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Summary

Some comorbidities, such as hypertension, are associated with higher von Willebrand factor (VWF) levels in the general population. No studies have been conducted to assess this association in patients with von Willebrand disease (VWD). Therefore, we studied this association in patients with type 1 (n = 333) and type 2 (n = 203) VWD from the 'WiN" study. VWF antigen (VWF:Ag) was higher in type 1 VWD patients with hypertension [difference: 0·23 iu/ml, 95% confidence interval (CI): 0·11–0·35], diabetes mellitus (0·11 iu/ml, 95% CI: −0·02 to 0·23), cancer (0·14 iu/ml, 95% CI: 0·03–0·25) and thyroid dysfunction (0·14 iu/ml, 95% CI: 0·03–0·26) than in patients without these comorbidities (all corrected for age, sex and blood group). Similar results were observed for VWF collagen binding capacity (VWF:CB), VWF activity as measured by the VWF monoclonal antibody assay (VWF:Ab) and factor VIII (FVIII) coagulant activity (FVIII:C). In type 1 VWD, age was associated with higher VWF:Ag (0·03 iu/ml; 95% CI: 0·01–0·04), VWF:CB (0·02 iu/ml; 95% CI: 0·00–0·04), VWF:Ab (0·04 iu/ml; 95% CI: 0·02–0·06) and FVIII:C (0·03 iu/ml; 95% CI: 0·01–0·06) per decade increase. After adjustment for relevant comorbidities, these associations were no longer significant. Despite the higher VWF and FVIII levels, type 1 VWD patients with comorbidities had more bleeding episodes, particularly during surgery. There was no association between comorbidities and VWF/FVIII levels or bleeding phenotype in type 2 VWD patients. In conclusion, comorbidities are associated with higher VWF and FVIII levels in type 1 VWD and may explain the age-related increase of VWF and FVIII levels.

Keywords: VWD, VWF, diabetes, cancer, elderly.

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Von Willebrand disease (VWD) is the most common inherited bleeding disorder, and is characterized by mucocutaneous bleeding due to a deficiency of von Willebrand Factor (VWF) (Leebeek & Eikenboom, 2016). VWD can be caused by reduced levels (type 1), abnormal function (type 2) or complete absence (type 3) of VWF (Sadler *et al*, 2006).

VWF levels have a large inter- and intra-individual variability (de Maat *et al*, 2016). The inter-individual variability is mostly caused by genetic variations and blood group, while the intra-individual variability is mainly caused by environmental factors (Gill *et al*, 1987; Jenkins & O'Donnell, 2006; van Schie *et al*, 2011). Furthermore, an age-related increase of VWF levels has been observed in both healthy individuals and patients with type 1 VWD (Gill *et al*, 1987; Coppola *et al*, 2003; Tofler *et al*, 2005; Vischer *et al*, 2005; Sanders *et al*, 2014; Rydz *et al*, 2015; Albanez *et al*, 2016). Both in healthy individuals and patients with VWD, higher levels of VWF are observed after exercise, trauma or surgery and during infectious diseases, pregnancy or use of certain medication (Prentice *et al*, 1972; Clark *et al*, 1998; Sadler *et al*, 2000; James & Lillicrap, 2012; Kawecki *et al*, 2017). In individuals without VWD increase of VWF has been associated with several disorders, including hypertension, diabetes mellitus and hypercholesterolaemia (Conlan *et al*, 1993; Kessler *et al*, 1998; Seligman *et al*, 2000; Brunner *et al*, 2005). Furthermore, several studies have demonstrated increased VWF levels in patients with cancer, hyperthyroidism, chronic liver disease and renal failure (Danielsson *et al*, 1990; Liu *et al*, 1993; Holvoet *et al*, 1996; Auwerda *et al*, 2005, 2007; Franchini *et al*, 2013; Elbers *et al*, 2016; Leebeek, 2016).

Although VWF levels are altered in patients with the above-mentioned disorders, the association between these disorders and VWF levels in patients with VWD is unknown. Also, the relationship between comorbidities, mostly occurring at older age, and the age-related increase of VWF levels is unknown.

Therefore, our primary aim was to assess the association between comorbidities and VWF and factor VIII (FVIII) levels in patients with VWD type 1 and 2. The second aim was to study whether comorbidities can explain the previously reported age-related increase of VWF in VWD type 1 patients. Thirdly, we aimed to assess the association between comorbidities and the bleeding phenotype of VWD patients.

Materials and methods

Participants

We performed a nationwide cross-sectional study in patients with VWD in the Netherlands; the "Willebrand in the Netherlands" (WiN) study (de Wee *et al*, 2010, 2012). Patients with VWD were recruited between October 2007 and October 2009. The inclusion criteria were haemorrhagic symptoms or a family history of VWD, and historically lowest VWF antigen (VWF:Ag) and/or VWF ristocetin cofactor activity (VWF:RCo) ≤0·30 iu/ml and/or FVIII coagulant activity levels (FVIII:C) ≤0·40 iu/ml (for type 2N VWD). Patients were excluded if they had other haemostatic disorders. Blood and saliva samples were obtained at study inclusion.

For the current analyses, we excluded patients with type 3 VWD (defined as VWF levels <0·05 iu/ml and VWF propeptide (VWFpp) <0·05 iu/ml), because by definition there will be no increase of VWF levels in these patients (Sanders *et al*, 2015a). Furthermore, we excluded patients younger than 16 years old, patients with missing data on comorbidities, patients without centrally measured VWF levels or with centrally measured VWF levels during pregnancy, or desmopressin medication or clotting factor concentrate infusion 72 h prior to blood sampling. The study was performed according to the Declaration of Helsinki and approved by the Medical Ethical Committees of all participating centres. Informed consent was signed by all patients.

Assessment methods

The assessment methods used in the WiN study, have been described in detail previously (de Wee *et al*, 2010, 2012). Participants completed an extensive questionnaire, including questions on comorbidities, use of medication, self-administered version of the condensed Tosetto bleeding score and bleeding episodes that required haemostatic treatment in the year prior to inclusion (Tosetto *et al*, 2006; Bowman *et al*, 2008). We previously reported that the self-administered bleeding score is comparable to the expert-administered bleeding score (de Wee *et al*, 2012). We did not score for bleeding if patients received treatment prophylactically, for instance before surgery (Tosetto *et al*, 2008).

