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Von Willebrand factor propeptide (VWFpp) — potential biomarker in inherited von Willebrand disease and acquired von Willebrand syndrome

Ksenia Bykowska[1], Bernadeta Ceglarek[2], Adela Gwozdowska[2], Dariusz Zakrzewski[3], Beata Baran[1], Ewa Mendek-Czajkowska[4]

[1]Department of Hemostasis and Metabolic Disorders, Institute of Hematology and Transfusion Medicine in Warsaw

[2]Clinic of Hemostatic Disorders and Internal Medicine, Institute of Hematology and Transfusion Medicine in Warsaw

[3]Valvular Heart Disease Department, National Institute of Cardiology in Warsaw

[4]Department of Hematology, Institute of Hematology and Transfusion Medicine in Warsaw

Summary

Von Willebrand factor propeptide (VWFpp) is a fragment of a new synthesized VWF molecule that plays an important part in the biosynthesis of this protein. After completion of multimerisation of von Willebrand factor (VWF) dimers in the Golgi apparatus, as result of furin proteolysis VWFpp is truncated/disconnected from the mature VWF molecule to form a non-covalent complex with VWF. The VWFpp-VWF complex is stored in endothelial and platelet storage granules and released into the bloodstream where it dissociates into VWFpp and VWF.

Plasma VWFpp level and ratio of plasma VWF propeptide (VWFpp) to VWF antigen (VWF:Ag) i.e VWFpp /VWF:Ag ratio are important biomarkers of VWF synthesis/release/clearance. These biomarkers have distinct therapeutic utility as they help to identify patients with von Willebrand disease (VWD) in whom DDAVP (desmopressin 1-desamino-8-D-arginine vasopressin) is ineffective due to rapid VWF clearance. They also have significant diagnostic value because they help to differentiate between congenital VWD subtypes as well as between congenital VWD and acquired von Willebrand Syndrome (AVWS).

The purpose of the study was to determine the VWFpp level and the VWFpp/VWF: Ag ratio in patients with VWD and AVWS and to assess the significance of these biomarkers for diagnosis and management of congenital and acquired von Willebrand disease.

Our study involved 120 VWD patients; 21 with AVWS and 111 healthy controls. Study results confirm that VWFpp level and the VWFpp/VWF: Ag ratio have significant value for discrimination between severe type 1 VWD and type 3 VWD as well as between acquired and congenital VWD which has serious implications for therapy. The biomarkers are also useful for identification of patients in whom DDAVP treatment may prove ineffective. In type 1 VWD (< 30 IU/ml) the VWFpp/VWF: Ag ratio was elevated in 57% of patients while in the group of patients with threshold VWF values ('Low VWF', 30–50 IU/dL) the ratio was within normal, which may be suggestive of other underlying causes of VWF deficiency. Assays in patients with non-neutralizing anti-VWF antibodies (AVWS) have shown that the VWFpp/VWF: Ag ratio

Adres do korespondencji: Ksenia Bykowska, Laboratory of Von Willebrand Disease, Department of Hemostasis and Metabolic Disorders, Institute of Hematology Transfusion Medicine in Warsaw, Indiry Gandhi Street 14, 02–776 Warsaw, phone (22) 349 61 60, e-mail: kbykowska@ihit.waw.pl
Translation: mgr Krystyna Dudziak

can have significant impact on monitoring therapy and assessment of remission in patients with anti VWF antibodies.

Key words: Von Willebrand factor propeptide, biomarker, inherited von Willebrand disease, acquired von Willebrand Syndrome

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Introduction

Von Willebrand disease (VWD) is the most common bleeding disorder, extremely heterogeneous in both clinical and diagnostic terms. The disease may either be inherited or acquired (*acquired von Willebrand Syndrome*, AVWS) and in both disorders bleeding is caused by the deficiency of von Willebrand factor (VWF) which is a protein involved in blood clotting as well as in platelet adhesion and aggregation. However, the pathogenesis of VWF deficiency in each disorder is different.

Inherited von Willebrand disease (VWD)

Inherited von Willebrand disease was first described and differentiated from hemophilia in 1926 by the Finnish doctor Eric von Willebrand [1]. According to epidemiological investigations, VWF deficiency is responsible for this bleeding disorder in approximately 1% of the general population — but not all have clinically significant bleeding [2]. VWD is inherited in an autosomal dominant and less often recessive pattern [3]. The disease presents both phenotypic and genetic variations which is illustrated by the fact that until 1994 more than 20 variants have been described. In 1994, a simplified VWD classification was introduced which is valid until today with minor modification made in 2006 [4, 5]. The current classification includes 3 types and 4 subtypes of VWD. In VWD type 1 and 3 the defect is quantitative caused by partial impairment or complete lack of VWF synthesis, while in type 2 (2A, 2B, 2M, 2N), the defect is qualitative and is most often attributed to VWF gene mutations. Approximately 70% of VWD patients are diagnosed with type 1 and in half the number VWF half-life is shorter which may imply little or no effect of 1-desamino-8-days-arginine vasopressin (DDAVP) [6]. In VWD type 1, the shorter VWF half-life is characteristic of the 1C VWD variant [7]. VWD type 1C (Clearance) was first described in 2006 when shorter VWF survival in plasma was determined in four families [8]. Type 1 C VWD was characterized by shorter VWF half-life (4.4 fold shorter compared to healthy controls), a higher increase and a rapid decrease of VWF level after DDAVP, high VWFpp/VWF: Ag ratio as well as normal platelet count and VWF level. Laboratory tests also demonstrate reduced VWF: RCo and VWF: Ag levels in plasma. In VWD type 1 C, VWF synthesis, multimerisation and release are within normal. VWF multimers are usually larger than in normal plasma and multimer analysis shows abnormal VWF triplet pattern. Paradoxically, the presence of higher molecular weight multimers is the result of impaired VWF degradation by ADAMTS13 (*A Disintegrin And Metaloprotease with ThromboSpondin -1 motif*) due to rapid clearance rate of VWF from plasma [6, 7, 9–11].

In some clinical situations it is difficult to correctly differentiate VWD type 1C and severe VWD type 3 because VWF levels in type 1C can be as low as < 5 IU/dl [12].

