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BRIEF REPORT

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Von Willebrand factor multimer quantitation for assessment of cardiac lesion severity and bleeding risk

Christopher O. Austin MD[1] | Dong Chen MD, PhD[2] | Colleen S. Thomas MS[1] | Robert E. Safford MD, PhD[1] | Brian P. Shapiro MD[1] | Justin A. Bryan BS[1] | Jordan C. Ray MD[1] | Joseph L. Blackshear MD[1]

[1]Division of Cardiovascular Disease, Mayo Clinic, Jacksonville, FL, USA

[2]Division of Hematopathology, Mayo Clinic, Rochester, MN, USA

Correspondence
Christopher O. Austin, MD, Department of Cardiovascular Disease, Mayo Clinic Florida, Jacksonville, FL, USA.
Email: austin.christopher@mayo.edu

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Abstract

Background: von Willebrand factor (VWF) multimer quantitation has been utilized in the assessment of valvular heart disease, however, there is no standardized method for quantitation. We compared three methods of assessment which utilized a normal plasma control.

Methods: We analyzed 476 samples and their control plasma from 368 patients with valvular heart disease, hypertrophic cardiomyopathy, or LVAD therapy, and 27 normal subjects. VWF multimers were assessed as normalized VWF multimer ratios (NMR) of gel bands >15/2-15 (NMR15) or gel bands >10/2-10 (NMR10). Associations of VWF laboratory and multimeric assessments with cardiac lesion severity and acquired bleeding were investigated.

Results: Abnormal multimers were present in 78% of patients with moderate to severe hemodynamic abnormalities compared to 19% of patients with normal or mildly abnormal hemodynamics. NMR showed strong association with severe cardiac lesions (NMR15: OR 15.29, CI 9.04-27.18; NMR10: OR 14.18, CI 8.88-23.21). PFA-CADP was strongly associated with moderate to severe cardiac lesions (OR 14.91, CI 9.08-24.50). PFA-CADP and NMR15 showed excellent ability to discriminate ≥moderate (AUC 0.86, CI 0.83-0.89 and 0.83, CI 0.79-0.87 respectively) and severe cardiac lesions (AUC 0.84, CI 0.81-0.88 and 0.85, CI 0.81-0.88 respectively). NMR was less strongly associated with bleeding (OR 4.01 for NMR10, CI 2.49-6.58).

Conclusion: Quantification of VWF multimers may provide clinical utility in circumstances where clinical estimation of cardiac lesion severity is challenging, such as with dysfunctional prosthetic valves. The presence of abnormal VWF multimers is associated with bleeding, however further quantitation provided only modest improvement in risk stratification.

KEYWORDS

bleeding, laboratory diagnosis, protein multimerization, valvular heart diseases, von Willebrand factor

Essentials

- VWF multimers have an established association with valvular heart disease.
- Significant interlaboratory variation exists in VWF multimeric analysis.
- We describe a method to normalize VWF multimers for assessment of cardiac lesion severity and clinical bleeding.
- Normalized VWF multimer ratios improved the diagnostic capabilities of the assay.

1 | INTRODUCTION

Abnormalities of von Willebrand factor (VWF), typically with loss of the highest molecular weight multimers (HMW), have been identified in states of high intravascular shear stress, most often manifesting clinically through a bleeding diathesis. The prototypical description of this phenomenon is Heyde syndrome, the association of severe aortic stenosis (AS) and gastrointestinal bleeding due to angiodysplasia.[1] Though loss of HMW VWF multimers and associated bleeding was first described in AS, recent descriptions of a similar constellation of findings has been associated with a variety of cardiac lesions including aortic regurgitation, mitral insufficiency and hypertrophic cardiomyopathy.[2-4] Additionally, VWF abnormalities have been described after valve replacement and repair and have been attributed to prosthetic valve dysfunction, perivalvular leak, patient prosthesis mismatch,[5-7] and in the setting of mechanical support with left ventricular assist devices.[8] More recently, abnormalities of VWF multimers have been identified in the absence of clinical bleeding and studies suggest that VWF multimers may provide utility as a biological assay for cardiac lesions in real-time and chronic settings.[9] Although this emerging data supports the use of VWF multimers for assessment of some cardiac lesions, a pooled cohort of various valvular and non-valvular high-shear cardiac states has not been described. Furthermore, variation exists in the processing and interpretation of VWF multimers between clinical laboratories, potentially limiting the reproducibility of VWF assays and their clinical utility.

