Type IIB von Willebrand Disease: Role of Qualitative Defects in Atherosclerosis and Endothelial Dysfunction

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Summary: Objective. To verify whether a hereditary bleeding tendency, such as von Willebrand disease (vWD) type IIB, protects against the onset of atherosclerosis. Participants and Methods. Twenty-four patients with vWD type IIB and 24 healthy controls, matched for common atherosclerotic risk factors. All patients were evaluated by color Doppler ultrasound of the common carotid, carotid bifurcation, common femoral artery, brachial artery, and abdominal aorta, investigating intima-media thickness (IMT) and presence of plaques in each arterial district. Flow mediated dilation (FMD) of the brachial artery was used to test endothelial function. Results. vWD type IIB patients presented no significant difference in IMT in any arterial district. FMD showed no differences between the 2 groups. Conclusions. The quantitative clotting defect characteristic of vWD type IIB does not seem to protect against atherosclerosis.

Key Words: Atherosclerosis—Coagulation—von Willebrand factor—Ultrasound—Flow-Mediated dilation.

INTRODUCTION

Coagulation plays a pivotal role in the onset of atherosclerotic complications—that is, in the formation of occluding arterial thrombi and consequent ischemic syndrome.

Atherosclerosis and thrombosis appear to be closely related, and the term *atherothrombosis* has been coined to include both concepts and applied to considerations relating both to acute coronary syndrome and to peripheral and cerebral vascular disease (1).

Two separate processes are distinguishable in the activation of the coagulation system: primary hemostasis (consisting of the adhesion and aggregation of platelets, resulting in a clot of platelets) and secondary hemostasis (a cascade of clot-activating factors, resulting in the formation of insoluble fibrin from fibrinogen).

The von Willebrand factor (vWF) is fundamental to primary hemostasis because it mediates platelet adhesion to subendothelial surfaces and platelet aggregation; it also protects FVIII (Factor VIII) against proteolytic degradation in plasma.(2).

A vWF deficiency alters both primary and secondary hemostasis because FVIII levels are often lower than normal in von Willebrand disease (vWD).

Type IIB vWD is characterized by a structural vWF abnormality: this variant was first described in the early 70s and features an enhanced vWF binding to the platelet glycoprotein Ib receptor. A consequence of this functional alteration is a lower concentration of the largest vWF multimers in plasma, and the platelet count may drop episodically as a result of microaggregation (3).

A hyperfunctional adhesive molecule in the blood causes a bleeding tendency, though this may seem like a paradox (4).

Numerous studies have associated vWF with thrombotic complications in atherosclerosis. High levels of vWF are considered predictors of coronary

risk and adverse outcome, and ischemic stroke risk (5-7,8). High levels of vWF have also been found in patients with peripheral vascular disease (9). Some authors think that vWF could be used as an early marker of atherosclerotic disease, and a number of studies have suggested that vWF might have a role in atherogenesis (10).

Pigs with homozygous vWD are resistant to the onset of spontaneous and/or diet-induced atherosclerosis (11,12). A mouse model with a total vWF deficiency was consequently developed, reproducing type III vWD in humans, with a view to evaluating whether vWF-deficient mice (vWF -/-) bred from mice lacking the low-density lipoprotein receptor (LDLR -/-) and kept on a fat-rich diet developed fewer atherosclerotic lesions (13). This hypothesis was confirmed and the authors assumed that vWF affects atherogenesis via a platelet and leukocyte recruitment to the lesion in a flow-dependent manner, or its effect may be part of the endothelial response to shear stress (14). However, data available for humans are too few to clarify the potential role of vWF in atherogenesis.

In the 1960s, Silwer and colleagues examined vWD patients more than 40 years old and identified atherosclerotic lesions in their coronary arteries and aorta. On this basis, the authors claimed that vWF deficiency could not prevent atherosclerosis in the arterial districts investigated (15). Postmortem studies in 3 carriers of vWD (1 type IIB, 2 type III) revealed severe lesions in all vascular areas in the patient with type IIB, and few and mild lesions in all areas in the 2 patients with type III. There was no clinical or pathological evidence of thrombosis in their coronary or other arteries. The authors concluded that severe vWD does not completely protect against atherosclerosis, though intensive lifelong treatment with plasma concentrates may have influenced the situation in these patients (16,17).

Research on patients with a hereditary bleeding tendency (including those with vWD and with hemophilia A and B) identified a minimal protection against the development of femoral lesions versus controls but no protection in the carotid district (18).

