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Increasing levels of von Willebrand factor and factor VIII with age in patients affected by von Willebrand disease

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Abstract

Background: Increasing levels of von Willebrand factor (VWF) and factor VIII (FVIII:C) was associated with age in type 1 von Willebrand disease (VWD).

Objectives: To evaluate VWF and FVIII:C increase with age in a large group of patients with VWD and low levels of VWF, in whom levels were repeatedly measured.

Methods: Clinical charts from all patients evaluated at the A. Bianchi Bonomi Center between 1970 and 2018 were reviewed and data on VWF and FVIII:C collected. Patients affected by type 3, severe type 1 and 2N VWD were excluded. The repeated measurements were evaluated by linear mixed-effects models. A linear association between age and VWF/FVIII:C was shown after the age of 40 years in the linear mixed models and analyzed by calculating the regression slope coefficient (β).

Results: A total of 617 patients were included in the study (314 type 2, 112 type 1, 181 low VWF levels), with a median age at first measurement of 28 years (interquartile range 14/42) and a mean follow-up of 16 years (standard deviation 11). VWF and FVIII:C increased with age in the whole group. The increase became linear after the age of 40 years (3.68 and 7.44 IU/dL per decade for VWF:activity and FVIII:C). In type 2, FVIII:C increased with age, whereas an increase of both VWF:activity and FVIII:C were shown in patients with type 1 VWD and low levels of VWF.

Conclusions: A differential increase of VWF and FVIII:C with age was shown among in different ages and types of VWD.

KEYWORDS

age groups, coagulation factors, coagulation factor VIII, von Willebrand disease, von Willebrand protein

1 | INTRODUCTION

Von Willebrand factor (VWF) is a multimeric glycoprotein involved in primary and secondary hemostasis by mediating platelet adhesion and aggregation in the presence of high shear stress. It circulates in association with factor VIII (FVIII), shielding it from premature inactivation and clearance.[1] VWF levels in plasma are regulated by complex mechanisms that encompass both its secretion and clearance.[1,2] Higher levels of VWF are present in blood group non-O than in blood group O. VWF levels in normal individuals with blood group O overlap with VWF levels in patients with low levels of VWF or mild type 1 von Willebrand disease (VWD), making the diagnosis of type 1 VWD particularly challenging.[3,4] Indeed, a large study in

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monozygotic and dizygotic twin pairs found that 66% of VWF total variation in plasma was due to genetics, and 30% of it was ascribed to ABO blood group.[5] Several physiological and pathological conditions have been associated with increased levels of VWF, such as age, pregnancy, postpartum, oral contraceptive, inflammation, and cancer.[1] In particular, the effect of age on VWF and FVIII levels in normal individuals was shown in large studies, such as the Atherosclerosis Risk in Communities cohort study and the Glasgow MONICA survey.[6,7]

The complex regulation of VWF levels in plasma and the high variability of its levels across blood groups, age groups, and different physiological conditions make the diagnosis of mild forms of VWD difficult.[3,8] The ISTH classification update, published in 2006, distinguishes quantitative and qualitative defects of VWF as causes of VWD.[9] The quantitative defects are type 3 VWD (characterized by undetectable levels of VWF in plasma and platelets, and inherited with a recessive pattern) and type 1 VWD (with a variable reduction of VWF, normal activity/antigen ratio of VWF, normal or slightly altered multimer profile, and usually inherited with a dominant pattern). The qualitative defects, characterized by a decreased activity/antigen ratio, are called type 2 and are further divided in type 2A (with loss of high molecular weight multimers), type 2B (with increased affinity of VWF to platelet glycoprotein Ibα), type 2M (with normal multimeric pattern), and type 2N (decreased binding affinity of VWF to FVIII). The classification of VWD clearly recognized the challenge of type 1 VWD diagnosis, but does not state a threshold for the diagnosis of type 1 VWD and low levels of VWF. Current guidelines[10] and practice[11] use a cutoff of 30 IU/dL to discriminate between type 1 VWD and low normal VWF, which remains a risk factor for bleeding, and needs therefore to be treated accordingly.

The challenge of VWD diagnosis is further increased by the complexity of some of the assays needed for classification of the disease, such as the evaluation of the multimer profile or the ristocetin-induced platelet agglutination assay.[12]

The effect of age on VWF and FVIII plasma levels in patients affected by VWD is interesting for two reasons. First, it poses an additional difficulty in the diagnosis of mild forms of the disease and, second, it is a possible modifier of the clinical bleeding phenotype during life.

The aim of our study was to evaluate the association between age and VWF and FVIII levels in a large group of Italian patients affected by VWD and low levels of VWF, referred to a single center during a timeframe of almost 50 years.

Essentials

- VWF and FVIII increase with age in patients affected by VWD.
- VWF and FVIII increase in type 1 and in low levels of VWF patients.
- VWF and FVIII do not increase in type 1 Vicenza.
- FVIII increases in type 2 VWD patients.

2 | MATERIAL AND METHODS

2.1 | Participants

All patients evaluated at the A. Bianchi Bonomi Hemophilia and Thrombosis Center between 1970 and 2018 with a diagnosis of VWD or low levels of VWF were included. Low levels of VWF was defined as VWF:antigen (VWF:Ag) and VWF:ristocetin cofactor (VWF:RCo) between 30% and 50% at diagnosis. Clinical chart review yielded data on FVIII:C, VWF:Ag, and VWF:RCo for 736 patients. Exclusion criteria were type 3 VWD (n = 33), severe type 1 VWD (defined as VWF:Ag and FVIII:C < 5 IU/dL, n = 6), and VWD type 2N (n = 14), as well as carriership of type 3 (n = 35) and 2N (N = 24). Because VWF:Ag and VWF:RCo served to evaluate the association between VWF levels and age, 11 further patients with type 2 VWD were excluded because they had normal levels of VWF:Rco (eight patients with type 2B mutation p.Ile1309Val[13] and three patients with type 2M mutation p.Ser1731Thr, which affects only VWF collagen binding tests reported in the EAHAD coagulation factor variant database, http://dbs.eahad.org/, accessed July 2020). Further exclusions were patients with incomplete data (missing date of birth, date of measurement, or incomplete diagnostic workup, n = 23), leaving 617 patients for analysis.

