The Effects of Storage Time at 2–8 Degrees Celsius on the Stability of von Willebrand Factor in Thawed, Platelet-Poor Plasma

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ABSTRACT

Objective: To investigate VWF stability in thawed plasma by comparing immediately thawed samples with plasma stored at 2–8 °C for 24–96 hours.

Materials and Methods: Plasma from healthy subjects with normal coagulation times and VWF panels was stored at -20 °C for one week. After thawing (at 0 hours), VWF:antigen (VWF:Ag), VWF:glycoprotein Ib binding assay (VWF:GPIbM), and VWF:collagen binding assay (VWF:CB) were assayed. The remaining plasma was stored at 2–8 °C and assayed at 24, 48, 72, and 96 hours. Differences between levels at baseline and 24, 48, 72, and 96 hours were deemed significant when P was < 0.05.

Results: Thirty-five samples were enrolled, with 25 from healthy subjects (VWF:Ag levels > 0.50 kIU/L). Median levels (interquartile range) were as follows: VWF:Ag = 0.91 (0.72–1.06) kIU/L; VWF:GPIbM = 0.85 (0.69–1.04) kIU/L; and VWF:CB = 0.78 (0.62–0.97) kIU/L. VWF:Ag remained stable for 72 hours, while VWF:GPIbM decreased significantly after thawing. VWF:CB declined after 48 hours at 2–8 °C. Similar stability trends were observed in 10 additional samples from VWD patients (VWF:Ag = 0.42 (0.36–0.46) kIU/L).

Conclusion: VWF:Ag and VWF:CB are stable in thawed plasma for 72 hours. VWF:GPIbM is less stable and should not be kept longer than 24 hours. Immediate testing of VWF:GPIbM after thawing is recommended.

Keywords: Stability; Thawed plasma; VWF:Ag; VWF:CB; VWF:GPIbM (Siriraj Med J 2023; 75: 567-574)

INTRODUCTION

Von Willebrand disease (VWD) is the most common inherited bleeding disorder, resulting from quantitative or qualitative abnormalities of von Willebrand factor (VWF). Currently, a provisional diagnosis of VWD requires both clinical and laboratory criteria to be met. The clinical criteria include the presence of abnormal bleeding symptoms with or without a familial history of VWD. The laboratory criteria involve the presence of abnormal quantitative or qualitative VWF assays.[1,2] Bleeding symptoms can be assessed empirically or preferably through systematic evaluation using scoring systems such as the bleeding assessment tool of the International Society on Hemostasis and Thrombosis.[3] If the bleeding score exceeds the normal cutoff value, further investigations are recommended.[3] VWF panel assays include a quantitative measurement (VWF:antigen; VWF:Ag) and functional assessments of platelet- and collagen-binding abilities.[4]

A definitive diagnosis of VWD and its subtypes relies on accurate results from individual tests. For instance, the possibility of VWD subtypes 2A, 2B, or 2M is suggested by a ratio of < 0.7 between each of the following factors

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https://doi.org/10.33192/smj.v75i8.263320

and VWF:Ag: VWF:ristocetin cofactor (VWF:RCo), VWF:glycoprotein Ib binding by ristocetin (VWF:GPIbR), VWF:glycoprotein Ib binding by multimer analysis (VWF:GPIbM), and VWF:collagen binding (VWF:CB).[4]

Various external and environmental factors, such as age, blood group, and concurrent inflammation, can interfere with test results.[5,6] Additionally, pre-analytical processes, including blood collection, sample storage methods (immediate plasma spinning or whole blood), and storage temperature, can significantly impact the outcomes.[7] Given the limited number of laboratories capable of performing VWF assays, samples are often sent to referral laboratories. Immediate plasma spinning is recommended by Magnette et al due to the crucial role of pre-analytical processes.[7]

If assays can be conducted within 4 hours, plasma should be stored at 20–28 °C. Otherwise, freezing the plasma at -20 to -80 °C until testing is advised.[7,8] During transportation from a blood collection center to a referral laboratory, frozen plasma should be kept in dry ice.[8] Nevertheless, there is a risk of partially melted plasma inadvertently reaching the referral laboratory. Furthermore, frozen plasma, which requires three assays, is typically collected and transported in a single tube. In practical terms, conducting all three procedures on the same day may not be feasible, and the practice of repeated thawing-refreezing-thawing for testing on different days is not recommended.[9] As a result, thawed plasma is usually stored at 2–8 °C until the tests can be conducted. However, the stability of VWF in plasma over an extended duration remains uncertain.

