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# The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study

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**Summary.** *Objective:* The aim of this study was the validation of the criteria defining a significant mucocutaneous-bleeding history in type 1 von Willebrand disease (VWD). *Subjects and methods:* To avoid selection bias, 42 obligatory carriers (OC) of type 1 VWD were identified from a panel of 42 families with type 1 VWD enrolled by 10 expert centers. OC were identified by the presence of an offspring and another first degree relative with type 1 VWD (affected subjects, AFF). A standardized questionnaire was administered to evaluate hemorrhagic symptoms at the time of first examination, using a bleeding score ranging from 0 (no symptom) to 3 (hospitalization, replacement therapy, blood transfusion). Sensitivity, specificity, diagnostic likelihood ratios, positive and negative predictive values for the diagnosis of type 1 VWD were calculated from the data collected in OC and in 215 controls. *Results:* Having at least three hemorrhagic symptoms or a bleeding score of 3 in males and 5 in females was very specific (98.6%) for the bleeding history of type 1 VWD, although less sensitive (69.1%). None of the misclassified OC had life-threatening bleeding episodes after diagnosis. *Conclusions:* We suggest that the use of a standardized questionnaire and bleeding score may be useful for the identification of subjects requiring laboratory evaluation for VWD.

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## Introduction

von Willebrand disease (VWD) is the most frequent inherited bleeding disorder and should be always considered in patients with a bleeding diathesis without an apparent cause [1,2]. Although VWD is known for several decades, evidence-based criteria are not available for the diagnosis of its most frequent phenotype called type 1. This phenotype represents 80–90% of cases registered at specialized centers with a reported prevalence in general population up to 1% [3]. Type 1 VWD is caused by a partial quantitative deficiency of von Willebrand factor (VWF). In type 1 VWD, all the multimeric forms of VWF are present albeit in a reduced concentration and there is a concomitant decrease of VWF antigen (VWF:Ag) and VWF ristocetin cofactor activity (VWF:RCo). In contrast, type 2 VWD is characterized by a qualitative defect of the VWF molecule, manifested by disproportionally reduced VWF:RCo/Ag ratio, often accompanied by an abnormal multimeric pattern, or by a reduced affinity of the VWF molecule with FVIII. The genetic transmission is generally co-dominant or dominant [4]. In rare instances, VWD is transmitted in a recessive manner with an almost complete VWF deficiency in homozygous patients; this phenotype, called type 3, is usually easily identified [5].

The diagnosis of VWD type 1 in the individual patient rests on the demonstration of the presence of hemorrhagic symptoms, reduced VWF level without the evidence of qualitative defect and autosomal inheritance [6]. The latter two criteria are usually easily defined, as inheritance is assumed when at least another family member shares a similar phenotype with the propositus and a quantitative deficiency is defined as a VWF level below the ABO blood group adjusted reference range [7]. In contrast, criteria defining a significant bleeding history have been suggested [6], but never validated as to their sensitivity and specificity for the diagnosis of VWD. There are at least two reasons explaining the difficulty in defining what should be considered as 'a significant bleeding history'. Firstly, type 1 VWD shows a highly variable heterogeneity of clinical (and laboratory) features, possibly because of additional genetic mechanisms [8] and resulting in a considerable overlap of hemorrhagic symptoms in affected patients and healthy controls [9–12]. In particular, at least one bleeding symptom is considered to be present in up to 25% of normal subjects [13]. Secondly, a major uncertainty exists as to set useful criteria for a clinically meaningful diagnosis of type 1 VWD [2]. For instance, even applying seemingly conservative criteria (at least two family members each with two hemorrhagic symptoms and reduced VWF level), up to 1% of the normal population can be diagnosed as affected by type 1 VWD [3]. However, most of these families will never seek medical assistance in the future, and often do not show consistent linkage with the VWF locus [14]. Clearly, these criteria cannot be applied in a clinical setting and there is a critical need to define better, more stringent criteria, possibly resulting in a more specific and clinically useful diagnosis.

In this paper, we aimed at establishing minimal clinical criteria for the identification of subjects affected by VWD, in a learning set of families, provided by a panel of international expert centers with a historically validated diagnosis of type 1 VWD. Using a standardized questionnaire, hemorrhagic symptoms were evaluated in obligatory carriers (OC) of the disease to avoid the selection bias resulting from more symptomatic cases and compared with those observed in age- and sex-matched normal controls to calculate the predictive value of the proposed diagnostic tool.