We report a diverse cohort of prospectively studied patients with high shear cardiac lesions including aortic stenosis, aortic or mitral regurgitation, normal prosthetic heart valves, dysfunctional prosthetic heart valves, hypertrophic obstructive cardiomyopathy, and left ventricular assist devices in which we assessed the association of VWF multimer abnormalities and the hemodynamic severity of these cardiac lesions as well as bleeding histories. Second, we investigated the use of various normalized VWF multimer ratios (NMR) as a novel assessment of cardiac lesion severity and bleeding risk compared to traditional measures such as VWF antigen to activity ratio and platelet function analyzer-100.

2 | METHODS

We identified patients with cardiac lesions from our multispecialty practice between 2010 and 2016 as previously described.[2-4,7] The study protocol was approved by the Mayo Foundation Institutional Review Board is registered at clinicaltrials.gov (NCT01334801). The protocol allowed inclusion of data from patients who had valve disorders and had VWF laboratory testing performed for the clinical indication of acquired bleeding and was further expanded to include patients with valvular or obstructive cardiac lesions that did not have bleeding. Patients were identified as having aortic stenosis (AS), aortic regurgitation (AR), mitral regurgitation (MR), surgical aortic valve replacement (SAVR), transcatheter aortic valve replacement (TAVR), mitral valve repair or replacement (MVR/rep), prosthetic valvular dysfunction following SAVR or MVR/rep, or hypertrophic obstructive cardiomyopathy (HOCM). A control group of subjects known to have no cardiovascular lesions, primarily consisting of physicians-in-training and laboratory technologists, as well as a group with left ventricular assist devices (LVAD) with known severe cardiovascular lesions, were identified for comparison. After written informed consent was obtained the patients provided research blood samples. Patients were asked to complete a bleeding questionnaire (Appendix) modified from the consensus conference on the assessment of von Willebrand disease as previously described.[2,10] For patients with positive bleeding questionnaire responses, further chart review and patient history acquisition were performed to identify clinically significant bleeding, and to determine whether Heyde syndrome (transfusion-dependent anemia resulting from GI bleeding and endoscopically documented angiodysplasia) was present. Bleeding was defined as any positive response on the questionnaire or from the medical history, while non-significant bleeding was considered to be present if only incidental bruising, epistaxis, or blood in the stool without clinically relevant events during the preceding 3 years, remote surgery with related transfusion, or in postmenopausal women with a remote history of menorrhagia.

Clinical data, including symptoms, concomitant medical problems, prescribed medications, and physical findings were recorded. Patients were excluded from the analysis if they had inadequate echocardiographic images for diagnosis, declined research blood sampling, were taking thienopyridines, or had a hemoglobin <8 g/dL. Objective grading of cardiac lesion severity was made by echocardiographic assessment per current American College of Cardiology and American Society of Echocardiography guidelines, including qualitative assessment of regurgitant volume and valvular or intracardiac gradient in obstructive disease. Subjects with LVADs were assumed to have severe lesions. Assessment of the severity of AS, AR, MR, prosthetic valvular dysfunction, and HOCM have been described previously.[2-4,7] All echocardiograms were reviewed for accuracy (J.L.B. and R.E.S).

