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Regular Article

Comparison of Von Willebrand factor (VWF) activity VWF:Ac with VWF ristocetin cofactor activity VWF:RCo

Ulrich Geisen [a], Barbara Zieger [b], Lea Nakamura [b], Andreas Weis [c], Jürgen Heinz [c], Jan Jacques Michiels [d], Claudia Heilmann [e,*]

[a] Institute of Clinical Chemistry and Laboratory Medicine, Freiburg, Germany
[b] Laboratory for Hemostaseology, Department of Pediatrics and Adolescent Medicine, Freiburg, Germany
[c] Dept. of Hematology and Oncology, University Freiburg Medical Center, Freiburg, Germany
[d] Goodheart Institute & Foundation Rotterdam, European Bloodcoagulation Science Center, Rotterdam, The Netherlands
[e] Department of Cardiovascular Surgery, Heart Center University of Freiburg, Freiburg, Germany

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ABSTRACT

Introduction: Ristocetin cofactor activity of Von Willebrand factor (VWF:RCo) and the ratio VWF:RCo to its antigen VWF:Ag are used as routine screening to estimate VWF function and to detect types of Von Willebrand disease (VWD) caused by loss of high molecular weight multimers. However, the VWF:RCo test is prone to analytic imprecisions due to various reasons. We compared an assay for VWF activity (VWF:Ac) with VWF:RCo putting emphasis on the ratios to VWF:Ag.
Materials and Methods: We evaluated 942 samples from 432 patients and evaluated three groups in detail: normal patients (normal multimers, VWF:Ag and VWF:RCo >0.5 U/ml, VWD type 1 excluded; n = 258), VWD type 1 (n = 76) and acquired Von Willebrand syndrome (AVWS, n = 326). In addition, 30 healthy subjects were analysed.
Results: VWF:Ac and VWF:RCo correlated well (Pearson´s r = 0.96, $p < 0.01$), so did their ratios to VWF:Ag (Pearson´s r = 0.82, $p < 0.01$). We calculated the normal range of VWF:Ac/VWF:Ag for healthy subjects as 0.8-1.16. In comparison, the reference range (mean ± 2std) derived from normal patient samples was 0.73-1.14. The corresponding ranges for VWF:RCo/VWF:Ag came to 0.74-1.23 (healthy) and 0.62-1.25 (normal patients). The ratios showed similar results regarding VWD type 1. The sensitivity for AVWS was higher with VWF:Ac/VWF:Ag than with VWF:RCo/VWF:Ag (97.5% versus 84.7%).
Conclusions: The data suggest that the results obtained with the VWF:Ac assay are comparable to that of the VWF:RCo assay. An AVWS was more reliably detected by VWF:Ac/VWF:Ag. We assume that the VWF:Ac assay could replace VWF:RCo for routine screening for AVWS, especially when prompt evaluation is required.

Introduction

Ristocetin cofactor activity of Von Willebrand factor (VWF:RCo) and the ratio to the VWF antigen (VWF:Ag) VWF:RCo/VWF:Ag are used worldwide as a routine method to estimate VWF function and as one step to detect Von Willebrand disease (VWD) type 1 or 3 and some subtypes of VWD type 2, particularly those with loss of high molecular weight (HMW) multimers [1]. The VWF:RCo assay measures the binding of VWF to GPIb receptors of fixed platelets. However, VWF:RCo tests are prone to analytic imprecisions due to interference by hemoglobin, bilirubin and triglyerides, human anti-mouse antibodies or rheumatoid factor, and VWF:Ag excess. Varying platelet preparations and technical difficulties may contribute to changing quality. Moreover, a broad array of genetic variances and polymorphisms at the ristocetin binding site may preclude sensitivity for diagnosis of congenital VWD type 2. [1–3] Thus, the VWF:RCo activity assay shows low accuracy, especially at low VWF:Ag concentrations [4]. A new test for the VWF activity has been introduced in 2011. The assay tests binding of VWF to recombinant GPIb which in turn binds to particle-fixed anti-GPIb antibodies. [5] Due to the dissimilar principles of the two assays, different readings can be assumed. We aimed at the validation of this new parameter VWF:Ac and its ratio to VWF:Ag, VWF:Ac/VWF:Ag in