Clinical chart review also provided information on comorbidities (high blood pressure, diabetes, or cancer), present at the time of FVIII and VWF measurements. Levels of FVIII and VWF were measured during follow-up visits or before surgery. Measurements during pregnancy, puerperium, bleeding episodes, after surgeries, or treatment with desmopressin and VWF/FVIII concentrate were excluded. The interval between measurements was not fixed.

2.2 | Laboratory measurements

All measurements were collected from the clinical charts and were performed in a timeframe of 48 years. FVIII:C was invariably measured with a one-stage assay, with various partial thromboplastin time reagents. The assay was first performed manually and subsequently on automated coagulometers.

VWF:Ag was measured with immunoradiometric assay[14] and electro-immunodiffusion assay[15] before June 15, 1989, then with an ELISA[16] until September 30, 2008, and subsequently with an immunoturbidimetric assay, which uses latex particles coated with a rabbit polyclonal antibody specific for human VWF.[17] VWF:Ag was measured by enzyme immunoassay/immunoradiometric assay in 189 samples (7%), by ELISA in 1217 (48%) and by immunoturbidometric assay in 1146 (45%).

VWF platelet dependent activity[18] was measured as VWF:RCo using aggregometry with formalin fixed platelets and ristocetin until October 15, 2002,[14] eventually modified with the use of an automated

coagulometer,[19] until January 31, 2011. Subsequently, an automated system using latex beads and ACL-TOP coagulometer[20] was used to measure the VWF activity until January 15, 2012 (VWF:Ab method, which uses latex beads coated with a monoclonal antibody directed against a VWF A1 domain epitope, HemosIL von Willebrand Factor Activity), and finally an automated method that uses the coagulometer, ristocetin, and latex beads coated with recombinant glycoprotein Ib (VWF:GpIbR, HemosIL von Willebrand Factor Ristocetin Cofactor Activity).[21] VWF platelet-dependent activity was measured by aggregometry in 790 samples (31%), modified aggregometry in 823 (32%), VWF:Ab in 105 (4%), and VWF:GPIbR in 834 (33%).

2.3 | Statistical methods

Categorical data are presented as frequencies and percentages, and continuous data with medians and interquartile ranges.

FVIII:C and VWF distributions were visually inspected finding a normal distribution for FVIII:C and a slightly skewed distribution for VWF, as expected in a population of patients affected by VWD; no transformations were deemed necessary.

Spaghetti plots of a random sample of 125 patients were prepared to visually evaluate the trend of VWF:Ag, VWF activity, and FVIII:C in the follow-up of single individuals.

To analyze the association between age and levels of VWF and FVIII:C, we used linear mixed models, in which the fixed effect of age was modeled using restricted cubic splines, and a random intercept and slope for age were used to account for the repeated measurements per individuals. Both an unadjusted analysis and an analysis adjusted for the following parameters: age at first measurement, sex, blood group (O or non-O), presence of comorbidities (high blood pressure, diabetes, cancer), and test type (in case of VWF:Ag and activity) were performed. In particular, regarding age at first measurement, this could be a confounder because we can expect that patients diagnosed later in life would have a milder disease and higher levels of VWF. Potential effect modification by VWD type (low levels of VWF, type 1, type 1 Vicenza, or type 2) was analyzed by adding an interaction term between age and VWD type to the model.

Because after the age of 40 years, a linear association between VWF/FVIII levels and age was shown in the linear mixed models, we refitted the model using only measurements obtained after the age of 40 years, with age as a linear covariate and calculated the regression slope β coefficient per decade and its 95% confidence intervals (CI).

The statistical analysis was performed using STATA statistical software, version 16.

3 | RESULTS

The study population is shown in Figure 1. The diagnoses were 181 affected by low levels of VWF, 81 by type 1 VWD, 31 by type 1 Vicenza, 314 by type 2 VWD (134 type 2A, 67 type 2B, and 116 type 2M), and 10 unclassified patients. Patients characteristics at diagnosis are reported in Table 1.

Female sex was more often present in low levels of VWF and type 1 VWD (respectively 65% and 63%), than for type 2 VWD (52%). Blood group O was also more present in patients affected by low levels of VWF (77%) and type 1 VWD (70%), than for type 2 VWD (53%). Comorbidities (cancer, diabetes, and high blood pressure) were present in 22% of the whole population, at any time during follow-up.

The mean follow-up time of patients was 16 years (standard deviation [SD] 11). Patients had on average 3 measurements over time (range 1-6). The median age was 28 years (interquartile range 14-42) at first measurement, which was similar in patients with low levels of VWF, type 1, and type 2 VWD.

At first measurement, mean levels of VWF activity were 23 IU/dL (SD 14), with lower levels in type 1 (20 IU/dL, SD 11), type 1 Vicenza (8 IU/dL, SD 5), and type 2 VWD (15 IU/dL, SD 16), than in the patients with low levels of VWF (42 IU/dL, SD 8). Mean FVIII:C was 55 IU/dL (SD 24) in the whole group of patients, and lower levels were found in type 1 (47 IU/dL, SD 26), type 1 Vicenza (30 IU/dL, SD 22) and type 2 VWD (52 IU/dL), than in the group with low levels of VWF (68 IU/dL).

