent in patients with VWD than low VWF ($P = .043$) and bleeding from minor wounds which was more common in patients with type 2 VWD ($P = .022$). The patient with type 3 VWD had both mucocutaneous and deep tissue bleeding. Most (92.5%) patients experienced more than single bleeding event before consultation.

Bleeding into deep tissues was found in 10 patients: 1 of type 3, 3 of type 2, 2 of type 1 VWD, and 4 of low VWF. Most of them were after minor trauma or with underlying lesions. In females (N = 36), the most common gynecologic hemorrhages were hypermenorrhea (52.8%) followed by postpartum bleeding (22.2%). There was no statistical difference of these 2 events among groups.

Among 67 patients with treatment information, cryoprecipitate, FVIII/VWF concentrate, tranexamic acid, and desmopressin were given in 44.8%, 14.9%, 23.9%, and 6.0%, respectively. Cases with BS $\geq$3 were more likely to receive VWF replacement compared with those with lower BS (67.3% vs 0%, $P < .001$). Local hemostatic measures by suturing and gauze packing were often applied for postdental extraction hemorrhages (64.7%). The patients with hypermenorrhea were treated by multiple modalities including tranexamic acid (36.8%), oral contraceptive pills (26.3%), dilation and curettage (21%), and VWF replacement (21%). However, 2 patients (1 of low VWF and 1 of type 2A VWD) eventually underwent hysterectomy to terminate their chronic refractory uterine bleeding.

Bleeding Score

The median BS calculated by the condensed MCMDM-1VWD bleeding questionnaire was 6 (IQR: 3-10). Bleeding score of patients with low VWF (median: 4, IQR: 2-8) was not significantly different from those of type 1 VWD (median: 3, IQR:

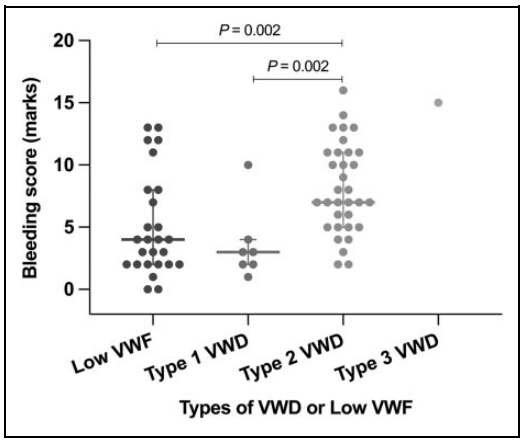


Figure 2. The scatter plot of bleeding scores of 67 patients classified by types of VWD and low VWF. VWD indicates von Willebrand disease.

2-4, $P = .36$). Type 1 had less severe BS compared with patients with type 2 VWD (median: 7, IQR: 5-11, $P = .002$). The BS of patient with type 3 VWD was high at 15.

In all patients, BS significantly related to VWF:RCo or VWF:GPIbM ($R^2 = 0.149$, $P = .002$; Goodman and Kruskal $\gamma = -0.272$, $P = .001$) and VWF:CB ($R^2 = 0.133$, $P = .003$; $\gamma = -0.267$, $P = .001$). The scatter plot of BS by each type of VWD and low VWF is illustrated in Figure 2.

By using BS $\geq$3 as the definition of an increased bleeding risk,[14,15] 53 (79.1%) patients with high BS displayed significantly lower VWF activities compared with those with normal BS: median GpIb binding activity of 10.0 IU/dL (95% CI: 15.66-25.97) versus 44.0 IU/dL (95% CI: 34.85-48.12, $P <$ .001) and median VWF:CB of 11.5 IU/dL (95% CI: 16.09-26.67) versus 38.0 IU/dL (95% CI: 29.02-43.41, $P = .008$), respectively (Figure 3A, B).

As the cutoff VWF level of 30 IU/dL could not differentiate the bleeding severity, the diagnostic accuracies of VWF activities for the increased risk of bleeding were consequently evaluated by ROC analyses (Figure 3C, D). Glycoprotein Ib binding activity <36.5 IU/dL provided the discriminating power of 74.5% sensitivity and 76.9% specificity, while VWF:CB <34.5 IU/dL showed 71.2% sensitivity and 78.6% specificity (Figure 3E).

The combination of GpIb binding activity <36.5 IU/dL and VWF:CB <34.5 IU/dL significantly improved the diagnostic yield of the elevated hemorrhagic risk by 80.5% accuracy (area under the ROC curve, 95% CI: 68.4-92.5, $P < .001$), 70.0% sensitivity (95% CI: 56.2-80.9), 92.3% specificity (95% CI: 66.7-99.6), 97.2% positive predictive value (95% CI: 85.8-99.9), 44.4% negative predictive value (95% CI: 27.6-62.7), and the positive likelihood ratio of 9.1 (Fisher exact $P < .0001$).

