Clinical evaluation of the Sebia Hydragel von Willebrand factor assay in comparison to Electrophoresis and blotting based Multimer analysis

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BACKGROUND and AIMS

Laboratory diagnosis of von Willebrand disease (VWD) requires measuring von Willebrand Faktor (VWF) by immunological (von VWF-Antigen (VWF:Ag), VWF-Collagen-binding (vWF:CB)) and functional tests (VWF activity (vWF-Act.), ristocetin cofactor activity VWF:RCo)). A reduced VWF ratio of functional and immunological assays often indicates a qualitative VWF defect. Knowledge of VWD subtype determines or modifies therapeutic options. The gold standard to identify qualitative VWD is the analysis of the multimers' distribution.

However, as conventional multimer analysis is a time and personal consuming process (2 – 3 days) and limited to specialized centers, urgent therapeutic decision making often has to be performed without its results.

Recently, a new rapid test (< 6 hours) Hydragel 5, von Willebrand Multimers (Sebia, Lisses, France) has been developed and is now commercially available. We investigated the impact of the Hydragel semi-automated system (in comparison to the conventional method.

PATIENTS and METHODS

Citrated plasma samples were loaded in a simple 2% agarose gel system (no stacking and running gel) and electrophoresed on the Hydrasys 2 Scan within 110 minutes. Multimers were probed in gel by immunofixation using horse-radish peroxid (HRP) conjugated rabbit anti-VWF (90 minutes). Visualization of multimers was achieved by colorimetry using commercially available TTF1/TTF2 Sebia reagents. Curves were produced using GelScan and Sebia Phoresis software.

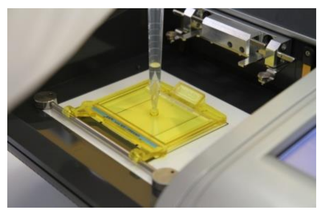
Conventional multimer analysis involves preparation low- and intermediate-resolution gels combined with an optimized visualization system.

We analyzed 101 patients with suspected or confirmed VWD. Clinical and laboratory phenotype were determined by standardized questionnaire and VWF parameter, respectively.

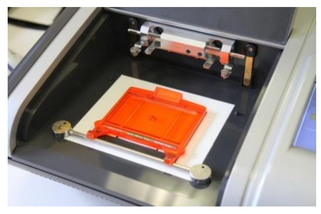
Ethical approval. The study was approved by the Ethical Committee of the ÄkNo, Dusseldorf, Germany.

1. **Electrophoresis of plasma sample in an 'uniform' SDS agarose gel**

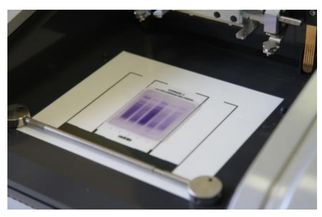
2. **Direct Immunofixation of multimers in gel using anti-VWF human antibodies**



3. **Gel staining: gel incubation with a secondary antibody (labelled with HRP / horse radish peroxydase) followed by an incubation with the specific TTF1/TTF2 substrate >> blue color**



4. **Gel scan / visual inspection and densitometry analysis**



RESULTS

Kits of Hydragel 5 von Willebrand Multimer (H5VWM) were ready to use and reagents were provided. Results were received within one working day (< 6 hours). Based on laboratory findings we found 22 patients with VWD type 1 and 12 patients with VWD type 2. The discrepant findings were shown in table 1: H5VWM failed diagnosing in a patient with cardial acquired VWD. As H5VWM can not visualize triplet structure, VWD type 2M can not be excluded by H5VWM (normal distribution of large multimers). False-positive results using the conventional assay were suspected likely due to transportation artefact in four cases. In one case clinically and laboratory based diagnosing of VWD was not sufficient with conflicting results of the multimer analysis. Probably different timepoints of blood samples explained discrepant findings for multimer analysis in a patient with essential thrombocythaemia (ET).