A recent study on individuals with type III vWD found no statistically significant difference in intima-media thickness (IMT) in the carotid and femoral districts, no differences for atherosclerotic plaque, and no differences for the extent of stenosis (19).

The goal of the present study was to evaluate the presence and extent of atherosclerotic lesions in asymptomatic patients with type IIB vWD with a view to clarifying the impact of a qualitative vWF defect on the development of atherosclerotic lesions.

METHODS

Study Design

We examined 24 vWD type IIB patients and 24 healthy individuals matched for age, gender, and the principal risk factors for atherosclerosis. The study lasted 12 months, from January 2003 to January 2004. Patients with a hereditary bleeding tendency were referred to our institute for clinical and hematological tests; controls were recruited from among the employees at our clinic. We chose to investigate the impact of the qualitative vWF defect characterizing vWD type IIB on atherosclerosis in the asymptomatic phase, so all patients with signs or symptoms of atherosclerotic disease (ie, angina pectoris, IMA, claudicatio intermittens, TIA, ictus) at the time of our investigation were ruled out, based on the available data on their medical history and physical diagnosis. All individuals eligible for our study were invited to participate and gave their informed consent.

Measurement of Risk Factors

Risk factors for atherosclerosis were evaluated on the basis of clinical findings, biochemistry, and a standardized questionnaire, recording the data on a card. We considered the following: age and gender; smoking habit (classified as the daily use of 10 or more cigarettes a day at the time or up until less than a month earlier); hypertension (systolic values >160 mm Hg and/or diastolic values >90 mm Hg on at least 2 occasions in a supine position after a 20-minute rest or the use of anti-hypertensive medication); obesity (body mass index [BMI] > 26 kg/m^2); diabetes mellitus (plasma glucose >126mg/dL after overnight fasting on at least 2 occasions, or >200 mg/dL 2 hours after 75 g oral glucose challenge, and/or the use of antidiabetic drugs).

The lipid profile was obtained by measuring total HDL and LDL cholesterol, and triglyceride concentrations: hyperlipidemia was classified as fasting venous concentrations of cholesterol >240 mg/dL and/or triglycerides >250 mg/dL on at least 3 occasions, and/or the use of lipid-lowering drugs (20).

Each patient with vWD was matched with a healthy control for age, gender, BMI, hypertension,

smoking habit, diabetes mellitus, and dyslipidemia: age and BMI were expressed as quantitative variables and hypertension, smoking habit, diabetes mellitus, and dyslipidemia as qualitative dichotomous variables (ie, presence/absence based on previously described parameters).

Ultrasound Assessment

Patients meeting the inclusion criteria underwent ultrasound assessment of the arterial system comprising the common carotid arteries, carotid bifurcation, common femoral arteries, brachial arteries, and abdominal aorta using an ATL Apogee 800 Plus (USA) instrument, a 7.5 MHz probe for B-mode echography, and a 6 MHz probe for pulsed Doppler.

The carotid arteries were assessed first with patients lying on the couch, having rested for at least 15 minutes, and with their neck rotated through 45°.

All carotid district arteries (common, bifurcation, internal, and external) were divided into 3 segments (inferior, median, and superior) with a 60° angle of incidence. The same procedure was used to study the leg arteries, taking longitudinal and transversal scans to assess the common femoral artery, bifurcation, and superficial or deep femoral artery, and dividing all arteries into 3 segments (inferior, median, and superior).

The same instrument was used for the abdominal aorta but with a 3.5 MHz probe for the echogram and a 6 MHz probe for the pulsed Doppler to examine the aorta below the origin of the celiac tripod and iliac arteries. The brachial artery was studied in the antecubital fossa.

An ultrasound technician conducted the tests, which were all videotaped and reassessed by another technician for validation; any discrepancies between them were blindly evaluated by a senior expert.

Two parameters were evaluated in each arterial district: IMT (in mm) and the presence and extent of atherosclerotic lesions, expressed as the percentage of stenosis in the vascular lumen. IMT was measured on images magnified 4 times to clearly display the far wall, and only where the characteristic "double line pattern" was identifiable, using the technique described by Pignoli et al in 1986 (21). Only the far wall was investigated because it is usually easier to measure (22).

