VALIDATION OF THE DIAGNOSTIC CRITERIA OF TYPE 1 VON WILLEBRAND DISEASE: AN INTERNATIONAL MULTICENTER STUDY

STUDY OUTLINE AND INSTRUCTIONS

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A study fostered by the SSC Subcommittee on von Willebrand factor of the International Society on Thrombosis and Hemostasis

SUMMARY

1. OVERVIEW OF THE STUDY

1.1 OUTLINE OF THE STUDY

During the SSC meeting in Barcelona (1996), a consensus was reached on some guidelines for the diagnosis of type 1 von Willebrand disease (vWd). Most experts agreed that these guidelines should be evaluated in a patient population. In particular, bleeding history was recognized as a critical point in the definition of the spectrum of the disease. The following project is aimed at formally evaluating the discriminant power of history and laboratory measurements in diagnosis of type 1 vWd.

1.2 BACKGROUND

Von Willebrand disease (vWd) is the most prevalent inherited hemorrhagic disorder, with an estimated prevalence of 1 case every 100 subjects among Caucasians. Although the prevalence of clinically important vWd may be lower, vWd should be always suspected whenever a patient reports a history of hemorrhagic symptoms.

Most cases of vWd, probably up to 90%, are represented by type 1. Although the general definition of type 1 vWd is clear, consisting in a mild to moderate bleeding disorder, transmitted in autosomal dominant fashion, with quantitatively reduced vWf:Ag and proportionally decreased dependent or related activities (vWf:RCof; VIII:C), the actual diagnosis in the individual patient is often troublesome.

A first difficulty pertains to the laboratory diagnosis. Most of these cases respond to DDAVP, showing that stored vWf is quantitatively and functionally normal and that it can be released after adequate stimulation. Conceivably, many factors like hormonal status, physical stress, smoke, age, inflammation, surgery, pregnancy and puerperium greatly influence the actual circulating level of vWf. ABO blood group also exerts a significant effect. As a consequence, up to 30 to 40% of “cases” may be either non-penetrant or show a variably low expressivity, even within families with established type 1 disease. Laboratory phenotype may

vary from severely reduced to nearly or completely normal values and fluctuate over time.

The genetic basis for classical dominant type 1 vWd has been elucidated in a very few families. In some cases with apparent recessive type 1, compound heterozygosity has been identified. Furthermore, locus heterogeneity cannot be excluded and mutations outside the vWf locus have been demonstrated in animal models.

The identification of a mutation does not imply that the mutation itself is responsible for the clinical situation; another unidentified intragenic mutation may be actually more important in compound heterozygotes. Furthermore, extragenic mutation (i.e., at a different locus) could exert an effect. Haplotype assignment using intragenic polymorphisms might help in the identification of the family members carrying the mutated allele, but it has a variable rate of success in tracing the affected allele. Thus, although genotype analysis may be important in identifying carriers within families, it is less useful in explaining symptoms and should not be incautiously used as the only evidence for prophylaxis (e.g., before surgery).

Finally, history taking, criteria for establishing personal and family bleeding diathesis and laboratory measurements are subjected to investigator or laboratory dependent variability (measurement bias) thus further increasing the heterogeneity of type 1 vWd phenotype.

In summary, no definite diagnostic criteria exist to diagnose vWd. The diagnosis of vWd is a difficult one because is compounded by a number of issues:

- Difficulty in identifying subjects with a *clear-cut, definite hemorrhagic history.* This is fundamental, because history is the first step upon which all subsequent actions should be taken;
- Difficulty in the *interpretation of laboratory results*, since there is wide overlap between normal and affected subjects for phenotype-based tests (e.g., FVIII:C, vWf:Ag, vWf:RCof, bleeding time);
- Even using genotype analysis, it is clear that *some mutations may be silent* in some affected, raising the suspicion that a genetic heterogeneity may be present within the same family so that screening of the entire gene in all family members is required;
- In a great proportion of cases *a complete family study should be obtained*, which is not always feasible for a variety of reasons.

Despite the above-mentioned difficulties, it is important to diagnose vWd with confidence, since this diagnosis has several implications for the patient. In particular, a precise diagnosis may be required for:

- diagnosis and treatment of a hemorrhagic patient (e.g., a bleeding patient after surgery)
- prophylaxis in subjects with a personal or familial history of bleeding
- counseling in “bleeding” families or in relatives of vWd patients
- legal/insurance issues.

