Determinants of plasma levels of von Willebrand factor and coagulation factor VIII

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Chapter 3

Von Willebrand Factor and its Propeptide: The Influence of Secretion and Clearance on Protein Levels and the Risk of Venous Thrombosis

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Summary

Background and Objectives. Elevated levels of Factor VIII (FVIII) are associated with an increased risk of thrombosis. FVIII levels are determined mainly by von Willebrand Factor (VWF). We investigated the contribution of secretion and clearance rates to elevated VWF antigen (VWF:Ag) and to thrombosis risk. VWF is secreted in equimolar amounts with its propeptide which has a shorter half-life. VWF propeptide can be used as a measure of VWF secretion and allows estimation of VWF half-life.

Methods and Results. We have measured VWF propeptide, VWF:Ag, FVIII:Ag and FVIII activity (FVIII:C) in the Leiden Thrombophilia Study. In controls high VWF propeptide was associated with high VWF:Ag, FVIII:Ag and FVIII:C. In contrast to mature VWF:Ag, VWF propeptide was not influenced by blood groups. Using an ELISA based assay we showed that the VWF propeptide lacks ABO antigens. Levels were higher in men and increased with age. Long VWF half-life was also associated with high VWF:Ag, FVIII:Ag and FVIII:C. VWF half-life was influenced by blood group (10hrs in O versus 12hrs in non-O individuals), but not by sex and only slightly by age. VWF propeptide was higher in thrombosis patients than in controls. VWF half-life was similar in patients and controls (11.4 and 11.1 hrs, respectively).

Conclusions. Both secretion and clearance rates are important determinants of VWF and FVIII levels. However, mainly high VWF and FVIII levels caused by increased secretion seem associated with thrombosis. ABO blood group influences clearance rates of VWF rather than VWF secretion rates.

Introduction

Several studies have shown that elevated plasma levels of von Willebrand factor (VWF) and factor VIII (FVIII) are important risk factors for venous thrombosis[1-7]. The risk of thrombosis seems affected mainly by FVIII, but because VWF is the main determinant of FVIII levels, understanding the regulation of VWF levels is important.

VWF is a glycoprotein that circulates in plasma as large multimers[8]. The protein functions as an adhesion molecule to bind platelets at sites of vascular damage.

Furthermore, VWF is the carrier protein of FVIII, which is a cofactor in secondary hemostasis. Binding of FVIII to VWF protects it from degradation in or premature clearance from the circulation. Plasma levels of FVIII depend strongly on levels of VWF. Therefore, cellular secretion as well as clearance rates of VWF may be important determinants of FVIII levels.
Levels of VWF and consequently levels of FVIII strongly depend on ABO blood group[9]. Individuals with non-O blood groups generally have higher levels of VWF and FVIII than individuals with blood group O. VWF carries ABO blood group antigens[10]. It is not exactly known how the presence of ABO blood group antigens influences the lifecycle of VWF. Blood group is believed to influence the clearance rate of VWF from the circulation, but may also play a role in the secretion of VWF[11-13].
VWF is synthesized by endothelial cells and megakaryocytes. The primary translation product undergoes a number of post-translational modifications, including glycosylation, multimerization and endoproteolytic cleavage of a propeptide. Both mature, highly polymerized VWF and the propeptide are targeted to Weibel-Palade bodies (WPb), endothelial cell-specific storage organelles. The contents of WPb are released into the circulation only after exposure of the endothelium to specific stimuli[8,13-16]. In vitro studies have shown that partially processed, functionally incompetent VWF is released in a constitutive manner. The physiological significance of this pathway is poorly understood, however[8,14].
Upon stimulation mature VWF and propeptide are released in equimolar amounts[8,17]. Once in the blood, mature VWF and its propeptide become completely dissociated and have a different life span[8,17,18]. VWF propeptide is important for intracellular trafficking and processing of VWF[19,20-22], but it is not clear yet whether it has a separate function in the circulation as well[8]. The VWF propeptide is cleared from the circulation at a much faster rate than mature VWF[23], with a half-life of approximately 2 hours. Mature VWF has a half-life between 10 and 12 hours. It is not clear yet whether the VWF propeptide carries ABO antigens like the mature VWF and whether its levels are influenced by ABO blood group[24].