Abbreviations: AVWS, acquired Von Willebrand syndrome; BCS, Behring Coagulation System; FVIII:C, coagulation factor VIII; GPIb, platelet receptor glycoprotein Ib; HMW, high molecular weight; U/ml, units per milliliter; VWD, Von Willebrand disease; VWF, Von Willebrand factor; VWF:Ac, VWF activity; VWF:Ag, VWF antigen; VWF:Ac/VWF:Ag, ratio of VWF:Ac to VWF:Ag; VWF:CB, VWF collagen binding capacity; VWF:CB/VWF:Ag, ratio of VWF:CB to VWF:Ag; VWF:RCo, VWF ristocetin cofactor activity; VWF:RCo/VWF:Ag, ratio of VWF:RCo to VWF:Ag.

* Corresponding author at: Department of Cardiovascular Surgery, Heart Center, University of Freiburg, Hugstetter Str. 55, 79106 Freiburg, Germany. Tel.: +49 761 270 72200; fax: +49 761 270 72201.
E-mail address: claudia.heilmann@universitaets-herzzentrum.de (C. Heilmann).

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Table 1
Descriptive statistics of VWF associated values for all 942 plasma samples of 432 patients.

	VWF:Ac (U/ml)	VWF:RCo (U/ml)	VWF:Ag (U/ml)	VWF:Ac/ VWF:Ag	VWF:RCo/ VWF:Ag	VWF:CB (U/ml)	VWF:CB/ VWF:Ag
n	942	942	942	916[1]	916[1]	471	471
mean	1.70	1.70	2.33	0.79	0.78	1.58	0.84
standard deviation	1.16	1.21	1.63	0.19	0.22	1.34	0.46
25% percentile	0.85	0.81	0.96	0.64	0.63	0.70	0.42
75% percentile	2.28	2.16	3.21	0.93	0.94	1.90	1.22
median	1.43	1.46	1.96	0.82	0.78	1.14	0.82
minimum	0.05	0.10	0.05	0.10	0.13	0.01	0.11
maximum	6.60	6.56	9.42	1.35	1.44	9.88	3.23

[1] not calculated for 26 data sets due to levels <0.2 U/ml for VWF:Ac, VWF:RCo and/or VWF:Ag.

comparison to VWF:RCo and VWF:RCo/VWF:Ag in routine patients and furthermore at establishing a reference range for VWF:Ac/VWF:Ag. An additional normal range was derived from healthy subjects.

Materials and Methods

Patients and subjects

The patient data set derives of 942 plasma samples from 432 patients. Patients were recruited from the hemostaseologic outpatient clinic and the in-hospital consultant service of our University Medical Center and from the coagulation monitoring program for patients with mechanical circulatory support and heart transplantation at the Department of Cardiovascular Surgery of the Heart Center. VWF:CB was measured in 471 samples simultaneously with VWF:Ac and VWF: RCo analyses. VWF multimer analyses of 462 plasma samples from 264 patients were available.

The full set (n = 942) included 258 data sets of 234 patients with normal multimers, VWF:RCo >0.5 U/ml, VWF:Ag >0.5 U/ml and clinical exclusion of VWD type 1 (normal). The reference ranges for the ratios of VWF:Ac, VWF:RCo and collagen binding activity of VWF (VWF:CB) to VWF:Ag were derived from these patients. Moreover, the full set contained 98 data sets of 62 patients with inherited Von Willebrand disease (VWD): 76 data sets of 48 patients with VWD type 1 and 22 data sets of 14 patients with other classifications. The latter were not analysed in detail with respect to the low number. Further, 326 data sets of 89 patients with acquired Von Willebrand syndrome (AVWS) were identified: the causes of AVWS were mechanical circulatory support or severe aortic valve stenosis in 310 data sets of 76 patients and hematologic disorders in 16 data sets of 13 patients. Patients were classified according to the criteria of the International Society of Thrombosis and Haemostasis (ISTH) [1]. The full set comprised also 260 data sets of 47 patients who were tested for various reasons but did not belong to one of the groups described above. In addition, samples from 30 healthy volunteers (15 females, 15 males, age 43.1 ± 10.7 years) were analysed for VWF:Ac, VWF:RCo, VWF:Ag and the ratios VWF:Ac/VWF:Ag and VWF:RCo/VWF:Ag. These subjects reported no previous bleeding tendencies, and c-reactive protein was <5 mg/l. Their data were used to calculate the normal range.