Over-diagnosis of von Willebrand's disease may be nonetheless undesirable since it is unduly alarming to the subject, it may negatively influence the subject's lifestyle and impair his/her social role and may result in unnecessary and possibly harmful treatment.

1.3 AIM OF THE STUDY

The aim of this retrospective study is to

- Standardize *clinical and laboratory assessment* in order to minimize the measurement biases
- Evaluate the discriminant power (sensitivity and specificity) of the diagnostic procedures (either history or laboratory alone or their combination), not only to validate criteria or standards for the *positive diagnosis of vWd*, but, perhaps equally important, to validate criteria for the *exclusion of vWd*
- Produce a descriptive picture of this bleeding disorder in symptomatic families
- Form the basis for future prospective studies (e.g., genetic diagnosis)

1.4 METHODOLOGY

The current study addresses the problem of vWd diagnosis by comparing obligatory carriers in families with type 1 vWd, as diagnosed by specialized Centers, with normal controls. Carriers of type 3 vWd are also included for separate analysis.

1.4.1 OBLIGATORY CARRIERS FROM VWD TYPE 1 FAMILIES.

Families to be studied are selected from Centers scattered all over the world and diagnosed to suffer from vWd by experienced clinicians. This approach has been chosen to avoid as much as possible a circular reasoning: to validate diagnostic criteria, one should have a gold standard for diagnosis and for vWd this could only be a time-honoured diagnosis (i.e., families whose symptoms, laboratory values, inheritance, clinical response to treatment are consistent with what experts define as vWd, see Appendix 1). We suggest that obligatory carriers be studied instead of probands to avoid a selection bias. Cases with possible type 1 vWd are not useful for the identification of obligatory carriers. As an example, since a proband is always diagnosed as vWd also on the basis of abnormal vWf level, the presence of an abnormal vWf phenotype may be falsely considered as necessary and sufficient condition for vWd diagnosis. By contrast, an obligatory carrier may have or not clinical symptoms or laboratory abnormalities; hence he/she is well suited for the analysis and interpretation of the predictive value of clinical and laboratory data. Obligatory carriers are selected as outlined in the subsequent section.

1.4.2 OBLIGATORY CARRIERS FROM VWD TYPE 3 FAMILIES.

Obligatory carriers of type 1 vWd are heterozygous for at least a single mutation in the vWf gene (or in an as yet unidentified extragenic site). It is well known that vWd type 1 frequently is caused by frameshift, nonsense mutations or deletions that overlap with those identified in type 3 vWd. Possession of a single mutant allele does not consistently cause bleeding symptoms, suggesting a possible compound heterozygosity in most symptomatic cases. Type 3 vWd is a (usually) recessive disorder in which vWf antigen is virtually

undetectable due to mutations in both maternal and paternal alleles. Parents or offspring of patients with type 3 vWd (obligatory carriers by definition) may be phenotypically normal or may have bleeding symptoms and meet criteria of type 1 vWd. Accordingly, a clear distinction between type 1 and type 3 carriers can not be invariably made and for these reasons a study comparing bleeding history and laboratory values in obligatory carriers of type 3 vWd with obligatory carriers of type 1 and with normal subjects is appropriate. A description of bleeding history of both type 3 patients and type 3 obligatory carriers could also be useful.

In addition to families with clear autosomal dominant vWd or clear recessive type 3 with very low vWf level in the propositus, other type 1 families have a less clear inheritance pattern at the phenotypic level and resemble more to type 3 with no or only mildly affected transmitters of the disease. In these families, an obligatory carrier is not identifiable with a reasonable certainty at a phenotypic level and thus these kindreds should be excluded from the study, since they lack an older and younger affected member. This unavoidable exclusion of some vWd families (unless full genotyping is performed in all suspected vWd families, which is clearly impossible at the moment) should not undermine the study. In fact, obligatory carriers as here identified carry at least one mutated allele and are not selected on the basis of their symptoms. Thus, we believe that they constitute a suitable learning set of patients upon whose symptoms and laboratory measurements minimal criteria for discrimination from normal people can be set. This means that both patients with one or two mutated alleles will be hopefully caught.

