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Clinical features and laboratory patterns in a cohort of consecutive Argentinian patients with von Willebrand's disease

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Background and Objectives. von Willebrand's disease (vWD) is a bleeding disorder with variable clinical expression. Our aim was to classify patients with vWD and to determine the phenotype in their relatives.

Design and Methods. The types and subtypes, blood group frequency and its relevance, bleeding sites, response to the desmopressin (DDAVP) test, transfusion requirements and clinical features in type 1 and 2A families were determined in 1,885 patients.

Results. Our findings were: type 1: 91%, type 2A: 3.1%, severe vWD: 1.3%; type 2N: 1.6%; type *low intraplatelet*: 2.7%; combined 1+2N: 0.3%. Blood group O prevalence was 70.5%. Bleeding and transfusion requirements were not correlated to blood groups. The most frequent symptoms were: ecchymoses-hematomas and epistaxis and, in females over 13 years, also menorrhagia. Normal levels of factor VIII:C were found in 38.4% of the patients. DDAVP was infused in 567 patients with a good response in 80.6%. About 9% of our patients needed transfusion therapy. The diagnosis of von Willebrand's disease is more likely in subjects belonging to families with type 2A disease than in members of families with type 1 vWD in spite of these being symptomatic.

Interpretation and Conclusions. These observations provide a good strategy to identify, classify and treat vWD patients without performing molecular assays.

Key words: vWD variants; DDAVP; symptoms; laboratory assays

Von Willebrand's disease (vWD) is a life-long bleeding disorder that appears to be inherited in an irregular fashion as an autosomal dominant trait, although a recessive pattern has also been described.[1–3] Clinical expression is variable and is characterized by mucocutaneous hemorrhages (epistaxis, ecchymoses, easy bruising), with a special constellation of symptoms in females (menorrhagia, bleeding after vaginal delivery or Cesarean section). Patients may have a mild, moderate or severe bleeding tendency from early childhood depending on the severity of the von Willebrand factor (vWF) or factor VIII (FVIII:C) defect.[4] vWD is caused by a quantitative or qualitative defect of vWF, a high molecular weight glycoprotein that plays a role in the early phases of hemostasis by promoting platelet adhesion to the subendothelium[5,6] and platelet aggregation under high shear conditions.[7] Furthermore, vWF is crucial in the intrinsic pathway of coagulation since it is also the carrier of FVIII:C in plasma.[4,8] vWD has been subdivided into three categories which reflect their physiopathology. Type 1 and 3 vWD refer to partial or virtually complete deficiency of vWF while type 2 vWD refers to a qualitative deficiency. The revised classification introduced in 1994 by Sadler[9] suggested subdividing type 2 vWD into 4 variants (2A, 2B, 2M, 2N) according to specific details of the phenotype. Recently, Lethagen[10] reported that a substantial number of patients, formerly assigned to type 1, whose circulating vWF contains mutant subunits in addition to a quantitative reduction, should be reclassified as type 2. He suggested that some of these patients would be helped by desmopressin (DDAVP) treatment. Moreover, Castaman[11] described a lack of association between genotypes, bleeding history and ristocetin cofactor activity (vWF:RCo) in many families diagnosed as having mild type 1 vWD. It is known that normal individuals with the 0 blood group have lower levels of FVIII:C and/or vWF:Ag[12-14] than those with a non-0 blood group. Although the reasons are uncertain, the physiologic basis for this difference is most likely related to differences in the type or amount of vWF glycosyla-

tion,[15] leading to a shortened plasma survival.[16] Therefore, the *in vitro* characterization of vWD does not offer a guide to treatment. Our aim was to analyze vWD patients with a clear personal or family history of bleeding associated with low vWF:RCo levels, and to determine the phenotype expression in their relatives and their response to DDAVP.

