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. © 2001, Ferrata Storti Foundation

Key words: vWD variants; DDAVP; symptoms; laboratory assays
plasma vWF variant 17,18 was defined as occurring in vWF:RCo below 10 U/dL. As occurring in those patients with vWF:Ag and binding capacity/vWF:Ag ratio. Severe vWD was defined disproportionately low levels of FVIII:C and low FVIII:C with proportionately low vWF:Ag and vWF:RCo but with low. Combined 1+2N cases were diagnosed in patients el and the FVIII:C binding capacity/vWF:Ag ratio were Normandy (2N) variant was suspected when FVIII:C lev-

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 lev-

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 × g for 15 min. Platelet-poor plasma was transferred to another tube and centrifuged at 3,000 × 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 Ivy21,22 or Mielke21 method (with a commercial device: Simplex 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 method22 (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 elsewhere25 (normal value: 50-150 U/dL). vWF:RCo was measured by the modified method of Mackerlane26 (normal value: 50-150 U/dL). The multimeric pattern of vWF was analyzed as described elsewhere.21 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.18,22 i-vWF:Ag (normal value: 0.1-0.4 U/109 platelets) and i-fibrinogen (normal value: 30-90 µg/109 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-

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
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 procedures. 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 and 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*=0.005). FVIII:C levels were slightly 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 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 (Table 1). 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±±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 O 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: 97.1±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.
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 2a 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: vWF:RCo: 16.4±7.2 U/dL; vWF:Ag: 88.2±27.1 U/dL; FVIII:C: 22.5±15.1 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:Ag: 40.28±6.39 U/dL; vWF:RCo: 40.28±6.39 U/dL; FVIII:C: 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
in a family were diagnosed as having type 1 vWD; in one case 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 families of members 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.

### Table 5. Laboratory tests in non-diagnosed relatives.

<table>
<thead>
<tr>
<th>Type of vWD in the families</th>
<th>FVIII:C</th>
<th>Patients with low values</th>
<th>PR</th>
<th>% pts. with low values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60.7</td>
<td>37.8%</td>
<td>23.4</td>
<td>56.3</td>
</tr>
<tr>
<td>2A</td>
<td>74.2</td>
<td>22.2%</td>
<td>17.4</td>
<td>62.5</td>
</tr>
<tr>
<td>2N</td>
<td>44.9</td>
<td>58.3%</td>
<td>16.4</td>
<td>66.7%</td>
</tr>
</tbody>
</table>

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.

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

Discussion

In agreement with other authors, type 1 was the most frequent variant of vWD in our patients. The prevalence of severe type vWD in our series was similar to, higher than, and lower than that reported by other authors. 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 and a family history or a causative mutation. However, these criteria are difficult to fulfill in many patients. It seems appropriate 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.

In agreement with Nitu-Whalley, 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.

Considering the age of patients at the time of admission, in agreement with other authors, 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. 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 type 1 population were males. This could be because the vWD 2N phenotype mimics hemophilia A; thus, many cases of mild hemophilia should be re-evaluated.

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-O blood groups. 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. 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. We found lower levels of vWF:Ag in type 1 vWD patients with group O compared to in patients with non-O blood group. Like other authors, 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%. Previous reports on a small sample of type 1 vWD 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. Some authors 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%).

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, 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. 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 and in the adherence of platelets to the subendothelial surface. Normal levels of intraplatelet vWF:RCo are associated with normal BT and decreased clinical bleeding in type 1 vWD whereas low levels were associated with a poor response to DDAVP. In agreement with Fressinard, 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 PT 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 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 WDV 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, MJ S, 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.

References