After digitizing into 640 × 580 peak cells with 256 gray levels, images were saved on the computer and analyzed offline. The highest values

were recorded for each arterial system investigated (carotid, aortic, femoral, and brachial).

Atherosclerotic plaque was defined as a protrusion into the vessel lumen of at least 2 mm, as measured from the border between the adventitial and medial layers.

The degree of atherosclerotic plaque was classified, based on the NASCET study (23) as follows: class 1 (0%–30% obstruction), class 2 (31%–50% obstruction), class 3 (51%–69% obstruction), class 4 (70%–99% obstruction), and class 5 (complete obstruction).

Flow-Mediated Brachial Artery Dilation

Endothelial dysfunction is considered to be one of the earliest signs of atherosclerosis, and brachial artery flow-mediated dilation (FMD) measurements are widely accepted as a good indicator of endothelial function (24-27). We used the procedures described in the Guidelines of the International Brachial Artery Reactivity Task Force (28)

We chose to investigate the brachial artery of the nondominant arm to avoid any influence of daily activity on the vessel's diameter at rest and capacity for vasodilation. Vascular reactivity is affected by many factors, such as temperature, food intake, drug use, and sympathetic stimuli. After fasting for 12 hours, patients were evaluated at rest in a quiet room at a controlled ambient temperature; any vasoactive medication was withheld for at least 4 half-lives wherever possible; tobacco was avoided for at least 6 hours.

Patients lay supine with their arm in a comfortable position for brachial artery imaging in the longitudinal plane 3 to 5 cm above the antecubital fossa. Only images with clear anterior and posterior intimal interfaces between the lumen and the vessel wall (near and far walls, respectively) were considered. The surface of the skin was marked and the arm was kept in the same position throughout the study. First, a sphygmomanometer cuff was placed around the forearm distal to the target artery. A baseline image was acquired to evaluate resting brachial artery diameter; then a pneumatic tourniquet was inflated to a pressure of 250 mm Hg and maintained for 5 minutes, during which time the operator constantly monitored the vessel on the screen.

A reactive hyperemia was then induced by suddenly deflating the cuff, causing a rise in shear stress and consequent NO vasodilation. Longitudinal scans of the brachial artery were taken continuously from 60 seconds before up until 120 seconds after deflating the cuff. The arterial diameter after

reactive hyperemia was measured between 45 and 60 seconds after deflating the cuff because this is considered the period of maximal response.[29,30] Fifteen minutes later, another resting scan was recorded to confirm vessel recovery. The change in diameter caused by FMD was expressed as the percentage change with respect to the initial resting scan (percentage FMD).

All patients went through the procedure without complications; most patients reported some warmth and a "pins-and-needles" sensation in their hand and forearm after the sudden cuff deflation; all symptoms disappeared within a few minutes.

We preferred to avoid using nitrate because it can cause significant hypotension and pose a risk in patients with bradycardia (31,32).

Statistical Analysis

IMT and FMD findings were compared using Student's two-tailed *t* test for matched data. Any atherosclerotic plaques were compared using the McNemar test. The Fisher test was used to compare the number of plaques as a function of the degree of stenosis generated. Statistical significance was defined at $P < .05$.

RESULTS

Table 1 shows the general characteristics and presence of the established atherosclerosis risk factors for patients and controls.

The cutoffs for each risk factor are explained in the section Methods.

Table 2 shows the mean IMT of the arterial districts evaluated. We found no significant difference in the mean thickness at any of the sites investigated. A visual comparison of IMT between patients and healthy controls is shown in Figs. 1 and 2.

At least one atherosclerotic plaque was found in 3 vWD patients (12.5%, 95% CI 0%–25.6%) versus 5 healthy controls (20.8%, 95% CI 4.8%–36.8%). Comparison with the McNemar test showed that this difference was not significant.

There were 3 plaques in all in vWD patients—all at the carotid bifurcation—versus 6 plaques in controls—1 in the common carotid, 1 at the carotid bifurcation, and 4 in the common femoral artery. No difference emerged between the 2 groups in terms of the number of atherosclerotic lesions divided by the extent of stenosis produced, as shown in Tables 3 and 4.