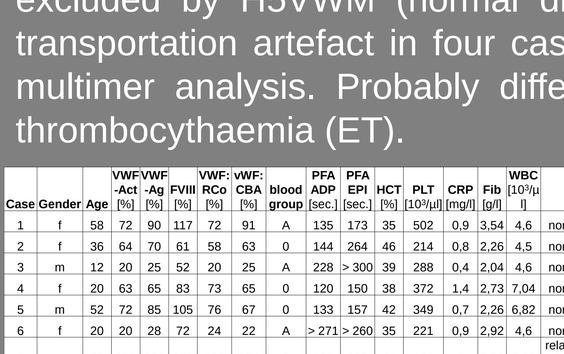
Case	Gender	Age	VWF-Act [%]	VWF-Ag [%]	FVIII [%]	VWF:RCo [%]	vWF:CBA [%]	blood group	PFA ADP [sec.]	PFA EPI [sec.]	HCT [%]	PLT [10³/µl]	CRP [mg/l]	Fib [g/l]	WBC [10³/µl]	H5VWM	probable diagnosis	conventional multimer analysis	rating	possible explanation for discrepancy	clinical information
1	f	58	72	90	117	72	91	A	135	173	35	502	0,9	3,54	4,6	normal distribution	normal	VWD type 2	discrepancy	transportation artefact	
2	f	36	64	70	61	58	63	0	144	264	46	214	0,8	2,26	4,5	normal distribution	normal	VWD type 2a/2b?	discrepancy	transportation artefact	
3	m	12	20	25	52	20	25	A	228	> 300	39	288	0,4	2,04	4,6	normal distribution	VWD type 1	normal distribution	no discrepancy		
4	f	20	63	65	83	73	65	0	120	150	38	372	1,4	2,73	7,04	normal distribution	VWD type 1	normal distribution	no discrepancy		
5	m	52	72	85	105	76	67	0	133	157	42	349	0,7	2,26	6,82	normal distribution	VWD type 1	possible VWD type 2a	discrepancy	transportation artefact	
6	f	20	20	28	72	24	22	A	> 271	> 260	35	221	0,9	2,92	4,6	normal distribution	VWD type 1	VWD type 2a	discrepancy	transportation artefact	
7	m	52	277	305	170	190	254	0	135	205	44	121	7	2,78	7,02	relative decrease of large multimers	VWD type 2	VWD type 2	no discrepancy		
8	f	92	16	27	73	12	147	0	> 300	> 300	42	240	2,4	2,97	6,85	relative decrease of large multimers	VWD type 2	VWD type 2a (subtype IIe)	no discrepancy		
9	f	68	8	21	36	42	11	A	276	> 300	39	313	2,1	3,79	5,09	relative decrease of large multimers	VWD type 2	VWD type 2a (subtype IIe)	no discrepancy		
10	f	35	29	99	103	ND	ND	A	ND	ND	38	313	10,7	3,73	8	normal distribution	VWD type 2M	VWD type 2M	discrepancy	No alterations of HMWM in vWD type 2M	
11	f	34	108	154	150	113	116	A	142	201	46	367	0,5	4,74	6,2	relative decrease of large multimers	VWD type 2	VWD type 2	no discrepancy		pregnancy
12	f	74	46	146	132	ND	170	A	122	222	44	437	20,6	3,87	8,15	normal distribution	aquired VWD type 2 (ET)	VWD type 2 (2010)	discrepancy	different timepoints of multimer analysis	ET
13	f	54	121	190	246	116	99	A	195	>300	39	820	10	436	9,66	relative decrease of large multimers	aquired VWD type 2 (ET)	VWD type 2	no discrepancy		ET
14	f	65	91	140	102	ND	ND	A	ND	ND	49	1125	1,3	3,34	10,5	relative decrease of large multimers	aquired VWD type 2 (MDS)	VWD type 2a	no discrepancy		PV
15	m	75	39	69	53	25	44	A	> 300	> 286	35	184	3,6	3,45	6,82	relative decrease of large multimers	aquired VWD type 2 (MGUS)	VWD type 2a	no discrepancy		MGUS
16	m	61	132	152	129	92	128	A	119	ND	47	551	17,3	3,74	5,2	relative decrease of large multimers	aquired VWD type 2 (ET)	VWD type 2	no discrepancy		ET
17	f	63	119	160	56	103	132	0	ND	141	46	367	0,5	2,99	6,2	relative decrease of large multimers	aquired VWD type 2 (MDS)	VWD type 2	no discrepancy		MPS
18	m	36	32	45	43	33	47	0	248	> 300	45	224	1,7	2,39	6,4	normal distribution	cardial aquired VWD type 2	VWD type 2	discrepancy	H5VWM failed	aortic valve stenosis
19	m	66	132	120	109	101	115	B	152	> 300	43	151	1	2,4	6,8	relative decrease of large multimers	not classifiable	VWD type 2 and normal distribution in control	conflicting results	not classifiable	SSRI