Assuming that the inter-individual variation in its half-life is relatively small, plasma levels of VWF propeptide reflect the rate of VWF secretion at steady state[23,25-27]. Measuring concentrations of VWF propeptide and mature VWF at steady state then allows an estimation of the clearance rate of mature VWF.
To investigate the contribution of VWF secretion and clearance to VWF and FVIII levels, we measured VWF propeptide levels in the Leiden Thrombophilia Study (LETS). Levels of the VWF propeptide were interpreted as a measure of the rate of VWF secretion. Furthermore, we used the levels of mature VWF and the VWF propeptide to estimate the half-life of VWF, assuming a VWF propeptide half-life of two hours.
Using these variables, we studied the influence of VWF secretion and clearance on levels of VWF and FVIII. We also evaluated the contribution of both secretion and clearance of VWF to the risk of venous thrombosis.

Patients and Methods

Leiden Thrombophilia Study

The LETS consists of 474 consecutive patients and 474 controls. All patients were referred for anticoagulant treatment after a first objectively confirmed episode of deep vein thrombosis without an underlying malignancy. Pregnant women were excluded from the study. Use of medication, including oral contraceptives or hormone replacement therapy, was not an exclusion criterion. The controls were matched for sex and age and were acquaintances or partners of the patients. Mean age for both patients and control subjects was 45 years, ranging from 15 to 69 years for patients and 15 to 72 years for controls. Both groups consisted of 272 women (57.4%) and 202 men (42.6%). FVIII:C was measured in the plasma of all participants by a one-stage clotting assay[1]. FVIII:Ag and VWF:Ag were measured by ELISA in the plasma of the first 301 patients and 301 controls[1]. These 602 individuals were not selected, but merely the first 602 individuals to enter the study. Results are expressed as international units per ml (IU/ml). Blood samples from patients were drawn at least six months after the thrombotic event. The design of this study has previously been described in more detail[28,29].

Measuring VWF propeptide

VWF propeptide antigen levels were measured by ELISA as described previously[23]. In brief, microtiter wells were coated overnight at 4°C with the antibody CLB-Pro 35[23]. Subsequently, wells were washed and blocked with 1% Bovine Serum Albumin (BSA) at room temperature for 2 hours. Samples were diluted to three different concentrations and added to the wells after washing. Samples were incubated for two hours at 37°C to ensure protein binding. After incubation, wells were washed and the bound VWF propeptide was detected with the antibody CLB-Pro 14.3 coupled to peroxidase[23]. Pooled normal plasma, which was calibrated against a plasma sample in which the absolute amount of VWF propeptide had previously been determined with purified recombinant VWF propeptide as a gold standard, was used as a standard. The normal pooled plasma had a VWF propeptide concentration of 6.13 nM (see below), which corresponds to one unit per ml (U/ml).

Estimation of VWF half-life

To estimate the half-life of the mature VWF, two important assumptions were made. First, we assume that VWF propeptide half-life is relatively invariable (2 hours) for the whole LETS population, compared to the half-life of mature VWF and that the clearance of both polypeptides follows first-order kinetics. It has been shown previously that these are reasonable assumptions[23,26,27]. Second, we assume that both mature VWF and VWF propeptide levels were at steady state at the time of blood draw. Again, this is a reasonable assumption, even for cases, as it was shown previously that an acute phase reaction was absent in most LETS samples[30] and all LETS blood samples were drawn in resting state. When these conditions are met, steady state kinetics enable us to estimate VWF half-life for each individual[31]:

$$\text{Concentration Steady state} = \frac{\text{Infusion Rate}}{\text{Total Body Clearance}}$$

In case of VWF, the infusion rate is the rate of secretion. To calculate the half-life, we use the following formula, in which Total Body Clearance is the apparent volume of distribution (V_D) times ln2 divided by the half-life.