Spaghetti plots representing VWF:Ag, VWF activity, and FVIII:C trends during follow-up were drawn in 125 random patients with repeated measurements (Figure S1), showing an increase of levels with age, albeit with a large variability between individuals. Spaghetti plots of 46 patients with a prolonged follow-up (>20 years) from the same group of random patients were drawn separately (Figure S2).

Figure 2 shows the increase of VWF:Ag, VWF activity, and FVIII:C with age (unadjusted model and model adjusted for age at first measurement, sex, blood group, comorbidities, and test type). A mild increase was shown from the age of 20 years, and it became linear after the age of 40 years. In younger patients (<20 years), we did not observe an increase of levels with age, but this group of patients was small resulting in larger CIs. The four types of VWD presented, after the age of 40, a different steepness in their increase curves for all parameters (Figures 3-5). Table 2 shows the results of the analysis after the age of 40 years: the regression coefficients per decade (and 95% CI) of the unadjusted models are shown together with those obtained after adjustment for age at diagnosis, sex, blood group, comorbidities, and assay (singularly or together in the full model). The results of the single adjustments are shown in the Table S1. Patients affected by low levels of VWF presented the largest increase for all parameters; which was, in the linear part of the curve, after the age of 40 years for VWF activity 5.00 IU/dL per decade (unadjusted, 95% CI 2.11-7.89) and 5.02 IU/dL (full model, 95% CI 0.54-9.51), and for FVIII:C 7.62 IU/dL per decade (unadjusted, 95% CI 4.22-11.02) and 6.71 IU/dL (full model, 95% CI 2.67-10.74).

The increase per decade after the age of 40 years, in patients affected by type 1 VWD, was, for VWF activity 2.92 IU/dL (unadjusted, 95% CI −0.61 to 6.45) and 3.95 IU/dL (full model, 95% CI −1.42 to 9.32), and for FVIII:C 3.42 IU/dL (unadjusted, 95%

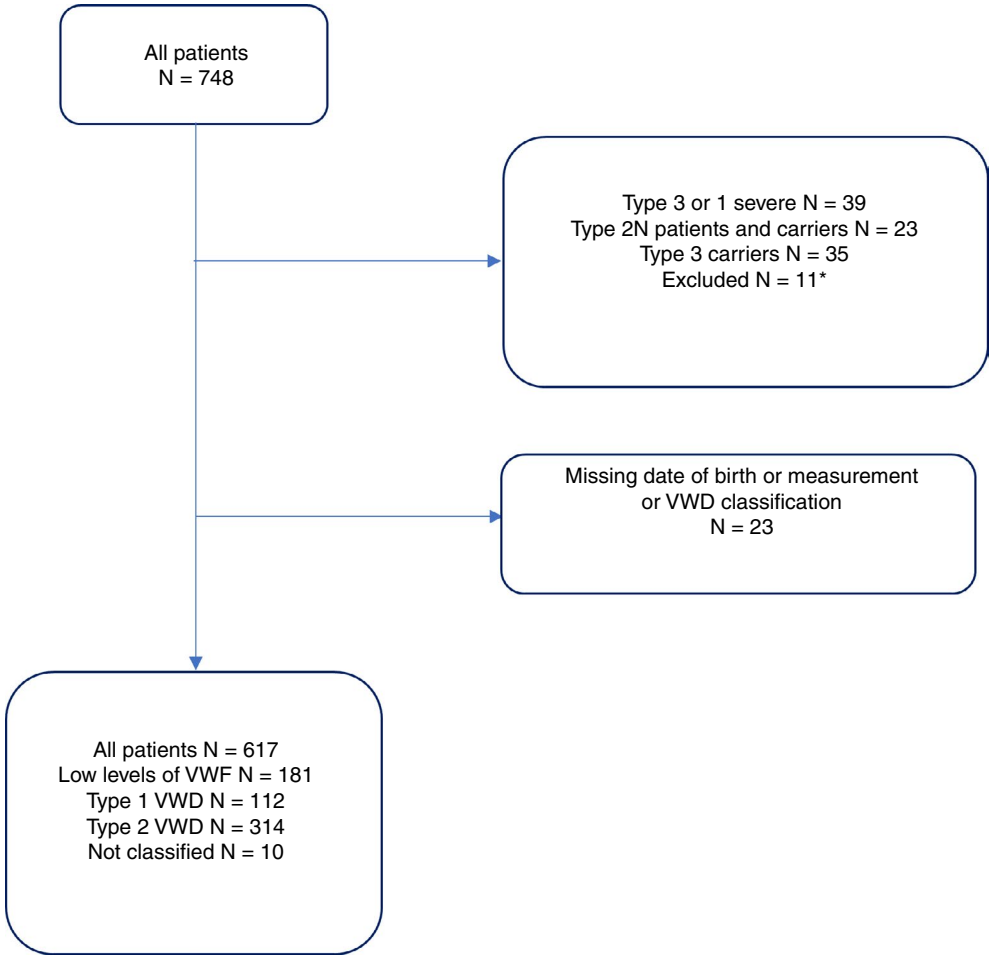

FIGURE 1 Study flow-chart.
*Excluded: eight patients with type 2B mutation p.Ile1309Val associated with normal levels of VWF (previously published) and three patients with type 2M mutation p.Ser1731Thr (that affects only VWF collagen binding test)