1.4.3 CONTROL SUBJECTS.

As control group, this study uses normal subjects randomly selected from the general population. Controls are age and sex matched with obligatory carriers, since age and sex may result in a different exposure to hemorrhagic challenges (e.g., in young adults and in children bleeding tendency could not have had time to become as manifest as in older subjects). There are two types of controls: clinical and laboratory controls.

Clinical controls are chosen to identify a “predictive” clinical history. These subjects are selected from the general population. From the same pool of subjects, laboratory controls are chosen to identify “predictive” laboratory values of tests. These subjects should have a

normal hemorrhagic history (that is, no significant history of hemorrhage). Thus, they may be used to compare their laboratory values with those of obligatory carriers of vWd.

1.5 DATA ANALYSIS

Sources of bias. The study is designed to minimize the selection bias, since the learning set of patients is composed by symptomatic and asymptomatic carriers and not only by symptomatic probands. Nevertheless, other potential sources of selection bias may be the inclusion in the learning set of very symptomatic families only (that is, families with a high penetrance) or incomplete family studies (that is, the analysis of the pedigree is limited only to most symptomatic siblings). To avoid this selection bias within pedigrees, each Center should possibly evaluate all families with a vWd diagnosis, irrespective of the penetrance of vWd within the family; moreover, each family should be evaluated thoroughly. In order to avoid over-representation of very large symptomatic families, only one obligatory carrier should be included for each family. It is the responsibility of each Center to select only one obligatory carrier within each family, using random selection criteria. Another possible bias is a measurement bias, due to differences in data collection between different Centers or to different data collection between obligatory carriers and controls. At this regard, since the researcher could not be blinded as to the carrier status, he/she should be absolutely fair about the data collection.

Sensitivity, specificity and predictive value of history. Sensitivity and specificity may be established for specific questions by comparing obligatory carriers and control subjects. After a preliminary analysis, questions may be combined into sets of questions to test the hypothesis of an improvement of sensitivity or specificity. It is important to remember, however, that the "true" positive and negative predictive value of each question or set of questions will greatly depend on the prevalence of vWd on the sample on which the history taking will be applied in practice. Hence, predictive values will be approximated for different clinical applications (e.g., screening in a general setting or screening of hemorrhagic patients) based on a presumptive prevalence.

Sample size. Hemorrhagic manifestations are rather commonly reported even by normal subjects. Hence, assumptions on sample size vary depending on the type of question. It is expected that for highly discriminant questions (e.g., profuse bleeding after tooth extraction) a significant difference in answer (β= 0.8, α=0.05) may be detected with 30 obligatory carriers and 30 controls. A minimum of 100 obligatory carriers may be necessary to detect differences in poorly discriminant questions. As described in the Appendix, in this study trivial bleeding symptoms are not considered and therefore a limited enrollment of carriers should not undermine the power of the ultimate analysis.

Statistical methods. The data analysis will be performed in two successive steps. First, the specificity and sensitivity will be computed for single questions. Subsequently, the most discriminating combinations of questions will be formed using two different approaches. The first, a parametric one, will use ROC curves determined from multiple logistic regression; the second method will be represented by the non-parametric approach given by the CART (Classification and Regression Trees) methodology. Both methods will be implemented using different *a priori* probabilities, to account for different clinical settings with varying vWd prevalence, as mentioned before.

1.6 FUTURE STUDIES

This investigation could offer, with an additional small effort, the opportunity for future useful studies aimed at clarifying the genetics of type 1 and type 3 vWd and the relationship between genotype and phenotype. For these studies, DNA samples should be voluntarely collected together with plasma samples from the obligatory carriers, affected family members and their mates and first degree relatives by those researchers willing to participate in genetic studies (see section 1.6).

Another future area of investigation will address the problem of the validation of diagnostic criteria in young (pediatric) patients.

2. OPERATIVE PROCEDURES

2.1 DEFINITION OF CARRIERS AND CONTROLS

2.1.1 DEFINITION OF TYPE 1 VWD OBLIGATORY CARRIERS

Obligatory carriers are identified on the basis of family trees. In the first example family (Family A), the father represents the *"older affected"* and the offspring the *"younger affected"*. Both are identified as affected from vWd on the basis of the presence of both the following criteria in each one:

- levels of vWf below the reference range;
- presence of at least two hemorrhagic symptoms.