$$\text{Secretion Rate} = \text{Concentration Steady state} * \frac{V_D * \ln 2}{\text{Half-life}}$$

Secretion Rate and V_D are equal for VWF and the VWF propeptide, which leads to the following:

$$\frac{[\text{VWF}]_{\text{Steady State}}}{\text{VWF half-life}} = \frac{[\text{Propeptide}]_{\text{Steady State}}}{\text{Propeptide half-life}} = \frac{[\text{Propeptide}]_{\text{Steady State}}}{2 \text{ hours}}$$

One unit per ml of VWF propeptide in the LETS corresponds to 6.13 nM. According to de Romeuf et al. one international unit of mature VWF per ml corresponds to 31 nM[24]. After converting all VWF propeptide and mature VWF concentrations in the LETS from (international) units to molar concentrations, the VWF half-life could be estimated for each individual. Throughout this paper we will refer to these estimates as 'VWF half-life', but it should be noted that these are just estimates calculated from the VWF/propeptide ratio after making two important assumptions.

ABO Antigens on VWF propeptide

Wells were coated overnight at 4°C with the antibody CLB-Pro 35. Subsequently, wells were washed and blocked with 1% Bovine Serum Albumin (BSA) at room temperature for 2 hours. Plasma was used from an all blood group A plasma pool, containing the plasma of 29 individuals and from a patient with acquired von Willebrand's disease (VWD) with blood group A. This patient had normal VWF propeptide levels (1.34 U/ml), but very low levels of mature VWF (0.09 IU/ml). The latter plasma was used to check for potentially falsely positive results caused by nonspecific binding of mature VWF. Wells were incubated with duplicates of seven dilutions of these plasmas (one in 20, one in 40 and so on until one in 1280) for two hours at 37°C to ensure VWF propeptide binding. We used four different secondary antibody combinations,

CLB-Pro 14.3 HRP to establish the presence of VWF propeptide, rabbit anti-VWF HRP to exclude the presence of mature VWF, murine anti-A/goat anti mouse HRP to determine the presence of A antigens on the VWF propeptide and anti-B/goat anti mouse HRP as a control in the blood group A plasma pool and the acquired VWD plasma. This experiment was repeated using plasma from a B plasma pool, containing the plasma of 5 individuals. Here, anti B/goat anti mouse HRP was used to determine the presence of B antigens on VWF propeptide. All experiments were repeated on blood group O plasma as well as a negative control.

Statistical Analysis

Protein levels and half-lives are presented as means with their 95% confidence intervals (CI95). The differences in levels between groups (Δ) are presented with their CI95s and were tested with ANOVA and Student's t-tests. Associations between continuous variables were tested with linear regression. To evaluate the effects of VWF propeptide levels and VWF half-life on the risk of thrombosis, odds ratios (OR) and their corresponding CI95s according to Woolf were calculated[32]. Odds Ratios were adjusted for levels of FVIII using logistic regression models.

Results

Levels of VWF propeptide and VWF half-life in healthy controls

In the controls, high levels of VWF propeptide were associated with elevated levels of both VWF and FVIII (Table 1). Mean VWF:Ag and FVIII:Ag levels were 1.52 and 1.41 IU/ml respectively for the highest quartile of VWF propeptide (>1.26 U/ml) versus 0.93 and 0.84 IU/ml for the lowest VWF propeptide quartile (<0.94 U/ml). Regression analysis showed a linear association of the VWF propeptide with VWF:Ag (Pearson's correlation coefficient (R) = 0.61, regression coefficient (ß) = 0.95 (CI95 0.81 to 1.09)), FVIII:Ag (R = 0.57, ß = 0.91 (CI95 0.76 to 1.06)) and FVIII:C (R = 0.51, ß = 0.61 (CI95 0.49 to 0.72)). Results were similar in cases. Stratification by ABO blood group did not influence the outcomes.