TABLE 1 Patient characteristics

	All (n = 617)	Low Levels VWF (n = 181)	Type 1 VWD (n = 81)	Type 1 Vicenza (n = 31)	Type 2 VWD (n = 314)
Sex female, no. (%)	354 (57)	117 (65)	51 (63)	17 (55)	163 (52)
Blood group 0, no. (%)	265 (63)	106 (77)	37 (70)	10 (48)	109 (53)
Age at first measurement, y, median (interquartile range)	28 (14-42)	27 (18-40)	26 (14-37)	26 (12-41)	30 (11-46)
VWF antigen at first measurement, IU/dL, mean (SD)	39 (23)	47 (13)	27 (13)	15 (16)	40 (26)
VWF activity at first measurement, IU/dL, mean (SD)	23 (18)	42 (8)	20 (11)	8 (5)	15 (16)
FVIII:C at first measurement, IU/dL, mean (SD)	55 (24)	68 (21)	47 (26)	30 (22)	52 (21)
Time between first and last measurement, y, mean (SD)	16 (11)	15 (9)	15 (10)	15 (11)	17 (12)
No. of repeated measurements, median (IQ range)	3 (1-6)	2 (1-5)	2 (1-5)	4 (1-6)	3 (1-7)
Comorbidities (cancer, diabetes, high blood pressure), no. (%)	82 (22)	17 (9)	9 (11)	8 (26)	48 (23)

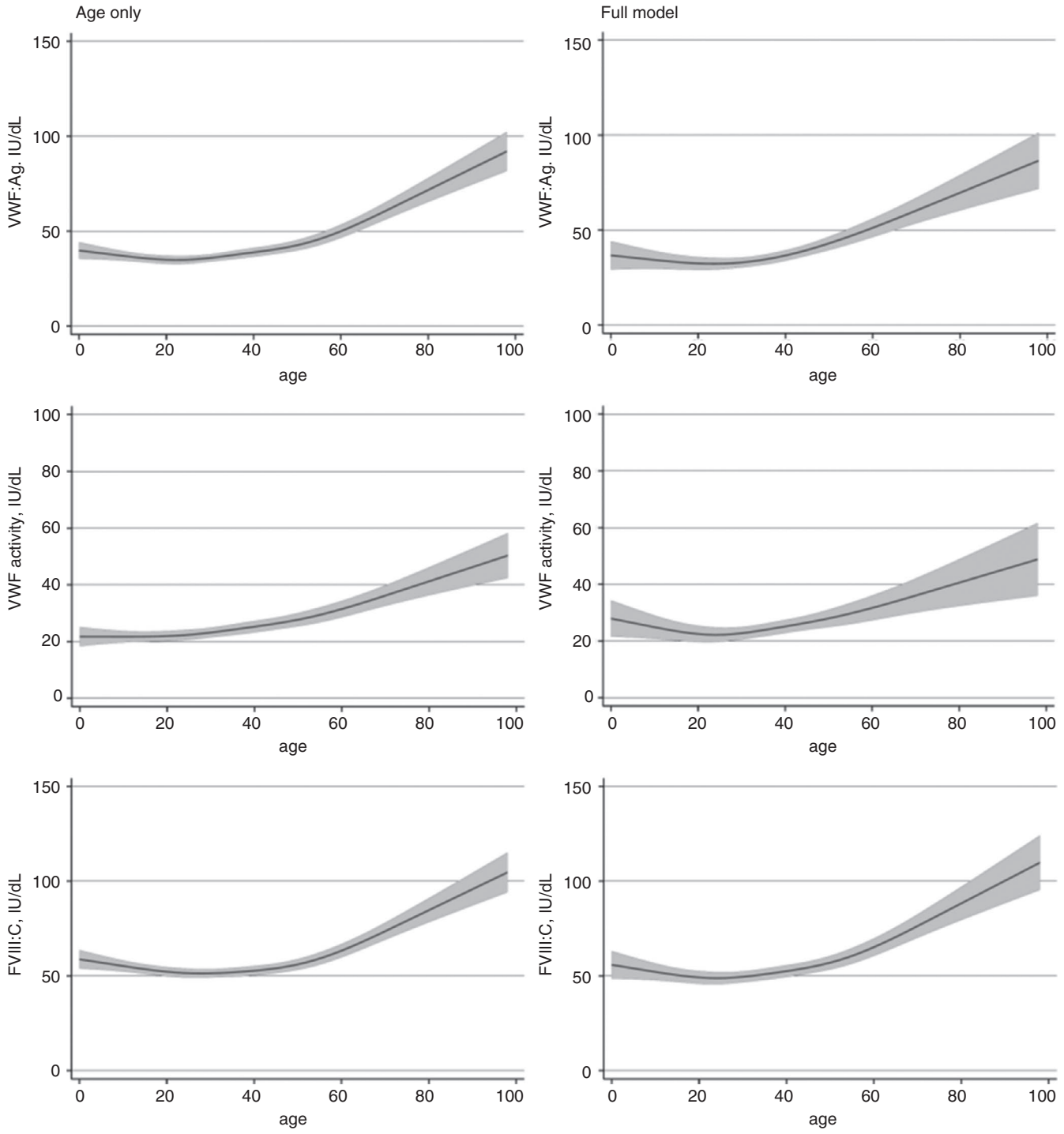

FIGURE 2 Increase with age of VWF:Ag, VWF platelet-dependent activity, and FVIII:C in the whole population. The unadjusted model is shown on the left side of the panel; on the right side, the full model is shown (adjusted for age at diagnosis, sex, blood group, comorbidities, and test type)

CI −0.33 to 7.17) and 5.22 IU/dL (full model, 95% CI 0.93-9.52). Finally, in patients affected by type 2 VWD an increase per decade after the age of 40 years, was shown for FVIII:C: 7.04 IU/dL (in the unadjusted model, 95% CI 5.11-8.97) and 8.21 IU/dL (full model, 95% CI 5.91-10.52) and for VWF:Ag (4.33 IU/dL in the unadjusted model, 95% CI 1.89-6.77), and 7.11 IU/dL (full model, 95% CI 5.91-10.52).