Acquired von Willebrand syndrome (AVWD)

Acquired von Willebrand syndrome is a rare bleeding disorder with symptoms similar to inherited von Willebrand disease [13–16]. It occurs in 0.04–0.13% of the general population [17]. Unlike VWD, AVWS appears regardless of age, most often in elderly people with no personal or family history of bleeding but associated with other underlying diseases [17]. The first reported case of AVWS was that of a 7-year-old boy with systemic lupus erythematosus described in 1968 [18]. In 2000, Federici et al. [19] published study results of 186 AVWS patients from 50 hematological centers. AVWS was reported to occur in 63% of hematologic malignancies, mostly lymphoproliferative (48%) and myeloproliferative (15%) diseases, cardiovascular disorders (21%), solid tumors (5%), autoimmune disorders (2%) and others (9%). Within the category of lympho- and myeloproliferative diseases, AVWS is most common in monoclonal gammopathies of unknown etiology (MGUS, *monoclonal gammapathy of undetermined significance*), multiple myeloma and essential thrombocythaemia (ET).

In some AVWS patients bleeding disorders are the first symptoms of the underlying disease. Recurrent bleeding — from mild to severe — occurs in approximately 20–33% of patients. Life threatening are particularly spontaneous bleedings during surgical procedures which occur in persons with no bleeding disorders in medical history [17, 19, 20].

The mechanisms responsible for AVWS are mainly:

— increased VWF clearance by autoantibodies;
— VWF adsorption on cancer cells, platelets and other surfaces;
— high ionic strength affecting the loss of HMW (high molecular weight) multimers;
— increased proteolytic degradation (plasmin, calpain, elastase, ADAMTS13) [21, 22].

Correct diagnosis and classification of VWF deficiency as well as determination of the cause have significant impact on therapy. The panel of available diagnostic tests for VWD is wide but still not sufficient enough, especially with regard to VWD types and subtypes. Laboratories worldwide are therefore working on the modification of old diagnostic methods as well as development of new methods for more precise assessment of the deficiency [23–28]. One such practice is determination of von Willebrand factor propeptide (VWFpp) using a method available only in high-tech laboratories) [11, 29–33].

Von Willebrand factor propetide

Von Willebrand factor propetide (VWFpp) was first described in 1978 by Montgomery and Zimmermann [34] as part of the von Willebrand factor precursor molecule (pre-pro VWF) which is formed during biosynthesis of this protein in endothelial cells and megakaryocytes [12, 35, 36]. The VWF precursor molecule is a 2,813-residue polypeptide consisting of a 22-residue signal peptide, a 741-residue VWF peptide (VWFpp) and the 2,050-residue mature subunit. Upon biosynthesis, VWF precursor molecule undergoes intracellular modifications. The signal peptide (pre) is disconnected, pro-dimers are formed in the endoplasmic reticulum through disulfide bonds between C-terminal cysteine 1908–2050-residues, which undergo glycosylation and sulfonation in subsequent stages of biosynthesis. Pro-dimers are trafficked into Golgi apparatus where multimerization continues with the formation of interchain disulfide bonds between cysteine residues within the N-terminal D3 domain of pro-VWF [37–39]. D1, D2 domains of VWFpp and D 'and D3 domains of N-terminal of the VWF monomer are involved in the multimerization process [40–45]. As a result of furin (membrane-bound Ca ion dependent endoprotease) proteolysis, VWFpp is released into the environment. Proteolysis occurs at position 763 VWF, between the end of VWFpp and D'VWF domain [44, 46].

Up to date, it was believed that VWFpp stored in endothelial cells and alpha granules forms a non-covalent complex with VWF and after release into circulation dissociates into VWFpp and VWF under the influence of physiological and pathological stimuli [23, 47]. In 2012, Madabhushi et al. [48] demonstrated that VWF and VWFpp form a non-covalent complex (VWFpp-D-D3-VWF) also in circulation and the interaction of VWFpp and the D'D3 VWF domain is an important hemostasis-regulatory mechanism. It reduces the accessibility of the VWF A1 domain for platelet GPIbα, limits VWF proteolysis by ADAMTS13, inhibits stabilization and factor VIII: C binding [49, 50].

VWFpp circulates as a noncovalent homodimer at concentrations of approximately 1 μg/ml as opposed to 10 μg/ml of mature VWF; the mean half-life for VWFpp and VWF is 2–3 hours and 12 to 24 hours respectively [24, 47, 51]. In normal plasma the concentration of VWFpp and VWF is 100% i.e. 1 ml of normal plasma contains 1 unit of VWF and 1 unit of VWFpp; the VWFpp/VWF: Ag ratio equals 1 regardless of the actual concentration. The ratio of both molecules released into the circulation is 1:1.

Differences in VWFpp and VWF half-life that reflect the clearance rate of VWF from plasma have been used in diagnostics of VWD [24–27, 30, 51] and AVWS [52–55]. VWFpp concentration, the VWFpp/VWF: Ag ratio as well as the VIII: C/VWF: Ag ratio are already recognized biomarkers of VWF synthesis, release and clearance [47, 56]. In AVWS cases of acquired VWF inhibitor, VWF is rapidly removed from circulation whereas VWFpp remains normal at significantly increased VWFpp/VWF:Ag ratio. Because VWFpp concentration does not depend on ABO blood group [57], its concentration in plasma is believed to be a more sensitive marker of endothelial cell activation/damage than VWF concentration. Elevated VWFpp levels occur in patients with hypertension, diabetes, systemic sclerosis, myocardial infarction as well as thrombotic thrombocytopenic purpura and haemolytic uremic syndrome [57–60].