Definitions

Presence of comorbidities was assessed at study inclusion, immediately before blood drawing for VWF level measurements. As the aim of the study was to assess the effect of comorbidities on VWF and FVIII levels, we only focused on comorbidities that are known to have an association with VWF or FVIII levels according to the literature. First, we analysed the effect of individual comorbidities on VWF and FVIII levels. All the disorders that seemed to be associated with higher VWF or FVIII levels in our VWD population were defined as relevant comorbidities. Secondly, we assessed the association between relevant comorbidities (as a group) and the age-related increase of VWF and FVIII levels in patients with type 1 VWD.

Patients were defined as having diabetes mellitus or thyroid dysfunction if reported on the questionnaire, or if they used medication that could only be used for these disorders, such as insulin, metformin or levothyroxine. We defined patients as having cancer when they reported to have a malignant disease. We did not define basal cell carcinoma as

cancer, because of the non-metastatic, semi-malignant characteristics (Bauer *et al*, 2015). Patients were defined as having hypertension or hypercholesterolaemia if reported on the questionnaire. Arterial thrombotic events were defined as a medical history of myocardial infarction, ischaemic stroke, transient ischaemic attack (TIA) or angina pectoris. These thrombotic events were previously evaluated by studying the medical charts of these patients (Sanders *et al*, 2013). We defined renal disease as intrinsic renal diseases, a renal transplantation in the past or a kidney removal due to an anomaly. Liver disease was defined as intrinsic liver diseases such as hepatitis or cirrhosis.

Laboratory measurements

At inclusion in the study, plasma levels of VWF:Ag, VWF collagen binding capacity (VWF:CB), VWF activity (measured by the VWF monoclonal antibody assay; VWF:Ab) and FVIII coagulant activity (FVIII:C) were centrally measured at the Erasmus University Medical Centre. VWFpp was centrally measured at the Leiden University Medical Centre (Leiden, the Netherlands) (Sanders *et al*, 2015a). We previously reported detailed information on blood sampling procedure and laboratory measurements (de Wee *et al*, 2012; Sanders *et al*, 2015a). VWF:Ag and VWF:CB were measured with an in-house enzyme-linked immunosorbent assay. For VWF:Ag we used polyclonal rabbit anti-human VWF antibodies and horseradish peroxidase conjugated anti-human VWF antibodies (DakoCytomation, Glostrup, Denkmark) for detection, while for VWF:CB we used collagen type 1 (Sigma-Aldrich, St Louis, MO, USA) for capture and horseradish peroxidase (HRP)-conjugated anti-human VWF antibodies (DakoCytomation) for detection. VWF:Ab was assessed with a latex immune assay on an automated coagulometer, in which monoclonal antibodies against the GP1bα binding site of VWF was used, reflecting the binding activity of VWF to GP1bα (HemosIL^{TM} von Willebrand Factor Activity; Instrumentation Laboratory BV, Breda, the Netherlands) (Sanders *et al*, 2015a). The VWF antibody assay was more recently suggested to be called VWF:Ab by the VWF subcommittee of the Scientific and Standardization Committee of the International Society for Thrombosis and Haemostasis (Bodo *et al*, 2015). Therefore, we have used VWF:Ab throughout the manuscript. We measured FVIII:C using a one-stage clotting assay (TriniCLOT, Biomerieux, Marcy l'Etoile, France) with FVIII-deficient plasma (Biopool, Umea, Sweden) and reference plasma (Precision biologic, Kordia, Leiden, the Netherlands).

Statistical methods

Categorical data are presented as frequencies and proportions. Continuous data are presented as median and interquartile range (IQR) or mean and (standard deviation). As type 1 and type 2 VWD have different mechanisms of disease pathogenesis, we analysed each separately.

VWF, FVIII and bleeding score were not normally distributed. However, the variables fulfilled the assumptions of linearity and homoscedasticity, whereas the sample size was large. Therefore, we used multiple regression analyses to calculate the effect of comorbidities on VWF and FVIII levels and bleeding score. For all comorbidities multiple regression analyses were adjusted for age, sex and blood group. The effect of an individual comorbidity on VWF levels was only adjusted for other comorbidities when the other comorbidities were relevant confounders (i.e. when addition of the other comorbidity to the regression model caused at least 10% change in the regression coefficient). For each of the disorders; hypertension, diabetes, arterial thrombotic events and hypercholesterolaemia, the other three disorders were analysed to identify whether they were confounders or effect modifiers. For hypertension and hypercholesterolaemia, we additionally investigated whether the use of anti-hypertensive drugs and statins acted as confounders or effect modifiers.

In the multiple regression models for analysing the association between comorbidities and the bleeding score, we adjusted for age, sex and blood group. We report linear regression outcomes as unstandardized β coefficient (difference) followed by the 95% confidence interval (CI). A *P*-value less than 0·05 was defined as significant. Statistical analyses were performed using SPSS Statistics version 24 (IBM Corp., Armonk, NY, USA).

Results

We included 536 patients in this study from the total WiN-study population of 837 patients. Figure 1 shows a consort diagram illustrating patient inclusion. Most patients were female (64%), had blood group O (62%) and had type 1 VWD (62%) (Table I). The mean age in type 1 and type 2 VWD were 47 (SD 15, range 17–81) and 46 (SD 16, range 16–83) years, respectively. Comorbidities were reported in 175 patients (33%), of whom 47 patients had two comorbidities, 19 patients had three comorbidities and 2 patients had four comorbidities. The most frequent reported comorbidity was hypertension (21·8%), followed by hypercholesterolaemia (9·5%) (Table II).

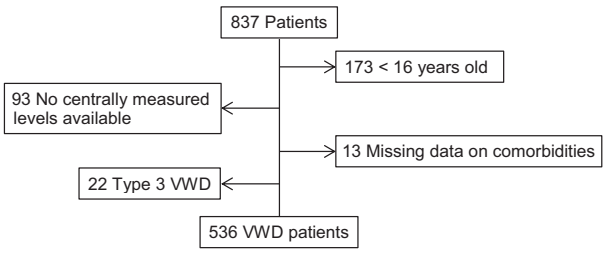

Fig 1. Consort diagram illustrating patient inclusion in the study. VWD, von Willebrand disease.

Table I. Baseline characteristics in type 1 and type 2 VWD.