## Patients and methods

### *Selection of participating centers*

Participation to the International Study was offered to internationally recognized centers among the members of the International Society on Thrombosis and Haemostasis, within the framework of the Scientific and Standardization Subcommittee on VWF. All the centers that responded were accepted. The study included separate enrolment and analysis of types 1 and 3 families, although this paper is limited to the families with type 1 VWD.

### *Selection of families*

The study protocol required that the participating centers evaluated all their families with a diagnosis of type 1 VWD who were actively followed and that all families with a family structure suitable for analysis of OCs were considered eligible, irrespective of the penetrance of VWD within the family or the severity of symptoms in the index case. Centers were required to include only historically validated, unrelated families.

### *Definition of type 1 VWD status*

A subject was considered as an OC if she/he had at least an affected offspring (younger affected) and at least another affected first degree relative (either father/mother or brother/sister, older affected). All these subjects should have been living and available for direct history taking at the time of enrolment. Family diagnosis and classification of members as affected by type 1 VWD was not centrally validated but left to the discretion of the referring center. Only one OC for family was allowed to be included.

### *Definition of controls*

Each participating center enrolled two normal controls, matched by age and gender with OC (either type 1 or type 3 VWD OC). Controls were eligible if in ostensible good health and if they had never been referred for hemostasis evaluation because of hemorrhagic symptoms. Laboratory screening for VWD was not required in controls, who were considered *a priori* as not carrying VWD. In the present study, we analyzed the data from all available controls to maximize sample size, as controls for both types 1 or 3 had a similar age and sex distribution. Informed consent was obtained from all participating subjects (family members and controls).

### *Questionnaire for bleeding history and bleeding score*

The same questionnaire was used for OC and controls, recording symptoms occurring before diagnosis or before enrolment, respectively. The questionnaire was administered by a doctor, who was aware of the diagnosis of the patient but unaware of his/her previous hemorrhagic history. For OC, hemorrhagic symptoms occurring after diagnosis were also collected. Instructions for history taking offered a descriptive threshold below which a specific symptom should have been reported as trivial. The average and the most severe presentation of each symptom were required. Specific symptoms included epistaxis, cutaneous symptoms, bleeding from minor wounds, oral cavity bleeding, gastrointestinal bleeding, postpartum hemorrhage, muscle hematomas/hemartrosis, bleeding after tooth extraction, bleeding after surgery and menorrhagia. The severity of each symptom was subsequently summarized, using a bleeding score system ranging from 0 to 3 (see Table 1 for details) and considering for each symptom the most severe

**Table 1** Bleeding score used in the study

| Symptoms | Assigned score |
|---|---|
| Epistaxis | 0 = no or trivial<br>1 = present<br>2 = packing, cauterization<br>3 = transfusion, replacement |
| Cutaneous symptoms | 0 = no or trivial<br>1 = petechiae or bruises<br>2 = hematomas<br>3 = medical consultation |
| Minor wounds | 0 = no or trivial<br>1 = present (1–5 episodes year$^{-1}$)<br>2 = medical attention<br>3 = surgery/blood transfusion |
| Oral cavity bleeding | 0 = no or trivial<br>1 = present<br>2 = medical attention<br>3 = surgery/blood transfusion |
| Gastrointestinal bleeding | 0 = no or trivial<br>1 = present<br>2 = medical attention<br>3 = surgery/blood transfusion |
| Postpartum hemorrhage | 0 = no or trivial<br>1 = present, iron therapy<br>2 = blood transfusion, dilatation-curettage, suturing<br>3 = hysterectomy |
| Muscle hematomas or hemarthrosis | 0 = no or trivial<br>1 = present<br>2 = medical attention<br>3 = transfusion, intervention |
| Tooth extraction (most severe episode) | 0 = no or trivial<br>1 = present<br>2 = suturing or packing<br>3 = transfusion |
| Surgery (most severe episode) | 0 = no or trivial<br>1 = present<br>2 = suturing or resurgery<br>3 = transfusion |
| Menorrhagia | 0 = no or trivial<br>1 = present<br>2 = consultation, pill use, iron therapy<br>3 = transfusion, hysterectomy, dilatation-curettage, replacement therapy |

occurrence. The questionnaire is available at http://www.med.vnc.edu/isth/ssc/collaboration/bleeding_type1_vwd.pdf