These criteria comply with the definition of vWd given by the SSC on vWf at the Barcelona meeting (1996). Given this family tree, the carrier (arrow) is considered an obligatory one, whatever may be his clinical or laboratory situation (symptomatic/asymptomatic; vWf deficient/vWf normal).

In the second pedigree (Family B), sister and offspring of the carrier (arrow) are the older and younger vWd affected according to the above mentioned SSC criteria. Even though the carrier is not an obligatory one from a strict point of view, we believe that enough evidence is provided by the pedigree to consider him as such, since two first-degree relatives in two generations are affected. In order to avoid a possible misclassification, one should also demonstrate a normal vWf level in the mate of the carrier.

In summary, a subject is considered as an obligatory carrier when he/she accomplishes both the following criteria:

- be the father/mother of an affected child;
- having at least one first degree relative (either father/mother or brother/sister) affected.

The study design allows the inclusion of families with type 1 subjects only when vWd is transmitted in an autosomal dominant way within families. Autosomal recessive type 3 families in which parents have no symptoms or doubtful symptoms are considered in a separate paragraph (see section 2.1.3).

In some families, a possible hemorrhagic history may be present also in the mate of the carrier or in both of his/her parents, thus confounding a clear mendelian autosomal dominant transmission. In this case, a brief description of symptoms should be given in the

appropriate space of the Data Collection Forms for Type 1 family, section *Family Data and Pedigree*.

2.1.2 TYPE 1 VWD FAMILIES WITH A SPECIFIC MUTATION

An additional criterion that may be used to select obligatory carriers relies on the identification of a specific mutation. Suppose that in the second pedigree (Family B) a specific mutation is identified in both the older and younger affected subjects, also presenting symptoms and reduced vWf levels. Then, if the same specific mutation is present in any first-degree relative of the older affected he/she may be considered obligatory carrier and may be included in the study. It is important to stress that for the purpose of this study and to avoid a comparison bias with the former approach, this genetic criteria may be applied only in the presence of at least two subjects in the family complying with the clinical definition of vWd (symptoms and reduced vWf), both presenting the same mutation. This approach would yield a greater number of carriers.

2.1.3 DEFINITION OF TYPE 3 VWD OBLIGATORY CARRIERS

Operative definition of type 3 patients. Any symptomatic patient (moderately to severely affected) with undetectable (less than 3 IU/dl) vWF:Ag level. Intentionally, this definition does not require a recessive transmission of symptoms (i.e., bleeding history in parents is not considered for inclusion), but it implicitly assumes that the propositus having such a great deficiency of vWf should be double heterozygous or homozygous for a defect affecting the quantitative expression of vWF gene.

Definition of type 3 vWd obligatory carriers. In the first example (see figure *Example Family C*), the parents are considered obligatory carriers. In the second example (see figure *Example Family D*), the offspring of the propositus are considered obligatory carriers as well as the parents of the propositus. For the purpose of the study, select preferably the first situation (see figure *Example Family C*) in which the parents are adults and can provide an informative history as to bleeding symptoms. The second situation (Family D) is less frequent and informative due to the young age of the offspring of the type 3 patient in most Centers. Of course, if the parents of the propositus are available, they will be selected as obligatory

carriers.

2.1.4 DEFINITION OF CONTROLS

Suitable controls are age and sex-matched subjects in an ostensible good health and considering themselves as normal. In practice, every Center can chose the controls (two for each obligatory carrier in type 1 families and 1 for each carrier in type 3 families) among staff personnel, friends of patients, blood donors, subjects referred for minor problems or for check-up not related to hematological problems or their friends or, preferably, from census list. The only limitations are:

1? Controls should be in an ostensible good health and not taking (at least on a regular basis) drugs affecting hemostasis;

2? They should answer negatively to this simple question: Have you ever been referred to a specialized Center for bleeding? Having been referred to a general physician for bleeding should not cause exclusion.

In such controls, reporting of hemorrhagic symptoms will prompt a laboratory investigation for vWd or other hemostatic disorders. The results should be reported to the coordinating Center, but these controls should not be excluded from analysis.

