Increase of von Willebrand factor with aging in type 1 von Willebrand disease: fact or fiction?

Aging is associated with an upregulation of the blood clotting system with increased levels of von Willebrand factor (VWF), amongst others. While many studies have shown a progressive increase of VWF levels in healthy individuals as a function of aging, very few data are available on VWF-related parameters in patients with von Willebrand disease (VWD). We followed a cohort of patients diagnosed with type-1 VWD (VWD-1) over a 25-year period and found a significant increase of VWF-related parameters as a function of time. However, while in patients with mild VWD the increase was evident, in patients with more severe VWD no increase was observed. Therefore, our data suggest that aging is not associated with an increase of plasma VWF levels in patients with a definite diagnosis of VWD-1 (<0.3 IU/ml).

The world population is growing old, and aging is associated with significant changes in hemostasis with a progressive shift towards hypercoagulability. Most of the blood clotting factors, such as fibrinogen, FV, FVII and FX, tend to increase during the lifespan[1] and platelet reactivity and *in vivo* platelet activation are enhanced in healthy older individuals.[2] Similar observations have been made for VWF, however, given the considerable influence that chronic inflammatory conditions and endothelial perturbation have on VWF biosynthesis and release, its changes with aging appear to be particularly marked.[3]

While the effect of aging on the hemostatic system of healthy subjects has been well characterized, its influence on VWF levels of patients with VWD is poorly characterized, with only one study - in a small group (n=31) of patients with different subtypes of VWD followed for a median of 11 years - suggesting that patients with VWD-1 experience age-related increases of VWF which can result in the complete normalization of hemostatic parameters.[4]

The aim of our study was to evaluate the changes in VWF activity, VWF antigen, Factor VIII (FVIII) levels and ristocetin-induced platelet aggregation (RIPA, defined as the minimal concentration of ristocetin leading to full platelet aggregation/agglutination),[5] in a relatively large group of patients studied for a mild bleeding diathesis and with blood clotting test results compatible with a diagnosis of VWD-1 who were repeatedly evaluated at our center between January 1990 and December 2015. Records of all patients fulfilling criteria for VWD-1 studied at our center during this 25-year span were reviewed, and patients who had been reevaluated at least once after initial diagnosis were included. VWD-1 was defined according to the following criteria: plasma VWF ristocetin cofactor activity (VWF:RCo) levels below the lower limit of our laboratory, i.e., two standard deviations from the average levels of 40 healthy, non-0 blood group donors, a consensual decrease of VWF-antigen (VWF:Ag), a VWF:RCo/VWF:Ag ratio >0.6, and a personal and/or familial history of mild mucocutaneous bleeding.[4-6]

VWF:RCo was assessed by light transmission aggregometry (LTA) using lyophilized platelets or with the HemosIL VWF:RCo assay (from 2011), VWF:Ag by enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies, RIPA by LTA in platelet-rich plasma (PRP), and FVIII levels by a one-stage coagulation assay.[7-9]

All laboratory results were tested for normality distribution (D'Agostino-Pearson normality test), and the differences between the first and last measurement were analyzed using the *t*-test for paired samples or the Welch's *t*-test, where appropriate. A regression analysis between the time elapsed from the first to last measurement and VWF:RCo, VWF:Ag, FVIII and RIPA variations was performed. A *P*-value <0.05 was considered as significant. Statistical analysis was carried out with the MedCalc version 12 (MedCalc Software bvba).

One hundred and ninety-five patients (108 women and 87 men, median age at first observation 28.7 years, range: 0.4-74.5 years) fulfilled the enrollment criteria (Table 1).

Each patient was studied an average of 3 times (range: 2 to 14) and the median duration of follow-up was 6.6 years (range: 0.4-24.7). Between the first and last measurement the mean VWF:RCo increased by 0.096±0.02 IU/ml, VWF:Ag by 0.14±0.033 IU/ml, FVIII by 0.094±0.035 IU/ml, and RIPA decreased by 0.3±0.1

Table 1. Description of the cohort at diagnosis.

	Overall VWD-1	Mild VWD-1	Moderate VWD-1	*P* (mild vs. moderate)
n	195	143	52	
Female (%)	58.6	60.7	56.6	ns
Blood Group 0 (%)	60	61.1	49	*P*=0.04
VWF:Ag IU (mean±95% CI)	0.37 (0.34 – 0.41)	0.47 (0.45 – 0.48)	0.23 (0.18 – 0.28)	*P*<0.0001
VWF:RCo IU (mean±95% CI)	0.33 (0.31-0.36)	0.43 (0.42 – 0.45)	0.18 (0.15 – 0.19)	*P*<0.0001
VWF:RCo/VWF:Ag ratio (mean±95% CI)	0.85 (0.77-0.94)	0.92 (0.62-1.22)	0.82 (0.78-0.86)	*P*=ns
RIPA (mg/ml) (mean±95% CI)	1.42 (1.22 - 1.61)	1.3 (1.14 - 1.45)	1.76 (1.18 - 2.34)	*P*=0.001
FVIII:C IU (mean±95% CI)	0.56 (0.57 – 0.71)	0.64 (0.57 – 0.71)	0.46 (0.39 – 0.53)	*P*=0.008

VWD: von Willebrand disease; VWF: von Willebrand factor; VWF:Ag: VWF-antigen; VWF:RCo: VWF ristocetin cofactor activity; RIPA: ristocetin-induced platelet aggregation; FVIII: Factor VIII; CI: confidence intervals; ns: not significant.

mg/ml^{-1} (all $P<0.05$) (Figure 1, inset).

