Evaluation of a new turbidimetric assay for von Willebrand factor activity useful in the general screening of von Willebrand disease

We evaluated a new assay (HemosIL™VWF Activity on ACL-Futura) in the screening of VWD. Samples from healthy donors and previously diagnosed VWD patients were blindly analyzed by this new activity assay and standard VWF:RCo. Results agreed and both assays showed a similar sensitivity for the screening of VWD.

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Von Willebrand disease (VWD), the most frequent inherited bleeding disorder, is caused by a deficiency and/or abnormality of the von Willebrand factor (VWF).[1,2] The revised classification of VWD identifies two major categories, characterized by quantitative (types 1 and 3) or qualitative (type 2) VWF defects.[2] The most common test of VWF activity, the assay of VWF:RCo together with VWF:Ag is traditionally considered the first step in the diagnosis of VWD, with the VWF:RCo/Ag ratio being recommended to discriminate type 1 from type 2A, 2B and 2M VWD.[1,2] Given the complexities of the aggregometric test, several alternative laboratory methods have been proposed to measure VWF activity. Monoclonal antibody-based ELISA,[3,4] Elisa-based *VWF:RCo*,[5,6] rapid assays for VWF:RCo using coagulometers,[7] and assays measuring the binding of VWF to collagen.[8] The aim of this study was to evaluate the novel fully automated HemosIL™VWF Activity assay on the ACL-Futura automated coagulometer (Instrumentation Laboratory) as a potential screening test for the diagnosis of VWD.[9,10] The assay uses latex sensitized with monoclonal antibodies (RFF VIII:R/1)[3] directed against the platelet binding site of VWF (GPIb receptor). The activity of VWF is determined by measuring the increase of turbidity produced by the agglutination of the latex particles as a consequence of the interaction between the GPIb receptor of VWF and the monoclonal antibodies. The lower limit of linearity has been reported at 12.5IU/dL.[9] A set of 57 normal controls and 70 VWD patients from two Hemophilia Centers were blindly analyzed in the R&D laboratory of Biokit with HemosIL™VWF:Ag, HemosIL™VWF Activity and VWF:RCo (Dade Behring) performed on an optical aggregometer. All methods were used to analyze samples which were then classified as normal or VWD type 1, 2, or 3 using the same criteria in both activity assays. The cut-off declared by the HemosIL based on ABO group was used to define normality or VWD, and the Activity/Antigen (Act/Ag) ratio was used to define quantitative (type 1) or qualitative (type 2) VWD using 0.7 as cut-off.[1] Sample values below detectability were classified as type 3.

After classification and database lock, blindness was disclosed by the two Hemophilia Centers where the patient samples were classified according to the clinical and laboratory findings. Table 1 shows the mean VWF concentration in IU/dL±SD (standard deviation) obtained for each assay. The first column shows the Classification according to the Haemophilia Centers. Nine samples from the Vicenza group which correspond to type 1 according to the last classification scheme,[2] are shown separately. The last columns show the mean Act/Ag ratios. The mean VWF:Ag value for the VWD type 3 samples was below detectability, hence this group is not shown in this Table. Comparing the mean values of the two activity assays, only the type 2A samples show a highly significant difference (p=0.0006). Comparing the mean values of the Act/Ag ratios for both activity assays, the HemosIL shows a higher Act/Ag ratio than the VWF:RCo for type 2A samples (p=0.002), while the other subtypes of VWD show no difference.