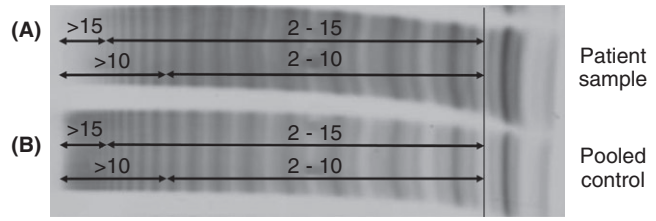

FIGURE 1 Assessment of Von Willebrand multimers by normalized multimer ratio. Gel columns of patient sample (A) and pooled control (B) are displayed. Multimer ratios are calculated by dividing the measured density of the sample range of interest (either bands >10 or bands >15) by the remaining bands in the column. The normalized multimer ratio calculated by dividing the patient multimer ratio for the band of interest by the pooled control multimer ratio for the band of interest

Qualitative loss of HMW VWF multimers was assessed with in-gel western blot gel electrophoresis[11] and VWF antigen (VWF:ag), VWF latex immunoturbidic activity (VWF:act), and quantitative loss of VWF ratios of multimers >15/2-15 mers and >10/2-10 mers were assessed in patients citrated frozen plasma samples, as previously described.[2] A control column utilizing pooled plasma from patients without cardiac lesions or known VWF abnormalities was used to normalize VWF multimers. NMR for each HMW VWF multimer range were calculated using the following equation:

$$\text{NMRX} = \frac{\text{density of subject multimers >X/density of control multimers 2 - X}}{\text{density of control multimers >X/density of control multimers 2 - X}}$$

where X equals the HMW multimer cutoff of interest. The first multimer band is ignored in this formula as it often contains proteins unassociated with VWF, and has the highest quantitative inter-observer variability of all the bands. We specifically evaluated NMR with HMW cutoffs and 10 (NMR10) and 15 (NMR15) multimers respectively (Figure 1). Platelet function analyzer 100 (Siemens, Deerfield, IL, USA) collagen ADP closure time (PFA-CADP) was assessed in citrated whole blood samples.

Numerical data were summarized as the sample median (interquartile range [IQR]). Comparisons between groups of patients were performed with the Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables. The diagnostic utility of VWF-related tests for detecting moderate or severe cardiac lesions was explored by estimating the area under the receiver operating characteristic (ROC) curve; sensitivity, specificity, positive predictive value and negative predictive value were estimated for predefined cutoff levels when available.[2] Odds ratios were calculated via 2 × 2 contingency analysis of categorical variables, defined by presence of clinic outcome (lesions severity or bleeding, respectively) and predefined cutoff of the assay in question. *P*-values of <.05 were considered statistically significant, and were not adjusted for multiple testing. Analyses were performed with JMP (version 13.1; SAS Institute, Cary, NC, USA) and R statistical software (version 2.14.0; R Foundation for Statistical Computing, Vienna, Austria).

3 | RESULTS AND DISCUSSION

The baseline characteristics of 395 subjects in this cohort that contributed 476 blood specimens are shown in Table 1. We performed comprehensive VWF testing on 159 blood specimens from patients diagnosed with HCM, 65 with AS, 84 with native aortic or mitral regurgitation, 63 with normally functioning prosthetic valve replacement or repair, 43 from patients with dysfunctional heart valve replacement or repair, 35 with LVADs, and 27 subjects without known cardiac lesions. The majority of patients with valvular lesions were treated with antithrombotic agents (aspirin: 58%; warfarin: 38%). Nearly all LVAD patients were treated with coumadin (97%) and aspirin (91%) and were the group most likely to report a history of bleeding (60%). Patient characteristics and laboratory findings stratified by cardiac lesion severity are described in Table 2. The group without cardiac lesions was younger as the majority of these subjects were physicians-in-training that were recruited as a control group for a separate study, otherwise there were no differences in age or sex amongst patients with cardiac lesions. Patients identified as having moderate or severe cardiac lesions reported more bleeding, were more anemic, and had abnormal VWF laboratory profiles ($P < .01$ for all except VWF activity). The presence of VWF multimer abnormalities demonstrated a linear relationship with cardiac lesion severity (Chi Square = 210, $P < .001$) (Figure 2A). Abnormal VWF multimers were not seen in the patients with structurally normal hearts (0%) but were present in mild (21%), moderate (64%), and severe (88%) cardiac lesions. Bleeding status was available for 347 of 476 samples. Patients that reported bleeding were more likely to be female, anemic, and have moderate or severe cardiac lesions (Table 2). Abnormal VWF multimers were present in 65% of bleeders compared to 46% of non-bleeders.