Design and Methods

Subjects

We analyzed the prevalence of vWD in 2,339 individuals referred to our Department from 1982 to July 1999 because of a personal and/or family history of hemorrhages or abnormal pre-surgery clotting assays. In those individuals with normal plasma FVIII:C, vWF:Ag and vWF:RCo levels but with a prolonged bleeding time (BT) or low platelet retention to glass beads (PR) and a personal or family history of bleeding, intraplatelet vWF:Ag (i-vWF:Ag) was also assayed. The age and position in the family were established for each person. Exclusion criteria were pregnancy, infectious or inflammatory diseases and estrogen-containing contraceptive pill use. The criteria used to define vWD were intentionally restrictive in order to avoid overdiagnosis. In all cases, without considering ABO groups, vWF activity was below 50 U/dL with the exception of subjects with vWD types 2N and *low intraplatelet* in whom the plasma vWF:RCo and vWF:Ag levels were normal. Type 2A variant was defined in those patients with reduced vWF:RCo, normal or slightly reduced levels of vWF:Ag and absence of large multimers of vWF. Probable type Normandy (2N) variant was suspected when FVIII:C level and the FVIII:C binding capacity/vWF:Ag ratio were low. Combined 1+2N cases were diagnosed in patients with proportionately low vWF:Ag and vWF:RCo but with disproportionately low levels of FVIII:C and low FVIII:C binding capacity/vWF:Ag ratio. Severe vWD was defined as occurring in those patients with vWF:Ag and vWF:RCo below 10 U/dL. *Low intraplatelet* with normal plasma vWF variant[17,18] was defined as occurring in those patients with only low intraplatelet vWF content. Platelet content of fibrinogen was also measured to exclude the presence of platelet abnormalities such as a low content of α-granules by platelet activation. Bleeding symptoms and family history of hemorrhages were obtained by a detailed questionnaire. Specific symptoms investigated included: epistaxis, menorrhagia, ecchymoses, hemorrhages (after tooth extraction, surgery, *post-partum*), hematomas, hemarthrosis and hematuria. Each symptom was recorded only when sufficiently fully reported. Laboratory investigation was offered to all available members of the families. The blood group was required from each subject. When the first study was normal and the personal or famiy history of bleeding was strong, the studies were repeated at least 3 times. Intake of any antiplatelet drugs for 10 days before the study was avoided. There were no cases of consanguinity in any of the families.

Laboratory investigations

Blood samples. Blood was taken by venipuncture between 7:30 and 9:00 a.m. after the subjects had been resting for at least 30 min. Blood was collected in polypropylene tubes with 0.11 M trisodium citrate (1:10 v:v) for coagulation tests and FVIII:C binding capacity and 0.11 M trisodium citrate, 50 mM EDTA, 60 mM n-ethylmaleimide, 2,000 KIU/mL aprotinin (1:10 v:v) for vWF:Ag, vWF:RCo, and multimeric pattern assays. For the assays of platelet retention to glass beads, 53.7 mM EDTA was used as anticoagulant. The samples were centrifuged at 2,000 $\times$ g for 15 min. Platelet-poor plasma was transferred to another tube and centrifuged at 3,000 $\times$ g for 30 min. It was then aliquoted and frozen at –70°C until analyzed.

Phenotypic analysis and laboratory assays. BT was tested by the Ivy[19,20] or Mielke[21] method (with a commercial device: Simplate R, General Diagnostics, Morris Plains, NJ, USA). For the Ivy method, our normal value was up to 4.30 min, and for the Mielke method, the normal value was up to 9.30 min. The tests were stopped when profuse bleeding was still observed at 9 and 18 min, respectively. PR was assayed according to the Hellem II method[22] (normal value: 26-70%). Activated partial thromboplastin time (APTT)[23] was measured by using rabbit cephalin-kaolin (normal value: 34-50 sec). FVIII:C was assayed using a one-stage method (normal value: 50-150 U/dL).[24] vWF:Ag was measured by a quantitative immunoelectrophoresis technique as described elsewhere[25] (normal value: 50-150 U/dL). vWF:RCo was measured by the modified method of Macfarlane[26] (normal value: 50-150 U/dL). The multimeric pattern of vWF was analyzed as described elsewhere.[27] FVIII:C binding capacity was measured by polyclonal anti-vWF capture of patient vWF on polystyrene tubes with recombinant FVIII:C. Bound FVIII:C was measured using the chromogenic method. A ratio of bound FVIII:C/vWF:Ag over 0.8 was considered as normal.[28,29] i-vWF:Ag (normal value: 0.1-0.4 U/10^9 platelets) and i-fibrinogen (normal value: 30-90 µg/10^9 platelets) were assayed in the supernatant of frozen and thawed-washed platelets.