### *FVIII and VWF measurement*

Factor VIII procoagulant activity (FVIII:C) and VWF were measured at each participating center, with results expressed as IU dL$^{-1}$. All centers used a one-stage aPTT-clotting assay for FVIII:C, VWF:RCo using formalin-fixed platelets and aggregometry, ELISA using polyclonal antibodies for VWF:Ag. The median value of available data was included for analysis.

### *Statistical methods*

Based on the previous data on the prevalence of hemorrhagic symptoms in VWD patients and normal controls [1], we expected that a significant difference in the distribution of hemorrhagic symptoms would be observed by enrolling at least 30 OC and 30 controls ($\beta = 0.8$, $\alpha = 0.05$). A 1:2 ratio between OC and controls was, however, chosen by a protocol to maximize the statistical power. The data analysis was performed in two successive steps. Firstly, diagnostic likelihood ratio (DLR) for the presence (DLR+) or absence (DLR−) of a symptom were computed according to Pepe [15] as a measure of the informativeness of the symptom (i.e. how much the presence or absence of the symptom increments or decrements the likelihood of VWD). The higher the DLR+, the greater the likelihood of VWD for each symptom. Secondly, we tried to identify the best diagnostic approach using different criteria (see Results) and computing sensitivity, specificity, positive and negative predictive values (PPV, NPV) for each criteria. PPV and NPV were computed based on a VWD prevalence of 1%. Given this prevalence, we could not exclude that some controls could also be carrier of VWD, but this chance would affect our estimates in a conservative way (i.e. decrease the specificity of bleeding history). The STATA software package was used for all calculations [16]. Classification and regression trees (CART) analysis was also used to identify the most useful combination of symptoms identifying type 1 VWD OC [17]. The advantage of this computer-based non-parametric technique lies in its ability to automatically analyze all possible combinations of binary data (i.e. presence/absence of a symptom) in order to find the best splitting rules (i.e. the best diagnostic algorithm). Another major advantage of the CART model is that it does not require any assumption of independence, statistical distribution or analysis of interaction between the independent variables (symptoms) used to explain the dependent variable (VWD OC) [17]. CART analysis was carried out using the CART for Windows statistical package version 2.5 (Salford Systems, CA, USA).

To avoid a possible selection bias, all computations were performed by comparing symptoms and bleeding score only in OC vs. normal controls, without considering older and younger affected.

## Results

Ten centers participated to the study, enrolling a total of 42 type 1 VWD families fulfilling the study protocol. The characteristics of the study population are reported in Table 2. A total of 341 subjects were available for analysis: 42 OC (M/F: 18/24, median age: 47 years), 84 affected (M/F: 34/50, median age: 38 years) and 215 controls (M/F: 90/125, mean age: 49 years). FVIII/VWF plasma levels were very similar between OC and affected relatives, confirming a true autosomal dominant inheritance within the families. As matching of OC and controls was at time of enrolment, whereas hemorrhagic symptoms were analyzed until diagnosis in OC, OC had a lower chance of developing hemorrhagic symptoms during their life than controls (median age at diagnosis in OC 27 years, median age at enrolment in controls 49 years). However, we

**Table 2** Main characteristics of the study population

| | Control subjects ($n = 215$) | Type 1 obligatory carriers ($n = 42$) | Type 1 affected ($n = 84$) |
|---|---|---|---|
| Gender (M/F) | 90/125 | 18/24 | 34/50 |
| Age at diagnosis, median | – | 27 | 25 |
| Age at study, median | 49 | 47 | 38 |
| FVIII:C (U $dL^{-1}$), mean ± SD | – | 44.4 ± 25.4 | 41.7 ± 22.7 |
| VWF:Ag (U $dL^{-1}$), mean ± SD | – | 26.8 ± 17.7 | 26.6 ± 20.7 |
| VWF:RCo (U $dL^{-1}$), mean ± SD | – | 18.9 ± 18.2 | 19 ± 20.3 |