	Type 1 VWD $N = 333$	Type 2 VWD $N = 203$	Total patients $N = 536$
Age (years), mean (SD)	47 (15)	46 (16)	46 (15)
Female, *n* (%)	232 (70%)*	113 (56%)*	345 (64%)
Blood group O, *n* (%)	227 (68%)*	105 (52%)*	332 (62%)
VWF:Ag	0·39 [0·25–0·55]*	0·25 [0·16–0·35]*	0·31 [0·21–0·47]
VWF:CB	0·45 [0·26–0·68]*	0·08 [0·06–0·15]*	0·27 [0·10–0·54]
VWF:Ab	0·48 [0·26–0·72]*	0·08 [0·04–0·16]*	0·25 [0·11–0·57]
FVIII:C	0·68 [0·52–0·89]*	0·38 [0·27–0·49]*	0·54 [0·37–0·77]
Bleeding score	9 [5–15]*	12 [8–17]*	11 [6–16]

Data are presented as median [interquartile ranges], unless otherwise specified. FVIII:C, factor VIII coagulant activity; SD, standard deviation; VWD, von Willebrand disease; VWF:Ab, von Willebrand factor activity as measured by a monoclonal antibody assay; VWF:Ag; von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding capacity.
*$P < 0·05$ comparing patients with type 1 and type 2 VWD.

Hypertension

There were 117 patients (22%) with hypertension (Table II). Patients with hypertension were older and more often female (Table II). Ninety seven of the 117 patients (83%) used anti-hypertensive drugs.

Type 1 VWD patients with hypertension had higher VWF:Ag, VWF:CB, VWF:Ab and FVIII:C, with a difference of respectively 0·23 iu/ml (95% CI: 0·11–0·35), 0·24 iu/ml (95% CI: 0·08–0·39), 0·26 iu/ml (95% CI: 0·09–0·44) and 0·19 iu/ml (95% CI: 0·03–0·35) when compared to patients without hypertension (corrected at least for age, sex, blood group and anti-hypertensive treatment, Fig 2). These findings were observed in both young (<65 years) and elderly (≥65 years) VWD patients, although we did not find a significant outcome in elderly type 1 VWD patients, due to the small number of patients (Figure S1). In patients with type 1 VWD and hypertension, use of anti-hypertensive drugs was associated with lower VWF:Ag, −0·18 iu/ml (95% CI: −0·33 to −0·03), and tended to be associated with lower VWF:Ab, −0·21 iu/ml (95% CI: −0·43 to 0·00) (both corrected for age, sex and blood group). In patients with type 2 VWD, no association between hypertension or use of anti-hypertensive drugs and VWF or FVIII levels was found (Fig 2; Table SI).

Diabetes mellitus

Twenty VWD patients (3·7%) had diabetes mellitus at the time of inclusion in the WiN study. Patients with diabetes were older and were less often blood group O compared to patients without diabetes (Table II). Fourteen patients had type 2 diabetes; the type of diabetes was not reported in the other six patients.

Type 1 VWD patients with diabetes had higher VWF:Ab and FVIII:C compared to patients without diabetes, with a difference of 0·23 iu/ml (95% CI: 0·05–0·40) and 0·27 iu/ml (95% CI: 0·11–0·44), respectively. A similar association, although not significant, was observed for VWF:Ag and VWF:CB, respectively 0·11 iu/ml (95% CI: −0·02 to 0·23) and 0·15 iu/ml (95% CI: −0·01 to 0·31) (Fig 3). In patients with type 2 VWD no association between diabetes and VWF or FVIII levels was found (Fig 3; Table SI).

Cancer

Twenty-three VWD patients (4·3%) had cancer at inclusion in the study; these patients were generally older (Table II). Prostate cancer was the most common form of cancer, occurring in five patients, followed by breast cancer in four patients.

Type 1 VWD patients with cancer had higher VWF:Ag and VWF:Ab, with a difference of 0·14 iu/ml (95% CI: 0·03–0·25) and 0·19 iu/ml (95% CI: 0·03–0·35), respectively. A similar trend was seen for VWF:CB and FVIII:C in cancer patients, respectively: 0·14 iu/ml (95% CI: −0·01 to 0·29) and 0·14 iu/ml (95% CI: −0·02 to 0·30) (Fig 4; Table SI). Patients with type 2 VWD tended to have a FVIII:C of 0·16 iu/ml (95% CI: −0·01 to 0·32) higher compared to type 2 VWD patients without cancer. However, this could be attributed to one patient who had a FVIII:C of 1·76 iu/ml (Fig 4).

Thyroid dysfunction

Five patients (0·9%) had hyperthyroidism and 13 patients (2·4%) had hypothyroidism at inclusion in the WiN study. Patients with hyperthyroidism and hypothyroidism were significantly older than patients without these disorders (Table II). All patients with hypothyroidism used thyroid-stimulating hormones (levothyroxine). The analyses for both disorders were only performed in patients with type 1 VWD, due to the low number of patients in type 2 VWD (Table II).

VWF:CB and VWF:Ab tended to be higher in patients with type 1 VWD with hyperthyroidism, although this was not significant, than in patients without hyperthyroidism, with a difference of 0·21 iu/ml (95% CI: −0·04 to 0·47) and 0·27 iu/ml (95% CI: −0·01 to 0·54), respectively (Fig 5;

Table II. Baseline characteristics for each comorbidity.

	Total	Hyper-tension	DM	Cancer	Hyper-thyroidism	Hypo-thyroidism	ATEs	Hyper-cholesterolaemia	Liver disease
Number of patients									
Total	536	117	20	23	5	13	23	51	10
Type 1, *n* (%)	333 (62%)	75 (64%)	14 (70%)	15 (65%)	5 (100%)	10 (77%)	14 (61%)	32 (63%)	2 (20%)
Type 2, *n* (%)	203 (38%)	42 (36%)	6 (30%)	8 (35%)	0 (0%)	3 (23%)	9 (39%)	19 (37%)	8 (80%)
Age, years	46 (15)	59* (10)	60* (12)	57* (13)	59* (4)	61* (12)	61* (12)	58* (12)	48 (16)
Female, *n* (%)	345 (64%)	90* (77%)	12 (60%)	13 (57%)	5 (100%)	11 (85%)	12 (52%)	36 (71%)	5 (50%)
Blood group O, *n* (%)	332 (62%)	73 (62%)	8* (40%)	14 (61%)	2 (40%)	9 (75%)	14 (61%)	33 (65%)	4 (40%)

Data are presented as mean (standard deviation), unless otherwise specified. ATEs, arterial thrombotic events; DM, diabetes mellitus.
*$P < 0{\cdot}05$ comparing patients with and without the mentioned disorder.