Standard plasma. The local standard plasma used for the different assays was made by pooling the plasmas from 20 healthy donors, from a single sample in citrate-n-ethylmaleimide-EDTA-aprotinin as anticoagulant and stored at –70°C until analyzed. APTT and FVIII:C were assayed in each donor. Plasmas with FVIII:C less than 80 U/dL and more than 110 U/dL were discarded. The standard pool was calibrated for FVIII:C, vWF:Ag and vWF:RCo activity against the International Reference Preparation for Factor VIII:C Related Activities in Plasma (IRP) (87/718), National Institute for Biological Standards and Control, London, UK.

DDAVP infusion

DDAVP was infused intravenously into 567 patients over a period of 20 min at a dose of 0.3 µg/kg body wt, in saline solution. Blood samples were obtained for PR, APTT, FVIII:C, vWF:Ag, vWF:RCo assays before and 1, 2

Table 1. Incidence of the variants of vWD in our patients.

	Overall		Males		Females		With symptoms	Family history
Type of vWD	n	%	n	%	n	%	%	%
Overall	1885	100	743	39.4	1142	60.6	87.7	56.9
1	1714	91	666	38.9	1048	61.1	87.3	55.1
2A	59	3.1	30	50.8	29	49.2	90.6	78.8
2N	31	1.6	22	70.9	9	29.1	100	73.3
Severe	24	1.3	6	25.0	18	75.0	100	73.7
Low intraplatelet	51	2.7	14	27.5	37	72.5	88.2	71.4
Combined 1+ 2N	6	0.3	5	83.3	1	16.6	100	100

Table 2. Frequency of clinical symptoms in vWD patients.

Clinical symptoms	Overall %	Males %	Females total %	>13 yr %
Ecchymoses-hematomas	50.4	48.7	51.3	51.4
Epistaxis	38.1	41.7	36.9	35.6
Menorrhagia	–	–	–	47.0
Gingival bleeding	26.1	22.8	28.1	29.1
Bleeding after tooth extraction	28.6	25.3	30.6	33.9
Bleeding after surgery	19.5	16.5	21.5	25
Post-partum hemorrhage	–	–	–	12.9
Hemarthrosis	6.3	15.2	0.9	0.4
Hematuria	4.7	8.4	3.1	1.5

and 24 hours after DDAVP infusion. BT was also measured. A good response implied that all abnormal parameters reached normal levels. The response was inadequate if neither FVIII:C nor vWF reached plasma levels required for hemostasis (>50 U/dL) or if neither BT nor PR reached normal values. No response meant that all the parameters remained abnormal or FVIII:C did not reach plasma levels required for hemostasis (>50 U/dL).

Statistical analysis

Normal ranges for FVIII:C, vWF:Ag and vWF:RCo (mean values ± 2 standard deviations) were calculated according to standard proceedures. We performed the mean-test using the EPI 6.04 from the CDC of Atlanta (USA). Comparison of the means of the data was performed using a repeated one-way analysis of variance followed by Student's t-test for unpaired data. Probability values less than $p<0.05$ were considered statistically significant. The chi-squared test was used to compare the different prevalences.

Results

From the total population of 2,339 individuals studied, we diagnosed vWD in 1,885, with a predominance of females (60.6%) The majority of the patients (71.1%) were over 13 years old. In children below 2 years of age, the percentage of males with vWD was higher than that of females (94.5%) ($p=0.00001$) but over 13 years the percentage of females rose to 68.7% ($p=0.00009$). The classification of vWD patients, according to the laboratory tests, is shown in Table 1.