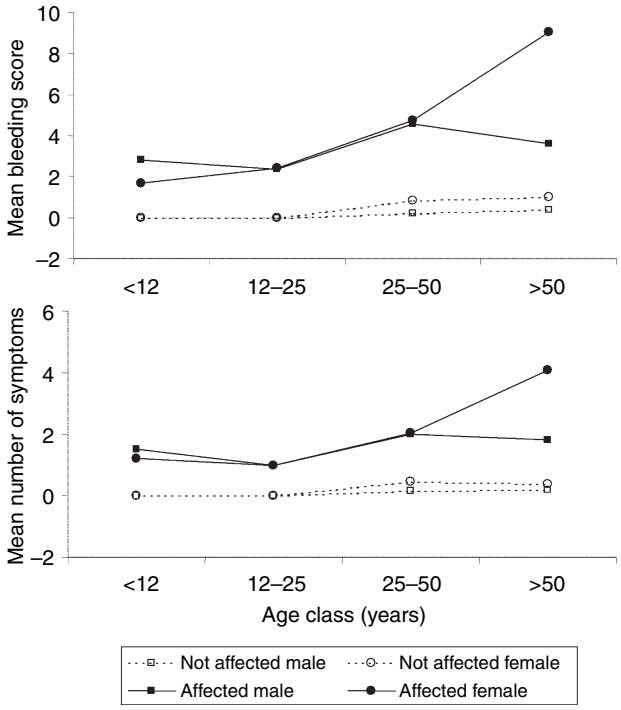


**Fig. 1.** Influence of age in the referral of hemorrhagic symptoms. Elder affected (either OC or affected family members) report an increased number of hemorrhagic symptoms and an higher bleeding score.

did not adjust for this differential exposure in successive computations as it would influence our results in a conservative way by actually lowering the discriminant power of the bleeding history.

Age, gender and affection status independently influenced the reported hemorrhagic symptoms from both a qualitative (mean number of hemorrhagic symptoms) and quantitative (mean score level) aspect ($P < 0.001$ for all variables, $\chi^2$and $F$ statistics, see Fig. 1). The median familial bleeding score (i.e. the median of bleeding score computed in OC, older and younger affected of the same family) was similar among the studied families, with 34/42 (80.9%) of the families showing a median bleeding score between 4 and 8.

Hemorrhagic symptoms were analyzed using a qualitative (presence/absence of a specific hemorrhagic symptom), a quantitative (bleeding score) assessment or with a 'mixed model', using both the preceding analyses.

**Table 3** Number of hemorrhagic symptoms according to the different categories of study subjects

| No of symptoms | Control subjects (%) | Type 1 obligatory carriers (%) | Type 1 affected (%) |
|---|---|---|---|
| 0 | 165 (76.7) | 5 (11.9) | 10 (11.9) |
| 1 | 33 (15.4) | 5 (11.9) | 23 (27.3) |
| 2 | 16 (7.4) | 11 (26.2) | 16 (19.1) |
| 3 | 1 (0.5) | 7 (16.7) | 19 (22.6) |
| 4 | – | 7 (16.7) | 8 (9.5) |
| 5 | – | 6 (14.2) | 7 (8.3) |
| 6 | – | 1 (2.4) | 1 (1.2) |

### *Results of qualitative analysis*

From 215 normal controls, only one female (0.46%) reported three hemorrhagic symptoms, whereas two symptoms were reported by 7.4% of the remaining normal subjects (Table 3). About 77% of normal subjects had never suffered from bleeding, in comparison with 11.9% of both OC (5/42) and affected (10/84). Absence of symptoms in 10 affected members, mostly of young age, was because of a negative history at first examination. Symptoms mostly associated with OC of VWD (in terms of higher DLR+) were bleeding after surgery and cutaneous bleeding (Table 4). For a cut-off of three or more hemorrhagic symptoms, the sensitivity and specificity for the diagnosis of OC were 50.0 and 99.5 percent, respectively (Table 5). Had a cut-off of two or more hemorrhagic symptoms been chosen, the sensitivity and specificity for the diagnosis of OC would have been 76.1 and 92.1 percent, respectively.