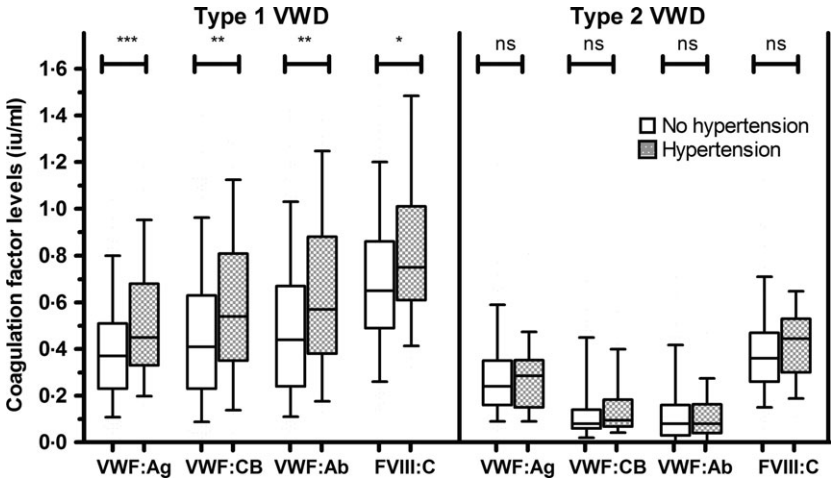

Fig 2. The association between hypertension and VWF and FVIII. Number of patients with and without hypertension are respectively in type 1: $n = 75$ vs. $n = 255$, type 2: $n = 42$ vs. $n = 160$. Multiple regression outcomes in type 1 VWD corrected for; age, sex, blood group and anti-hypertensive treatment. VWF:Ab and FVIII:C additionally corrected for diabetes. Multiple regression outcomes in type 2 VWD corrected for; age, sex, blood group and anti-hypertensive treatment. VWF:Ag, VWF:CB and FVIII:C additionally corrected for hypercholesterolaemia. Moreover, FVIII:C also corrected for arterial thrombotic events. The association between hypertension and VWF levels was only adjusted for other comorbidities when the other comorbidities were relevant confounders (see methods). Data presented as boxplots with median and interquartile ranges, and 5–95 percentiles. *$P < 0{\cdot}05$. **$P < 0{\cdot}01$. ***$P < 0{\cdot}001$. FVIII:C, factor VIII coagulant activity; ns, not significant; VWD, von Willebrand disease; VWF:Ab, von Willebrand factor activity as measured by a monoclonal antibody assay; VWF:Ag; von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding capacity.

Table SI). Remarkably, type 1 VWD patients with hypothyroidism also had higher FVIII:C [0·20 iu/ml (95% CI: 0·01–0·40)], and tended to have higher VWF:Ab [0·18 iu/ml (95% CI: −0·02 to 0·38)] (Fig 5).

Arterial thrombotic events and hypercholesterolaemia

Twenty-three patients (4·3%) had previously suffered an arterial thrombotic event at the time of inclusion in the WiN study. Fifty-one patients (9·5%) were reported to have hypercholesterolaemia. Detailed information on the type of arterial thrombotic events in our study population was reported previously (Sanders *et al*, 2013). Of the patients with hypercholesterolaemia, 27 (53%) used statins at the time of inclusion. Patients with arterial thrombotic events and hypercholesterolaemia were older than patients without these conditions (Table II). No association was found between prevalent arterial thrombotic events, hypercholesterolaemia or use of statins and VWF or FVIII levels (data not shown).

Liver- and renal disease

Chronic liver disease was present in ten VWD patients (1·9%) and renal disease was present in four patients (0·7%) at inclusion in the study (Table II). Because of the small number of patients we could only analyse patients with type 2 VWD with chronic liver disease. In this group, three patients had hepatitis B and five patients hepatitis C. Type 2 VWD patients with liver disease did not have different VWF

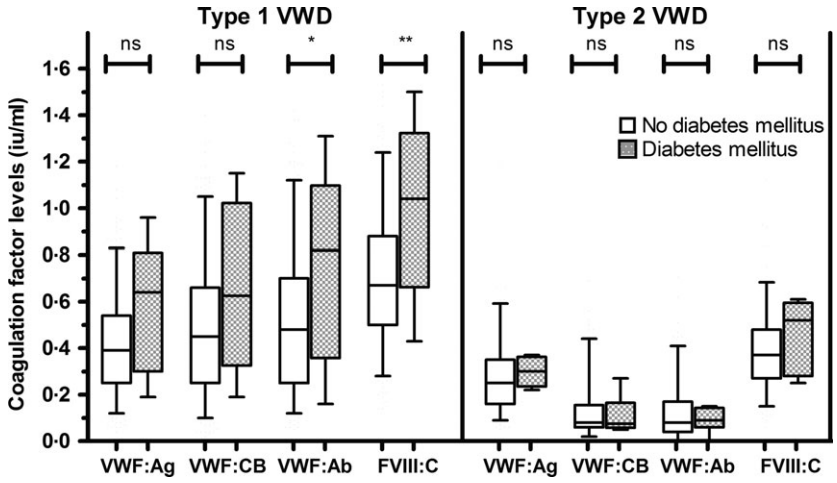

Fig 3. The association between diabetes mellitus and VWF and FVIII. Number of patients with and without diabetes are respectively in type 1: $n = 14$ vs. $n = 319$, type 2: $n = 6$ vs. $n = 197$. Multiple regression outcomes in type 1 VWD corrected for; age, sex and blood group. VWF:Ag. VWF:CB and VWF:Ab additionally corrected for hypertension. Moreover, VWF:Ag also corrected for hypercholesterolaemia. Multiple regression outcomes in type 2 VWD corrected for; age, sex and blood group. VWF:Ag, VWF:Ab and FVIII:C additionally corrected for hypertension. Moreover, VWF:Ag, VWF:CB and FVIII:C for hypercholesterolaemia and FVIII:C for arterial thrombotic events. The association between diabetes and VWF levels was only adjusted for other comorbidities when the other comorbidities were relevant confounders (see methods). Data presented as boxplots with median and interquartile ranges, and 5–95 percentiles. *$P < 0{\cdot}05$. **$P < 0{\cdot}01$. FVIII:C, factor VIII coagulant activity; ns, not significant; VWD, von Willebrand disease; VWF:Ab, von Willebrand factor activity as measured by a monoclonal antibody assay; VWF:Ag; von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding capacity.

or FVIII levels compared to type 2 patients without liver disease (data not shown).