There were 126 patients (6.7%) with only abnormal laboratory findings of vWD without personal bleeding manifestations or a family history. These patients consulted us because of abnormal pre-surgical findings. The prevalences of family and personal symptoms are shown in Table 1. Low values of PR were observed in 80% of our patients, while low levels of FVIII:C were found in only 61.6% (patients with 2N and combined 1+2N variants were not considered); low vWF:Ag and prolonged BT were found in 62.2% and 58.4%, respectively. The distribution of patients according to blood groups was: 70.5% had group 0, 24.8% had group A, 4.1% had group B and 0.6% had group AB.

The frequency of clinical symptoms is shown in Table 2. Ecchymoses-hematomas and epistaxis were the most common clinical symptoms. Considering the age of the patients, menorrhagia was the second most frequent clinical symptom in females over 13 years old. Hemophilia B and vWD was found in 22 patients. Storage pool deficiency was diagnosed in 27 patients, whereas abnormal platelet release reaction was diagnosed in 100, Ehlers Danlos in 6, Noonan syndrome in 4 and Rendu Osler disease in 2 patients.

Patients with type 1 variant

Type 1 vWD was diagnosed in 1,714 patients, with a high prevalence in females (Table 1); 87.3% had bleeding symptoms and 70% had blood group O. Laboratory test results according to blood groups are shown in Table 3. vWF:Ag was significantly lower in group O than in non-O ($p=0.005$). FVIII:C levels were slightly lower in the patients with blood group O ($p=0.08$). There were no differences in vWF:RCo, BT and PR between patients with O and non-O blood groups, although more patients with blood group O had low PR than did patients with a non-O group (83.3% vs 76.9%; $p=0.019$).

Patients with a diagnosis of 2A, 2N, severe, "low intraplatelet" or combined 1+2N variants

Type 2A vWD was diagnosed in 59 patients, with a similar distribution in males and females (Table1). Bleeding symptoms were present in 90.6% of these patients and 76% had blood group O. Prolonged BT and low PR were found in 88.7% and 86.3% of these patients, respectively. Results were: vWF:RCo: 9.4±12.6 U/dL; vWF:Ag: 55.6±26.4 U/dL; FVIII:C: 43.2±18.9 U/dL.

Type 2N vWD was functionally diagnosed in 31 patients, with a high prevalence in males (Table 1). All patients had clinical symptoms and only 59% had group 0 blood. Prolonged BT and low PR were found in 16% and 53.3% of the patients, respectively. Results were: FVIII:C: 19.7±19.4 U/dL; vWF:Ag: 97.13±39.1 U/dL; vWF:RCo: 85.09±31.3 U/dL. The ratio of bound FVIII:C/vWF:Ag was 0.49±0.238.

Table 3. Laboratory tests in patients with type 1 vWD considering the blood groups.

Blood group		vWF:RCo U/dL	vWF:Ag U/dL	FVIII:C U/dL	Pts with prolonged BT	PR patients mean	PR patients % low values
O n: 714	×	38.2	45.7	43.9		13.0	
	SD	11.5	22.3	22.4	46.4%	13.3	83.3%
Non-O n: 288	×	39.0	50.4	46.9		13.2	
	SD	13.7	27.4	30.1	46.1%	14.1	76.9%
p O vs non-O		0.349	0.005	0.080	0.985	0.815	0.019

X: mean; SD: standard deviation; vWF.RCo: ristocetin cofactor activity; vWF:Ag: von Willebrand antigen; BT: bleeding time, the percentage of patients with prolonged values is given; PR: platelet retention, the mean and SD and also the percentage of patients with low values are given.

Table 4. Laboratory tests in patients with and without transfusion requirements.