Discussion

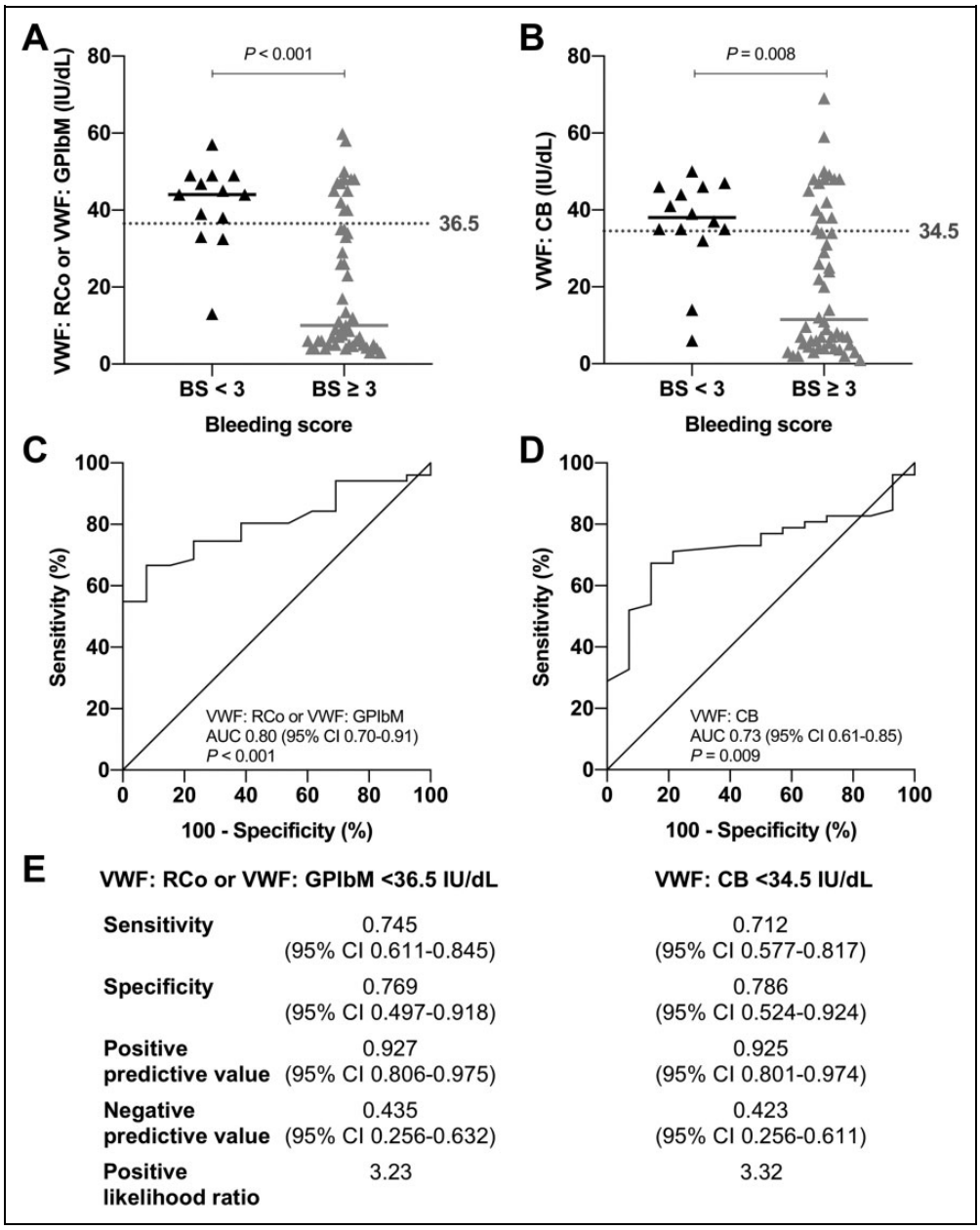
In this study, VWD has been infrequently diagnosed in Thais. A large population-based study is required to accurately determine the disease burden because type 1 VWD and low VWF may not come for medical attention due to minor symptoms.[7] Only 8 (9.6%) patients in our study fit the current diagnostic criteria for type 1 VWD, while the other 34 (41%) patients were reassigned as low VWF for VWF levels of 30 to 50 IU/dL.[8] Type 2 was the most common form of VWD similar to a previous study from Thailand[33] and the majority of them were type 2A. The proportions of types and subtypes of VWD are variable among studies and countries.[7,33-37] The Taiwanese cohort[37] showed a larger fraction of type 1 VWD (75.4%), as the cutoff VWF level of <50 IU/dL was used.

Bleeding symptoms were more severe in type 2 than type 1 VWD probably due to the lower VWF activities. From a previous study in Europe, the condensed MCMDM-1VWD BS $\geq$4 could differentiate patients with type 1 VWD from healthy controls by 100% sensitivity and 87% specificity.[11] In this study, however, type 1 VWD exhibited the median BS of 3 indicating that the cutoff of BS $\geq$4 may be too high for Thais. The patients with BS $\geq$3 revealed significantly lower VWF activities. If we used the cutoff BS $\geq$3 for children[15] and male adults and BS $\geq$4 for female adults according to the sex-specific 97.5th percentiles of BS in healthy Thais,[14] the number of patients with increased bleeding risk (N = 53) remained identical and all relevant statistical analyses were similar to those determined by "BS $\geq$3 for all participants." Therefore, BS $\geq$3 was applied for a practical purpose.