We compared the stability of VWF:Ag, VWF:GPIbM, and VWF:CB in thawed plasma that was obtained from patients with VWD and healthy controls and stored at 2–8 °C for up to 96 hours.

MATERIALS AND METHODS

Plasma samples

The research received approval from the Institutional Review Board (COA no. Si 575/2017). Between November 2017 and September 2019, a total of 35 participants were enrolled, comprising 25 healthy subjects and 10 patients with either VWD type 1 or type 2A. Briefly, 1-milliliter samples were collected from 3.2% citrated plasma. Following blood collection, platelet-poor plasma (PPP) was obtained by centrifugation at 2,000 g for 15 minutes at room temperature. All samples from healthy subjects exhibited normal prothrombin time, activated partial thromboplastin time, VWF level, and function. To simulate typical sample transportation from other hospitals, each 1-milliliter sample was stored at -20 °C for a week before the experiment, with a time interval of within 4 hours between blood collection and plasma freezing. Thawed samples were immediately subjected to VWF panel assays. One hundred microliters of the remaining plasma from each sample were aliquoted into individual Eppendorf tubes and stored at 2–8 °C for 24, 48, 72, and 96 hours, with additional VWF panel assays conducted at those specific time points.

VWF assays

Quantitative analysis of VWF:Ag was performed using enzyme-linked immunosorbent assay, following the method described by Ingerslev.[10] The platelet-binding function of VWF:GPIbM, as described by Bodo et al, was assessed by coating plastic beads with gain-of-function recombinant glycoprotein Ib (Innovance VWF Ac).[11] The addition of plasma, serving as a source of VWF, initiated aggregation of the plastic beads, with the percentage of light transmission directly correlating with the platelet-binding capacity of VWF.[11] Finally, VWF:CB was measured through enzyme-linked immunosorbent assay, where a microtiter plate was coated with human collagen type 3 (SouthernBiotech).[12] The optical density directly correlated with the binding affinity of VWF to collagen.[12]

Statistical analyses

VWF panel results for continuous variables following a normal distribution are presented as the means and standard deviations. Nonnormally distributed continuous variables are reported as medians with interquartile ranges. Categorical variables are summarized as the number and percentage of samples. We defined the threshold of allowable bias of VWF to be 6.9% (calculated as the VWF level at 0 hours minus allowable errors). Decreases in the measured values of VWF that exceeded 6.9% were considered clinically significant for VWF instability.[13]

To compare the VWF results of thawed plasma stored at different time points with the defined threshold, a paired t-test was used for normally distributed outcomes. The Wilcoxon signed-rank test was employed for two related samples with a nonnormal distribution. The mean percentage change of each VWF test was evaluated in comparison to the threshold level across different time points using repeated-measures ANOVA.

Statistical significance was defined as a P value of < 0.05 for all performed tests. The analyses were conducted using PASW Statistics (version 18; SPSS Inc, Chicago, IL, USA).

RESULTS

Twenty-five samples were collected from normal

subjects, with a mean age of 40±18 years. Among these samples, 20 out of 25 (80%) were obtained from female individuals. Immediately after blood collection and prior to freezing the PPP, the VWF:Ag, VWF:GPIbM, and VWF:CB levels of each sample were above 0.50 kIU/L. After thawing the PPP, immediate assays (conducted at 0 hours) revealed median levels of VWF:Ag, VWF:GPIbM, and VWF:CB of 0.91 (0.72–1.06), 0.85 (0.69–1.04), and 0.78 (0.62–0.97) kIU/L, respectively (Table 1).

Significant decreases were observed in VWF:Ag levels, declining from 0.91 kIU/L at 0 hours to 0.67 kIU/L at 96 hours ($P < 0.001$; Table 1). Furthermore, at 96 hours, 80% of the samples had VWF:Ag levels below the threshold value. Regarding the VWF:GPIbM assay, a rapid decline in stability was observed after 24 hours, with levels decreasing from 0.85 kIU/L (0 hours) to 0.73 kIU/L (24 hours), yielding a *P* value of 0.001 (Table 1). More than 90% of the samples stored at 4 °C for 96

TABLE 1. Levels of VWF:Ag, VWF:GPIbM, and VWF:CB in thawed plasma from 25 healthy individuals at 0 hours and after storage at 2–8 °C for 24, 48, 72, and 96 hours.