Patients		vWF:RCo U/dL	vWF:Ag U/dL	FVIII:C U/dL	Pts. with prolonged BT	PR %	Pts. group O
Non-transf.	×	39.3	51.9	49.2		13.8	
(n: 1768)	SD	16.6	29.8	23.2	51.2%	14.4	68.2%
Transfused	×	33.7	48.8	27.8		13.8	
(n: 176)	SD	16.4	31.4	23.6	63.5%	14.0	70.4
p transf. vs. non-transf.		0.0000	0.188	0.0000	0.0152	0.405	0.6525

X: mean; SD: standard deviation; vWF.RCo: ristocetin cofactor activity; vWF:Ag: von Willebrand antigen; BT: bleeding time, the percentage of patients with prolonged values is given; PR: platelet retention, expressed as mean and SD and also as percentage of patients with low values. Transf.: transfused.

Severe vWD was diagnosed in 24 patients, with a high prevalence in females (Table 1). All patients had bleeding symptoms, only 62.5% had blood group O. Prolonged BT and low PR were found in 100% and 95.7%, respectively. Results were: FVIII:C: 22.5±15.1 U/dL; vWF:Ag: 2.5±3.7 U/dL; vWF:RCo: 1.1±0.8 U/dL.

Type *low intraplatelet* with normal plasma vWF variant was diagnosed in 51 patients, with a high prevalence in females (Table 1). Eighty-eight percent of these patients had bleeding symptoms and 58.7% had blood group O. Prolonged BT and low PR were found in 36.2% and 90.2%, respectively. Results were: i-vWF:Ag: 0.068± 0.018 U/10^9 platelets; i-fibrinogen: 52±20 µg/10^9 platelets; FVIII:C: 62.9±21.5 U/dL; vWF:RCo: 76.9±25.5 U/dL; vWF:Ag: 88.2±27.1 U/dL.

Combined 1+2N vWD was diagnosed in 6 patients (5 males); all had bleeding symptoms and 66.6% had blood group O. Prolonged BT and low PR were found in all these patients. Results were: FVIII:C: 10.7±8.6 U/dL; vWF:RCo: 40.28±6.39 U/dL; vWF:Ag: 45.43±10.5 U/dL.

Analysis of patients with increasing number of bleeding sites

We evaluated the percentage of patients according to the number of bleeding sites. There was a high prevalence of females with 3 or more bleeding sites. Nevertheless, the incidence of obstetric-gynecologic bleeding was independent of the number of bleeding sites. The prevalence of blood group O was the same in patients with no bleeding and in those with one or more bleeding sites (67.6% vs 69.9%).

Requirement of replacement therapy

The requirements of replacement therapy were evaluated (those patients with associated hemophilia B were not considered); only 176 (9.3%) patients needed transfusion; 109 patients were males and 67 females (p=0.000). The most frequent hemorrhagic episodes that required replacement therapy were: hemarthrosis (knee: 50; ankle: 30; elbow: 27; shoulder: 7; hip: 6; feet: 6), intramuscular bleeding (thigh: 34; psoas: 14; calf muscles: 13; arm-forearm: 8; buttocks: 7; knee: 5), bleeding after tooth extraction: 30, epistaxis: 32, bleeding after surgery: 22, menorrhagia: 16 and post-partum hemorrhages: 9. The prevalence of blood group O was 70.4% in transfused patients vs 68.2% in those not needing transfusion (p=0.6525). As shown in Table 4, vWF:RCo and FVIII:C were significantly lower in transfused patients. Furthermore, there were more patients with prolonged BT among those requiring transfusions (p=0.0152). Considering each variant individually, the requirements of transfusion therapy in our patients were as follows: 29.1% of patients with severe vWD, 33.3% with combined phenotype 1+2N, 22% of patients with type 2A, 10.1% of patients with type 1 disease; 8.8% of patients with type 2N vWD and 1.9% of those with *low intraplatelet* variant. Factor VIII:C concentrates were used in 104 episodes, cryoprecipitates in 48, plasma in 12, DDAVP in 12 and red blood cells in 45. Twenty-six patients were transfused but the blood component used was not identified. DDAVP had been previously tested in 56 of the patients with transfusional requirements: 31 with good response, 23 with inadequate response and 3 with no response.