No significant difference was found in the diameter of the brachial artery at rest (3.93 ± 0.64 mm

TABLE 1. General Characteristics of Patients With vWD Type IIB and Healthy Controls

	vWD	Healthy Controls
Age (years)	47.71 ± 20.28	49.95 ± 19.63
Hypertension	33.3%	33.3%
Hyperlipidemia	4.2%	4.2%
BMI (kg/m²)	25.54 ± 4.59	25.06 ± 4.40
Smoking	20.8%	20.8%
Diabetes mellitus	12.5%	12.5%
Gender (% males)	54.2%	54.2%

TABLE 2. Intima-Media Thickness (IMT) of the Arterial Districts Evaluated. Wall Thickness Is Expressed in mm as Mean ± Standard Deviation (SD); $P > .05$

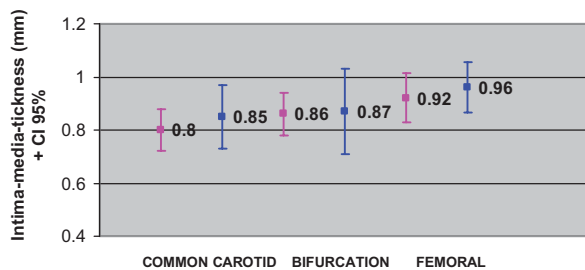| Arterial Districts	vWD	Healthy Controls
Common carotid	0.80 ± 0.19	0.85 ± 0.32
Bifurcation	0.87 ± 0.38	0.86 ± 0.21
Brachial	0.69 ± 0.09	0.72 ± 0.13
Femoral	0.92 ± 0.23	0.96 ± 0.23
Abdominal aorta	2.72 ± 1.19	2.75 ± 0.69

FIG. 1. Visual comparison of intima-media thickness (IMT) in patients with von Willebrand disease (vWD) type IIB versus healthy controls for the common carotid, carotid bifurcation, and femoral arterial districts. IMT is expressed in mm and 95% confidence intervals.
P > .05.
a. vWD carriers.
b. Controls.

in vWD patients and 3.98 ± 0.67 mm in controls). After reactive hyperemia, the percentage of FMD did not change significantly in either group, the percentage of vasodilation being 15.2 ± 3.1% in vWD patients versus 14.1 ± 2.9% in controls ($P > .05$), as shown in Fig. 3.

DISCUSSION

Our findings indicate that vWD type IIB (which is characterized by a qualitative vWF defect) affords no protection against atherosclerosis.
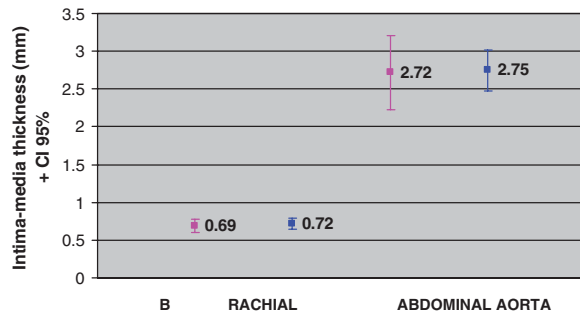
FIG. 2. Visual comparison of intima-media thickness (IMT) in patients with von Willebrand disease (vWD) type IIB versus healthy controls for brachial and abdominal aorta arterial districts. IMT is expressed in mm and 95% confidence intervals.
P > .05.
a. vWD carriers.
b. Controls.

TABLE 3. Distribution of Atherosclerotic Plaques by Class of Stenosis: Carotid District

| Class | Extent of Obstruction | | vWD | Controls |
	From (%)	To (%)		
1	0	30	3 (100%)	2 (100%)
2	31	50	0	0
3	51	69	0	0
4	70	99	0	0
5	Complete		0	0

TABLE 4. Distribution of Atherosclerotic Plaques by Class of Stenosis: Femoral District

| Class | Extent of Obstruction | | vWD | Controls |
	From (%)	To (%)		
1	0	30	0	3 (75%)
2	31	50	0	1 (25%)
3	51	69	0	0
4	70	99	0	0
5	Complete		0	0