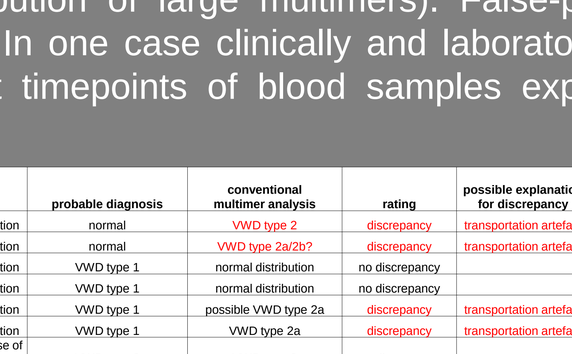
Table 1: Patients with discrepant findings between conventional multimer analysis and H5VWM, and all patients with clinical confirmed diagnosis of VWD type 2:

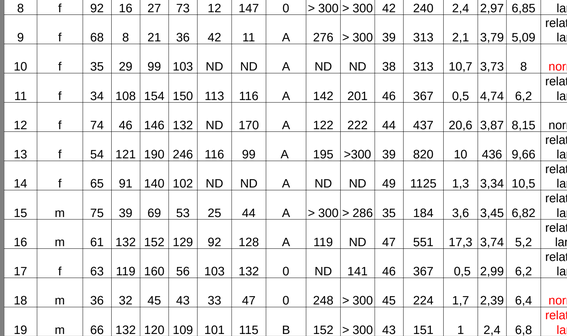
- False diagnosis of VWD type 2 probably due to transportation artefact in 4 patients (cases 1, 2, 5 and 6)
- Concordant results for both assays in 9 patients with VWD type 2 (cases 7, 8, 9, 11, 13, 14, 15, 16 and 17)
- H5VWM failed diagnosing in one patient with acquired (aortic valve stenosis) VWD type 2 (case 19)
- Alterations of triplet structure that may allow subclassification of VWD type 2M, cannot be seen with the H5VWM system (case 10). Type 2M is characterized for normal multimer distribution.
- Discrepant findings in case 12 were probably explained by different timepoints of multimer analysis (e.g. before and after treatment of ET)
- Diagnosing and classification of VWD failed due to conflicting clinical and laboratory results in case 19

Abbreviations:

ÄkNo: Ärztekammer Nordrhein, CT: control, ET: essential thrombocythaemia, Fib: Fibrinogen (Clauss), HCT: hematocrit, H5VWM: Hydragel 5 von Willebrand Multimer, MGUS: monoclonal gammopathy of undetermined significance, HMWM: high-moecular-weight multimers, MPS: myeloproliferative syndrome, ND: not determimed, PFA: platelet function analyzer, PLT: platelet count, SSRI: selective serotonin reuptake inhibitor, VWF: von Willebrand factor, WBC: white blood cells







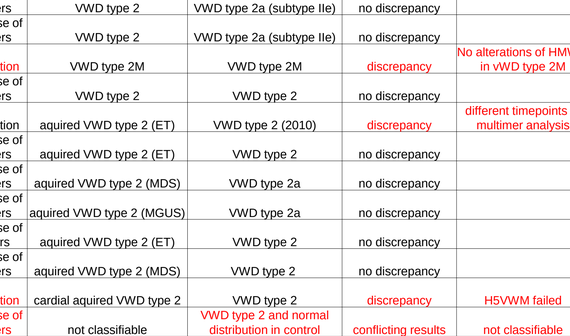


Figure 1: densitometry analysis of obtained gels in a control (CT) plasma and in patients with VWD type 1 (case 3), acquired VWD type 2 (case 14) and VWD type 2 and pregnancy (case 11).

CONCLUSIONS

- This study confirms the reliability of H5VWM in lab's routine. It detects diminution or loss of multimers in the majority of samples. For more complex samples it helps the lab in orientating the decision towards more specialized laboratory tests (i.e. visualization of triplets, genotyping).
- The risk of false-positive results, e.g. due to transport to the extern specialized lab, could be excluded.
- VWD type 2M can not be detected by H5VWM, as this subtype is characterized by alterations of triplet structure and not by any decrease of high-molecular-weight multimers (HMWM).
- The new assay was easy and rapid to perform (< 6 hours) and could be performed on a commercially available instrument (Hydrasys 2 and GelScan or Hydrasys 2 Scan).
- Due to the contemporary results it could be possibly useful particularly for patients with urgent surgery or even in emergency situations when VWD type 2 is suspected (e.g. by decreased VWF ratio) and before patients VWD subtype is determined by conventional method.

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VWF and von Willebrand Disease

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