We estimated the half-life of VWF, which ranged from 4.6 to 20.1 hours in the LETS. Longer VWF half-life, indicating decreased VWF clearance, was associated with elevated levels of both VWF and FVIII (Table 1). Mean VWF:Ag and FVIII:Ag levels were 1.56 IU/ml and 1.28 IU/dl respectively for the highest quartile of VWF half-life (>12.8 hours) versus 0.84 IU/ml and 0.87 IU/dl for the lowest half-life quartile (<9.2 hours). Again, regression analysis showed a linear association of the VWF half-life with VWF:Ag (R = 0.71, ß = 0.10 (CI95 0.09 to 0.11)), FVIII:Ag (R = 0.38, ß = 0.05 (CI95 0.04 to 0.07)) and FVIII:C (R = 0.41, ß = 0.04 (CI95 0.03 to 0.05)).

Both secretion and clearance of VWF are determinants of levels of VWF and FVIII. However, secretion and clearance rates of VWF did not influence each other. Linear regression showed no association between levels of VWF propeptide and VWF half-life (R = 0.096, ß = -1.089 (CI95 -2.372 to 0.195)).

Table 1. Quartiles of VWF propeptide and VWF half-life and VWF & FVIII levels in controls.

Quartiles of VWF	Mean VWF:Ag	Mean FVIII:Ag	Mean FVIII:C
1 *(0.44-0.94)*	0.93 *(0.87-0.99)*	0.84 *(0.78-0.90)*	0.96 *(0.90-1.01)*
2 *(0.94-1.06)*	1.12 *(1.05-1.19)*	0.97 *(0.90-1.04)*	1.04 *(0.99-1.10)*
3 *(1.06-1.26)*	1.32 *(1.25-1.39)*	1.14 *(1.06-1.21)*	1.18 *(1.12-1.25)*
4 *(1.26-2.03)*	1.52 *(1.43-1.61)*	1.41 *(1.30-1.51)*	1.33 *(1.27-1.40)*
Quartiles of VWF	**Mean VWF:Ag**	**Mean FVIII:Ag**	**Mean FVIII:C**
1 *(4.6-9.2)*	0.84 *(0.79-0.90)*	0.87 *(0.80-0.95)*	0.96 *(0.90-1.01)*
2 *(9.2-11.0)*	1.13 *(1.07-1.20)*	1.01 *(0.93-1.09)*	1.07 *(1.01-1.13)*
3 *(11.0-12.8)*	1.32 *(1.25-1.40)*	1.16 *(1.08-1.25)*	1.19 *(1.13-1.25)*
4 *(12.8-20.1)*	1.56 *(1.48-1.63)*	1.28 *(1.18-1.38)*	1.29 *(1.22-1.35)*

*Quartiles based on levels in controls only.

Levels of VWF propeptide and VWF half-life and known determinants of VWF and FVIII

The LETS controls with blood group O had lower levels of VWF and FVIII than those with non-O blood groups (Table 2). The half-life of VWF was also influenced by ABO blood group. Non-O individuals had a longer mean half-life of approximately 12 hours compared to O individuals, whose mean half-life was approximately 10 hours.

In contrast, levels of VWF propeptide were similar in both O and non-O controls, 1.08 and 1.13 U/ml respectively (Table 2), which is consistent with previous reports[24,33].

Table 2. Protein levels and VWF half life for ABO blood groups O versus non-O and women versus men in controls.

Mean Value	O	non-O	Mean Difference (Δ)	CI95 of Δ
VWF:Ag IU/ml	1.06	1.33	0.26	0.18 to 0.35
FVIII:Ag IU/ml	0.94	1.19	0.25	0.16 to 0.34
FVIII:C IU/ml	1.00	1.19	0.19	0.16 to 0.27
VWF propeptide U/ml	1.08	1.13	0.05	-0.003 to 0.11
VWF half-life Hrs.	10.02	11.98	1.96	1.36 to 2.57
Mean Value	**Women**	**Men**	**Mean Difference (Δ)**	**CI95 of Δ**
VWF:Ag IU/ml	1.26	1.34	0.08	0.02 to 0.15
FVIII:Ag IU/ml	1.15	1.27	0.12	0.05 to 0.19
FVIII:C IU/ml	1.21	1.18	-0.03	-0.07 to 0.01
VWF propeptide U/ml	1.14	1.21	0.07	0.02 to 0.11
VWF half-lifeHrs.	1.21	1.18	0.16	-0.28 to 0.60