### *Results of quantitative analysis*

The score for each hemorrhagic symptom is reported in Table 4. For each hemorrhagic symptom, a clear-cut difference in the distribution of the score was present between controls and both affected and OC. No clear differences were apparent between the affected and OC. In normal controls, a score below or equal 3 for males and 5 for females corresponded to the 98.8th and 99.2th percentile, respectively. When these gender-adjusted reference limits were used, the sensitivity and specificity of the score for the diagnosis of OC was 64.3 and 99.1 percent, respectively (Table 5). Again, had a less-restrictive cut-off been chosen (a score higher than 2 in males and females),

**Table 4** Diagnostic values of hemorrhagic symptoms. For each symptom, the distribution of score values and the diagnostic likelihood ratio (positive and negative) are reported. The $\chi^2$ *P* tests the null hypothesis that the score distribution is similar among the OC and affected (older and younger) and between OC and controls

| | Score | | | | $\chi^2 P$ | | DLR | |
|---|---|---|---|---|---|---|---|---|
| Symptoms | 0 | 1 | 2 | 3 | OC vs. controls | OC vs. affected | DLR + | DLR− |
| Menorrhagia | | | | | | | | |
| Obligatory carriers | 5 | 4 | 7 | 5 | < 0.001 | 0.897 | 4.3 | 0.35 |
| Affected | 11 | 6 | 9 | 7 | | | | |
| Controls | 101 | 3 | 9 | 8 | | | | |
| Surgery | | | | | | | | |
| Obligatory carriers | 11 | 3 | 6 | 3 | < 0.001 | 0.100 | 19.1 | 0.58 |
| Affected | 26 | 0 | 9 | 7 | | | | |
| Controls | 170 | 2 | 1 | 1 | | | | |
| Tooth extraction | | | | | | | | |
| Obligatory carriers | 7 | 5 | 16 | 0 | < 0.001 | 0.670 | 13.2 | 0.29 |
| Affected | 13 | 8 | 24 | 1 | | | | |
| Controls | 171 | 3 | 7 | 0 | | | | |
| Epistaxis | | | | | | | | |
| Obligatory carriers | 17 | 14 | 11 | 0 | < 0.001 | 0.113 | 5.1 | 0.51 |
| Affected | 38 | 32 | 9 | 3 | | | | |
| Controls | 192 | 20 | 3 | 0 | | | | |
| Cutaneous bleeding | | | | | | | | |
| Obligatory carriers | 11 | 20 | 9 | 2 | < 0.001 | 0.844 | 19.1 | 0.32 |
| Affected | 25 | 35 | 19 | 2 | | | | |
| Controls | 207 | 8 | 0 | 0 | | | | |
| Wounds | | | | | | | | |
| Obligatory carriers | 21 | 17 | 2 | 2 | < 0.001 | 0.968 | 10.2 | 0.51 |
| Affected | 45 | 31 | 3 | 5 | | | | |
| Controls | 205 | 9 | 0 | 1 | | | | |
| Postpartum | | | | | | | | |
| Obligatory carriers | 7 | 3 | 3 | 1 | < 0.001 | 0.608 | 13.3 | 0.43 |
| Affected | 9 | 6 | 3 | 5 | | | | |
| Controls | 102 | 1 | 2 | 0 | | | | |

**Table 5** Comparison of different clinical criteria for the diagnosis of VWD. For five different criteria (see text for details), the sensitivity, specificity, positive and negative predictive values (PPV and NPV) and positive and negative diagnostic likelihood ratios (DLR + and DLR−) are reported. VWD prevalence is assumed to be 1%

| Criteria | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | DLR + | DLR− |
|---|---|---|---|---|---|---|
| 1. Qualitative (more than two symptoms) | 50.0 | 99.5 | 52.1 | 99.5 | 100.0 | 0.5 |
| 2. Quantitative (score > 3 or 5 in males and female) | 64.2 | 99.1 | 41.1 | 99.6 | 71.4 | 0.4 |
| 3. Mixed: criteria 1 and 2 | 45.2 | 100 | 100 | 99.5 | ∞ | 0.6 |
| 4. Mixed: criteria 1 or 2 | 69.1 | 98.6 | 33.3 | 99.6 | 49.3 | 0.3 |
| 5. CART model | 80.1 | 91.6 | 8.9 | 99.8 | 9.5 | 0.2 |

the sensitivity and specificity for the diagnosis of OC would have been 78.5 and 86.9 percent, respectively.