Biomarkers of VWF synthesis and clearance

Von Willebrand's disease is very heterogenous therefore a correct diagnosis requires

several laboratory diagnostic tests, some of which can be performed only in high-specialized laboratories. One such test is the measurement of VWF propeptide (VWFpp) and determination of the VWFpp/VWF: Ag ratio and the factor VIII: C/VWF: Ag ratio. Implementation of this assay into routine diagnostics of VWD [9, 13] has been discussed for many years now. The VWFpp test performed in various laboratories is based on the assumption that 1 unit corresponds to the concentration of VWFpp in 1 ml of plasma. Studies that indicate the importance of VWFpp assessment for the diagnosis of both congenital and acquired von Willebrand disease are becoming more frequent. VWFpp is believed to be an important biomarker of VWF synthesis, release and clearance in circulation. Lower values or absence of VWFpp may imply impairment of these processes but may also suggest the type of causative mutation [8–10, 25, 40, 41, 61]. The advantage of VWFpp assay is its independence of blood type; there are no ABO antigens on the VWFpp molecule while they are present on the VWF molecule [24]. In the assessment of VWF synthesis, release and clearance, we rely mostly on VWFpp/VWF: Ag ratio and VIII: C/VWF: Ag ratio and not on VWFpp level. [11, 61]. Elevated VWFpp/VWF: Ag ratio occurs in patients with increased VWF clearance (VWD 1C) while the normal ratio may appear in patients with normal clearance but with impaired secretion/release and intracellular retention of VWF.

The VIII: C/VWF: Ag ratio is another important biomarker of VWF synthesis and clearance (apart from the VWFpp/VWF: Ag ratio). Unlike VWF and VWFpp, which appear independently in circulation, factor VIII forms a non-covalent complex with VWF (1 VIII molecule to 50 VWF monomers). The complex with VWF protects factor VIII against proteolytic degradation [62]. Each VWF monomer has 1 binding site for VIII: C, which implies that there are many free sites capable of binding VIII: C. The concentration of coagulation factors is expressed in units per 1ml of plasma, therefore the ratioVIII: C/VWF: Ag in normal plasma is 1. Reduction/absence of VWF is believed to automatically cause factor VIII deficiency (type 2N VWD). Increased VIII: C/VWF: Ag ratio occurs when synthesis or release are defective; VIII: C/VWF: Ag ratio is within normal when VWF deficiency is caused by accelerated VWF clearance [61]. Elevated VIII: C/VWF: Ag ratio at impaired VWF synthesis/release is explained by the fact that all synthesized VIII: C can bind to VWF because VWF has many unoccupied VIII: C binding sites. As consequence, reduction of VWF by 50% (heterozygotes of the null allele) causes a twofold increase in the VIII: C/VWF: Ag ratio. The same mechanism explains why in 2N VWD heterozygotes, the ratioVIII: C/VWF: Ag equals 1 although the binding capacity of facor VIII: C is impaired. 2N heterozygotes have 50% normal subunits and 50% subunits with reduced or no factor VIII binding. The 50% of normal monomers bind the whole factor VIII and so VIII: C/VWF: Ag ratio is not reduced.

Material and methods

Tests were performed in a total 252 persons:

1. 111 controls (VWF concentration 50–100 IU/dL);
2. 120 VWD patients (VWF:RCo concentration < 5–50 IU/dL);
3. 21 AVWS patients, including:
   - 3 MGUS patients (P-1, P-2, P-3);
   - P-1-A-tests performed in regression following combined (cyclophosphamide + dexamethasone + immunoglobulin) therapy,
   - P-2 three tests performed:
     - P-2-A — at the start of treatment which involved 2 courses of bortezomide + doxarubicin + dexamethason-PAD,
     - P-2-B — during therapy, after 6 courses of PAD followed by transplantation of peripheral blood stem cells (PBSCT),
     - P-2-C — following treatment, during complete remission which lasted 292 days,
   - P-3-A tests performed after dexamerhazon pulse therapy,
   - 11 patients with essential thrombocytosis (ET) and 7 patients with aortic stenosis (SA). With the exception of the 7 last patients under the care of the Institute of Cardiology in Anin, all were patients of the Institute of Hematology and Transfusion Medicine in Warsaw.

Factor VIII activity (VIII: C), antigenic level of von Willebrand factor (VWF: Ag), ristocetin cofactor (VWF: RCo) assays were performed with Siemens reagents on the BCS XP Siemens analyzer. According to Krizek et al. [63], VWF multimers and von Willebrand factor propeptide were determined using ELISA assay and Sanquin reagents (Amsterdam).

Table 1. Characteristics of the control group (n = 111)

Research	Range	Average value ± SD	Median
VIII:C [IU/dL]	57.1–162.1	114.3 ± 21.11	113
VWF:Ag [IU/dL]	58.3–158.42	98.4 ± 24.6	96
VWF:RCo [IU/dL]	51.5–139.4	88.3 ± 23.5	83.4
VWFpp [IU/dL]	40–136	78.7 ± 23.2	74
VIII:C/VWF:Ag	0.55–1.68	1.20 ± 0.29	1.2
VWF:RCo/VWF:Ag	0.58–1.34	0.89 ± 0.16	0.88
VWFpp/VWF:Ag	0.49–1.98	0.82 ± 0.23	0.79

VWF:RCo — ristocetin cofactor; VWF:Ag — antigen VWF; VWFpp — propeptide VWF; factor VIII — VIII:C

Table 2. Results of the tests of VWD patients (n = 7) with concentration of VWF:Ag < 5 IU/dL

Nr	VIII:C [%]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWF:Rco/ /VWF:Ag	VWFpp [IU/dL]	VWFpp/ /VWF:Ag
1	2.37	2.37	< PD*	< PD*	< PD*	< PD*
2	2.36	< PD*	< PD*	< PD*	< PD*	< PD*
3	2.0	< PD*	< PD*	< PD*	< PD*	< PD*
4	15.0	< PD*	< PD*	< PD*	< PD*	< PD*
5	16.81	4.6	1.79	0.38	72	15,6
6	19.25	< PD*	< PD*	< PD*	< PD*	< PD*
7	< PD*	< PD*	< PD*	< PD*	< PD*	< PD*

*PD — results below detection level

Results

Tests involved 252 persons: 111 controls; 120 VWD patients and 21 AVWS patients aged 17–70.

Control group

The parameters for the control group were as follows: VWF:RCo from 114.3 ± 21.1 IU/ /dL; VWF:Ag — 98.4 ± 24.67 IU/dL; VWF:RCo/VWF:Ag ratio 0.89 ± 0.16; VWFpp level 78.72 ± 23.26 IU/dL; VWFpp/VWF:Ag ratio — 0.81 ± 0.22 (Table 1). Based on the test results, we accepted the following normal values: for VWFpp — values from 40–136 IU/dL, for VWFpp/VWF:Ag ratio — 0.49–1.98 and VIII:C/VWF:Ag ratio — 0.55–1.68. VWFpp/VWF: Ag ratio of 2.0 was the threshold value in our study.