Responses to DDAVP infusion

The responses to DDAVP infusion were evaluated in 567 patients. Their ages at the time of receiving the infusion were from 2 to 5 years (9 patients), 6 to 10 years (41 patients), 11 to 20 years (197 patients), 21 to 40 years (230 patients), and older than 41 years (90 patients). A good response was present in 457/567 patients (80.6%); the high prevalence of females within this group was significant (89.8% vs 65.2%; p= 0.000). The response was inadequate in 91/567 patients (16%) and 19/567 patients (3.4%) had no response. A good response was achieved in 84.6% of patients with type 1 vWD; an inadequate response was found in 54.5% and 31.6% of patients with type 2 A and *low*

Table 5. Laboratory tests in non-diagnosed relatives.

Type of vWD in the families		*FVIII:C U/dL*	*pts. with low values*	*Patients with prolonged BT*	*PR mean %*	*% pts. with low values*
1	×	60.7	37.8%	36.5%	23.4	56.3
	SD	30.6			20.4	
2A	×	74.2	22.2%	42.9%	17.4	62.5
	SD	31.4			9.4	
2N	×	44.9	58.3%	44.5%	16.4	66.7%
	SD	40.9			13.7	

X: mean; SD: standard deviation; FVIII:C was expressed as U/dL and the percentage of patients with low levels is also given; BT: bleeding time, the percentage of patients with prolonged values is given; PR: platelet retention, expressed as mean and SD. The percentage of patients with low values is also given.

Table 6. Families with 8 and more members studied, number of subjects affected and symptomatic relatives.

	Type 1		Type 2A	
Number of families	4		4	
Number of members	40		41	
Diagnosed members	12/40	30.0%	36/41	87.8%
with symptoms	5/12	41.6%	30/36	83.3%
Non-diagnosed members	28/40	70%	11/28	39.3%
with symptoms	5/41	12.2%	0/5	0.0%

intraplatelet, respectively. No response occurred in 100% and 66.7% of patients with severe type vWD and 2N, respectively.

Phenotype of affected and unaffected members in families with vWD types 1 and 2A

In order to analyze the phenotype expression of affected and unaffected relatives of our population of vWD patients, we studied a total of 1,188 relatives belonging to 410 families; 736 patients were diagnosed and classified as follows: type 1 vWD: 657 patients; type 2A: 44; type 2N: 27 and type severe: 8. It was not possible to reach a diagnostic classification in the other 452 members; 430 of them belonged to families affected by vWD type 1 and 50.3% were symptomatic; 9 members belonged to families with type 2A vWD and 33% had clinical symptoms; 13 members belonged to type 2N families and 66.7% had symptoms.

We could to study the parents and sisters of only 6 patients with severe vWD; in 2 cases, their mothers were diagnosed as having vWD type 1; in 2 cases both parents were diagnosed as having type 1 vWD; in one case the mother was classified as having type 2A; in another case the sister also had severe vWD and in the 6th case the mother only showed prolonged BT.

In non-diagnosed members, we found that 53% of the subjects with low PR, 61.4% with low FVIII:C and 59.5% with a prolonged BT were symptomatic. Results of laboratory tests of relatives and their distribution according to the type of vWD in the families are shown in Table 5. PR was the most frequently abnormal test in non-diagnosed relatives.

Furthermore, with the purpose of analyzing the phenotype of the disease in members of families with established vWD we selected 8 families (4 with type 1 vWD and 4 with type 2A vWD) in which it was possible to study 8 or more members. The number of members with and without diagnosis in each family, type of vWD in the affected members and clinical symptoms in non-diagnosed members were considered. From a total of 40 members belonging to the type 1 families, vWD was diagnosed in 30% of them; from the 41 members of type 2A families, 87.8% of the members were shown to be affected (Table 6). The frequency of symptomatic members was similar in both groups of families. We found that 39.3% of non-diagnosed members belonging to families with type 1 vWD were symptomatic, whereas none of the non-diagnosed members belonging to families with vWD type 2A was symptomatic.