The influence of sex, a known determinant of levels of VWF and FVIII, was studied as well among the controls. Results are shown in the second part of Table 2. Both VWF:Ag and FVIII:Ag were higher in men than in women, for FVIII:C no difference was observed. The VWF propeptide was also higher in men, indicating an increased VWF secretion in men compared to women. VWF half-life was not different between men and women.

Finally, we studied the effect of age on VWF secretion and clearance rates in healthy controls. Linear regression shows that both levels of VWF propeptide and VWF half-life increase with age. The association between age and VWF propeptide ($R = 0.30$, ß = 0.006 (CI95 0.004 to 0.008)) however, was stronger than that between age and VWF half-life ($R = 0.12$, ß = 0.03 (CI95 0.001 to 0.05)). The R^2s for both variables indicate that almost 10 % of all variation in VWF secretion and only 1% of all variation in VWF clearance can be explained by age.

ABO blood group

In contrast to the association between VWF level and ABO blood group, we did not observe an association between VWF propeptide and ABO blood group (Table 2). The ABO dependence of the VWF level is thought to be related to the presence of ABO oligosaccharide structures on the N-linked oligosaccharide chains of VWF[10,34]. We hypothesized that the lack of association between ABO blood group and VWF propeptide levels can be explained by the absence of ABO antigens on the VWF propeptide. However, there are no previous reports excluding the presence of ABO antigens on the VWF propeptide. Therefore, we have studied whether or not ABO antigens are present on VWF propeptide using an ELISA based method.

In both blood group A pooled plasma, normal blood group B plasma and in the plasma of a patient with blood group A and acquired VWD, we could measure normal amounts of VWF propeptide. A secondary antibody against mature VWF showed that no VWF had bound the primary anti-VWF propeptide antibody in either plasma sample (data not shown). Even though both the blood group A pooled plasma and the acquired VWD plasma samples came from blood group A individuals only, no A antigen was present on the VWF propeptide in either sample. Also, no B antigen was present on VWF propeptide in the blood group B plasma pool, indicating that ABO antigens are expressed only on mature VWF and not on the VWF propeptide. Both during these experiments and in the past, under similar conditions, the presence of A and B antigens could be demonstrated on mature VWF[35].

Levels of VWF propeptide and VWF half-life in cases versus controls

Patients had higher VWF propeptide levels than controls (Table 3a). In the LETS, patients had a mean VWF propeptide level of 1.22 versus 1.11 U/ml in controls. Stratification by ABO blood group did not influence this observation. In agreement with this observation, higher levels of VWF propeptide were associated with an increased risk of venous thrombosis (Table 3b). There was a clear dose-response relationship between VWF propeptide levels and thrombotic risk. Individuals with VWF propeptide levels in the highest quartile

had an increased risk of thrombosis (odds-ratio (OR) 2.6, 95% confidence-interval (CI95) 1.7-4.1) compared to those in the lowest quartile. A large proportion of this risk increase disappeared after adjustment for FVIII:Ag (OR 1.4, CI95 0.8-2.3).

Table 3a. VWF, FVIII, VWF propeptide & half-life in cases versus controls.

Mean Value	Cases	Controls	Mean Difference (Δ)	CI95 of Δ
VWF:Ag IU/ml	1.36	1.21	0.15	0.09 to 0.21
FVIII:Ag IU/ml	1.31	1.08	0.23	0.16 to 0.30
FVIII:C IU/ml	1.28	1.11	0.17	0.14 to 0.23
VWF propeptide U/ml	1.22	1.11	0.11	0.07 to 0.16
VWF half-life Hrs.	11.43	11.14	0.29	-0.14 to 0.72

VWF half-life was similar in patients and controls (11.43 and 11.14 hrs, respectively) (Table 3a). Stratification by ABO blood group did not influence this observation. Also, VWF half-life only marginally influenced thrombosis risk (OR 1.4, CI95 0.9-2.3 in the highest versus the lowest quartile) and no dose-response relationship was observed (Table 3b). The marginal increase in risk completely disappeared after adjustment for FVIII:Ag (OR 0.9, CI95 0.6-1.6).