We measured IMT using B-mode ultrasound as an indicator of generalized atherosclerosis because Pignoli and colleagues demonstrated its feasibility in vivo as far back as 1986: data obtained by this method do not differ from those obtained by microscopic measurements on arterial wall biopsies (21). Changes in myointimal wall thickness may precede the onset of atherosclerotic lesions, and ultrasound assessment of IMT of the common carotid appears to be a feasible, reliable, valid, and cost-effective method for studying atherosclerosis progression and regression (22).
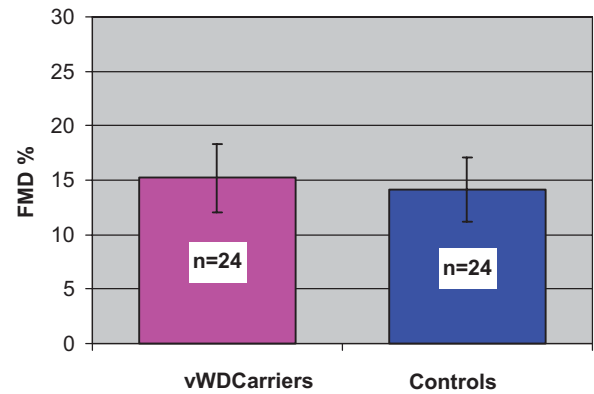

FIG. 3. Flow-mediated dilation (FMD) of the brachial artery expressed as a percentage of resting diameter ± SEM. P > .05.

The relationship between IMT and exposure to atherosclerotic risk factors, such as hypertension and hypercholesterolemia, is well known (30). Common carotid IMT reflects the presence and extent of atherosclerotic lesions in the coronary as well as in the peripheral system (33–35). A 10-year prospective study has stressed that ultrasound of the carotid and femoral arteries could be used for screening asymptomatic individuals to evaluate the risk of cardiovascular events (36).

The common femoral artery is an excellent site to consider, yielding reliable and reproducible data (37). The popliteal artery seems to be a good site to measure as a model of a medium-sized arterial vessel, whereas this cannot be said of the brachial artery (38).

There are currently no studies suggesting a correlation between abdominal aorta IMT and generalized atherosclerosis, whereas an inverse linear relationship has been found between common carotid IMT and brachial artery FMD (29).

Our work investigated the common carotid, carotid bifurcation, and common femoral artery, all districts known to be important in the study of generalized atherosclerosis. We also considered the brachial artery and abdominal aorta to obtain a wider spectrum of evaluation, though the significance of these sites remains unclear.

FMD stresses the endothelial function, one of the first parameters known to change in atherosclerotic disease. No significant difference emerged between vWD type IIB carriers and healthy controls, however, prompting us to suggest that the qualitative defect typical of vWD IIB has no important influence on endothelial function or, more generally, on parietal reactivity.

Few studies are available on the role of clotting defects in human atherosclerosis. Šrámek and colleagues compared common carotid and common femoral artery IMT in patients with coagulation disorders (hemophilia A or B, or vWD) versus a control population and found that a clotting defect afforded some small degree of protection against femoral but not against carotid atherosclerosis (18). Their patient classification may have been inadequate, however, because they did notsay which types of vWD they investigated but only distinguished between moderate vWD and all other defects.

A subsequent work investigated only vWD type III (complete absence of vWF) and ruled out any effect of this coagulation disorder in terms of preventing atherosclerotic plaque; the authors concluded that vWF has no significant role in human atherogenesis (19). It is only possible to speculate that type III vWD patients might be protected against arterial occlusion despite any presence of atherosclerotic lesions.

Patients with vWD are treated with clotting factors during bleeding episodes (39). We wondered whether this might have influenced our results by transforming a clotting disorder carrier into a person with a normal coagulation profile. Data available at this juncture seem to exclude this possibility, however (40).

Other studies suggest a role of vWF in the pathogenesis of intimal hyperplasia (41). Discrepancies observed in humans in other studies could have different explanations.

1. First, there is the vessel studied. In pigs and mice, the protective effect against atherosclerosis has only been shown for the aorta, whereas in humans the sites suitable for study are the common carotid, carotid bifurcation, and common femoral artery, whereas no data are available on the aorta so no comparison can be drawn.
2. Second, there is the technique employed. The accurate work of Methia et al (41,42) used immunohistochemical and histological analyses from postmortem aortic samples and quantified parameters such as cell density, number of leukocytes recruited in the outer wall, expression of adhesion molecules on the endothelium, and rate of fatty strikes after a lipid-rich diet. On the other hand, all the studies involving humans used B-mode ultrasound, which can identify atherosclerotic disease not in the preliminary stage of fatty strikes but only after an increase in IMT. The methods are consequently not comparable.
3. There is the entity of clotting defect. The mice considered were totally lacking in vWF, so they could be compared with type III vWD patients; our study investigated vWD type IIB, which is characterized by a qualitative coagulation defect that coincides with a mild bleeding tendency, so no direct analogies with the animal models can be attempted.

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