Discussion

In agreement with other authors,[2,14,30-34] type 1 was the most frequent variant of vWD in our patients. The prevalence of severe type vWD in our series was similar to,[33] higher than,[35] and lower than that reported by other authors.[30,34,36,37] The most frequent symptoms in our population were ecchymoses-hematomas, epistaxis, bleeding after tooth extraction and menorrhagia in females over 13 years. About 56.9% of our patients had a family history; 87.7% of the patients had bleeding symptoms. Only 6.7% had neither a personal history nor a family history of bleeding symptoms. To be considered as having type 1 vWD, the subject should have vWF:RCo below the blood group specific normal range, bleeding symptoms[11] and a family history or a causative mutation.[38] However, these criteria are difficult to fulfill in many patients. It seems appropiate to make a category of *possible* type 1 vWD if only two criteria are present: tests compatible with type 1 vWD and either bleeding history or inheritance.[40] In agreement with Nitu-Whalley,[39] we consider that the low levels of vWF:RCo and personal bleeding history are of prime importance in the diagnosis and treatment of vWD. Asymptomatic patients with low values of vWF:RCo should be regarded with caution, as many of them might not have had a hemostatic challenge to manifest a bleeding tendency. In these cases, when the diagnosis of vWD can neither be confirmed nor excluded and the risk of bleeding is unknown, empirical treatment is recommended.[39,40]

Considering the age of patients at the time of admission, in agreement with other authors[14] we found that

94.5% of the children below 2 years were males; between 3 and 12 years the frequency of males and females were similar but over 13 years, females were more frequent. Since patients with type 1 vWD have minor bleeding episodes, menorrhagia was the reason for the first clinical consultation and the most frequent clinical symptom in young females, as indeed previously reported by other authors.[41,42] The prevalence of females was evident in subjects with type 1, severe and *low intraplatelet* variants. Menstruation, post-partum and obstetric-gynecologic complications explain why more females than males are symptomatic over 13 years of age and makes it essential to search for a diagnosis, including the measurement of intraplatelet vWF when plasma levels are normal. On the other hand, 70.9% of our 2N type population were males. This could be because the vWD 2N phenotype mimics hemophilia A; thus, many cases of mild hemophilia should be re-evaluated.[43]

It has been described that the mean level of vWF in normal subjects with blood group O is lower than that in subjects with non-0 blood groups.[2,13,39,44] The reasons for this are uncertain but it is a source of some ambiguity in diagnosis. Most people with blood group O and two normal vWF alleles have plasma vWF levels below the normal range and clinical symptoms of mild vWD. Some authors suggest that normal ranges of vWF should be defined separately for O and non-O subjects.[2] Other authors suggest that the use of ABO adjusted ranges for vWF levels might not be essential for diagnosis and consider that the bleeding history is of prime importance in clinical diagnosis and treat type 1 vWD patients accordingly.[39] We found lower levels of vWF:Ag in type 1 vWD patients with group 0 compared to in patients with non-0 blood group. Like other authors,[39] we think that patients with low levels of vWF and clinical symptoms must be considered as vWD, independently of the ABO group. Moreover, our patients with group O neither bleed more frequently nor need more transfusions than those with other blood groups at matched vWF levels in plasma. Interestingly, 70.5% of our patients have blood group O whereas the frequency of this group in our normal population is 50.9%.[45] Previous reports on a small sample of type 1 vWD[13,14,39] showed similar results.

It is important to bear in mind that 71.1% of our patients were diagnosed over 13 years old; in spite of being a congenital illness, clinical manifestations of type 1 vWD are not especially evident in childhood.