Storage time after thawing (hours)	Amount (median and IQR) (kIU/L)	Number of samples with VWF lower than threshold (%)	Amount of decrease from threshold (median and IQR) (kIU/L)	% decrease from threshold (mean and 95% CI) (%)	*P*
VWF:Ag (N=25)					
0	0.91 (0.72 to 1.06)	-	-	-	-
Threshold (hour 0-allowable error)	0.85 (0.67 to 0.99)	-	-	-	-
24	0.86 (0.66 to 1.10)	9 (36)	0.05 (-0.03 to -0.14)	-0.71, (-7.62, 6.2)	.834
48	0.83 (0.65 to 1.04)	13 (52)	-0.004 (-0.11 to -0.11)	-4.64, (-13.24, 4.01)	.279
72	0.82 (0.71 to 1.05)	12 (48)	- 0.02 (-0.07 to -0.20)	-1.37, (-10.87, 8.13)	.769
96	0.67 (0.52 to 0.87)	20 (80)	-0.14 (-0.24 to -0.03)	-22.44, (-30.04, -14.85)	**<0.001**
VWF:GPIbM (N=25)					
0	0.85 (0.69 to 1.04)	-	-	-	-
Threshold (hour 0-allowable error)	0.79 (0.64 to 0.97)	-	-	-	-
24	0.73 (0.63 to 0.93)	15 (60)	-0.04 (-0.06 to -0.02)	-14.464 (-22.296, -6.632)	**0.001**
48	0.73 (0.62 to 0.93)	15 (60)	-0.01 (-0.11 to -0.02)	-14.747 (-22.357, -7.138)	**0.001**
72	0.69 (0.57 to 0.87)	18 (72)	-0.07 (-0.20 to -0.03)	-20.965 (-28.629, -13.301)	**<0.001**
96	0.66 (0.53 to 0.86)	23 (92)	-0.10 (-0.25 to -0.02)	-26.190 (-35.185, -17.195)	**<0.001**
VWF:CB (N=25)					
0	0.78 (0.62 to 0.97)	-	-	-	-
Threshold (hour 0-allowable error)	0.73 (0.58 to 0.90)	-	-	-	-
24	0.74 (0.61 to 0.90)	11 (44)	0.02 (-0.1 to -0.13)	-4.111 (-13.249, 5.027)	0.362
48	0.77 (0.61 to 0.97)	13 (52)	-0.004 (-0.1 to -0.21)	-1.647 (-13.061, 9.766)	0.768
72	0.69 (0.61 to 0.82)	15 (60)	-0.04 (-0.16 to -0.12)	-10.169 (-20.846, 0.507)	0.610
96	0.61 (0.51 to 0.74)	20 (80)	-0.10 (-0.20 to -0.04)	-19.580 (-29.223, -9.937)	**<0.001**

Abbreviations: 95% CI, 95% confidence interval; IQR, interquartile range; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor: antigen; VWF:CB, von Willebrand factor: collagen binding assay; VWF:GPIbM, von Willebrand factor: glycoprotein Ib binding assay

hours displayed VWF:GPIbM levels below the defined threshold.

In contrast, VWF:CB remained stable for up to 96 hours of storage, with levels declining significantly from 0.78 kIU/L at 0 hours to 0.61 kIU/L at 96 hours ($P < 0.001$; Table 1). Twenty of the 25 samples (80%) contained VWF:CB levels below the threshold value at 96 hours (Table 1).

Another set of experiments involved 10 plasma samples obtained from patients diagnosed with VWD type 1 or type 2A. The median levels of VWF:Ag, VWF:GPIbM, and VWF:CB at 0 hours were 0.42 (0.36–0.46), 0.20 (0.16–0.33), and 0.25 (0.19–0.53) kIU/L, respectively (Table 2). The VWF:Ag level experienced a significant decrease from the defined threshold after 48 hours of storage, declining from 0.42 kIU/L to 0.23 kIU/L at 72 hours ($P < 0.001$; Table 2). Similarly, the level of VWF:GPIbM dropped from 0.20 kIU/L to 0.13 kIU/L at 48 hours ($P < 0.001$; Table 2). All patients exhibited decreased levels of VWF:Ag and VWF:GPIbM below the threshold value after 48 hours. Regarding VWF:CB, stability was observed up to 48 hours, with the median level declining to 0.19 kIU/L at 72 hours and to 0.12 kIU/L at 96 hours ($P = 0.003$ and < 0.001, respectively; Table 2). All patients displayed VWF:CB levels below the threshold level at 96 hours.