The comparison of HemosIL™ VWF Activity to the reference method VWF:RCo shows a Passing & Bablok fitted curve y=0.95x+1 and Pearson correlation of r=0.956 (p<0.0001). The ability of the activity assays as screening tests to distinguish between VWD patients from non VWD was evaluated. One out of 70 VWD samples which corresponded to a type1 VWD patient, was classified as Normal by both activity assays. Thus the sensitivity for the VWD was 98.6% for both. From the remaining 69 VWD samples which resulted in a VWD type from both activity assays, we compared their ability to discriminate between quantitative and qualitative deficiencies. Table 2 shows the classification according to the HemosIL and the VWF:RCo assays for each group of samples. Sensitivity for qualitative VWD was 94.7% (95%CI: 86.2%-99.9%) for the HemosIL and 100% (95%CI:90.7%-100.0%) for the VWF:RCo. Sensitivity for quantitative VWD was 71.0% (95%CI:52.0%-85.5%) and 64.5% (95%CI:45.4%-80.8%) for the HemosIL and the VWF:RCo respectively. The overall agreement was 84.1% for both assays.

In conclusion, unlike other indirect tests based on monoclonal antibodies against epitopes of VWF A1 domain,[3,4] the HemosIL™VWF activity correlates with

Table 1. Descriptive statistics of samples analyzed with HemosIL™ VWF Antigen, HemosIL™ VWF Activity and VWF:RCo.

Classification of VWD according to the Hemophilia Centers	*N*	*VWF:Ag*	*VWF Activity*			*Ratio Act/Ag*		
		HemosIL	*HemosIL*	*VWF:RCo*	*p*	*HemosIL*	*VWF:RCo*	*p*
Normal	57	103.3±38	94.5±41	100.5±47	0.05	0.90±0.1	0.96±0.21	0.01
Type 1	13	30.9±24	29.9±26	23.9±19	0.03	0.82±0.4	0.76±0.3	ns
Type 1Vicenza	9	15.8±12	10.9±8	8.3±4	ns	0.67±0.3	0.61±0.3	ns
Type 2A	14	37.0±18	15.4±9	8.8±5	0.0006	0.44±0.2	0.27±0.2	0.002
Type 2B	12	38.5±14	13.9±8	13.8±7	ns	0.35±0.1	0.36±0.1	ns
Type 2M	12	19.9±15	7.7±4	5.2±2	0.05	0.43±0.3	0.32±0.2	ns

Data are expressed as mean IU/dL±SD. The mean values of the activity assays and the Act/Ag ratios are compared for each sample group. ns: not significant.

Table 2. Sample classification according to HemosIL™ VWF Activity and VWF:RCo.

Classification according to the Hemophilia Centers			*Classification in the R&D lab for the HemosIL VWF activity*				*Classification in the R&D lab for the VWF:RCo*			
Clinical status	*VWF deficiency*	*# samples*	*Norrmal*	*VWD type 1*	*3*	*2*	*Normal*	*VWD type 1*	*3*	*2*
Normal		**57**	**54**	0	0	3	**53**	1	0	3
Type 1	quantitative	**13**	1	**8**	0	4	1	**8**	0	4
Type 1 Vicenza	quantitative	**9**	0	**4**	**1**	4	0	**2**	**1**	6
Type 3	quantitative	**10**	0	0	**9**	1	0	0	**9**	1
	Total quantitative	**32**	1	**12**	**10**	9	1	**10**	**10**	11
Type 2A	qualitative	**14**	0	1	0	**13**	0	0	0	**14**
Type 2B	qualitative	**12**	0	0	0	**12**	0	0	0	**12**
Type 2M	qualitative	**12**	0	1	0	**11**	0	0	0	**12**
	Total qualitative	**38**	0	2	0	**36**	0	0	0	**38**

Classification according to the Hemophilia Centers and the R&D lab for the HemosIL VWF Activity and VWF:RCo. In bold, samples correctly classified.

the standard VWF:RCo in all type 1 and 2 VWD variants. Compared with the classical aggregometric assay for VWF:RCo, the new assay has the advantage of being much faster and fully automated, although it has a sensitivity limit of 12.5 IU/dL. HemosIL™ VWF activity, in combination with the HemosIL™ VWF:Ag appears to be a useful screening tool in the diagnostic evaluation of patients with a bleeding diathesis,[9,10] although additional VWF tests should always be performed to confirm and further characterize diagnosis.

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