4 | DISCUSSION

In our study, a mild increase of VWF:Ag, activity, and FVIII was shown in the whole group of patients after the age of 20 years. A linear increase was shown after the age of 40 years. The differential increase we observed before and after the age of 40 years could be due to physiological and pathological changes (such as menopause in

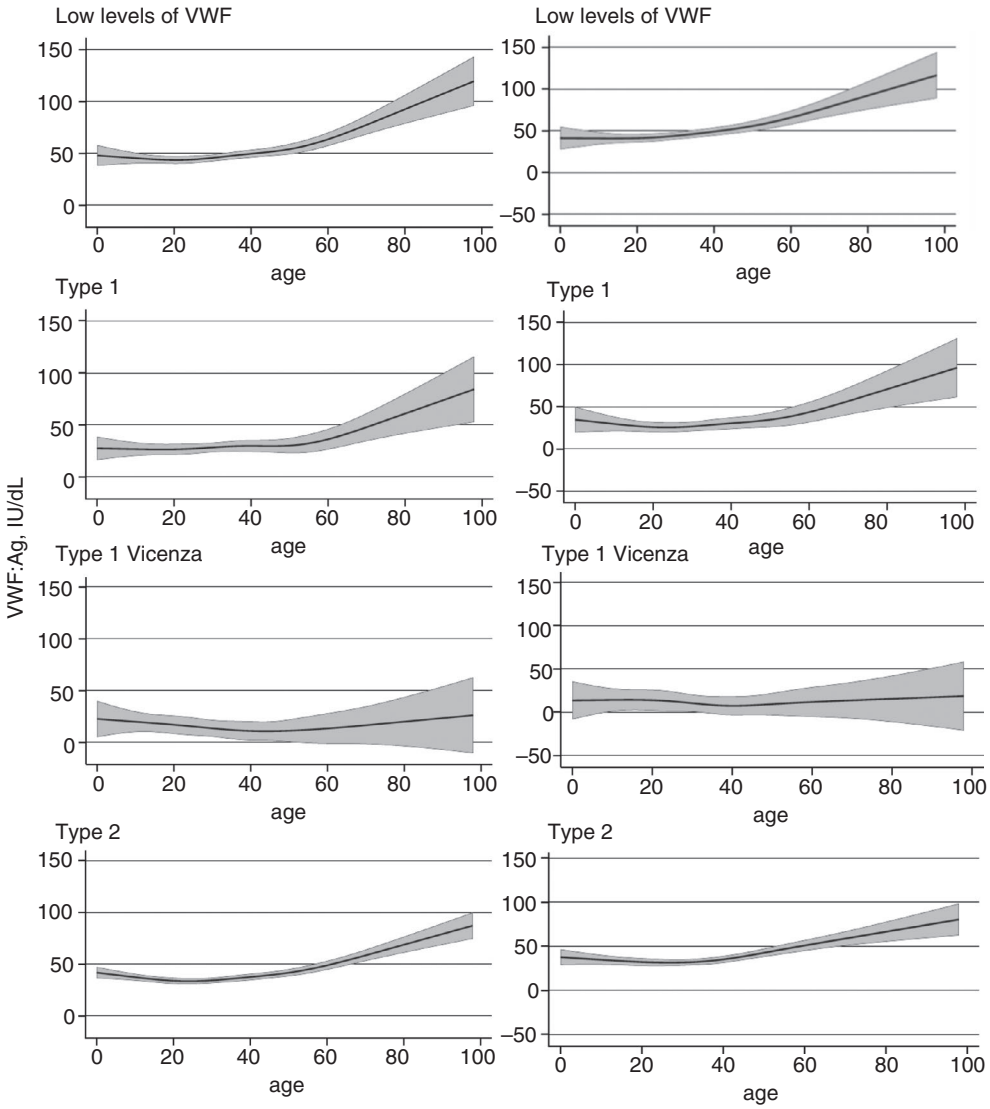

FIGURE 3 Increase with age of VWF:Ag in low levels of VWF, type 1, type 1 Vicenza, and type 2 VWD. The unadjusted model is shown on the left side of the panel; on the right side, the full model is shown (adjusted for age at diagnosis, sex, blood group, comorbidities, and test type)

women or decreased renal function). When we analyzed our data in subgroups, patients affected by low levels of VWF and type 1 VWD showed an increase of VWF:Ag, activity, and FVIII:C, while the small group of patients affected by type 1 Vicenza VWD did not show an increase of VWF and FVIII. Finally, patients affected by type 2 showed an increase of FVIII:C and VWF:Ag, but not of VWF activity. In type 2 VWD patients, the increase of FVIII:C was particularly important, comparable with that seen in patients affected by low levels of VWF and type 1 VWD.