We explored the diagnostic utility of traditional VWF laboratories and NMR for the identification of moderate to severe cardiac lesions as well as clinically significant bleeding (Table 3). NMR showed strong association with severe cardiac lesions (NMR15: OR 15.29, CI 9.04-27.18; NMR10: OR 14.18, CI 8.88-23.21). ROC analysis identified NMR15 as the best individual laboratory evaluation for the identification of severe cardiac lesions (AUC 0.85, CI 0.81-0.88). Prolonged PFA-CADP was strongly associated with moderate to severe cardiac lesions (cutoff > 140 s: OR 14.91, CI 9.08-24.50) and was superior to NMR15 (PFA-CADP: AUC 0.86, 0.83-0.89; NMR15: AUC 0.84, CI 0.80-0.87) for identification for patients with moderate to severe cardiac lesions. For assessment of bleeding, NMR10 and NMR15 were equally useful, with AUC 0.72 (CI 0.66-0.75) and 0.71 (CI 0.66-0.76) respectively. NMR10 was a better screening test than NMR15 (Sensitivity 68% vs 56%), however no particular test was more strongly associated with bleeding than others.

A growing body of data supports the concept that VWF can be viewed as an intravascular sensor of shear stress. The descriptions of aortic stenosis, bleeding, and abnormalities of VWF multimers have now been supplemented with extensive data in left ventricular assist devices, native mitral and aortic regurgitation, prosthetic valve

TABLE 1 Cohort demographics and routine laboratory assessment by cardiac lesion severity

	No cardiac lesions	Aortic stenosis	Hypertrophic cardiomyopathy	Mitral or aortic regurgitation	Normal heart valve replacement or repair	Dysfunctional heart valve replacement or repair	Left ventricular assist device
Patient specimens[a]	27	65	159	84	63	43	35
Age, y (IQR)	44 (39-54)	79 (70-84)	65 (51-72)	71 (61-80)	74 (66-81)	72 (68-81)	65 (53-71)
% Female	40.7	35.4	52.8	41.2	44.4	44.2	11.4
% Non-white	7.4	4.6	11.3	15.3	14.3	4.7	20
Cardiac lesion severity[b]							
Normal/mild	27	17	81	28	60	4	0
Moderate	0	17	31	34	1	28	0
Severe	0	31	47	22	2	11	35
Bleeding history[d]	0	21	81[e]	25	13	12	21
Anemia[c]	0	4	6	12	1	13	4
Hemoglobin, mg/dL	13.6	13	12.9	12.7	12.7	11.3	12
(IQR)	(12.1-15.1)	(10.9-15.0)	(10.9-15.0)	(10.4-15.0)	(11.1-14.3)	(9.2-13.4)	(10.3-13.7)
Hematocrit	39.6	38.3	38.3	38	37.5	34.1	36.9
(IQR)	(35.5-43.8)	(32.5-44.2)	(32.6-44.1)	(31.6-44.4)	(33.1-41.9)	28.3-40.1)	(31.4-42.4)
Platelet	222	194	195	217	237	198	200
(IQR)	(163-281)	(127-262)	(127-262)	(147-288)	(99-375)	(125-270)	(141-260)
Antithrombotic agents							
Warfarin	0	10	11	15	46	26	34
Aspirin	3	43	36	40	41	25	32

IQR, interquartile range.