2.1.5 PEDIATRIC CASES

The pedigree approach is of course not suitable for inclusion of carriers of pediatric age. However, a useful information for pediatric cases could be derived from the descriptive histories of the affected members. Of course, pediatric cases can be used to identify obligatory carriers. The molecular approach would clearly allow a more extensive inclusion of pediatric cases.

Future studies should be encouraged to specifically address the validation of diagnostic criteria in young patients.

2.2 GUIDELINES FOR HISTORY TAKING

Personal and family histories are an essential component in the diagnosis of von Willebrand disease since they establish the presence or absence of a bleeding diathesis and its severity. These clinical features are largely independent from the results of laboratory tests. To be valuable, history should not be a simple list of symptoms as spontaneously described by the patient or his/her relatives. On the contrary, it should be the result of a careful medical interview conducted by an expert physician posing critical questions to the patients. Occurrence, frequency, severity and other inherent characteristic of every bleeding episode should be fully investigated. However, absence of bleeding in circumstances in which it could be expected is as well as important as its presence for the purpose of establishing a bleeding diathesis. These circumstances should also be fully investigated.

For the purpose of this study it is essential that every symptom or its absence should be firmly established and thoroughly described. Thus, the questionnaire used in this study represents only a simple guide, aiming at standardizing the art of history taking. It is not intended as a mean to exonerate the investigator to exert his/her own criticism in interpreting the patient's description by simply filling in the questionnaire based on patient's rough answers. The investigator should also appreciate the perception of the symptoms by the patient.

It is clearly impossible to report in detail every symptom, e.g. every episode of epistaxis or menorrhagia. Thus, necessarily the investigator should try to offer the most accurate and significant overall picture by describing the average episode and its frequency. Moreover, the most severe episode(s) and its frequency should be reported separately, as suggested by the questionnaire.

A similar approach is required to collect the history from control subjects.

It is mandatory that obligatory carriers, affected relatives and controls should be interviewed directly unless clinical records of exceeding quality are available (which is usually not the case). In any case, clinical records should be reviewed to check the results of the interview especially for episodes occurred in the past and to control the consistency of patient's answers. Many normal healthy subjects consider their bleeding and bruising

excessive. For example, Miller et al (Blood 54,137,1979) found that 23% of normal subjects had “positive” bleeding histories. Similarly, Wahlberg et al (Methods Inform Med 19, 194, 1980) using a self-administered questionnaire in normal subjects showed that as many as 57% reported nose bleeding, 52% bleeding from the gums and 22% a tendency to bruises. Using more conservative criteria, however, a familiar bleeding diathesis could not be found in more than 5% of normal subjects (Rodeghiero et al, Blood 69,454,1987)

It is important to distinguish between real symptoms in a clinical sense and episodes of trivial importance simply reported by overzealous subjects. To this purpose and in order to assure standardisation, we will offer a descriptive threshold cut-off below which a specific bleeding episode does not reach the level of a "symptom" and should not be reported in the questionnaire but only marked as "trivial" in the appropriate box.

Epistaxis: Any nosebleed, especially if not occurring during pre-puberal age only, which could not be managed by the patient him/herself OR longer than 5 minutes OR requiring medical attention. Very frequent and disturbing bleedings (at least one every week) could be recorded even if not meeting the above criteria in the "Note" space of the appropriate box of the questionnaire.

Bruising/ Hematoma: Any spontaneous bruise/hematoma larger than 3 centimetres or considered disproportionate to trauma by the investigator

Petechiae: No further requirements

Minor cutaneous wound: Any prolonged bleeding, longer than 5 minutes, caused by superficial cuts (e.g. by razor, knife or scissors)

Gum bleeding: Any spontaneous bleeding lasting for a minute or longer causing frankly bloody sputum or any profuse bleeding after tooth brushing

Tooth eruption: Any bleeding requiring assistance or supervision by a physician

Bites to lips, cheek and tongue: Any bleeding longer than 5 minutes or causing a swollen tongue or mouth

Hematemesis, melena and hematochezia: No further requirement

Tooth extraction: Any bleeding occurring after leaving the dentist’s office or a prolonged bleeding at the dentist’s office causing a delay in the procedure