The requirements of transfusion in our patients were very low (9.3%) compared with those reported by other authors, i.e. only 22.4%[46] and 33%[47] of patients with severe vWD had never been transfused. The transfusion requirements were related not only to the low FVIII:C and vWF:RCo levels (such as in patients with type 2N and combined 1+2N variants) but also to the prolonged BT. It was more frequent in males ($p<0.000$) but this can be overestimated considering that the search for Normandy variant patients is more frequent in suspected hemophiliacs.

When the number of bleeding sites was considered, 33% of the patients had 3 or more sites of hemorrhages, with a higher prevalence of females, independently of obstetric-gynecologic bleeding and transfusion requirements.

In agreement with other authors,[48-50] good response to DDAVP was found in 80.6% of our patients, inadequate response in 16% and no response occurred in the remaining 3.4%. Good response to DDAVP was more frequent in females. To date, DDAVP is the best and safest therapy.

We found a large group of patients with bleeding symptoms, normal levels of plasma vWF:Ag and vWF:RCo but with low intraplatelet vWF:Ag content as described first by Weiss.[17] A defective release or content from cellular compartments of vWF was excluded in these patients by measuring intraplatelet fibrinogen content. The exact role of platelet vWF has not been defined although several studies have indicated that it plays a key role in primary hemostasis[48,51,52] and in the adherence of platelets to the subendothelial surface.[53,54] Normal levels of intraplatelet vWF:RCo are associated with normal BT and decreased clinical bleeding in type 1 vWD[48,55] whereas low levels were associated with a poor response to DDAVP.[48] In agreement with Fressinaud,[54] we found low PR in 90.2% of these patients and DDAVP infusion did not correct this abnormality. The above concepts could explain why 31.6% of these patients have an inadequate response to DDAVP in spite of having normal plasma vWF levels, unlike patients with type 1 variant, in whom only 15% have an inadequate response.

The sensitivity of each of the assays for the diagnosis of vWD and their predictive values in screening patients with hemorrhagic diatheses are at present unknown. It should be borne in mind that 38.4% of patients show normal levels of FVIII:C, therefore, the diagnosis of vWD should not be discarded in patients with clinical symptoms but normal FVIII:C. In patients with a diagnosis of vWD, PR was the most frequently abnormal laboratory assay (besides vWF:RCo). In spite of normal routine screening tests, we consider that vWF:RCo, vWF:Ag, PR and BT should be included among the first level tests for evaluation of subjects with a mild bleeding diathesis. In our experience, PR prompts a diagnosis of vWD. We, thus, recommend its use because it is easy and inexpensive, although difficult to standardize.

Some authors[56] have described a penetrance of 58% in the classic vWD. We observed a similar phenotype expression (30%) in the type 1 variant having a lower incidence of symptomatic members with a diagnosis of vWD, but a higher phenotype expression in type 2A (87.8%).

These results indicate the possibility of diagnosing and treating a substantial group of patients with vWD even in the absence of molecular information, which is not always available in the most frequent type of vWD, the type 1 variant.

Contributions and Acknowledgments

AIW planned the study, collected the clinical and laboratory data, reviewed the literature and wrote the manuscript. SSM was the clinician responsible for referring patients to our Department and their clinical diagnosis and management. She also contributed to the formulation of the study. AIW, ANB, MJS, CEF and ACK performed the phenotypic analysis and laboratory assays. MAL supervised the entire study and revised the final version of the manuscript. All authors contributed equally to the discussion and interpretation of results and approved the final version of the paper.

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Potential implications for clinical practice

Personal bleeding history and low vWf:RCo are of prime importance in the diagnosis and treatment of vWD. Asymptomatic subjects with low vWf:RCo should be regarded with caution, since many of them might not have had a hemostatic challenge sufficient to evidence the bleeding tendency. When the diagnosis of vWD can neither be confirmed nor excluded, the risk of bleeding is unknown. Patients with low vWF and clinical symptoms must be considered as having vWD, independently of the ABO group. In spite of normal levels of FVIII:C, when patients have clinical symptoms, the diagnosis of vWD should not be discarded.

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