Stratification by sex did not influence the effects of VWF propeptide levels or VWF half-life on the risk of thrombosis.

To ensure the latter findings were not influenced by VWF secretion, we looked at VWF half-life in cases and controls for each quartile of VWF propeptide separately. Only in the lowest VWF propeptide quartile was the mean VWF half-life slightly longer in cases than in controls, 12.5 versus 11.3 hrs. respectively (Δ = 1.2 hrs (CI95 0.002 to 2.4)). For the higher three VWF propeptide quartiles, there was no difference in VWF half-life between cases and controls.

Table 3b. VWF propeptide quartiles and VWF half-life quartiles and the risk of venous thrombosis.

Quartiles of VWF propeptide* *(range)*	**Cases**	**Controls**	**Odds Ratio *(CI95)***
1 *(0.44-0.94)*	54	80	1
2 *(0.94-1.06)*	41	71	0.9 *(0.5-1.4)*
3 *(1.06-1.26)*	80	79	1.5 *(0.9-2.4)*
4 *(1.26-2.03)*	126	71	2.6 *(1.7-4.1)*
Quartiles of VWF half-life* *(range)*	**Cases**	**Controls**	**Odds Ratio *(CI95)***
1 *(4.6-9.2)*	47	75	1
2 *(9.2-11.0)*	91	75	1.9 *(1.2-3.1)*
3 *(11.0-12.8)*	97	76	2.0 *(1.3-3.3)*
4 *(12.8-20.1)*	66	75	1.4 *(0.9-2.3)*

*Quartiles based on half-lifes in controls only.

Discussion

A novel aspect of this study is that we have assessed both the VWF secretion rate and VWF clearance rate on the basis of steady state VWF propeptide concentrations. The plasma level of VWF propeptide can be used as a tool to study the secretion of VWF, considering equimolar secretion of the mature VWF and its propeptide[8,16,17,]. In addition, the clearance of VWF can be evaluated using the molar concentrations of VWF propeptide, VWF and the half-life of the VWF propeptide. We measured VWF propeptide levels in the LETS to study the effects of VWF secretion and clearance on levels of VWF and FVIII and on the risk of venous thrombosis.

To study VWF clearance effects, we estimated VWF half-life. As described in the methods we had to make assumptions to allow this estimation. The assumptions that clearance of VWF and VWF propeptide follows first order kinetics and that both proteins were at steady state at time of blood draw are based on previous results from both the LETS and other studies. The most crucial assumption however, is that the half-life of the VWF propeptide is more or less constant in all LETS individuals. We believe that this assumption is

supported by previous data as well. VWF propeptide half-life has been shown to be relatively invariable before[23,26,27], although in relatively small groups of individuals, both men and women. Furthermore, in our study, VWF propeptide levels were independent of ABO blood group, which is a major determinant of VWF level variations. Nonetheless, there is no proof that VWF propeptide half-life is indeed constant, so therefore, the calculated VWF half-lifes should be interpreted as estimations only.

In the controls of the LETS, high levels of VWF propeptide were associated with higher levels of VWF and FVIII. As could be expected, increased secretion of VWF indeed leads to higher plasma levels of both VWF and FVIII. Similarly, decreased clearance rates were associated with elevated levels of VWF and FVIII. The magnitude of the effects of both secretion and clearance of VWF on levels of VWF and FVIII was more or less the same (Table 1).

Even though secretion and clearance of VWF both influenced protein levels of VWF and FVIII, they did not affect each other. Elevated levels of VWF propeptide were not associated with either in- or decreased VWF half-life and vice versa. This makes the existence of a direct feedback system regulating VWF levels unlikely. Levels of VWF and FVIII are therefore influenced independently by both secretion and clearance of VWF.