TABLE 2. Levels of VWF:Ag, VWF:GPIbM, and VWF:CB in thawed plasma from 10 patients with von Willebrand disease at 0 hours and after storage at 2–8 °C for 24, 48, 72, and 96 hours.

Storage time after thawing (hours)	Amount (median and IQR) (kIU/L)	Number of samples with VWF lower than threshold (%)	Amount of decrease from threshold (median and IQR) (kIU/L)	% decrease from threshold (mean and 95% CI) (%)	*P*
VWF:Ag (N=10)					
0	0.42 (0.36 to 0.46)	-	-	-	-
Threshold (hour 0-allowable error)	0.39 (0.34 to 0.43)	-	-	-	-
24	0.42 (0.26 to 0.55)	4 (40)	0.01 (-0.03 to -0.06)	-5.60 (-20.43, 9.22)	0.414
48	0.33 (0.25 to 0.56)	6 (60)	-0.01 (-0.11 to -0.02)	-9.53 (-33.60, 14.54)	0.394
72	0.23 (0.15 to 0.40)	10 (100)	-0.14 (-0.17 to -0.04)	-39.17 (-52.25, -26.08)	**<0.001**
96	0.20 (0.15 to 0.26)	10 (100)	-0.19 (-0.21 to -0.15)	-54.80 (-63.31, -46.29)	**<0.001**
VWF:GPIbM (N=10)					
0	0.20 (0.16 to 0.33)		-	-	-
Threshold (hour 0-allowable error)	0.18 (0.15 to 0.30)		-	-	-
24	0.15 (0.09 to 0.23)	8 (80)	-0.06 (-0.16 to -0.003)	-27.57 (-58.12, 2.98)	0.072
48	0.13 (0.08 to 0.18)	9 (90)	-0.07 (-0.18 to -0.04)	-43.78 (-60.03, -27.53)	**<0.001**
72	0.10 (0.05 to 0.15)	10 (100)	-0.09 (-0.21 to -0.06)	-58.53 (-71.91, -45.15)	**<0.001**
96	0.06 (0.04 to 0.12)	10 (100)	-0.11 (-0.22 to -0.10)	-69.67 (-80.60, -58.73)	**<0.001**
VWF:CB (N=10)					
0	0.25 (0.19 to 0.53)	-	-	-	-
Threshold (hour 0-allowable error)	0.24 (0.17 to 0.49)	-	-	-	-
24	0.33 (0.21 to 0.45)	4 (40)	-0.03 (-0.13 to -0.01)	9.19 (-14.31, 32.70)	0.399
48	0.29 (0.18 to 0.46)	4 (40)	0.02 (-0.07 to -0.02)	4.07 (-21.09, 29.22	0.723
72	0.19 (0.12 to 0.34)	8 (80)	-0.05(0.01 to -0.16)	-29.81 (-46.76, -12.85)	0.003
96	0.12 (0.05 to 0.21)	10 (100)	-0.17 (0.08 to -0.29)	-64.09 (-76.77, -51.41)	**<0.001**

Abbreviations: 95% CI, 95% confidence interval; IQR, interquartile range; VWD; von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor: antigen; VWF:CB, von Willebrand factor: collagen binding assay; VWF:GPIbM, von Willebrand factor: glycoprotein Ib binding assay

DISCUSSION

Quality assurance systems in laboratories typically encompass various processes, such as blood collection, sample storage, laboratory methods, analyses, and reporting.[8] Although standard recommendations for pre-analytical processes in VWF assays are well established, they may not always be fully followed due to various limitations.[7]

Referral laboratories commonly encounter partially melted plasma during transportation from blood collection centers, leading to inevitable sample rejection. Furthermore, conducting all von Willebrand factor (VWF) assays on the same tube of frozen plasma within a single day is often impractical. However, repeating a blood collection is often impractical, and in certain instances, the assays may still need to be performed upon request from the external laboratory. Since the duration of sample storage can affect thrombin generation[14], the ideal situation would be to conduct assays within 4 hours at the blood collection center.