The association between the number of comorbidities and VWF and FVIII levels

All comorbidities that had an association with higher VWF and FVIII levels were defined as relevant comorbidities: hypertension, diabetes mellitus, cancer and thyroid dysfunction. Patients with renal disease were excluded from this analysis, due to the insufficient number of patients (<5). In total, 146 patients (27%; 96 with type 1 VWD and 50 with type 2 VWD) had any of these relevant comorbidities. Patients with relevant comorbidities were more often female (74·0% vs. 61·1%, $P = 0{\cdot}006$) and were older [58 years (11) vs. 42 years (14), $P < 0{\cdot}001$], than patients without relevant comorbidities. There was no difference in blood group (61·4% vs. 62·3% blood group O, $P = 0{\cdot}839$).

VWF and FVIII levels were strongly associated with the number of comorbidities in patients with type 1 VWD. As the number of relevant comorbidities increased, VWF:Ag, VWF:CB, VWF:Ab and FVIII:C increased for presence of each additional comorbidity (Fig 6). VWF:Ag increased from 0·36 iu/ml [0·23–0·49] in patients without comorbidities to 0·44 iu/ml [0·29–0·62] in patients with one comorbidity to 0·67 iu/ml [0·39–0·90] in patients with two comorbidities ($P < 0{\cdot}001$ corrected for age, sex and blood group). Similar results were observed for VWF:CB, VWF:Ab and FVIII:C (Fig 6).

The age-related increase of VWF and FVIII levels in type 1 VWD

In patients with type 1 VWD, age was associated with higher VWF:Ag, VWF:CB, VWF:Ab and FVIII:C, with a respective increase per decade of 0·03 iu/ml (95% CI: 0·01–0·04), 0·02 iu/ml (95% CI: 0·00–0·04), 0·04 iu/ml (95% CI: 0·02–0·06) and 0·03 iu/ml (95% CI: 0·01–0·06) (Fig 7; Table SI). After adjustment for relevant comorbidities no significant association was observed between age and VWF:Ag, VWF:CB, VWF:Ab or FVIII:C, respectively 0·01 iu/ml (95% CI: −0·01 to 0·03), 0·00 iu/ml (95% CI: −0·02 to 0·02), 0·02 iu/ml (95% CI: −0·01 to 0·04) and 0·01 iu/ml (95% CI: −0·01 to 0·04) (Fig 7; Table SI). Moreover, in patients without relevant comorbidities ($n = 234$) there was no association between age and VWF:Ag, VWF:CB, VWF:Ab or FVIII:C, with a difference per decade of 0·01 iu/ml (95% CI: −0·01 to 0·02), 0·00 iu/ml (95% CI: −0·03 to 0·03), 0·01 iu/ml (95% CI: −0·02 to 0·04) and 0·01 iu/ml (95% CI: −0·02 to 0·04), respectively (Figure S2). Blood group was not a relevant confounder in these analyses and was not different between patients with or without relevant comorbidities (66·3% vs. 69·2% blood group O, $P = 0{\cdot}547$). Nevertheless, the age-related increase in VWF and FVIII was more pronounced in patients with a non-O blood group compared to blood group O, which was only significant for VWF:CB [0·04 iu/ml vs. 0·02 iu/ml increase per decade ($P = 0{\cdot}023$)]. Even when we assessed the increase of VWF levels as a percentage of the mean VWF levels in patients with blood group O and a non-O blood group, the age-related increase of

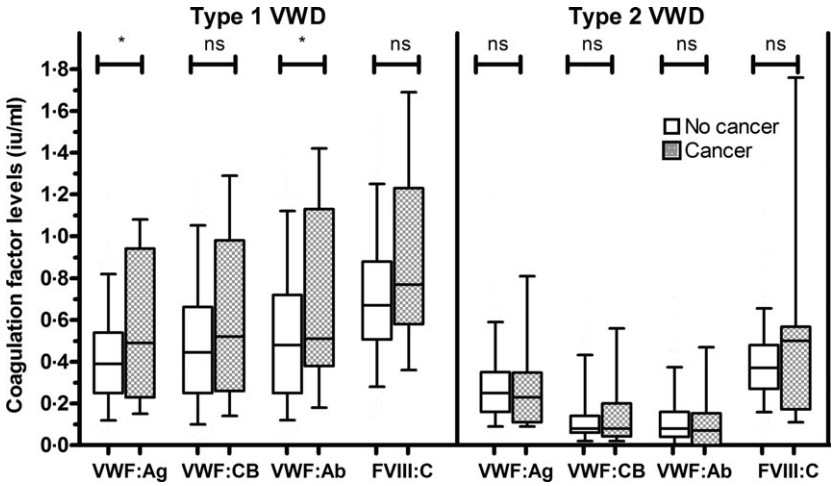

Fig 4. The association between cancer and VWF and FVIII. Number of patients with and without cancer are respectively in type 1: $n = 15$ vs. $n = 318$, type 2: $n = 8$ vs. $n = 195$. Multiple regression outcomes corrected for age, sex and blood group. Data presented as boxplots with median and interquartile ranges, and 5–95 percentiles. $^*P < 0{\cdot}05$. FVIII:C, factor VIII coagulant activity; ns, not significant; VWD, von Willebrand disease; VWF:Ab, von Willebrand factor activity as measured by a monoclonal antibody assay; VWF:Ag; von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding capacity.