[a]Patients had more than one specimen if an intervention expected to impact turbulent flow was performed; result was documented by follow-up echocardiogram allowing reassessment of hemodynamic severity.

[b]See text for definition.

[c]Hb < 10.

[d]See text for definition.

[e]Overall, 89 patients with HCM had 159 specimens obtained at the time of hemodynamic assessment, and 26/89 patients had a history of bleeding.

TABLE 2 Baseline cohort demographics and von Willebrand laboratory assessment by cardiac lesion severity and patient reported bleeding

	Cardiac lesion severity			Patient reported bleeding		
	Normal or mild (n = 219)	Moderate or severe (n = 257)	*P*-value	No (n = 219)	Yes (n = 127)	*P*-value
Age, y (IQR)	68 (52-77)	71 (63-80)	.990	71 (57-80)	72 (64-79)	.237
Females (%)	70 (43)	92 (39)	.399	73 (35)	58 (49)	.014
Bleeding (%)	33 (25)	94 (45)	<.001	115 (52)	94 (74)	<.001
Hemoglobin, g/dL (IQR)	13.3 (12.1-14.3)	12.6 (10.9-13.8)	<.001	13.3 (12-13.9)	11.8 (10-13.4)	<.001
Anemia (%)	3 (2)	37 (16)	<.001	9 (5)	28 (24)	<.001
PFA-CADP, s (IQR)	90 (75-114)	173 (124-298)	<.001	110 (84-166)	175 (112-291)	<.001
VWF multimers > 15 (IQR)	0.16 (0.14-0.19)	0.11 (0.08-0.14)	<.001	0.15 (0.12-0.17)	0.11 (0.08-0.16)	<.001
VWF multimers > 10 (IQR)	0.44 (0.38-0.50)	0.34 (0.29-0.41)	<.001	0.41 (0.36-0.46)	0.35 (0.28-0.43)	.003
VWF antigen, IU/dL (IQR)	136 (94-179)	146 (115-196)	.005	138 (104-183)	173 (118-210)	<.001
VWF activity, % (IQR)	119 (89-154)	113 (91-150)	.944	112 (92-147)	129 (100-172)	.013
Activity to antigen ratio	0.88 (0.83-0.95)	0.78 (0.7-0.87)	<.001	0.86 (0.79-0.93)	0.78 (0.69-0.90)	<.001
Patients with activity to antigen ratio < 0.8 (%)	31/219 (14)	138/257 (54)	<.001	56/219 (26)	67/127 (53)	<.001
Multimers abnormal (%)	41 (19)	200 (78)	<.001	99 (46)	83 (65)	<.001
NMR15 (IQR)	0.84 (0.73-0.97)	0.62 (0.47-0.72)	<.001	0.76 (0.64-0.89)	0.63 (0.44-0.77)	<.001
NMR10 (IQR)	0.87 (0.77-0.98)	0.70 (0.60-0.80)	<.001	0.81 (0.70-0.93)	0.70 (0.59-0.80)	.002

IQR: interquartile range; NMR10: normalized multimer ratio, high molecular cutoff band = 10; NMR15: normalized multimer ratio, high molecular cutoff band = 15; PFA-CADP: platelet function analyzer collagen plus adenosine diphosphate; VWF: von Willebrand factor.