Our results confirm previous studies.[22-25] A Dutch cross-sectional study on VWD (WIN-study, enrolling 804 patients, all characterized by historical levels of VWF ≤ 30 IU/dL or FVIII:C ≤ 40 IU/dL) with centrally measured FVIII:C and VWF activity (VWF:Ab),[22] found an increase per decade of 4.1 IU/dL (95% CI 2-6.3) and 3.7 (95% CI 1.6-5.9) for VWF:Ab and FVIII:C, respectively, in patients affected by type 1 VWD. In patients affected by type 2 VWD, no increase of VWF:Ab was shown (β 1.0 IU/dL per decade, 95% CI −0.6 to 2.6), whereas FVIII:C increase was 2.1 IU/dL per decade (95% CI 0.2-4.0). Another study by Rydz et al[23] described 31 patients older than 30 years affected by type 1 VWD (with VWF:Ag and/or VWF:RCo < 50 IU/dL), with at least two measurements of FVIII and VWF (collected at least 5 years apart). More than 50% of this group showed a normalization of FVIII and VWF levels with age and the increase per decade was 20 IU/dL (95% CI 13-27) for VWF:RCo and 20 (95% CI 11-29) for FVIII:C. Similar increases were described by Abou-Ismail et al[24] in 126 patients affected by type 1 VWD or low VWF, with at least two repeated measurements and 5 years of follow-up (β coefficient per decade 24 IU/dL for VWF:RCo and 14 IU/dL for FVIII). An Italian study[25] of 195 patients with repeated measurements, affected by type 1 VWD or low VWF, found a lower increase (β coefficient per decade 4.7 IU/dL for VWF:RCo and 10.2 IU/dL for FVIII:C). Unfortunately, a direct comparison between the studies is not feasible because of the differences in the enrolment criteria (different VWF cutoff

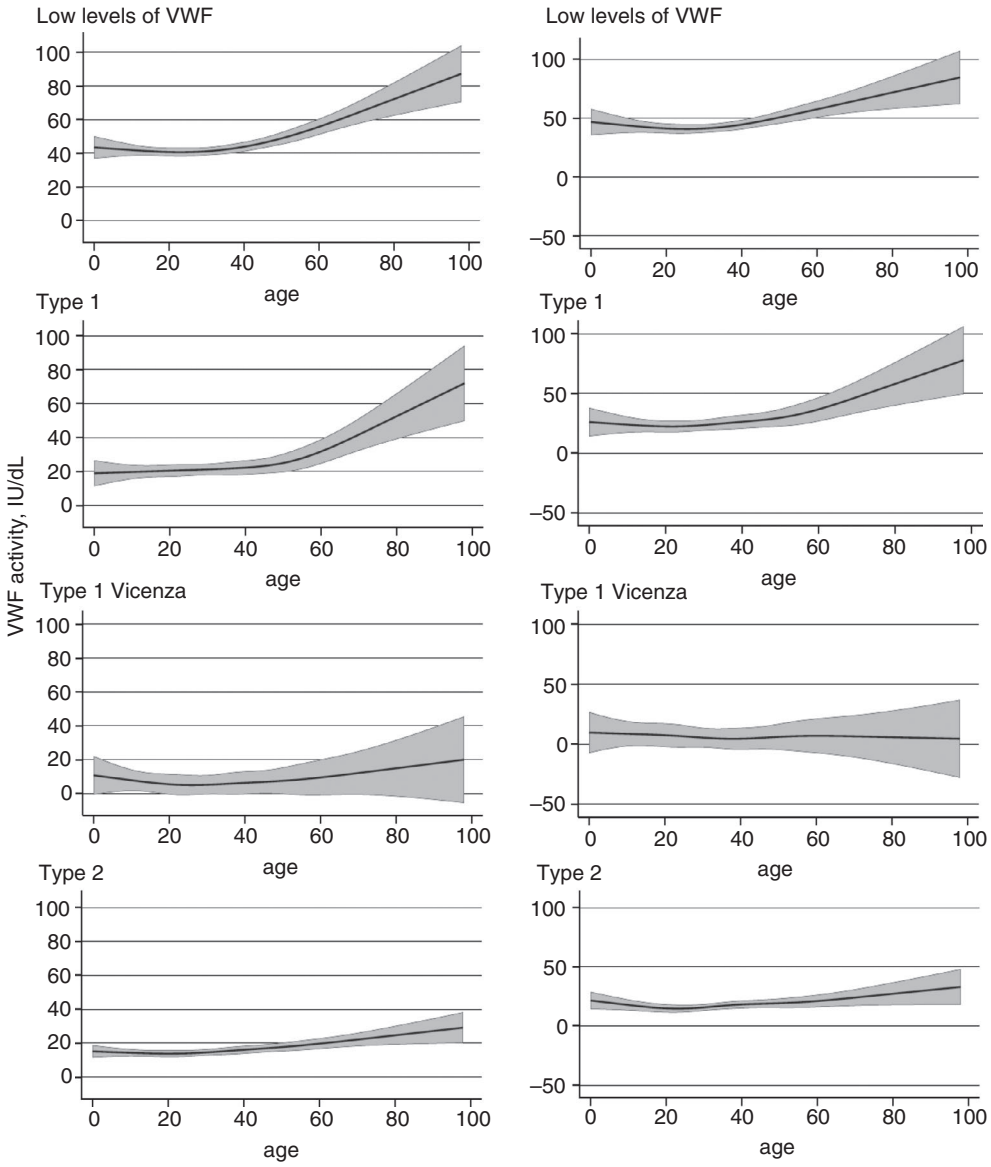

FIGURE 4 Increase with age of VWF platelet dependent activity in low levels of VWF, type 1, type 1 Vicenza, and type 2 VWD. The unadjusted model is shown on the left side of the panel; on the right side, the full model is shown (adjusted for age at diagnosis, sex, blood group, comorbidities, and test type)

were used, so that some studies enrolled also patients affected by low VWF), in the mean age of patients (that we showed being relevant, with the use of restricted cubic splines), in the type of study (cross-sectional with centralized laboratory evaluation or longitudinal with repeated measurements), in the type of endpoint (increase per decade of VWF or normalization of the levels), and in the type of assay used to measure the VWF activity endpoint (VWF:Ab, VWF:RCo, VWF:GpIbR). In our study, we did not evaluate a possible normalization of levels (VWF activity and FVIII:C > 50 IU/dL), described by some authors[23,24] because normal ranges vary with age and blood group (O or non-O); for this reason, we preferred to evaluate only the increase of factors with age.