Inherited von Willebrand disease

Patients (n = 7) with VWD and VWF:Ag < 5 IU/dL and VWF:RCo < 10 IU/dL (Table 2)

In 6 patients VWFpp level and VWFpp/ /VWF:Ag ratio were below detection threshold (type 3 VWD). In one patient, VWFpp was normal (72%) while VWFpp/VWF: Ag markedly elevated — 15.6 (n-2.0). T, which implied significantly increased VWF clearance and was suggestive of VWD type 1C.

VWD type 1 patients with VWF:RCo of 10– –20 IU/dL (n = 15) (Table 3)

VWFpp was reduced (n = 40–136 IU/dL) in 12 (80%) patients, while the VWFpp/VWF: Ag ratio was elevated/increased (> 2.0) in 6 (40%). In 13 patients (86.6%) the Factor VIII: C/VWF: Ag ratio was increased as compared to control group.

VWD type 1 patients (n = 5) with VWF: RCo of 20–30 IU/dL (Table 4).

In one patient (20%) VWFpp was reduced and in two (40%) the VWFpp/VWF:Ag ratio was increased. Factor VIII: C/VWF:Ag was elevated in 4 (80%) patients.

VWD type 1 patients (n = 16) with VWF: RCo of 30–40 IU/dL (Table 5).

In 6 (37.5%) patients, VWFpp level was reduced; the VWFpp/VWF:Ag ratio was reduced in

Table 3. Test results of type 1 VWD patients (n = 15) and concentration of VWF:RCo 10–20 IU/dL

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VIII:C/ /VWF:Ag	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag
1	26.99	16.32	11.77	24	1.65	0.72	1.47
2	23.71	12.16	11.61	32.8	1.94	0.95	2.69
3	31.02	17.51	13.36	152	1.77	0.76	8.68
4	51.89	19.85	17.98	22	2.6	0.9	21.1
5	42.49	13.67	10.38	24	3.1	0.75	1.75
6	67	22	16	7.2	3.04	0.72	0.32
7	55	16	13	6	3.43	0.81	0.375
8	71	27	20	18	2.62	0.74	0.66
9	42	15.8	15	30	2.65	0.94	1.89
10	27	12.9	12	96	2.09	0.93	7.44
11	41.19	13.34	11.32	6	3.06	0.84	0.44
12	32.6	18.34	14.47	60	1.77	0.78	3.27
13	67.9	15.45	11.25	36	4.39	0.72	2.33
14	55.45	19.42	16.41	24	2.85	0.84	1.23
15	40.85	20.02	16.52	20	1.64	0.82	0.99
Range	27–67.9	12.16–27	11.25–20	6–152	1,64–3.43	0.72–0.94	0.99–8.68
Average ± SD	45.07 ± 15.67	17.31 ± 3.95	14.07 ± 2.84	2.30 ± 2.49	2.74 ± 1.11	0.81 ± 0.08	2.30 ± 2.49
Median	42	16.32	13.36	1.47	2.85	0.81	1.47

Table 4. Test results of type 1 VWD patients (n = 5) and concentration of VWF:RCo 20–30 IU/dL

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VIII:C/ /VWF:Ag	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag
1	67.29	33.69	29.98	68	1.99	0.88	2.01
2	64.1	27.61	25.27	32	2.32	0.91	1.15
3	65.33	29.16	21.67	68	2.24	0.74	2.33
4	65.23	31.52	27.45	52	2.06	0.87	1.64
5	56.71	37.26	27.07	56	1.52	0.72	1.5
Range	56.71–67.29	27.61–37.26	21.67–29.98	32–68	1.52–2.32	0.72–0.91	1.15–2.33
Average	63.73 ± 4.08	31.84 ± 3.8	26.28 ± 3.08	55.2 ± 14.8	2.06 ± 0.31	0.82 ± 0.08	1.72 ± 0.45
Median	65.23	31.52	27.07	56	2.06	0.87	1.64

1 patient (6,25 %). In one patient (6.25%) VIII:C was slightly reduced; in 8 (50%) VIII:C/VWF:Ag ratio was elevated.

VWD type 1 patients (n = 26) with VWF: RCo of 40–50 IU/dL (Table 6).

For all patients with VWF:RCo of 40–50% the VWF:RCo/VWF: Ag ratio was ≥ 0.7. In 8/26 (30.7%) patients VWFpp was reduced; VIII:C/ /VWF:Ag was elevated in 9/26 (34.61%) patients (normal range 0.55–1.68); VWFpp/VWF:Ag ratio was within normal range for 26/26 patients; for 3/26 (11.5%) a correlation was observed between VWFpp reduction and VIIII: C/VWF:Ag ratio.

VWD type 2A patients (n = 11) VWF:RCo < 10 IU/dL and VWF:Ag > 10 IU/dL (Table 7)

In 10 (90.9%) patients, the VWFpp level was normal. In 10 (90.9%) patients VWFpp/VWF: Ag and VIII: C/VWF: Ag ratios were elevated (> 2.0).

VWD type 2A patients (n = 19) with VWF:RCo — 10–20 IU/dL (Table 8)

In 3 patients (15.7%) VWFpp was reduced, elevated in 1 person, normal in others. The ratio VWFpp/VWF: Ag was increased in 6 people (31.57%). Factor VIII: C/VWF: Ag was elevated in 15 patients (78.9%).