Surgical bleeding: Any bleeding stated as abnormally prolonged by the surgeon or causing a delay in discharge or requiring some supportive treatment

Menorrhagia: See the questionnaire

Post-partum hemorrhage: As for surgery

For every symptom try to fill in the questionnaire summarizing it on the basis of the average presentation that is the most frequent one and describe if appropriate its most severe presentation

2.3 BLOOD SAMPLING

2.3.1 AIM OF BLOOD SAMPLING

Blood samples are collected to:

- obtain plasma specimens for evaluating sensitivity and specificity of established or new laboratory tests. This is mandatory for all participating Centers.
- obtain DNA samples for future genetic studies (see section 1.6), aimed at evaluating a possible role of molecular diagnosis. DNA samples should be collected only by those researchers interested in genetic or haplotyping studies.

2.3.2 FAMILY MEMBERS TO SAMPLE IN TYPE 1 FAMILIES

It is mandatory that plasma should be obtained from the carrier and the two affected family members used to identify the carrier. If a significant bleeding is present in the mate of the obligatory carriers, he/she should also be sampled as well (see section 2.1.1).

Facultatively, researchers interested in future genetic studies are invited to collect plasma and DNA samples from all the relatives and mates depicted in Families A and B. In general, for type 1 vWd first-degree relatives and mate of obligatory carriers should be evaluated.

2.3.3 FAMILY MEMBERS TO SAMPLE IN TYPE 3 FAMILIES

It is mandatory to obtain plasma from the affected (homozygous) propositus and the parents (obligatory carriers) in the example Family C. In example Family D, the affected (homozygous) propositus, the spouse if symptomatic and the offspring (obligatory carrier(s)) should be sampled.

Facultatively, plasma and DNA should be collected from all the subjects depicted in the example families C and D.

2.3.4 SAMPLING FROM CONTROL SUBJECTS

Plasma from the two control subjects for each family should be obtained. It is not required to keep DNA samples of those control subjects.

2.3.5 INFORMED CONSENT

Patients and control subjects should be fully informed of the study protocol and procedures according to local regulations. Anonymity of patients and controls is assured throughout the study, by coding of the subjects. Approval from the local Ethical Committee should be obtained whenever required, by the participating center.

2.3.6 BLOOD SAMPLING PROCEDURES

The following steps are suggested for patients, carriers and control subjects.

1? The subject should be resting for at least 30 minutes before venipuncture

2? Sampling should be delayed if there is evidence of strenuous exercise before sampling or fever (body temperature greater than 38 °C) in the last three days before sampling

3? For female subjects, please record the day of menstrual cycle, information on pregnancy status or type of estroprogestin-containing pills taken by the subject

4? Blood should be collected into 1:10 trisodium citrate (3.8 % or 3.2%; please specify) and centrifuged within two hours from venipuncture for 20' at 2,000 g. Platelet-poor plasma should be immediately snap-frozen in dry ice-acetone or liquid nitrogen and stored at -80 °C. Store at least five 1 ml aliquots of plasma for shipping in tightly screwed vials. Label each vial according to the codes used to collect the questionnaire (Center code plus Family code plus Subject code). Use A1, A2, A3, etc. as Subject code to identify the **A**dditional family members

5? For those wishing to collect DNA, leukocyte genomic DNA can be isolated according to the procedure currently implemented by the laboratory. Alternatively, RBCs and white cells buffy coat remaining in the tube after centrifugation for plasma can be immediately put a - 30 °C.

2.3.7 SAMPLES CENTRALIZATION, STORAGE AND MEASUREMENTS

Plasma samples necessary for full phenotype characterization should be sent to the coordinating laboratory (Hematology Dept., S. Bortolo Hospital, I-36100, Vicenza, Italy), via express delivery, according to the instructions that will be sent to each Center separately. A minimum of two aliquots of plasma should be provided to the coordinating center for each subject. The coordinating center will cover the shipping costs.

At the coordinating laboratory, the following measurements will be performed:

- ABO (reverse) typing
- vWf:Ag, vWf:Rcof, FVIII:C measurement.

Results will be expressed with reference to International Standard; a quality-control process of storage, shipment and assay will be established. Results of plasma testing will be made immediately available to each participating Center.