We could calculate a β regression coefficient, after the age of 40 years, when the association became linear. We found a differential increase of VWF activity in the 4 groups of patients, highest in patients affected by low levels of VWF and absent in patients with type 2 VWD (low levels of VWF adjusted β coefficient 4.85, 95% CI 0.40-9.30; type 2 adjusted β coefficient 1.10, 95% CI −2.25 to 4.45). An increase of FVIII:C with age was observed in all patient groups except type 1 Vicenza, with the highest increase observed in type 2 VWD. Type 2 patients showed an increase of VWF:Ag but not of VWF activity. This could be due to lower baseline levels of VWF activity compared with the antigen because a relative increase is shown for both parameters.

The differential increase of VWF activity and FVIII:C with age in different patient subgroups is clinically relevant, in particular regarding the choice of treatment in case of bleeding or in case of interventions that need a prophylactic treatment. Different concentrates (recombinant and plasma-derived concentrate containing only VWF,

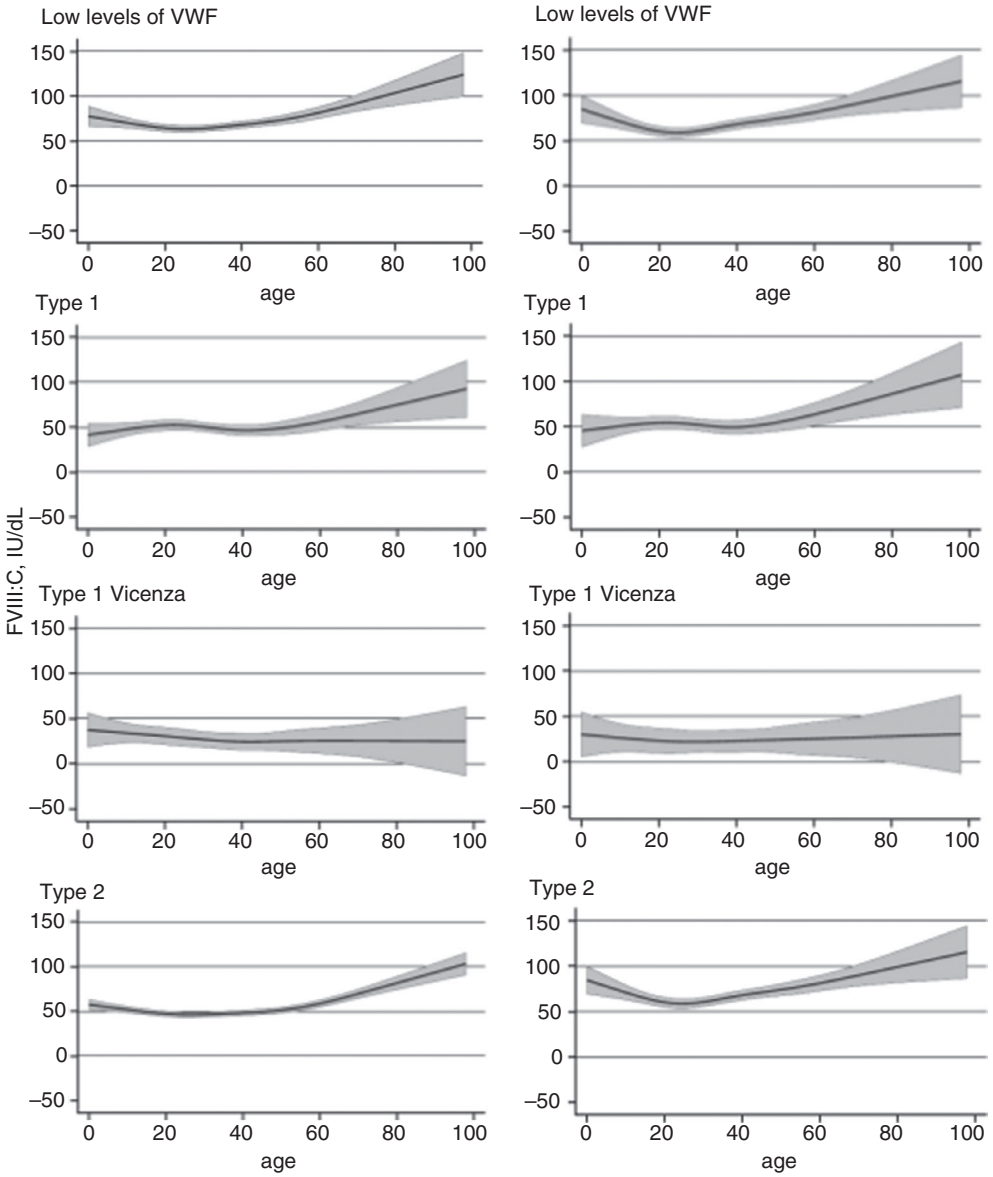

FIGURE 5 Increase with age of FVIII:C in low levels of VWF, type 1, type 1 Vicenza, and type 2 VWD. The unadjusted model is shown on the left side of the panel; on the right side, the full model is shown (adjusted for age at diagnosis, sex, blood group, comorbidities)

and plasma-derived concentrates with different ratio between VWF and FVIII) are now available for the treatment of these patients[26] and an accurate choice cannot rely only on levels measured at diagnosis, but should take into account a recent evaluation of levels of VWF activity and FVIII. Moreover, in case of repeated treatments, an accurate follow-up of VWF activity and FVIII levels is needed, especially in older patients.