The Clinical Laboratory Standards Institute recommends centrifuging citrated whole blood (WB) immediately after collection to separate PPP. The PPP should be kept at room temperature, and assays should be conducted within 4 hours. If that is not possible, the PPP should be stored at -80 °C until ready for analysis.[15,16] Frozen plasma intended for long-term storage or transportation to referral laboratories should be maintained at temperatures ranging from -20 to -80 °C.

A study by Zhao et al demonstrated that frozen plasma stored at -80 °C remained stable for a year in terms of fibrinogen, thromboplastin time, and prothrombin time. However, activated partial thromboplastin time remained stable for only 6 months, while Factors VIII and IX remained steady for only 1 month.[17] After thawing frozen plasma, assays should be conducted immediately.[15] However, there is limited research on the stability of VWF in thawed plasma.

The diagnosis of VWD relies on accurate laboratory test results, particularly for subtype-classification tests. However, various challenges can affect these assays, especially during the transportation of samples from blood collection centers to referral laboratories.

Limited studies have investigated the stability of VWF in different scenarios. Improper sample preparation can alter both the quantity and function of VWF. For instance, Favaloro et al demonstrated that VWF:Ag in whole blood (WB) or platelet-poor plasma (PPP) remained stable at room temperature (20–25 °C) for up to 6 days.[18] Unfortunately, frozen or thawed plasma and VWF activity were not assessed in their study. Zürcher et al reported that VWF:Ag and VWF:RCo in WB or PPP were stable at room temperature (2 °C in winter and 17–29 °C in summer) for up to 2 days.[19] However, the stability of frozen or thawed plasma was not investigated. Other studies by Gosselin et al and Linskens et al found that VWF:RCo remained stable for 16 and 48 hours, respectively, when plasma was immediately centrifuged after collection and stored at 22–28 °C.[20,21]

The aforementioned studies have shown that VWF stored in either WB or PPP remains stable at room temperature for up to 2–6 days. However, it is important to note that this finding may not apply to tropical countries, where room temperatures can reach 33–36 °C during the summer. Despite this, it is worth mentioning that most laboratories in Thailand still adhere to the guidelines set forth by the Clinical Laboratory Standards Institute for the storage and transportation of samples to referral laboratories for VWF assays.[7]

Furthermore, the studies conducted thus far have focused on the stability of VWF in WB or immediately spun plasma, without considering frozen and thawed plasma. Limited studies have specifically examined the stability of VWF in frozen and thawed plasma. One study demonstrated that VWF:Ag remained stable for up to 6 days after the plasma was thawed and stored at 4±2 °C, which is similar to our findings.[22] Unfortunately, no functional assays were conducted in the earlier investigation.

Regarding long-term storage, most previous studies primarily investigated thawed fresh frozen plasma or thawed lyophilized plasma. Several studies have shown that the VWF:Ag of thawed fresh frozen plasma remains stable for up to 6 days at 4 °C.[22-24] However, those studies did not assess the platelet or collagen binding activities of VWF. Furthermore, the studies focused on VWF:RCo as the platelet binding activity of VWF, without considering VWF:GPIbR or VWF:GPIbM. Table 3 summarizes the stability of VWF with various preparations and storage conditions from previous studies.

In our study, we observed that VWF:Ag and VWF:CB exhibited stability in thawed plasma obtained from normal subjects when stored at 2–8 °C for up to 72 hours prior to testing. However, we noted that VWF:GPIbM displayed lower levels of stability under the same storage conditions. We hypothesize that the cold temperature during freezing at -80 °C might affect platelets present in PPP and impair VWF function, as previous studies have shown that ice can damage platelets and impair VWF when WB is stored on ice.[25-27] Considering the stability of thawed plasma from VWD patients, whose initial VWF values were lower than normal, VWF:Ag and VWF:CB appeared to be less stable than in normal subjects. Interestingly, VWF:GPIbM tended to be more

TABLE 3. Studies on the stability of von Willebrand factor with different preparations and storage conditions