We encourage all Centers to send also the remaining plasma aliquots and the DNA samples (these latter collected on a voluntary basis). This would allow a better management of future activities, with the understanding that the material will be used only after written consent of the sending Center (see next paragraph). For those Centers that can not obtain permission to send outside biological material of patients from their Ethical Committee, we recommend that they store the remaining plasma aliquots and/or DNA within their lab, possibly at -80° C (plasma) and -30° C (DNA) for five years.

2.3.8 POLICY FOR SAMPLES AVAILABILITY

Plasma and DNA aliquots may be made available for scientific research purposes to all researchers participating in the present study, after specific, written arrangements between members of the Steering Committee. Similarly, any proposal for further specific study from any participating Center should be approved by the Steering Committee. It is mandatory that any further plasma testing or DNA analysis shall not be performed or the samples made available by the organizing Center to anyone without the written permission of each participating Center.

2.4 STUDY FLOW-CHART

Your Center, participating to the study, is essentially required to:

1? Read this document carefully

2? Identify the appropriate vWd type 1 and type 3 families from your Center patient files, according to the criteria reported in Appendix 1. Please note that families with propositi with severe or mild symptoms are similarly important. Code each family with a sequential two-digit number (e.g., family 01, 02, 03, …)

3? Draw a family tree, using the Pedigree section of the Data Collection Forms, extending in type 1 families to all the first-degree relatives of the obligatory carrier chosen for the study; include also always the mate of the obligatory carrier. If the mate of the carrier has any bleeding symptom, please describe briefly the symptoms in the appropriate space of the Data Collection Forms, Type 1 family. Please note that general frameworks for type 1 and type 3 vWd family trees are provided with the Pedigree section of the Data Collection Forms.

4? Identify with an arrow in the Pedigree the obligatory carrier(s) that will be evaluated in each family. Choose only one carrier for each type 1 family. It is the responsibility of each Center to select only one obligatory carrier within each type 1 family, using random selection criteria, when more than one may be present within a family. For type 3 families, identify in the Pedigree the parents of the affected as FIRST and SECOND carrier, if the family fits the Example Family C, or randomly choose and identify a maximum of 2 obligatory carriers (as FIRST and SECOND) in the offspring , if the family fits the Example Family D; see *Definition of Carriers 2.1.1, 2.1.2 and 2.1.3;* see also *Data analysis*, 1.5 for a review of potential biases

5? Select two control subjects matched to each type 1 carrier or to first and second type 3 carrier (see *Definition of Controls, section 2.1.4)*

6? Obtain informed consent from both carriers and controls

7? Answer to the general part of the Data Collection Forms (white sheets) according to the data already present in your clinical files

8? Collect by interview for each family the complete bleeding history from the obligatory carrier and from the two affected members for type 1 families or from the affected and the two carriers for type 3 families (thus, collect the bleeding history from no more than three members for each family). Collect the bleeding history also from two controls, according to the above-mentioned criteria (see *Guidelines for History Taking, section 2.2*). The investigators should collect directly the bleeding history from: 1) the family members on which carriership was established (*older* and *younger affected members* in type 1 and the *affected* or *propositus* in type 3); 2) the *obligatory carrier(s); 3)* the two *control subjects*. For instance for Example Family A collect directly the history of father, obligatory carrier and offspring (son); for Example Family B, collect the history of sister, obligatory carrier and offspring (daughter); for Example Family C, collect the history of the parents and of the propositus; for Example Family D, collect the history of propositus (father), mother and offspring (carrier). Complete bleeding history of other family members is not required.

9? Bleeding history is split *before* and *after* diagnosis for each subject. The date of diagnosis is based on the time of the first visit of the affected or carrier at a specialized center independently from the time on which a specific diagnosis was established.

10? Give information regarding laboratory measurements (if available) in obligatory carrier(s) and in family member(s) on which carriership was established as present in the patient's file. No new test is required.

11? Collect plasma samples in family members as indicated in the example figures A, B, C, D and in controls (see *Blood Sampling, section 2.3). Facultative sampling* of plasma and DNA is encouraged for those researchers willing to participate in future genetic studies (see 2.3.6). Label the samples as indicated in the last page of the Data Collection Forms.

12? Ship completed data collection forms to the coordinating center.

13? Ship collected specimen after specific arrangements with the coordinating center.