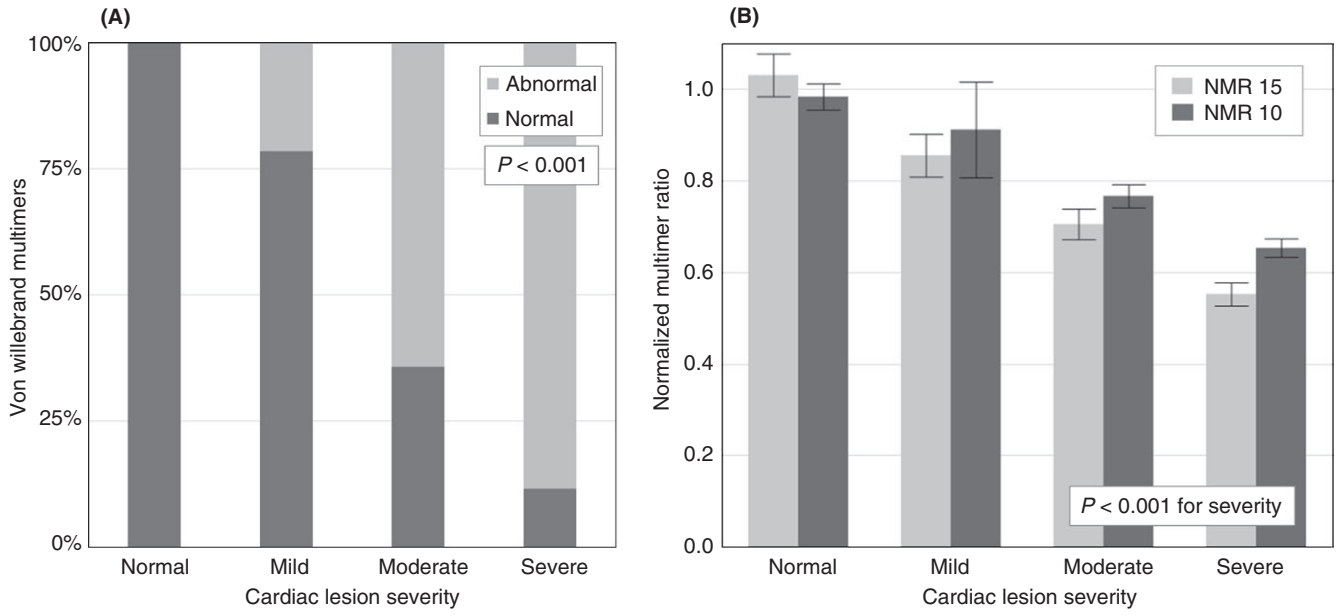

FIGURE 2 Presence of abnormal von Willebrand multimers by cardiac lesion severity (A). Normalized multimer ratio (NMR) with interquartile range (25% and 75%) by cardiac lesion severity (B)

dysfunction, including use of PFA-CADP in the acute assessment of hemodynamic success of TAVR, and hypertrophic cardiomyopathy. Additional key observations reveal resolution of VWF abnormalities after valve replacement (but not aortic valvuloplasty), mitral valve replacement or repair for regurgitation, and septal reduction therapy in hypertrophic cardiomyopathy. Several important implications of the consistent association of VWF abnormalities and high-shear cardiac disorders have potential clinical utility. First, in situations in which assessment of cardiac lesion severity is difficult such as the use of intraprocedural PFA-CADP for quantitation of post-TAVR paravalvular

TABLE 3 Diagnostic utility of Von Willebrand assessment for the identification of cardiac lesion severity or clinically significant bleeding, NMR adjusted for age and sex