Levels of VWF and FVIII are strongly influenced by ABO blood group. Blood group O individuals have lower levels than non-O individuals. We found that the estimated half-life of VWF was influenced by ABO blood group. VWF half-life in LETS controls with blood group O was estimated to be almost 2 hours shorter than in those with a non-O blood group. Most likely, the ABO antigens present on the mature VWF influence the clearance rate of the molecule[12,13]. Levels of VWF propeptide were not influenced by ABO blood group and were similar in O and non-O individuals. ABO antigens present on the mature VWF therefore are unlikely to influence the secretion rate of VWF, rather these structures influence the clearance of mature VWF. We did not detect A or B antigens on the VWF propeptide itself. The observation that VWF propeptide levels were not influenced by ABO blood group, supports our findings that these antigens are in fact absent from the VWF propeptide and restricted solely

to the mature VWF polypeptide.

Elevated levels of FVIII have been recognized as an important risk factor of venous thrombosis. Therefore, it can be expected that determinants of elevated FVIII levels also increase the risk of venous thrombosis. Levels of VWF propeptide, which are associated with levels of FVIII, were indeed higher in cases than in controls. There was a dose-response relationship between levels of VWF propeptide and the risk of venous thrombosis, just as there was a strong dose-response relationship between levels of VWF propeptide and levels of FVIII (and VWF). After adjustment for FVIII levels, the risk largely disappeared. This is a corroboration of previous findings in the LETS described by Koster et al[1], where FVIII and not its determinant VWF appeared responsible for increases in the risk of thrombosis.

However, for the VWF half-life, which was also associated with levels of FVIII, there was not such a dose response relationship with the risk of venous thrombosis. The risk appeared slightly elevated in the second and third quartiles of VWF half-life compared to both the lowest and highest quartile. However, only a marginal effect on risk was observed for the highest half-life quartile, which was associated with the highest mean FVIII levels. This marginal risk increase disappeared completely after adjustment for FVIII levels. It may seem that these results contradict the previous findings in the LETS described by Koster et al, however, before the measurement of the VWF propeptide, we were not able to make the distinction between high FVIII and VWF levels caused by increased secretion and those caused by decreased clearance. When taken high levels of FVIII as a whole, we of course observe the same increase in risk as described previously[1].

It seems contradictory that two determinants of elevated levels of VWF and FVIII, secretion and clearance of VWF, should have a different effect on thrombosis risk. However, blood samples from cases have been collected after a thrombotic event. Therefore, it is not possible to discriminate between high FVIII levels as a cause or a consequence of the thrombosis. It now appears that elevated levels of FVIII are mainly a risk factor of venous thrombosis if associated with increased secretion and less if associated with decreased

clearance. An obvious and credible explanation could be that in the LETS, where blood samples were collected several months after the thrombotic event, elevated levels of FVIII reflect mainly the consequence of thrombosis and less the cause of thrombosis. However, high FVIII has also been identified as an important risk factor for venous thrombosis in a large prospective study, in which blood samples were drawn well before a thrombotic event[7]. A more plausible explanation would then be that high FVIII is in the causative pathway but that the majority of the increased VWF and FVIII levels in LETS patients are the consequence of increased secretion of VWF due to post-thrombotic endothelial damage. Or, perhaps FVIII levels caused by increased VWF secretion merely reflect the condition of vascular endothelium and therefore associate with venous thrombosis. In this last scenario, increased levels of VWF and consequently of FVIII are merely epiphenomena of an increased thrombotic risk caused by endothelial damage. That would be consistent with the fact that high FVIII is a predictor of a recurrent thrombosis[4].
In summary, both secretion and clearance of VWF are important determinants of levels of both VWF and FVIII. However, it appears that mainly increased VWF secretion increases the risk of venous thrombosis, whereas decreased VWF clearance does not or less so. Secretion and clearance rates of VWF do not influence each other. ABO blood group influences levels of VWF and FVIII via the clearance of VWF, rather than via VWF secretion.

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