Upon arrival of clinical data and plasma samples, the coordinating center will proceed to:

1? Store the clinical data on a computer database

2? Store plasma samples in a biologic bank at -80 °C; samples may be distributed according to the policy stated in section 2.3.8

3? Perform specific assays (FVIII:C, vWf:Rco; vWf:Ag, ABO reverse)

4? Data analysis (see section 1.5)

5? Manuscript preparation, together with the members of the Steering Committee

2.5 PUBLICATION POLICY

The preliminary draft of the manuscript is prepared by the Study Coordinator and submitted to the members of the Steering Committee. The manuscript will report: description of symptoms in carriers and controls; analysis of clinical and laboratory discriminating features; establishment of minimal criteria for further investigation or diagnosis of suspected vWd patients.

2.6 WEB SITE

Additional informations/updates on this Study Protocol, as well as a copy in Word 6.0 format, may be obtained at the following Internet address:

http://www.hemato.ven.it/research/vwf/index.htm

E-mail concerning the study protocol may be sent to *rodeghiero@hemato.ven.it*

3. FIGURES

APPENDIX: CONSENSUS CRITERIA FOR DIAGNOSIS OF VWD TYPE 1

Modified from the original approved in Barcelona; use these criteria to select your families.

Possible type 1 vWd patients are not eligible for the study.

Significant mucocutaneous bleeding symptoms:

1? Nose bleeding, ≥ 2 episodes without a history of trauma not stopped by short compression of <10 min., or ≥1 episode requiring blood transfusion
2? Cutaneous hemorrhage and bruisability with minimal or no apparent trauma, as a presenting symptom or requiring medical treatment
3? Prolonged bleeding from trivial wounds, lasting ≥ 15 min. or recurring spontaneously during the 7 days after wounding
4? Oral cavity bleeding that requires medical attention, such as gingival bleeding, or bleeding with tooth eruption or bites to lips and tongue
5? Spontaneous gastrointestinal bleeding requiring medical attention, or resulting in acute or chronic anemia, unexplained by ulceration or portal hypertension
6? Heavy, prolonged, or recurrent bleeding after tooth extraction or other oral surgery such as tonsillectomy and adenoidectomy, requiring medical attention
7? Menorrhagia resulting in acute or chronic anemia, or requiring medical treatment, not associated with structural lesions of the uterus
8? Bleeding from other skin or mucous membrane surfaces requiring medical treatment (e.g., eye, ear, respiratory tract, genitourinary tract other than uterus).

Criteria for bleeding symptoms - A significant mucocutaneous bleeding history requires that at least two symptoms in the absence of a blood transfusion history, or one symptom requiring treatment with blood transfusion, or one symptom recurring on at least three distinct occasions

Criteria for family history - A positive family history compatible with vWd type 1 requires that at least one first degree relative, or at least two second degree relatives, have a personal history of significant mucocutaneous bleeding and laboratory tests compatible with vWd type 1 (discussed below). When available, the use of vWf mutations or genetic markers linked to the vWf locus may permit the analysis of more remote relatives, and may allow asymptomatic relatives with low vWf levels to provide evidence for inheritance.

Laboratory tests in suspected vWd:

Screening Tests - complete blood count with differential and platelet count, PT, aPTT, vWf:RCo, vWf:Ag

Screening or Confirmatory Tests - factor VIII level, ABO blood type

Confirmatory Tests - RIPA, vWf multimers

Criteria for laboratory tests - Laboratory tests results are compatible with vWd type 1 if the levels of both vWf:RCo and vWf:Ag are < 2 SD below the population mean and ABO type adjusted mean on ≥ 2 determinations. If the tests are performed, RIPA must not indicate abnormal sensitivity to low concentrations of ristocetin, and the plasma vWf multimer distribution must be normal

CRITERIA FOR CATEGORY vWd TYPE 1

vWd type 1: vWd type 1 is an inherited bleeding disorder due to quantitative deficiency of vWf. The diagnosis therefore is based upon criteria for symptoms, vWf deficiency, and inheritance, all of which must be satisfied. These include: significant mucocutaneous bleeding, laboratory tests compatible with vWd type 1, and either a positive family history for vWd type 1 or an appropriate vWf mutation.