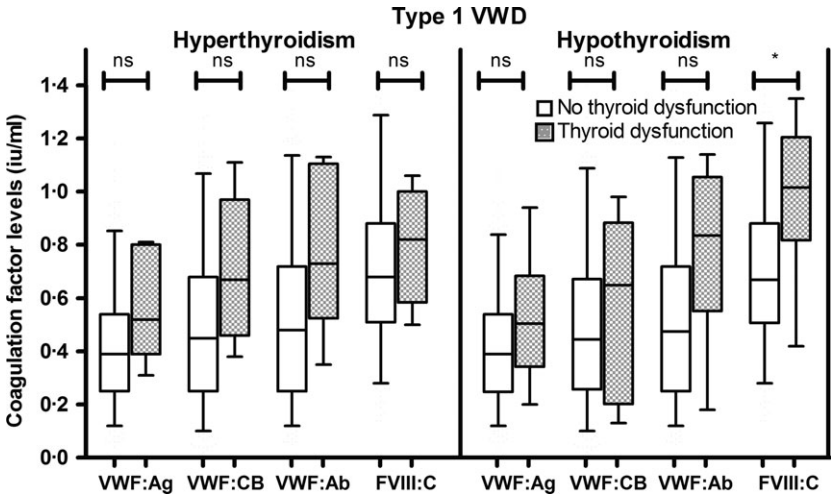

Fig 5. The association between thyroid dysfunction and VWF and FVIII. Number of patients with and without hyperthyroidism are respectively $n = 5$ vs $n = 327$. Number of patients with and without hypothyroidism are respectively $n = 10$ vs. $n = 322$. Multiple regression outcomes corrected for; age, sex, blood group and hypo- or hyperthyroidism. Data presented as boxplots with median and interquartile ranges, and 5–95 percentiles. $^*P < 0{\cdot}05$. FVIII:C, factor VIII coagulant activity; ns, not significant; VWD, von Willebrand disease; VWF:Ab, von Willebrand factor activity as measured by a monoclonal antibody assay; VWF:Ag; von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding capacity.

VWF levels was more pronounced in patients with a non-O blood group, which, for VWF:CB was 8·7% (non-O) vs. 3·5% (O) increase per decade ($P = 0{\cdot}023$). In both groups the age-related increase of levels could be explained by the presence of relevant comorbidities.

Pathophysiology of increased VWF levels

In type 1 VWD patients, no association was found between VWFpp and the presence of relevant comorbidities, 0·04 iu/ml (95% CI: −0·05 to 0·14, corrected for age, sex and blood group). The VWFpp/VWF:Ag ratio was −1·23 (95% CI: −2·01 to −0·45) lower in VWD patients with relevant comorbidities, compared to patients without comorbidities (corrected for age, sex and blood group). No association between FVIII:C/VWF:Ag ratio and relevant comorbidities was found, −0·18 iu/ml (95% CI: −0·40 to 0·04). Also no association was observed between VWFpp or the VWFpp/VWF:Ag or FVIII:C/VWF:Ag ratios and comorbidities in patients with type 2 VWD.

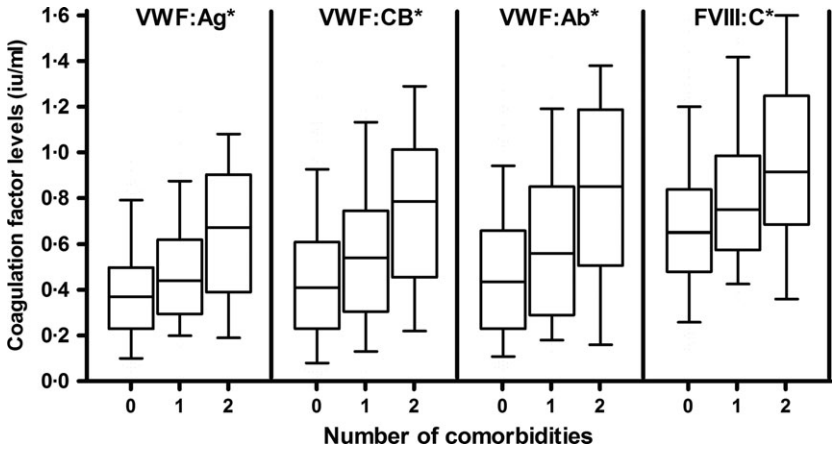

Fig 6. The number of comorbidities and VWF and FVIII levels in type 1 VWD. There were 236 patients with 0 relevant comorbidities, 77 patients with 1 relevant comorbidity and 18 patients with two relevant comorbidities. Only 2 patients had three relevant comorbidities and are excluded from the figure. Data presented as boxplots with median and interquartile ranges, and 5–95 percentiles. *$P < 0{\cdot}001$, Multiple regression outcomes corrected for age, sex and blood group. FVIII:C, factor VIII coagulant activity; VWD, von Willebrand disease; VWF:Ab, von Willebrand factor activity as measured by a monoclonal antibody assay; VWF:Ag, von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding capacity.

The effect of comorbidities on bleeding phenotype

Type 1 VWD patients with comorbidities more often had a bleeding episode in the year prior to inclusion in the study, 29·2% vs. 18·4% ($P = 0{\cdot}030$). Additionally, patients with comorbidities had a total bleeding score of 1·9 (95% CI: 0·1–3·8) higher than patients without comorbidities (corrected for age, sex and blood group). When analysed separately, patients with comorbidities had 0·6 (95% CI: 0·1–1·0) higher bleeding score for the surgery, whereas when surgery was excluded the bleeding score was not significantly different (1·4, 95% CI: −0·3 to 3·0) (both corrected for age, sex and blood group). In patients with type 2 VWD, we did not find an association between comorbidities and the frequency of bleeding in the year prior to inclusion in the study, 48·0% vs. 38·8% ($P = 0{\cdot}252$), for either the total bleeding score (0·8, 95% CI: −1·6 to 3·3), or the bleeding score for surgery (0·1, 95% CI: −0·5 to 0·7) (both corrected for age, sex and blood group).

Discussion

In this study, we demonstrate that comorbidities are associated with VWF and FVIII levels in type 1 VWD patients. VWD type 1 patients with hypertension, diabetes mellitus, cancer and thyroid dysfunction had higher VWF and/or FVIII levels than VWD patients without these disorders. In patients with type 1 VWD, these comorbidities may explain the age-related increase of VWF and FVIII levels. Despite the higher VWF and FVIII levels, patients with comorbidities had more bleeding episodes in the year prior to inclusion of the study, and had a higher total bleeding score, which was mainly caused by more bleeding during surgery. Furthermore, VWFpp/VWF:Ag ratio was lower in patients with type 1 VWD with comorbidities, suggesting there is a slower clearance of VWF in patients with comorbidities. In type 2 VWD, we did not find an association between comorbidities and VWF or FVIII levels or bleeding phenotype.

In accordance with previous population studies in individuals without VWD (Brunner *et al*, 2005; Seligman *et al*, 2000; Auwerda *et al*, 2007; Elbers *et al*, 2016; Franchini *et al*, 2013.), we found that individuals with hypertension, diabetes, cancer and hyperthyroidism had higher VWF and FVIII levels than individuals without these disorders. In our study, this was observed in both young and elderly VWD type 1 patients.