Variable	Cutoff	N	Test+/Status− (%)	Test+/Status+ (%)	Odds ratio (95% CI)	AUC (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Detection of severe cardiac lesions using										
PFA-CADP	>121 s	470	114/323 (35)	126/147 (85)	10.32 (6.21-17.15)	0.84 (0.81-0.88)	85 (78-90)	65 (59-70)	52 (46-58)	91 (86-94)
PFA-CADP	>140 s	470	74/323 (23)	116/147 (79)	12.14 (7.59-19.42)	0.84 (0.81-0.88)	79 (71-84)	77 (72-81)	61 (54-68)	89 (84-92)
VWF multimers > 15	<0.15	474	132/327 (40)	128/147 (87)	9.93 (5.84-16.86)	0.83 (0.79-0.87)	87 (91-92)	60 (54-65)	49 (43-55)	91 (87-94)
VWF multimers > 10	<0.36[a]	474	79/327 (24)	97/147 (66)	6.04 (3.95-9.23)	0.80 (0.75-0.84)	66 (58-73)	76 (71-80)	55 (47-62)	83 (79-87)
VWF:Act/VWF:Ag	<0.8	473	70/326 (21)	99/147 (67)	7.47 (4.84-11.52)	0.76 (0.70-0.81)	67 (59-74)	78 (74-83)	58 (51-65)	84 (80-88)
NMR15	<0.71[a]	474	104/327 (31)	129/147 (88)	15.29 (9.04-27.18)	0.85 (0.81-0.88)	88 (82-92)	68 (63-73)	55 (49-61)	93 (89-95)
NMR10	<0.73[a]	474	67/327 (21)	115/147 (79)	14.18 (8.88-23.21)	0.84 (0.80-0.87)	79 (70-84)	79 (75-83)	63 (55-69)	89 (85-92)
Detection of moderate to severe cardiac lesions using										
PFA-CADP	>121 s	470	44/216 (20)	196/254 (77)	12.63 (8.15-19.59)	0.86 (0.83-0.89)	77 (71-82)	80 (74-84)	81 (76-86)	75 (69-80)
PFA-CADP	>140 s	470	24/216 (11)	166/254 (65)	14.91 (9.08-24.50)	0.86 (0.83-0.89)	65 (59-71)	89 (84-93)	87 (82-91)	68 (63-74)
VWF multimers > 15	<0.15	474	62/217 (29)	198/257 (77)	8.31 (5.50-12.55)	0.81 (0.77-0.85)	77 (72-82)	71 (65-77)	76 (70-81)	73 (66-78)
VWF multimers > 10	<0.36[a]	474	33/217 (15)	143/257 (56)	6.83 (4.39-10.61)	0.78 (0.74-0.82)	56 (50-62)	85 (79-89)	81 (74-86)	62 (56-67)
VWF:Act/VWF:Ag	<0.8	473	31/217 (15)	138/256 (54)	6.83 (4.37-10.70)	0.74 (0.70-0.79)	54 (48-60)	85 (80-90)	81 (75-86)	61 (56-67)
NMR15	<0.71[a]	474	41/217 (19)	192/257 (75)	12.71 (8.16-20.22)	0.83 (0.79-0.87)	75 (69-80)	81 (75-86)	82 (77-86)	73 (67-78)
NMR10	<0.73[a]	474	28/217 (13)	154/257 (60)	10.51 (6.56-17.39)	0.82 (0.77-0.84)	60 (54-66)	87 (82-91)	84 (78-89)	65 (59-70)
Detection of clinically significant bleeding using										
PFA-CADP	>121 s	342	95/218 (43)	87/124 (70)	3.11 (1.95-4.97)	0.68 (0.62-0.73)	70 (62-78)	57 (50-63)	48 (41-56)	77 (70-83)
PFA-CADP	>140 s	342	73/218 (33)	75/124 (60)	3.02 (1.91-4.77)	0.68 (0.62-0.73)	60 (51-68)	67 (60-73)	51 (43-59)	74 (68-80)
VWF multimers > 15	<0.15	345	98/219 (45)	86/126 (68)	2.74 (1.73-4.33)	0.67 (0.61-0.73)	69 (60-76)	56 (49-62)	47 (40-55)	75 (68-81)
VWF multimers > 10	<0.36[a]	345	60/219 (27)	66/126 (52)	3.02 (1.91-4.79)	0.65 (0.58-0.71)	53 (44-61)	73 (66-78)	53 (45-62)	73 (66-78)
VWF:Act/VWF:Ag	<0.8	345	56/218 (26)	67/126 (53)	3.33 (2.09-5.29)	0.65 (0.58-0.71)	53 (45-62)	74 (68-80)	55 (46-63)	73 (67-79)
NMR15	<0.65[a]	345	61/219 (28)	70/126 (55)	3.11 (1.95-5.03)	0.71 (0.66-0.75)	56 (47-64)	72 (66-78)	54 (45-62)	74 (58-79)
NMR10	<0.76[a]	345	82/219 (37)	85/126 (67)	4.01 (2.49-6.58)	0.72 (0.66-0.76)	68 (59-75)	62 (56-68)	51 (44-59)	77 (70-82)