### *Results of mixed analysis*

Two models were considered. In the first, a subject was considered a possible VWD if at least two hemorrhagic symptoms were present totalling a score > 3 or 5 in males and females, respectively. In the second, a subject was considered a possible VWD if either two hemorrhagic symptoms were present or if a score > 3 or 5 in males and females, respectively, was totalled. Sensitivity and specificity for these two models are reported in Table 5.

### *Results of CART analysis*

This analysis was focused only on the five most powerful symptoms as evaluated in univariate analysis (Table 4) to compensate for the relatively small sample size of OC. The best predictive model is reported in Fig. 2. For both males and females, referral of cutaneous bleeding (ecchimoses or bruises) was the best predictor of type 1 VWD among spontaneous symptoms. In those who underwent tooth extraction or surgery, postextraction bleeding was the best predictor followed again by cutaneous symptoms. This approach showed a PPV of 8.9% and a NPV of 99.8% (Table 5).

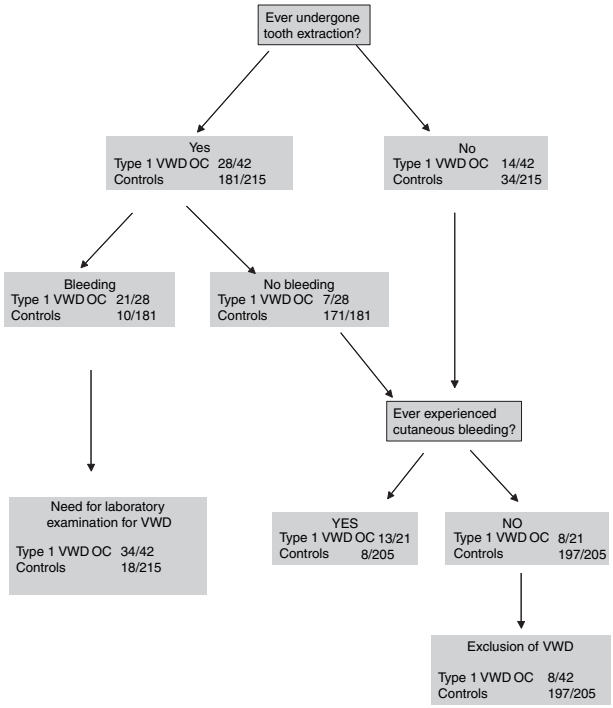


**Fig. 2.** Flow chart of a possible algorithm for the identification of type 1 VWD obligatory carriers, as suggested by CART analysis of the enrolled set of patients.

### *Bleeding events in misclassified subjects*

Table 6 reports the bleeding events occurred after diagnosis in the 15 OC that were not identified by the clinical criteria 4 or 5 of Table 5. Only 1/15 not identified OC (6.6%) developed haemorrhages that required plasma transfusion. Five additional subjects could potentially have benefited from desmopressin prophylaxis had they been diagnosed, but their bleeding was anyway controlled with standard procedures (e.g. suturing).

## Discussion

The minimal criteria defining a clear-cut hemorrhagic history in inherited bleeding disorder have never been formally validated. The main aim of this study was to establish a threshold identifying a significant bleeding history in OC of type 1 VWD. To this purpose, we investigated hemorrhagic symptoms before diagnosis in 42 OC belonging to families with a well-established, historically validated, diagnosis of type 1 VWD and we compared them with those of normal controls, to validate criteria both for the diagnosis and the exclusion of VWD. Various strategies were adopted to minimize unavoidable selection biases. Firstly, for each family both symptomatic and asymptomatic OC and not only symptomatic probands were included. Secondly, to avoid preferential inclusion of very symptomatic families, the study protocol required all participating centers to evaluate all families with a VWD diagnosis in active follow-up and with a family structure suitable for analysis of OC, irrespective of the penetrance of VWD within the family. Thirdly, to avoid over-representation of very large symptomatic families, only one OC was included for each family. Finally, a multicenter study was preferred to allow for different criteria of identification of patients.