We did not expect VWD patients with hypothyroidism to have higher VWF and FVIII levels than VWD patients with normal thyroid function, as hypothyroidism is known to be associated with reduced VWF levels and may even cause acquired von Willebrand syndrome (Dalton *et al*, 1987; Stuijver *et al*, 2014). This also seems contradictory, because hyperthyroidism is known to be associated with higher VWF levels (Liu *et al*, 1993; Elbers *et al*, 2016). However, it has been shown that VWF levels can increase after treatment with levothyroxine (Rogers & Shane, 1983; Stuijver *et al*, 2014). In our study, all patients with hypothyroidism used levothyroxine. Therefore, hypothyroidism may have resulted in lower VWF levels in our population before they were treated, but this effect could have been reversed by treatment with levothyroxine. In nine of 12 patients with hypothyroidism, VWD was clearly inherited, based on family history. Of the remaining patients, one patient had type 2 VWD. Therefore, in most patients acquired VWD due to hypothyroidism could be excluded.

Our findings suggest that the association between aging and increase in VWF and FVIII levels in patients with type 1 VWD may be explained by the presence of relevant comorbidities.

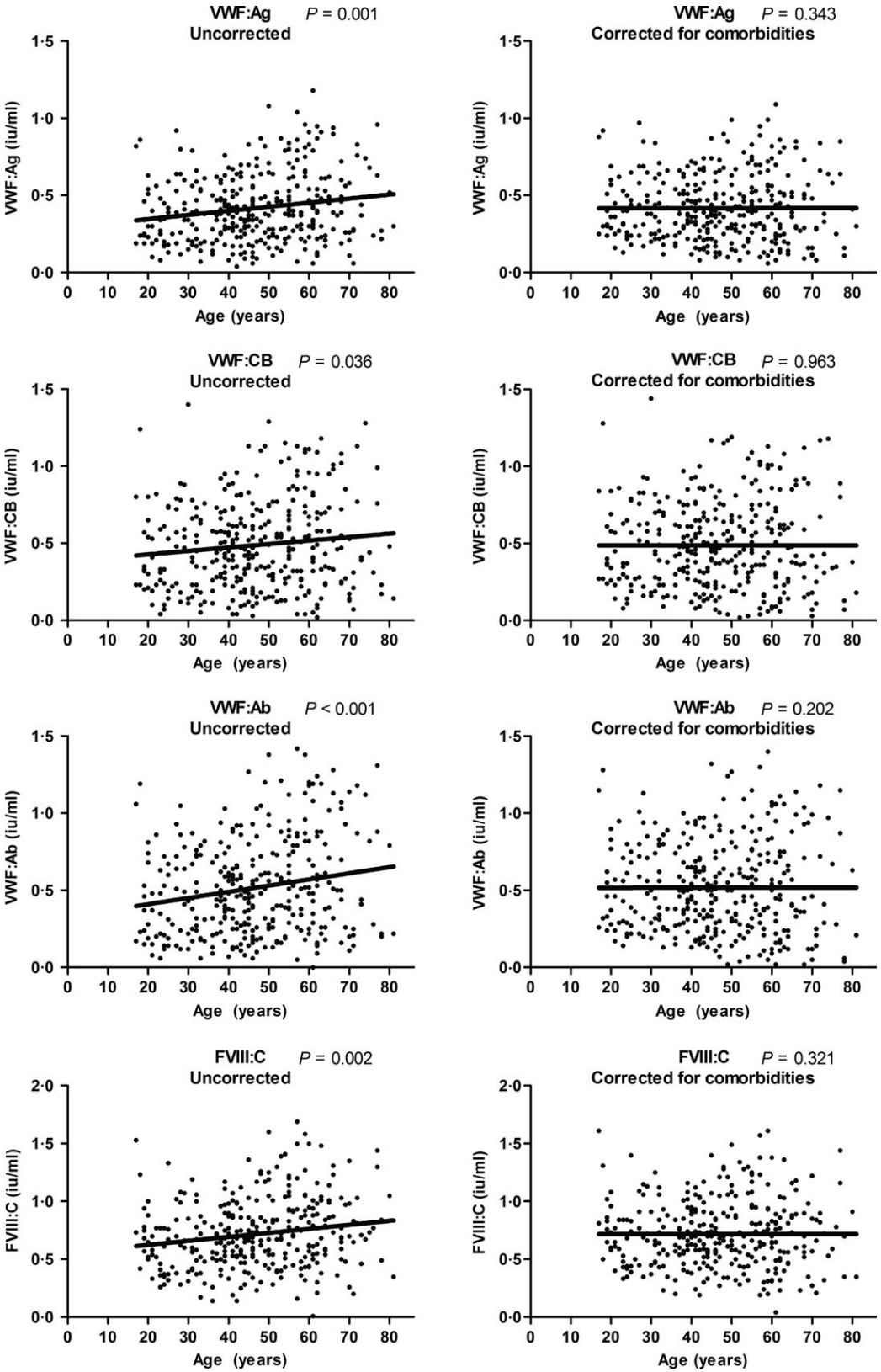

Fig 7. The effect of comorbidities on the age-related increase of VWF and FVIII levels in type 1 VWD. FVIII:C, factor VIII coagulant activity; VWD, von Willebrand disease; VWF:Ab, von Willebrand factor activity as measured by a monoclonal antibody assay; VWF:Ag, von Willebrand factor antigen; VWF:CB, von Willebrand factor collagen binding capacity.

The age-related increase of VWF and FVIII levels was demonstrated previously in several studies. However, many of these reports did not take comorbidities into account (Favaloro *et al*, 2005; Tofler *et al*, 2005; Vischer *et al*, 2005; Miesbach & Berntorp, 2011; Sanders *et al*, 2014; Rydz *et al*, 2015; Borghi *et al*, 2017; Abou-Ismail *et al*, 2018). Gill *et al* (1987) found that medication use was associated with higher VWF:Ag in VWD patients with blood group A. This could have been caused by the comorbidities, i.e. hypertension, for which the medication was used, as many patients (43%) used an anti-hypertensive drug. In a well-designed study, Coppola *et al* (2003) found an age-related increase of VWF:Ag in 74 healthy centenarians and 110 healthy controls younger than 82 years (range 21–86). However, they defined "healthy" based on the Senieur Protocol in which some disorders, including thyroid dysfunction, is defined as healthy (Ligthart *et al*, 1984). Moreover, although centenarians might not have a disorder, the combination of reduced function in several organ could have led to an increase of VWF or FVIII levels in a similar pattern as in patients with relevant comorbidities. Albanez *et al* (2016) found that the age-related increase of VWF and FVIII is strongest in patients with non-O blood group. Also, in our population, the age-related increase in VWF and FVIII levels seemed to be stronger in patients with non-O blood group. However, the association with comorbidities was independent of the blood group. Furthermore, there was no association between the previously reported *CLEC4M* and *STXBP5* mutations and the effect of comorbidities on VWF levels (data not shown) (Sanders *et al*, 2015b).