Laboratory methods

VWF activity (VWF:Ac, INNOVANCE VWF Ac®), ristocetin cofactor activity (VWF:RCo, BC Von Willebrand Reagent®), VWF antigen (VWF:Ag) and coagulation factor VIII (FVIII:C) (all Siemens Healthcare Diagnostics, Eschborn, Germany) were measured in sodium citrate plasma using the Behring Coagulation System XP® (BCS) according to standard protocols. Standard human plasma (Siemens Healthcare Diagnostics) was used for calibration. Measurements were performed within the routine laboratory analysis. The ratios VWF: Ac/VWF:Ag and VWF:RCo /VWF:Ag were calculated.

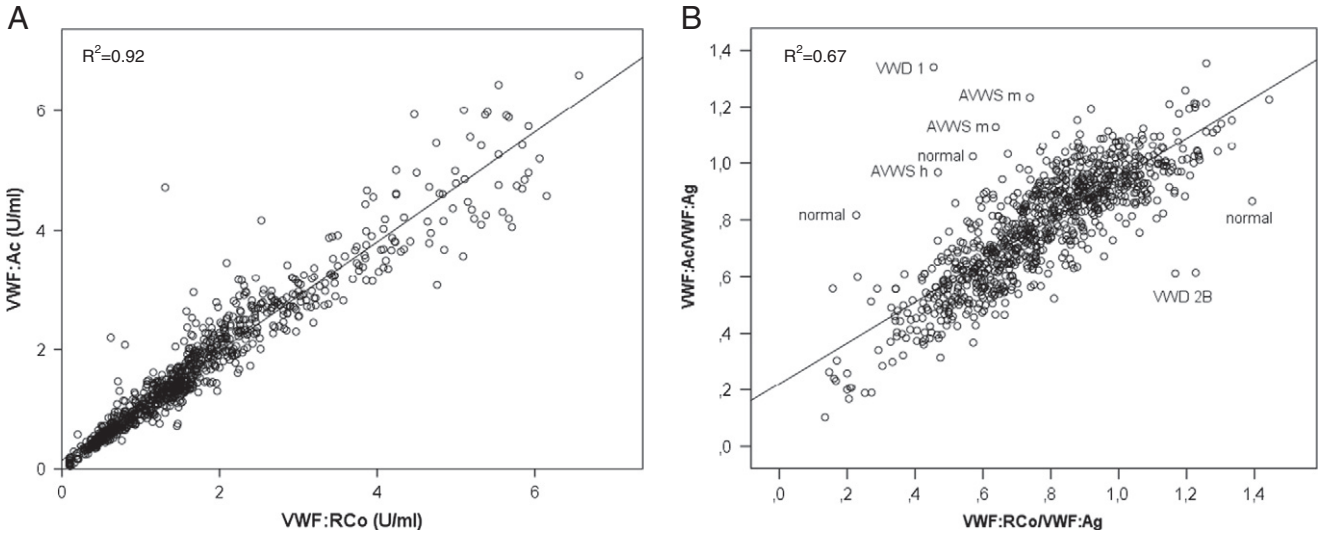

Fig. 1. Scatter plot of VWF:Ac against VWF:RCo values (A) and of the ratios VWF:Ac/VWF:Ag against VWF:RCo/VWF:Ag (B). The central lines represent the best linear fit. Outliers are marked: normal, VWD 1, VWD type 1, VWD 2B, VWD type 2B, AVWS m, acquired Von Willebrand syndrome due to mechanical circulatory support, AVWS h, acquired Von Willebrand syndrome due to hematologic disorder.