Regarding the possible pathologic mechanisms for VWF increase with age in VWD, a role for comorbidities was shown by Atiq et al[27] in a reevaluation of the Dutch cross-sectional study. In this study, the increasing levels of FVIII and VWF with age were associated with high blood pressure, diabetes, cancer, and thyroid dysfunction assessed by a self-reported questionnaire. Adjustment for these variables annulled the increase of VWF and FVIII in type 1 VWD. In our group of patients affected by type 1 VWD, a specific role for comorbidities could not be confirmed. This could be due to the small number of patients and therefore large CI or a difference in the ascertainment of comorbidities (self-reported questionnaire in the Dutch study and data from clinical charts in our study).

The small group of patients affected by type 1 Vicenza VWD included in our study did not show an increase of VWF and FVIII. Because these patients are characterized by a high clearance of VWF, we might hypothesize that only patients with a normal clearance of VWF can increase VWF and FVIII levels with age. Unfortunately, data on propeptide levels to confirm this hypothesis were not available for all patients.

How the increase of VWF and FVIII levels might influence the bleeding phenotype in older age (in particular decreasing the number of bleeding episodes) was evaluated only in the Dutch

TABLE 2 Regression slope β coefficient per decade and its 95% CI, calculated after the age of 40 y (after this age a linear regression was shown by the linear mixed models with restricted cubic splines)

	Patients, n	Measurements, n	All Patients β Coefficient per Decade (95% CI) IU/dL	Low VWF Levels β Coefficient per Decade (95% CI) IU/dL	Type 1 VWD β Coefficient per Decade (95% CI) IU/dL	Type 1 Vicenza VWD β Coefficient per Decade (95% CI) IU/dL	Type 2 VWD β Coefficient per Decade (95% CI) IU/dL
VWF antigen							
Not adjusted	276	893	5.43 (3.52-7.45)	8.36 (4.39-12.32)	4.69 (−1.42 to 10.81)	2.12 (−5.84 to 10.08)	4.31 (1.89-6.74)
Adjusted for age at 1st measurement, sex, blood group, comorbidities, and assay type	156	657	6.48 (3.43-9.54)	10.36 (5.36-15.36)	10.53 (3.47-17.59)	1.91 (−0.68 to 10.63)	7.00 (3.07-10.73)
VWF activity							
Not adjusted	275	901	2.32 (0.82-3.81)	4.96 (2.09-7.83)	3.79 (−0.63 to 8.21)	2.36 (−3.40 to 8.11)	0.60 (−1.21 to 2.41)
Adjusted for age at first measurement, sex, blood group, comorbidities, and assay type	155	660	3.68 (0.62-6.75)	4.85 (0.40-9.30)	5.54 (−0.92 to 12.01)	3.09 (−5.40 to 11.57)	1.10 (−2.25 to 4.45)
FVIII:C							
Not adjusted	270	1000	6.87 (5.31-8.43)	7.61 (4.26-10.95)	5.22 (0.67- 9.77)	0.53 (−5.73 to 10.95)	7.00 (5.11-8.90)
Adjusted for age at first measurement, sex, blood group, and comorbidities	151	712	7.44 (5.19-9.70)	6.59 (2.63-10.55)	6.96 (1.48-12.43)	2.38 (−4.72 to 9.48)	7.99 (5.24-10.75)

study, which reported more bleeding episodes in the patients with comorbidities. One of the limitations of our study is the lack of information on the bleeding phenotype in older age (to correlate it with the repeated measurements). These data were not available or difficult to evaluate for patients on chronic prophylaxis. Indeed, type 1 VWD and low VWF are associated with mild bleeding symptoms, frequently present only after some challenge such as surgery or trauma, making difficult a comparison of number of bleeding events in different time intervals. Moreover, some bleeding symptoms typical of VWD, such as easy bruising, can appear also in healthy older individuals resulting from hypodermis atrophy and skin thinning.

Another limitation of our study is the lack of information on the genetic mutations causing the disease. These data could be interesting because we could hypothesize a bigger increase in the levels of VWF and FVIII in patients without a genetic mutation (compared with those with a genetic mutation). Moreover, during such a long timeframe, several assays were used to measure VWF:Ag and activity; therefore, our analysis was adjusted for the type of assay. Finally, our data showed an increase of VWF and FVIII levels after the age of 40 years but data on menopause, detailed comorbidities, renal function, or body mass index (that could be mediators of the increase) were not available.

Among the strengths of our study are the repeated measurements in a large number of patients affected by different types of VWD or low levels of VWF. The analysis with a linear mixed model and restricted cubic splines enabled us to use all available data, showing an increase with age, which becomes linear only after the age of 40 years.

In conclusion, our study showed a differential increase of VWF:Ag, activity, and FVIII:C with age in different types of VWD or low levels of VWF and also in different age time frames.

CONFLICT OF INTEREST

Dr. Peyvandi received honoraria for participating as a speaker at satellite symposia organized by Bioverativ, Grifols, Roche, Sanofi, Sobi, Spark, and Takeda. Dr. Peyvandi reports participation at advisory board of Roche, Sanofi, and Sobi. Dr. Biguzzi received fees as speaker at satellite symposia organized by Takeda, paid through the institution.

AUTHOR CONTRIBUTIONS

Eugenia Biguzzi, Simona Maria Siboni, Frits R. Rosendaal, Astrid van Hylckama Vlieg, and Flora Peyvandi designed the research. Eugenia Biguzzi, Simona Maria Siboni, Saskia Le Cessie, and Astrid van Hylckama Vlieg collected and analyzed the data. Eugenia Biguzzi wrote the manuscript. Simona Maria Siboni, Luciano Baronciani, Frits R. Rosendaal, Astrid van Hylckama Vlieg, and Flora Peyvandi revised the paper for important intellectual content.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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