Given that for about 60% of the subjects studied a shift in the VWF:RCo assay used was performed during the observation time, we analyzed separately VWF:RCo changes for those in which the assay was changed and those for which it was not: changes were almost identical in the two groups (*data not shown*).

The variations of VWF-related laboratory parameters were positively correlated with the length of the time interval between the first and the last measurement, with an estimated rate of change of 0.047 IU/ml/10 years for VWF:RCo, 0.062 IU/ml/10 years for VWF:Ag, 0.102 IU/ml/10 years for FVIII, and 0.1mg/ml/10 years for RIPA. The increase was more evident in patients of a more advanced age at diagnosis (Figure 2).

Neither sex- nor blood group (0 *vs.* not 0)-related differences were observed for the changes in VWF:RCo and VWF:Ag during follow up (*data not shown*). However, when patients were subdivided for severity in mild (baseline VWF:Ag and VWF:RCo >0.3 <0.5 IU/ml, group 1, n=143) and moderate VWD (baseline VWF:Ag and VWF:RCo ≤0.3 IU/ml, group 2, n=52), a significant increase of VWF with aging was confirmed only in the mild VWD subgroup, while in the moderate subgroup no changes were observed (Figure 1A,B). Interestingly, the frequency of blood group 0 was significantly higher in the milder group than in the moderate group (61.1% *vs.* 49%, $P=0.04$), while the two groups were balanced with regard to sex distribution (Table 1).

Our study shows that while VWF levels increase progressively and significantly with aging in a VWD-1 patient population, defined according to broad standard criteria[4-6] no significant changes occur in type-1 VWD defined by more stringent criteria. The diagnosis of mild VWD is difficult and often no manifest genotype/phenotype correspondence is found,[10] with many cases remaining without clear evidence of disease. Our data suggest that the milder patients initially labeled as VWD-1 may not actually be VWD-1, and that the changes of VWF-related laboratory parameters in function of aging are indeed restricted to healthy individuals. In fact, gene mutations affecting the synthesis, processing and secretion of VWF are more frequently detected in the more severe VWD-1 cases,[10] and it is reasonable to presume that genetic defects of the synthesis or processing of VWF may not allow the expression of age-related increases.[4] Indeed, 117 out of 195 (60%) of the patients investigated in our series would not have been diagnosed with VWD-1 had they been studied only at the last observation point. Indeed, when subdividing patients by severity subgroup, while 94% of those initially labeled as mild had normal laboratory values at the last observation time, only 6% of those defined as moderate normalized.

It might be objected that mild mucocutaneous bleeding was among the diagnostic criteria in our entire patient cohort, nevertheless, minor bleeding is very frequent in