Table 5. Test results of type 1 VWD patients (n = 16) and concentration of VWF:RCo 30–40 IU/dL

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VIII:C/ /VWF:Ag	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag
1	72.44	35.03	39.51	32.8	2.06	1.12	0.93
2	72.59	45.28	36.6	60.8	1.6	0.8	1.34
3	101.38	82.61	34.25	152	0.81	0.7	1.83
4	67.31	48.67	35.04	60.8	1.38	0.71	1.24
5	73.19	40.03	40.52	46	1.82	1.01	1.14
6	68.81	38.78	34.16	10	1.77	0.88	0.25
7	73.32	39.48	34.6	26.8	1.85	0.87	0.67
8	32	35.08	36	20	0.91	1.02	0.57
9	62	41	33	72	1.51	0.80	1.75
10	76.6	38.7	39.11	76	1.97	1.01	1.96
11	91.5	38.67	39.89	24	2.36	1.03	0.62
12	76.1	36.90	31.78	28	2.09	0.87	0.77
13	61.38	42.94	39.55	60	1.42	0.92	1.39
14	73.14	37.62	34.82	52	1.42	0.92	1.38
15	60.89	44.14	39.7	60	1.37	0.89	1.51
16	96.39	37.92	39.38	72	2.54	1.03	1.82
Range	32–101.38	35.03–82.61	33–40.52	10–152	0.81–2.54	0.7–1.12	0.25–1.96
Average ± SD	72.44 ± 16.01	42.64 ± 11.28	36.74 ± 2.87	53.32 ± 33.48	1.68 ± 0.47	0.91 ± 0.12	1.19 ± 0.51
Median	72.86	39.13	36.3	56	1.68	0.9	1.29

VWD type 2A patients (n = 8) and VWF: RCo of 20–30 IU/dL (Table 9)

In 2 patients (25%) VWFpp was reduced and the VWFpp/VWF: Ag ratio was elevated in 1 (12.5%). For the whole group factor VIII: C was normal and the factor VIII: C/VWF: Ag ratio elevated in 5 patients (62.5%).

VWD type 2A patients (n = 13) and VWF: RCo of 30–40 IU/dL and partially reduced HMW VWF multimer fraction (Table 10)

In 5 patients (38%) VWFpp was reduced and elevated in 2 (15%); VWFpp/VWF:Ag ratio was elevated in 2 (15.38%) patients. In 4 (30.7%) patients the VIII: C/VWF; Ag ratio was elevated.

Acquired von Willebrand syndrome

Patients with AVWS due to appearance of antibodies in the course of monoclonal gammapathy (n = 3) (Table 11)

Patients with anti-VWF antibodies (P-1-A, P-2-A, P-3-A) at the beginning of therapy have reduced factor VIII: C level (< 10 IU/dL; 28.37 IU/dL; 22 IU/dL, VWF:RCo of < 10 IU/dL < 9.89 IU/dL, < 10 IU/dL respectively and VWF antigen concentration of 47 IU/dL, 14.67 IU/dL; 21 IU/dL presented normal or slightly increased VWFpp values. On the other hand, the VWFpp/VWF: Ag ratio was significantly elevated to 3.57, 5.7; 4.57 respectively (n = 0.49–1.98). Patient P-1-A with VIII: C below detection threshold presented reduced VIII: C/VWF: Ag ratio; for patients P-2-A and P-3-A the ratio was higher.

Patient P-2 was evaluated at various stages of (P-2-A; P-2-B; P-2-C) therapy and test results showed reduction of VWFpp/VWF: Ag ratio from 5.7 to 3.14 and of VIII: C/VWF: Ag ratio from 1.93 to 1.78 during treatment and return to normal values in complete remission.

Patients with AVWS in the course of essential thrombocythaemia (n = 11) (Table 12)

VIII: C activity was normal for all patients; VWF: RCo/VWF: Ag ratio < 0.7; VWFpp was normal or elevated; VWFpp/VWF: Ag ratio and VIII: C/VWF: Ag ratio were normal.

Table 6. Test results of type 1 VWD patients (n = 26) and concentration of VWF:RCo 40–50 IU/dL

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VIII:C/ /VWF:Ag	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag
1	77.43	46.35	47.09	36	1.67	1.01	0.77
2	88.14	57.29	46.47	60	1.53	0.81	1.04
3	94.96	50.29	46.13	36	1.88	0.91	0.71
4	98.55	59.37	43.3	48	1.65	0.72	0.80
5	116.46	57.18	47.73	80	2.03	0.83	1.39
6	97.69	57.18	47.73	44	1.7	0.83	0.76
7	108.86	62.23	41.18	68	1.74	0.7	1.09
8	100.52	54.66	46.73	40	1.83	0.85	0.73
9	73.19	40.03	40.52	46	1.82	1.01	1.14
10	55.35	41.89	45.95	44	1.32	1.09	1.05
11	88.96	51.18	45.49	48	1.73	0.89	0.93
12	96.67	64.45	47.94	40	1.49	0.74	0.62
13	108.41	64.39	40.95	76	1.68	0.73	1.18
14	92.89	47.92	43.54	38	1.93	0.90	0.79
15	92.51	41.95	40.83	26	2.2	0.97	0.61
16	81	54	41	44	1.5	0.75	0.81
17	93	57	43	30	1.63	0.75	0.52
18	64	58.85	48.22	34	1.08	0.81	0.57
19	89.98	54.92	44.97	52	1.63	0.82	0.94
20	90.53	56.26	43.28	36	1.6	0.76	0.63
21	57.75	53.73	48.14	36	1.07	0.89	0.67
22	75.95	56.81	48.3	48	1.33	0.85	0.84
23	80.4	51.76	44.81	52	1.55	0.86	1.0
24	73.63	51.23	48.6	44	1.43	0.94	0.85
25	86.38	62	44.35	68	1.39	0.71	1.09
26	94.14	66.1	49.3	72	1.42	0.74	1.08
Range	55.35–116.46	40.03–66.1	41–49.3	26–72	1.07–2.2	0.7–1.09	0.52–1.39
Average ± SD	87.59 ± 14.9	54.57 ± 6.95	45.21 ± 2.78	47.92 ± 14.47	1.60 ± 0.26	0.84 ± 0.10	0.86 ± 0.21
Median	90.25	55.59	45.72	44	1.63	0.83	0.82

Patients with lack of high molecular multimers VWF in the course of aortic stenosis (n = 11) (Table 13)

Seven (7) patients with aortic stenosis and absence of high molecular multimeters at high VWF: RCo concentrations prior to TAVI (transcatheter aortic valve implantation), presented normal or slightly reduced VWFpp/VWF: Ag ratio.