Very few hemorrhagic symptoms were found in the 215 age- and sex-matched normal controls with only 8% of normal subjects reporting two or more minor hemorrhagic symptoms, never requiring medical attention. It is likely that the use of a detailed, standardized questionnaire administered and reviewed by a physician was able to reduce the proportion of normal subjects reporting hemorrhagic symptoms. By comparison, previous studies reported a frequency of hemorrhagic symptoms in normal population ranging from two to as high as 51%, most of the symptoms being indeed trivial [18–20]. For most available studies, figures concerning severity and the number and combination of different symptoms in an individual subject are lacking.

The panel of families recruited into the study showed consistently low FVIII/VWF levels in OC and affected family members, confirming the dominant inheritance of disease, as required by the inclusion criteria. FVIII:C/VWF ratios were slightly increased, thus suggesting a 'true' genetic defect in these families [21]. In these families, the bleeding tendency was also strikingly similar, with a similar median familial bleeding score. Females showed the more severe pattern, in keeping with the occurrence of menstruation and parturition in their adult age [22]. The number of symptoms and bleeding score severity was similar between OC and affected members, but significantly higher in comparison with the normal subjects (Tables 3 and 4).

The main result of this study is that having at least three hemorrhagic symptoms, irrespective of their severity, represents a minimal criterion useful to define a bleeding history distinguishing VWD OC from normal subjects. This criterion results in a very good specificity (99.5%) and an intermediate sensitivity (50%). Taking into account both the severity and the number of symptoms in a semi-quantitative bleeding score, a higher sensitivity (64.2%) was observed without a substantial reduction of specificity (99.1%). The combinations of the qualitative and quantitative criteria resulted in an increase of specificity (100%) when both criteria were required, or in an increase of sensitivity (69.1%) when at least one was needed (Table 5). Cutaneous bleeding and surgical bleeding showed the highest probability of being associated with VWD (positive DLR, 19.0 for both symptoms) while conversely absence of bleeding after tooth extraction made the diagnosis of VWD very unlikely (negative DLR, 0.29). Indeed, a CART model shows that reporting of cutaneous symptoms for both males and females was the best predictor of type 1 VWD among spontaneous symptoms, while postextraction bleeding was the best predictor among OC who underwent tooth extraction (Fig. 2), with an overall good sensitivity (80.1%) but a low specificity (91.6%) (Table 5).

Distribution of bleeding being discrete, there is an unavoidable wide difference in sensitivity and specificity of the different possible combinations of symptoms and their severity and

**Table 6** Hemorrhagic symptoms in OC not identified by criteria 4 and 5 of Table 5

| Subject | Not identified by criteria# | Referred symptoms | |
|---|---|---|---|
| | | Before diagnosis | After diagnosis |
| 1 | 4 | Spontaneous cutaneous symptoms not requiring medical attention | Spontaneous cutaneous symptoms not requiring medical attention |
| 2 | 4, 5 | Minor epistaxis; bleeding from gums after brushing | Minor epistaxis; bleeding from gums after brushing; melena |
| 3 | 4, 5 | Bleeding from minor wounds | Spontaneous cutaneous symptoms not requiring medical attention; bleeding from minor wounds; bleeding from gums after brushing; hematochezia |
| 4 | 4 | Minor epistaxis, spontaneous cutaneous symptoms; bleeding from minor wounds; bleeding from gums after brushing | **Bleeding after tooth extraction requiring antifibrinolytics**; spontaneous cutaneous symptoms; bleeding from minor wounds; bleeding from gums after brushing |
| 5 | 5 | Minor epistaxis, spontaneous bleeding from gums; bleeding after tonsillectomy requiring resuturing | Minor epistaxis, spontaneous bleeding from gums requiring antifibrinolytics, **bleeding after tooth extraction requiring resuturing; bleeding after nose surgery requiring antifibrinolytics** |
| 6 | 4 | Spontaneous cutaneous symptoms, bleeding from gums after brushing, menorrhagia | Spontaneous cutaneous symptoms; bleeding from gums after brushing; menorrhagia requiring medical attention |
| 7 | 4 | Minor epistaxis, spontaneous cutaneous symptoms not requiring medical attention, bleeding from gums after brushing | **Melena requiring replacement therapy**; menorrhagia requiring medical attention; **bleeding after delivery requiring plasma transfusion**; spontaneous cutaneous symptoms |
| 8 | 5 | Minor epistaxis, bleeding after surgery, bleeding after delivery; menorrhagia | Bleeding after delivery; menorrhagia |
| 9 | 4, 5 | Bleeding from minor wounds, bleeding from gums after brushing | **Bleeding from minor wounds requiring surgical hemostasis**; bleeding from gums after brushing |
| 10 | 4 | Spontaneous cutaneous symptoms; bleeding from minor wounds requiring surgical hemostasis | Spontaneous cutaneous symptoms, bleeding from minor wounds; **bleeding after tooth extraction requiring resuturing** |
| 11 | 4 | Spontaneous cutaneous symptoms requiring medical consultation 25 episodes year$^{-1}$ | Spontaneous cutaneous symptoms, bleeding from minor wounds; Menorrhagia |
| 12 | 4 | Bleeding from minor wounds during $>10$ min. 1–5 episodes year$^{-1}$; bleeding after tooth extraction | Bleeding from gums after brushing; hematemesis, melena |
| 13 | 4, 5 | None | None |
| 14 | 4, 5 | None | None |
| 15 | 4, 5 | None | **Bleeding after tooth extraction requiring resuturing**; menorrhagia requiring medical attention |