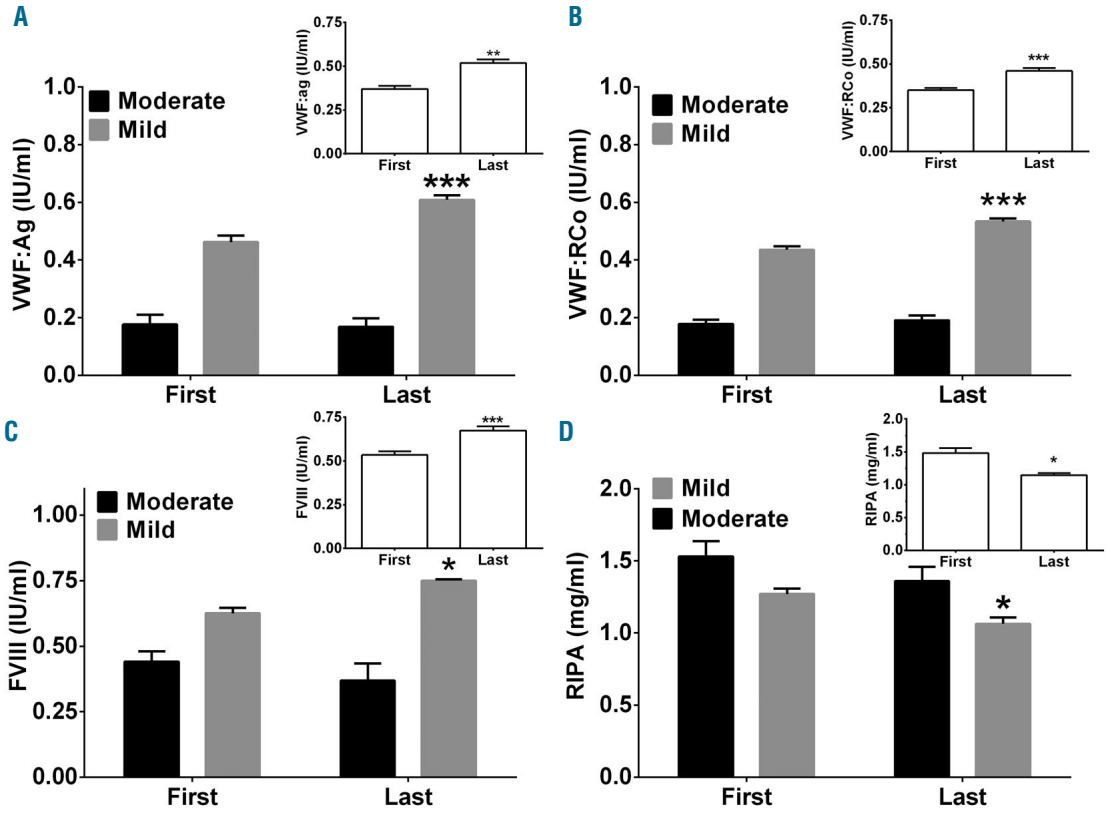

Figure 1. VWF:RCo, VWF:Ag, FVIII and RIPA levels at first and last meaurement. Mean VWF:Ag (A), VWF:RCo (B), FVIII (C) and RIPA (D) levels at first and last measurement in the moderate (black columns) and mild (gray columns) groups. Average levels in the whole cohort are shown in the respective insets. Data are expressed as means±S.E.M. *$P<0.05$ *vs.* First; **$P<0.005$ *vs.* First; ***$P<0.0001$ *vs.* First. VWF: von Willebrand factor; VWF:Ag: VWF-antigen; VWF:RCo: VWF ristocetin cofactor activity; FVIII: Factor VIII; RIPA: ristocetin-induced platelet aggregation.

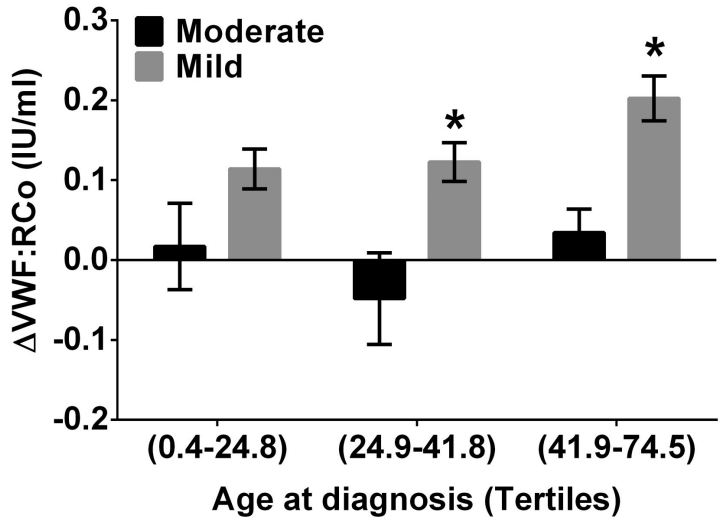

Figure 2. VWF:RCo increase by age at diagnosis. VWF:RCo change from first to last measurement (Δ VWF:RCo) in the moderate (black columns) and mild (gray columns) VWD-1 patients, divided into tertiles according to age at diagnosis. The age span for each tertile is indicated in brackets below the abscissa. The number of subjects was as follows: first tertile= 87; second tertile= 58; third tertile= 50. Mean duration of follow-up by tertiles was as follows: first tertile = 3 yrs; second tertile =5.3 yrs; third tertile = 5.2 yrs. *$P<0.05$ *vs.* first tertile. VWF:RCo: VWF ristocetin cofactor activity.

the general population and it may associate fortuitously with mildly reduced VWF levels.[6,9-11]

Our results have several implications. First, age-related normal reference ranges should be envisaged for VWF levels, as recently proposed for the platelet count.[12] Second, caution should be taken before assigning a diagnosis of VWD-1 to patients with only mildly reduced VWF levels,[11] particularly when the family bleeding history is not clear, or when VWF level reduction is not confirmed on repeated testing. Third, preoperative prohemostatic treatment should be prescribed with caution to aged patients with an old diagnosis of mild VWD-1 and no significant previous bleeds, and any therapeutic decision should take into account the current VWF levels and the thrombotic risk of prohemostatic interventions.[13]

Unfortunately, due to its retrospective nature, our study neither allowed for the systematic measurement of the severity of the bleeding phenotype of our cohort, for instance by a standardized bleeding assessment tool, nor for the exploration, in greater detail, of the effect of aging on VWF levels in patients with genetically-confirmed VWD and with different mechanisms of VWF reduction.

A collaborative prospective, long-term follow-up study, including a well-characterized and genotyped cohort of VWD patients undergoing a prespecified, periodic, standardized reevaluation of VWF-related laboratory parameters and of the bleeding phenotype, is warranted to definitively clarify the impact of aging on VWD-1 phenotype and the clinical relevance of mild VWD-1 diagnosis.[14,15]

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