Discussion

Our study results confirm those of other authors who demonstrated VWFpp and VWFpp/ /VWF: Ag ratio as well as VIII:C/VWF:Ag ratio to be important biomarkers of normal synthesis and VWF clearance which have significant value for differentiation of VWD types and discrimination between VWD and AVWS. Determination of VWFpp and VWFpp/VWF:Ag ratio has therapeutic significance because accelerated VWF clearance may impair the effectiveness of DDAVP treatment [64].

The tests were performed in 120 patients with inherited VWD) (type 1, 2, 3) and 21 patients with acquired von Willebrand syndrome. The results were compared to those of controls (111) where the parameters were as follows: VWFpp — 40–136 IU/dL; VWFpp/VWF: Ag ratio — 0.49–1.98 and VIII: C/VWF: Ag ratio 0.55–1.68 (Table 1).

Table 7. Test results of type 2A VWD patients (n = 11) (VWF:RCo < 10 IU/dL and VWF:Ag > 10 IU/dL)

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VIII:C/ /VWF:Ag	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag
1	95.5	44.72	7.01	120	2.1	0.15	2.68
2	109.99	41.27	3.12	108	2.6	0.075	2.61
3	110.74	50.5	9.23	128	2.1	0.18	2.53
4	44.46	13.82	1.67	44	3.2	0.12	3.18
5	41.73	20.45	5.14	46	2.04	0.25	2.24
6	32.28	10.4	1.4	76	3.1	0.13	7.3
7	38.4	16.78	7.2	96	2.2	0.42	5.72
8	37.38	16.41	8.31	72	0.43	0.50	4.38
9	19.89	10.16	2.82	68	1.95	0.27	6.69
10	49.93	23.94	9.55	36	2.08	0.39	1.5
11	33.81	17.96	8.33	104	1.88	0.46	5.79
Range	19.69–110.74	10.16–50.5	1.4–9.55	36–120	0.43–2.6	0.075–0.5	1.5–6.69
Average	55.81± 32.97	24.21 ± 14.38	5.79 ± 3.08	81.63 ± 31.78	2.15 ± 0.72	0.26 ± 0.15	4.05 ± 2.0
Median	41.73	17.96	7.01	76	2.1	0.25	3.18

Table 8. Patients (n = 19) with type 2A VWD and concentration of VWF:RCo 10–20 IU/dL

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VIII:C/ /VWF:Ag	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag
1	107.9	56.61	14.56	44	1.9	0.25	0.77
2	79.53	45.57	17.52	44	1.74	0.38	0.9
3	61.15	29.71	17.13	64	2.05	0.57	2.15
4	99.5	44.72	10.1	132	2.2	0.22	2.95
5	52.15	23.05	12.27	26	2.22	0.53	1.12
6	75.7	109.68	11.8	260	0.69	0.1	2.37
7	65.23	29.03	12.85	40	2.24	0.44	1.37
8	54.01	18.01	11.05	106	2.99	0.61	5.88
9	98	56.93	19	62	1.72	0.33	1.08
10	66	33	14	50	2.0	0.42	1.51
11	84	51	13	44	1.64	0.25	0.86
12	53	26	13	64	2.03	0.5	2.46
13	87.92	45.43	10.71	140	1.93	0.23	3.08
14	67.82	21.55	14.35	40	3.14	0.66	1.85
15	54.14	37.3	13.94	64	1.45	0.37	1.71
16	49.93	23.94	10.55	28	2.08	0.44	1.16
17	61.01	24.88	13.78	48	2.45	0.55	1.92
18	79.2	47.58	11.69	72	1.66	0.24	1.51
19	61	24.8	13.78	36	2.45	0.55	1.45
Range	49.9–260	23–109.6	10–17.52	26–260	1.4–5.88	0.1–0.66	0.86–5.88
1.9 ± Average ±SD	71.7 ± 55.6	39.4 ± 21	13.42 ± 2.4	71.7 ± 55.6	2.0 ± 0.53	0.5 ± 0.1	1.9 ± 1.1
Median	50	33	13	50	2.03	0.42	1.51

Table 9. Test results of type 2A VWD patients (n = 8) and concentration of VWF:RCo 20–30 IU/dL

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp	VIII:C/ /VWF:Ag	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag
1	91.29	42.63	26.46	66	2.14	0.62	1.54
2	116.84	76.91	26.1	60	1.51	0.33	0.78
3	62	64	26	68	0.96	0.4	1.06
4	92.05	50.6	26.43	48	1.81	0.52	0.94
5	85.45	40.02	24.19	32.8	2.13	0.6	0.82
6	90.85	43.95	29.86	48	2.06	0.67	1.09
7	66.13	33.4	22.21	18	1.97	0.66	0.54
8	62.95	47.34	27.5	68	1.32	0.58	2.47
Range	62–116.84	33.4–76.91	22.21–29.86	32–68	1.52–2.32	0.33–0.67	0.54–2.47
Average	83.44 ± 18.85	49.85 ± 14.1	26.09 ± 2.24	55.2 ± 14.8	2.06 ± 0.31	0.54 ± 0.12	1.15 ± 0.6
Median	88.15	45.64	26.26	56	2.06	0.59	1.0

Table 10. Test results of type 2A VWD patients (n = 13) concentration of VWF:RCo 30–40 IU/dL and partially reduced HMW VWF multimers fraction

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VIII:C/ /VWF:Ag	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag
1	87.71	56.46	36.09	28	1.55	0.63	0.92
2	61.87	35.47	39.42	36	1.74	0.55	1.01
3	57.77	126.45	37.62	352	0.45	0.29	2.78
4	101.38	82.61	34.25	144	1.22	0.41	1.74
5	75.08	49.82	33.37	38	1.5	0.66	0.76
6	115.58	51.57	33.74	32	2.24	0.64	0.62
7	117.2	55.44	32.53	24	2.1	0.58	0.43
8	87.86	47.44	32.34	42	1.84	0.68	0.88
9	86.44	57.02	39.64	42	1.51	0.69	0.73
10	75.08	49.82	33.37	48	1.5	0.67	0.96
11	61.87	61.87	36.78	44	1.0	0.59	1.19
12	80.87	63.15	38.09	84	1.28	0.6	2.2
13	85.7	59.3	31.5	80	1.44	0.53	1.34
Range	57.77–115.58	35.47–126.45	31.5–39.64	28–352	1.2–2.28	0.29–0.69	0.73–2.78
Average ± SD	84.18 ± 18.87	61.26 ± 22.36	35.28 ± 2.79	76.46 ± 88.96	1.49 ± 0.46	0.5784 ± 0.1153	1.19 ± 0.67
Median	85.7	56.46	34.25	42	1.5	0.6	0.96

The first part of the study consisted in analysis of the importance of VWFpp for differentiation of VWD types and subtypes. Our results were consistent with those of other authors [8–11].