PFA-CADP, platelet functional assay ADP cartridge closure time; VWF, Von Willebrand factor; Ag, antigen; Act, activity; NMR, normalized multimer ratio; AUC, area under the curve; CI, confidence interval; HMWM, high molecular weight multimer.

[a]Cutoffs for VWF multimers > 10, NMR15, and NMR10 for cardiac lesions and clinically significant bleeding were identified by multivariate logistical regression and ROC analysis with age and sex included as model effects as described in the manuscript.

regurgitation, allowing for procedural adjustment of TAVR.[9] The PFA-CADP cutoff of 140 seconds as identified by Van Belle et al. was particularly useful for the identification of moderate to severe cardiac lesions in our population. This concept of aiding severity assessment could easily be extended to the longitudinal follow-up of native valve regurgitation and tissue prosthetic valves which all fail eventually from obstruction, regurgitation, or infection. Second, many patients with cardiac disease receive antithrombotic therapies, as exemplified by our patient population, and if and when bleeding occurs, it is natural to suspect the anticoagulant has permitted bleeding and the association between the cardiac lesion and acquired von Willebrand syndrome may be overlooked. The laboratory assessment documenting PFA-CADP elevation and normalized multimer ratio depression in the ranges we have established for ≥moderate cardiac lesion severity provides an important clue to the etiology of bleeding, and suggests that cardiac repair, not endoscopic treatment of focal bleeding gastrointestinal lesions, may be more successful in the long term. Finally, and yet to be exploited, is the prognostic content resident in abnormal VWF tests in patients with cardiac disorders. From our data, it appears that NMR and PFA-CADP are best suited to this purpose.

The PFA-100 (Seimans Medical Solutions, Malvern PA, USA) is a single platform analyzer and can be utilized widely with center to center comparisons of PFA-CADP. The epinephrine cartridge is sensitive to aspirin and the ADP cartridge sensitive to thienopyridine but, except for patients with cardiac or peripheral vascular stents, this is not a major limitation. However, there is no standardization of how to quantify VWF multimers. We have proposed the NMR schema, but it is not a new concept, having been reported by Weintsein[12] and also in two recent analyses of van Belle and colleagues.[5,9] Some laboratories are not able to produce gels that clearly delineate gel bands greater than 10, limiting the usefulness of the proposed technique. Individual laboratories must decide if the proposed NMR technique is appropriate dependent on the quality of their assays. Although this study is retrospective in nature in a heterogenous population of mixed valvular disease and hypertrophic cardiomyopathy, we believe that our findings are likely to be a generalizable to a similar population of cardiovascular patients.

ADDENDUM

J. L. Blackshear wrote the protocol. J. L. Blackshear, R. E. Safford, and B. P. Shapiro identified and recruited patients. D. Chen oversaw the processing and interpretation of von Willebrand factor multimers. C.O. Austin and C. Thomas performed statistical analysis. C.O. Austin, J. L. Blackshear, R. E. Safford, J.C. Ray and D. Chen wrote the paper.

RELATIONSHIP DISCLOSURES

Joseph Blackshear received a research grant from Baxalta, Inc. Christopher Austin, Dong Chen, Colleen Thomas, Robert Safford, Justin Bryan, Jordan Ray, and Brian Shapiro have no conflicts of interest.

ORCID

Christopher O. Austin http://orcid.org/0000-0001-6237-1403

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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