Clinically relevant symptoms are reported in bold.

consequently these parameters cannot be more finely tuned by a receiver–operator curve (ROC) analysis. As shown in Table 5, we, however, favored combinations with sufficiently high specificity (above 98%) to avoid useless screening of healthy population. The sex-adjusted criteria discussed in the present study appear to be more conservative than the provisional ones [6]. In these latter criteria, a bleeding symptom requiring blood transfusion (equivalent to a score of 3 in the present study) or the presence of just two symptoms is in fact the requirements to define a bleeding tendency.

The choice of a specific criterion among those presented in Table 5 remains, however, open and dependent on the clinical setting. Based on our findings, we would speculate that if a screening study is undertaken (e.g. a population based survey) then very stringent criteria would be advisable (e.g. criterion No. 3 in Table 5). In contrast, more sensitive criteria may be preferred for family studies (e.g. criterion No. 4 or 5 in Table 5). A simple criterion may be adopted (e.g. criterion No. 1 of Table 5) for the use in non-specialized clinics, such as general practitioners. Finally, the adoption of less specific, but more sensitive criteria may be useful whenever more specific laboratory tests will become widely available.

The present study has some limitations. Firstly, we analyzed the data from OC belonging to VWD families cared for by specialized centers. These subjects may be more symptomatic or may refer bleeding symptoms more accurately than other less selected type 1 VWD patients. Secondly, the safety of this proposed approach to VWD diagnosis has not been prospectively validated, and therefore its usefulness as a screening tool remains uncertain. Prospective studies with a follow-up of subjects diagnosed with the proposed criteria are therefore needed before advising a wider clinical application [23]. At this regard, however, results of Table 6 could reassure the clinician about the partial sensitivity of the proposed criteria, as missing a potential diagnosis in less symptomatic OC may be devoid of major consequences. In contrast, the adoption of less stringent criteria is the most likely to produce irrelevant diagnoses, unduly labeling some subjects with a genetic stigma. At this

regard, the criteria proposed by the current study are more restrictive that those of the recently proposed provisional criteria [6]. Finally, as only five OC were < 14 years of age, the proposed criteria may not be as useful in the pediatric setting. Further study will be required to determine the usefulness of the proposed criteria in the younger children and maybe the present criteria need to be modified for young children.

In conclusion, we developed a semi-quantitative tool based on a standardized questionnaire for the identification of a significant bleeding history requiring further laboratory investigations for a definite diagnosis of type 1 VWD. This approach could be more generally applicable to the investigation of other inherited bleeding disorders with variable expressivity (e.g. mild platelet function disorders) to avoid the bias of subjective investigator evaluation of hemorrhagic symptoms and to reduce the need for expensive laboratory investigations.

## Contributions

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[1]Study initiation and coordination; [2]Data collection; [3]Analysis and interpretation of results; [4]Statistical analysis; [5]Lead authors of initial manuscript; [6]Revisions of draft manuscripts; and [7]Review and approval of the final manuscript.

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