Test results of VWD type 1 patients (Tables 2–6) revealed that the frequency of rapid VWF clearance, impaired synthesis/release of VWF depends on the degree of VWF deficiency. VWFpp/VWF: Ag ratio was elevated inabout 60% of patients with VWF: RCo 10–20 IU/dL and in 40% of patients with VWF: RCo of 20–30%; altogether in 57% of patients with VWD1 (< 30 IU/dL). On the other hand, for all patients with threshold values of VWF (Low VWF 30–50 IU/dL) it was found normal, which may imply a different underlying cause of VWF deficiency than in VWD1 patients (< 30 IU/dL).

In VWD type 2 patients (tables 7–10) elevated VWFpp/VWF: Ag and VIII: C/VWF: Ag ratios, impaired synthesis and rapid VWF clearance were

Table 11. Patients with AVWS caused by antibodies anti VWF in MGUS process

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VWFpp/ /Ag	RCo/ /VWF:Ag	VIII:C/ /VWF:Ag
P-1-A	< 10	47	< 10%	168	3.57	ND	Nd
P-2-A	28.37	14.67	< 9.89	84	5.7	ND	1.93
P-2-B	59	33.03	15.85	104	3.14	0.47	1.78
P-2-C	145.69	137.92	149.2	88	0.63	1.08	1.05
P-3-A	22	21	< 10	96	4.57	ND	

P-1; P-2; P-3 — patients; A — before the treatment; B — during the treatment; C — after the treatment

Table 12. Patients with AVWS during essential thrombocythaemia (ET)

Nr	VIII:C [IU/dL]	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VIII:C/ /VWF:Ag	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag
1	59	76	42	68	0.77	0.55	0.89
2	55	97	43	104	0.56	0.44	1.072
3	58	49	35	48	1.18	0.71	0.97
4	46	61	22	56	0.75	0.36	0.91
5	58	60	31	64	0.96	0.51	1.06
6	61	159	25	160	0.38	0.15	1.0
7	59	75	38	80	0.78	0.50	1.06
8	78	49	31	60	1.59	0.63	1.22
9	57	75	37	60	0.76	0.49	0.8
10	69	113	29	108	0.55	0.25	0.95
11	81	107	33	88	0.75	0.30	0.82
Range	55–81	49–159	22–43	56–160	0.38–1.59	0.15–0.71	0.8–1.22
Average	61.90 ± 10.23	83.72 ±33.08	33.27 ± 6.58	81.45 ± 32.55	0.82 ± 0.33	0.44 ± 0.16	0.97 ± 0.12
Median	59	75	33	68	0.76	0.49	0.97

Table 13. Patients with HMWM deficiency (n = 11) during the aortic stenosis

Nr	VWF:Ag [IU/dL]	VWF:RCo [IU/dL]	VWFpp [IU/dL]	VWF:RCo/ /VWF:Ag	VWFpp/ /VWF:Ag	HMW Multimers
1	312	331	228	1.06	0.73	Brak
2	165	141	72	0.85	0.43	Brak
3	210	166	102	0.79	0.48	Brak
4	162	99	92	0.61	0.61	Brak
5	215	129	160	0.6	0.74	Brak
6	153	131	120	0.85	0.78	Brak
7	600	203	144	0.33	0.24	Brak
Range	153–600	99–331	72–228	0.33–1.06	0.24–0.78	
Average ± SD	259.57 ± 159.63	171.42 ± 77.5	131.14 ± 52.25	0.72 ± 0.23	0.57 ± 0.19	
Median	210	141	120	0.79	0.61	

more common for those with VWF: RCo < 10 IU/dL and VWF: Ag > 10 IU/dL values (90 and 91% respectively). They were less frequent for patients with VWF:RCo 10–20 IU/dL — in 32 and 79% of patients respectively; for patients with VWF: RCo 20–30% — in 12 and 62% of patients and for patients with vVWF 30–40% they were least frequent and observed in 15 and 31% respectively.

Our studies which involved patients suspected of type 3 VWD confirmed that VWFpp and VWFpp ratio is an important differentiation marker between type 1 (severe) and severe type 3 VWD. Tests results of 6 patients (Table 2) suspected of severe VWD type (VWF < 5 IU/dL), confirmed the diagnosis in 5 cases (VWFpp < 1 IU/dL) while 1 patient with VWF: Ag — 4.6 IU/dL; VWFpp — 72 IU/dL and VWFpp/VWF: Ag ratio — 15.6 (n < 2) was diagnosed with type 1C VWD which presents with rapid VWF clearance.

Due to heterogeneity of inherited von Willebrand disease, proper diagnosis often requires detailed tests some of which can only be performed in high-specialized laboratories. More attention is therefore devoted to implementation of VWFpp and VWFpp/VWF:Ag ratio measurement into routine VWD diagnostics.

Discussions on the implementation of such assays into routine diagnostics of VWD [9, 13] have been ongoing for many years. Numerous publications point to the importance of VWFpp in diagnostics of both inherited and acquired von Willebrand disease. VWFpp is considered an important biomarker of VWF synthesis, release and clearance; normal VWFpp concentration implies normal synthesis, release and clearance of VWF, whereas reduced/absent VWFpp values indicate not only impairment of the above but are also suggestive of the type of causative mutation [8–10, 25, 40, 41, 61]. VWFpp assays have the advantage of being independent of blood type as no ABO antigens appear on the VWFpp molecule (they are present on the VWF molecule) [24]. In the assessment of VWF synthesis, release and clearance we rely mainly on VWFpp/VWF: Ag and VIII: C/VWF: Ag ratios [56, 61] and not on VWFpp levels. Increased VWFpp/VWF: Ag ratio is observed in patients with rapid VWF clearance (VWD 1C) while the normal ratio in persons with normal clearance but defect of release and intracellular retention.