Authors	VWF of interest	Types of samples	Storage temperature	Maximum stability duration
Studies on samples with a short-term storage after blood collection				
Favaloro EJ et al[17]	VWF:Ag	WB	RT (20-25 °C)	6 days
		PPP	RT (20-25 °C)	6 days
Zürcher M et al[18]	VWF:Ag and VWF:RCo	WB	RT (2 °C in winter and 17-29 °C in summer)	2 days (stable both VWF:Ag and VWF:RCo)
		PPP	RT (2 °C in winter and 17-29 °C in summer)	2 days (stable both VWF:Ag and VWF:RCo)
Gosselin RC et al[19]	VWF:RCo	PPP	Frozen in -70 °C freezer Dry ice	16 hours (stable both in freezer or dry ice)
Linskens EA et al[20]	VWF:RCo	PPP	RT (temperature not stated)	48 hours
Favaloro EJ et al[25]	VWF:Ag and VWF:CB	WB	22 °C	3.5 hours (stable both VWF:Ag and VWF:CB)
			0-4 °C (on ice)	3.5 hours (unstable both VWF:Ag and VWF:CB)
Böhm M et al[26]	VWF:Ag and VWF:RCo	WB	0-4 °C (on ice)	6 hours (unstable both VWF:Ag and VWF:RCo)
			RT (temperature not stated)	6 hours (stable both VWF:Ag and VWF:RCo)
		PPP	0-4 °C (on ice)	6 hours (stable both VWF:Ag and VWF:RCo)
			RT	6 hours (stable both VWF:Ag and VWF:RCo)
Studies on frozen samples with a long-term storage				
von Heymann C et al[21]	VWF:Ag	Fresh frozen plasma		4 °C 6 days
Buchta C et al[22]	VWF:Ag	Frozen solvent/detergent-treated plasma		4 °C 6 days
Schoenfeld H et al[23]	VWF:Ag	Lyophilized plasma		4 °C 6 days

Abbreviations: PPP, platelet poor plasma; RT, room temperature; VWF:Ag, von Willebrand factor: antigen; VWF:CB, von Willebrand factor: collagen binding assay; VWF:GPIbM, von Willebrand factor: glycoprotein Ib binding assay; VWF:RCo, von Willebrand factor: ristocetin cofactor assay; WB, whole blood

stable in VWD patients than in normal subjects. We speculate that the low initial VWF levels may mask the effect of storage time on VWF stability.

Based on the results of the study, we recommend the immediate assay of thawed plasma for all VWF panels. In cases where simultaneous assays are not feasible, priority should be given to the VWF:GPIbM assay due to its observed instability after thawing. Furthermore, we suggest that blood collection centers divide plasma into smaller aliquoted samples (200 µL/sample) before freezing. Each individual test can then be performed using a separate aliquot, either at the blood collection center's laboratory or at an off-site referral laboratory. This approach eliminates the need for repeated freezing and thawing of the original bulk sample. Last, if the initial results from a referral laboratory indicate values lower than the normal range, we recommend collecting a fresh plasma sample directly from the patient rather than transferring a sample from the blood collection center. The tests should be repeated before making a definitive diagnosis of VWD and its subtype.

A limitation of this study is the small number of samples from VWD patients. Further studies with a larger sample size are needed to draw definitive conclusions.

CONCLUSION

Transporting blood samples to referral laboratories for VWF assays poses challenges. Despite established transportation standards, there is still a risk of partially melted plasma reaching the referral laboratories, and sample rejection may not always be feasible. It has been observed that VWF:Ag and VWF:CB in thawed plasma can remain stable for up to 72 hours, whereas VWF:GPIbM displays less stability.

List of abbreviations

PPP, platelet-poor plasma; VWD, von Willebrand disease; VWF, von Willebrand factor; VWF:Ag, von Willebrand factor:antigen; VWF:CB, von Willebrand factor:collagen binding assay; VWF:GPIbM, von Willebrand factor:glycoprotein Ib binding by multimer analysis; VWF:GPIbR, VWF:glycoprotein Ib binding by ristocetin; VWF:RCo, VWF:ristocetin cofactor; WB, whole blood

Declarations

Ethics approval and consent to participate

This study was authorized by the Institutional Review Board of the Faculty of Medicine Siriraj Hospital, Mahidol University (approval no: 420/2560/EC4).

Consent for publication

This manuscript has been approved by all authors. A copy of the consent document is available for review from the Editor-in-Chief of Siriraj Medical Journal.

Availability of data and materials

The data sets used during the study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no conflicts of interest related to this study.

Funding

This study received a grant from the Routine to Research (R2R) Fund, Faculty of Medicine Siriraj Hospital, Mahidol University.

Authors' contributions

All authors designed the study. YN collected and analyzed all data and drafted the manuscript. TB performed all of the VWF assays, while TR1, TR2 and YN read and revised the manuscript.

ACKNOWLEDGMENT

The authors gratefully appreciate Ms. Khemajira Karaketklang for her assistance with the statistical analyses.

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