In type 1 VWD, the VWFpp/VWF:Ag ratio was lower in patients with relevant comorbidities, whereas there was no association with VWFpp or FVIII:C/VWF:Ag ratio. This finding is quite surprising, because we expected that VWF and FVIII levels rise in patients with comorbidities due to more endothelial VWF release. However, our results suggest that patients with comorbidities have higher VWF and FVIII levels due to a slower clearance of VWF, and that therefore VWF remains for a longer period in the circulation (Eikenboom *et al*, 2013). Potentially, low-density lipoprotein receptor-related protein-1 (LRP1), which has a significant role in VWF clearance, could have an important role in understanding this association, because LRP1 alterations are associated with several disorders, including cancer, hypertension and inflammation (Sendra *et al*, 2008; Gonias & Campana, 2014; Lenting *et al*, 2015).

Despite the higher VWF and FVIII levels, type 1 VWD patients with comorbidities presented with more bleeding episodes than patients without comorbidities. In particular, these patients experienced more perioperative bleeding. This may be caused by the fact that patients with type 1 VWD in whom VWF levels are normalized due to an age-related increase of VWF, may have been incorrectly less often prophylactically treated perioperatively, however we do not have detailed information on perioperative treatment in these individuals. Moreover, it is expected that patients with comorbidities more often undergo surgical interventions, such as surgical removal of tumours in patients with cancer or tissue removal in patients with a diabetic foot. Prospective studies are needed to further clarify the association between comorbidities and perioperative bleeding.

The primary strength of this study is the large number of patients with VWD that enabled us to assess the role of several comorbidities on VWF and FVIII levels. Therefore, we could correct the multiple regression models for various relevant confounders. We also included use of relevant medication, such as anti-hypertensive drugs and statins in the analyses. Additionally, we assessed the role of comorbidities on bleeding phenotype.

The most important limitation of this study is that we did not longitudinally asses VWF levels in patients, therefore we could not assess the association between comorbidities and the age-related increase of VWF levels intra-individually. In addition, the data on comorbidities is patient-reported. However, we also obtained information on medication use and, in most patients, the use of medication confirmed the presence of the patient-reported comorbidities. Moreover, we previously evaluated the medical records of patients that reported arterial thrombotic events. All patient-reported diagnosis were confirmed and no other arterial thrombotic events were found in a random sample of 237 VWD patients (Sanders *et al*, 2013). Furthermore, although the total sample size is large, for hyper- and hypothyroidism the numbers of patients were small. Therefore, for these comorbidities the analysis could potentially be overfitted. Another limitation of this study is that the bleeding phenotype was retrospectively assessed, which is less accurate than an assessment at diagnosis, for which it was originally developed. Furthermore, we do not have detailed information on comorbidity-related measurements, such as the blood pressure and HbA1c. However, this study aimed to assess the influence of comorbidities on VWF and FVIII levels, independent of disease control. To validate our findings a prospective study should be performed with measurement of VWF and FVIII levels over time in individuals who develop comorbidities during follow-up.

In conclusion, this is the first study on the association between comorbidities and VWF and FVIII levels in patients with VWD. Type 1 VWD patients with hypertension, diabetes mellitus, cancer and thyroid dysfunction had higher VWF and/or FVIII levels than VWD patients without these disorders. Comorbidities may explain the age-related increase of VWF and FVIII levels in patients with type 1 VWD. Despite the higher VWF and FVIII levels, patients with comorbidities had more bleeding episodes, particularly bleeding during surgery.

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Authorship

Contribution: FA designed the study, performed statistical analyses, interpreted data, and wrote the manuscript. JE, KF, EPM, KM, JM, BAPL, JGB and MHC designed the study, interpreted data and critically revised the manuscript. MPM participated in data statistical analyses, interpreted data and critically revised the manuscript. FWGL conceived and designed the study, interpreted data, and critically revised the manuscript. All authors gave their consent to the final version of the manuscript.

Conflict of Interest

F.W.G. Leebeek received research support from CSL Behring and Shire for performing the Willebrand in the Netherlands (WiN) study, and is consultant for uniQure, Novo Nordisk and Shire, of which the fees go to the institution. *J. Eikenboom* received research support from CSL Behring and he has been a teacher on educational activities of Roche. *K. P. M. van Galen* received unrestricted research support from CSL Behring and Bayer. *E.P. Mauser-Bunschoten* received unrestricted research/ educational support from CSL Behring, Bayer, Baxter, Griffols, Novo Nordisk, Pfizer, Biotest and Sanquin. *J.G. van der Bom* has received unrestricted research/ educational funding for various projects from the following companies: Bayer Schering Pharma, Baxter, CSL Behring, Novo Nordisk, and Pfizer. In addition, she has been a consultant to Baxter and Pfizer, and she has been a teacher on educational activities of Bayer Schering Pharma. *M.H. Cnossen* has received unrestricted research/educational and travel funding from the following companies: Pfizer, Baxter, Bayer Schering Pharma, CSL Behring, Novo Nordisk and Novartis, and serves as a member on steering boards of Roche and Bayer of which fees go to the institution. *K. Fijnvandraat* is a member of the European Haemophilia Treatment and Standardization Board sponsored by Baxter, has received unrestricted research grants from CSL Behring and Bayer, and has given lectures at educational symposiums organized by Pfizer, Bayer and Baxter. *K. Meijer* received research support from Bayer, Sanquin and Pfizer; speaker fees from Bayer, Sanquin, Boehringer Ingelheim, BMS and Aspen; consulting fees from Uniqure. *B. Laros-van Gorkom* has received unrestricted educational grants from Baxter and CSL Behring. None of the other authors has a conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (A) The association between hypertension and VWF and FVIII in patients younger than 65. (B) The association between hypertension and VWF and FVIII in patients aging 65 years or older.

Figure S2. No association between age and VWF and FVIII levels in patients without relevant comorbidities ($n = 234$).

Table SI. Multiple regression outcomes presented in a tabular form.

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