VWFpp/VWF: Ag and VIII: C/VWF: Ag ratio helps to differentiate between severe type 1 VWD and VWD type 3 as well as identify VWD type 1C.

Patients [25, 65] recognized as type 3 VWD with complete absence of VWFpp are believed to be either homozygous or heterozygous for null allele, whereas patients with measurable VWFpp may be heterozygous for missense mutation combined with accelerated VWF clearance. There is a difference between the two groups of patients with regard to severity/degree of the bleeding disorder. The *bleeding score* in patients suspected of type 3 and with measurable VWFpp is markedly lower than in patients with complete absence of VWFpp, therefore patients with measurable VWFpp should be reclassified as severe VWD type 1 (VWD 1C) [25, 65].

Proper identification of VWD 1C cases has therapeutic significance because in this large group of patients DDAVP treatment will prove ineffective. According to Zdziarska et al. [66] VWD 1C accounts for about 20% of all VWD type 1 cases, for over 70% of cases with VWF concentration of 2–10 IU/dL and almost 40% of cases with VWF: Ag ratio of 11–20 IU/dL.

VWFpp/VWF: Ag ratio not only indicates the VWF survival time but may also help to identify the molecular background of the defect [25, 61, 65].

Eikenboom et al. [61] demonstrated that in VWD type 1patients the VWFpp/VWF: Ag and VIII: C/VWF: Ag ratios are higher than in healthy members of their families and the values correlate with the type of causative mutation. In missense heterozygotes the VWFpp/VWF: Ag ratio is higher than in null heterozygous, while FVIII: C/VWF: Ag ratio is higher among VWF null heterozygotes.

Determination of VWFpp may also be relevant for differentiation between VWD type 2B and PT--VWD. Unfortunately, here we have no experience of our own, but Woods et al. [67] and Casonato et al. [24] described type 2B patients who presented elevated VWF clearance and increased VWFpp/ /VWF: Ag ratio, most probably due to increased clearance/removal of VWF-platelet complexes by macrophages (increased receptor 1 lipoprotein binding). VWFpp/VWF: Ag ratio in platelet-type von Willebrand disease (PT-VWD) is normal whereas in VWD 2B it is usually elevated, the authors suggest that the nature of hemorrhagic disorder can be inferred from VWFpp/VWF: Ag [66].

In the second part of our study we explored the importance of VWFpp and VWFpp/VWF: Ag ratio for differentiation between congenital von Willebrand disease and acquired VWS.

Tests were performed in patients with AVWS caused by: anti-VWF antibodies (patients with MGUS, Table 11); VWF adsorption on platelets (pa-

tients with essential thrombocythaemia, Table 12); deficiency of high molecular weight VWF multimers (in patients with aortic stenosis, Table 13). In 3 MGUS patients, the VWFpp and VWFpp/VWF: Ag measurements were performed at the beginning of treatment (P-1-A; P-2-A-; P-3-A) and in one (P-2), during and after the end of treatment (Table 11).

The study demonstrated that in all three patients (P-1; P-2; P-3), the underlying cause of AVWS was the appearance of anti-VWF antibodies in vivo. In vitro — in a mixture of normal and patient's plasma — did not inhibit VWF: RCo activity.

At the beginning of therapy, VWF: RCo < 10 for all 3 patients, VWFpp was normal, while the VWFpp/VWF: Ag ratio markedly elevated which suggested normal VWF synthesis and rapid VWF clearance. In one of these patients P-2 (with multiple myeloma IgG type lambda-IA), we followed the changes of VWFpp/VWF:Ag values from the beginning of therapy, in the course of therapy and after the treatment was terminated. The value of the ratio was observed to gradually decrease proportionally with VWF: RCo increase and improvement of the patient's clinical condition; VWFpp was within normal value. After two cycles of PAD combination therapy (bortezomib + ddxarubicin + dexamethasone) regression occurred (partial remission according to PAD) and the VWFpp/ /VWF: Ag ratio was 5.7; after 6 cycles the ratio decreased to 3.14 and the remission was partial. In tests performed during persisting remission following transplantation of peripheral blood stem cells VWFpp level and VWFpp/VWF: Ag ratio were normal (0.63; n = 0.49–1.98).

In 2014, Lee et al. [53] described changes in VWFpp/VWF: Ag ratio during the course of treatment of a patient with anti-VWF inhibitor antibodies. At first diagnosis, VWF: RCo, VWF: Ag ratio and FVIII were indeterminate, VWFpp/ /VWF: Ag ratio markedly increased. Following immunosuppression with prednisone and azathioprine VWF/FVIII level was normal as was the VWF multimeric pattern while the VWFpp/VWF: Ag ratios were still two-fold higher than normal. According to the authors, this was associated with residual antibody activity and VWF clearance which was still too high. Complete remission (no antibodies) was achieved after normalization of VWFpp/VWF: Ag.

It is always extremely difficult to assess the background of the bleeding disorder in patients with anti VWF antibodies. There are anti-VWF antibodies that neutralise VWF activity which are determined by residual activity of VWF in a mixture of normal and patient's plasma and more common anti-VWF antibodies that do not change VWF function but increase its clearance. In vitro activity of anti-VWF antibodies (in a mixture of normal and patient's plasma) will be normal taking into account the dilution with tested plasma (with inhibitor). On the other hand, in vivo VWF activity will rapidly decrease eg. after administration of VWF concentrate. The presence of anti-VWF antibodies is confirmed by VWFpp and VWFpp/ /VWF: Ag ratio [53].

Summary

To summarize, determination of VWFpp and VWFpp/VWEF: Ag ratio is of great therapeutic and diagnostic significance. It helps to differentiate VWD types and subtypes in cases of inherited VWD as well as to identify patients with shorter VWF survival for whom DDAVP treatment may prove ineffective [8–11, 61]. In the case of patients with AVWS due to presence of anti-VWF inhibitor antibodies, the VWFpp/VWEF: Ag ratio may be a valuable parameter of therapy effectiveness and indicator of complete remission [53].

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