Bleeding phenomena of the low VWF group were comparable to those of the low VWF Ireland Cohort (LoVIC).[38] They discovered that 77.8% of 126 patients had the condensed MCMDM-1VWD BS $\geq$3. Similar to another Asian cohort,[37] patients with VWD and low VWF were usually diagnosed in adulthood (80.7%), despite personal history of recurrent bleeding since young ages. When compared to the Westerners,[36] Asians showed less epistaxis (39%-40% versus 61%-66%) but more postdental extraction bleeding (53%-60% versus 31%-53%) and menorrhagia (67%-83% versus 32%-60%). For Thais, epistaxis, which was more frequent in VWD (56%), and minor wound bleeding, which was more often in type 2 VWD (52%), might indicate higher severity of symptoms.

Despite the negative correlations between VWF activities and BS, bleeding severities were not different between type 1 VWD and low VWF groups. This might be partly explained by concomitant platelet dysfunction found in 42.9% (6/14) of low VWF participants. Since the restrictive cutoff VWF levels <30 IU/dL could not recognize patients with BS $\geq$3 in this study, the higher cutoff VWF levels (VWF:RCo or VWF:GPIbM <36.5 IU/dL and VWF:CB <34.5 IU/dL) were found to be more clinically relevant.

As blood group O was found in 37% of general Thai population,[19] the high prevalence of blood group O in low VWF group was consistent with the LoVIC study.[38] However, it was unexpected that all our patients with type 1 VWD also had blood group O. The lack of A and B antigens on VWF not only decreases VWF synthesis/secretion but also increases VWF clearance causing proportionately decreased VWF antigen and activities.[39] Genetic testing will hopefully determine whether



E

	VWF: RCo or VWF: GPIbM <36.5 IU/dL	VWF: CB <34.5 IU/dL
Sensitivity	0.745 (95% CI 0.611-0.845)	0.712 (95% CI 0.577-0.817)
Specificity	0.769 (95% CI 0.497-0.918)	0.786 (95% CI 0.524-0.924)
Positive predictive value	0.927 (95% CI 0.806-0.975)	0.925 (95% CI 0.801-0.974)
Negative predictive value	0.435 (95% CI 0.256-0.632)	0.423 (95% CI 0.256-0.611)
Positive likelihood ratio	3.23	3.32

Figure 3. Scatter plots of (A) Gplb binding activity, (B) VWF:CB, classified by the bleeding risk (bleeding score [BS] $\geq$3 vs BS <3). Receiver operator characteristic (ROC) curve analyses (C, D) determining the best cutoff VWF levels (dashed lines in A-B) and the diagnostic parameters for each cutoff value (E) were listed. BS indicates bleeding score.

there are more *VWF* gene mutations which underlie the depressed VWF levels in type 1 VWD.

This study has limitations including incomplete investigations due to limited resources. All patients were tested for VWF:Ag, but the activity assays were missed in 4.8% of patients. Only 22 (26.5%) patients were investigated for VWF assays more than once. Low-dose RIPA and VWF multimer analysis were not performed in one-third of type 2 VWD participants. Nevertheless, there was no thrombocytopenia that suggested type 2B VWD.[27] Our institute was unable to perform VWF-FVIII binding activity (VWF:FVIIIB)[40] and *VWF* gene sequencing assays, and therefore, the diagnosis of type 2N VWD and *VWF* genetic variants could not be delivered to any patients. Although 62.7% of patients had family history of bleeding tendency, the preconceptional testing and prenatal diagnosis for VWD in the affected parturients[41] have not been established in our obstetric practice. Additionally, VWF levels after delivery were not routinely monitored. However, VWF replacement and tranexamic acid (28.6%) were promptly given for postpartum hemorrhages.

In summary, type 2 VWD, which is the most common form in Thailand, showed lower VWF activities and more severe bleeding symptoms compared with type 1 VWD. Notably, the patients with low VWF (VWF levels of 30 to 50 IU/dL) had

similar BS to those with type 1 VWD. von Willebrand factor activities of approximately <35 IU/dL may be used to identify patients with increased risk of bleeding.

Authors' Note

Ethical approval to report this study was obtained from the institutional review board of Faculty of Medicine, Chulalongkorn University (IRB No. 356/58). Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article. C.M. designed and performed the research, analyzed the data, and wrote the manuscript. B.A., Y.K., A.S., and M.M. designed the research, performed laboratory assays, and revised the manuscript. N.U. and D.S. designed the research and revised the manuscript. P.R. designed the research, analyzed the data, and revised the manuscript.

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Declaration of Conflicting Interests

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