Patients With Type 3 Severe von Willebrand Disease Are Not Protected Against Atherosclerosis
Results From a Multicenter Study in 47 Patients

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Background—The results of a number of studies in pigs and mice suggest that absence of von Willebrand factor (vWF) protects against the development of atherosclerosis. We studied whether patients with a complete deficiency of vWF (type 3 von Willebrand disease [vWD]) develop fewer atherosclerotic vessel wall changes than healthy controls.

Methods and Results—This study included 47 individuals with type 3 vWD and 84 healthy controls. Early atherosclerotic changes were assessed by measuring the thickness of the intima-media in the carotid and femoral arteries by B-mode ultrasonography. Advanced atherosclerotic changes were quantified by summing the maximal thickness of atherosclerotic plaques in the carotid and femoral arteries and were expressed as a plaque score. Established risk factors were determined to adjust for possible differences between the groups. We found no substantial difference in intima-media thickness between vWD patients and controls (adjusted difference for carotid artery 0.007 mm, 95% CI −0.022 to 0.036 mm; femoral artery 0.069 mm, 95% CI −0.056 to 0.19 mm). Similar proportions of patients and controls had atherosclerotic plaques (19% and 17%, respectively). No difference was found in the plaque score between groups (adjusted difference −0.22 mm, 95% CI −0.69 to 0.26). Among vWD patients, we found no effect of treatment with vWF concentrates on intima-media thickness or plaque score.

Conclusions—The results of this study indicate that vWF does not play a substantial role in human atherogenesis.

(Key Words: atherosclerosis ■ coagulation ■ von Willebrand factor ■ cardiovascular disease ■ carotid arteries)

Myocardial infarction is caused by occluding arterial thrombi on top of plaques.1 Hemostasis and coagulability therefore play an important role during the formation of occluding arterial thrombi. In the activation of the coagulation system, 2 separate processes can be distinguished: adhesion and aggregation of platelets, which results in a clot of platelets (primary hemostasis), and a cascade of activating clotting factors that results in the formation of insoluble fibrin from fibrinogen (secondary hemostasis). For an adequate primary hemostasis, von Willebrand factor (vWF) is essential.2 Furthermore, vWF stabilizes factor VIII, one of the clotting factors involved in secondary hemostasis. A deficiency of vWF therefore results not only in an impaired primary hemostasis but also in an impaired secondary hemostasis. The role of hemostasis in ischemic cardiovascular disease is generally thought to be in the formation of occluding arterial thrombi. In the process of arterial thrombus formation, vWF has a key role. In particular, in vessels with high shear stress (eg, arterioles and stenosed arteries), the presence of vWF is required for mural thrombi to be formed.3 Furthermore, increased vWF levels have been shown to be associated with coronary artery thrombosis.4 A number of studies, however, suggest that vWF might also play a role in the development of atherosclerotic plaques. Less atherosclerosis was found in pigs and mice that had a complete deficiency of vWF.5–9

The goal of the present study was to examine whether vWF plays a role in atherogenesis. Atherosclerotic vessel wall changes were determined in patients with a complete deficiency of vWF (ie, type 3 von Willebrand disease [vWD]) and in individuals with a normal coagulation profile.
Methods

Study Design
We included 47 patients with type 3 severe vWD and 84 healthy controls. Selection of the patients with severe vWD was based on the Italian part of a registry that was started in 1984 to register all patients with severe vWD in Europe. The patients were examined at the medical centers where they were used to be treated for their bleeding tendency (ie, the medical centers in Milan, Bologna, Vicenza, Florence, Rome, Naples, and Bari) and were all enrolled in the Italian National Registry of vWD. The controls were healthy individuals with a similar age and gender distribution. Each patient was asked to provide 2 healthy controls if possible (partner, friends, neighbors, or colleagues). For those who failed to provide the controls, healthy volunteers (colleagues, nurses, or friends) were asked to participate instead. The selection of the healthy volunteers was based on the distributions of age, gender, and the region of origin of the patients. The diagnosis of severe vWD was confirmed in all patients and was based on the original criterion of the registry in 1984, ie, vWF:Ag below 1 U/dL, determined by an immunoradiometric assay.

Statistical Analysis
Comparison of vessel wall thickness and exposure to various risk factors between patients with severe vWD and healthy controls was performed by the Student t test for continuous variables and by the \( \chi^2 \) test for discrete variables. Variables that were not normally distributed were transformed logarithmically. The relation between the studied variables and intima-media thickness was determined by linear regression analysis. The regression coefficient of a continuous variable indicates the increase or decrease of the intima-media thickness for every unit increase of the studied variables (eg, age and blood pressure). Discrete variables (eg, family history for cardiovascular disease or vWD status) were coded as 0 or 1 (negative=0, positive=1) and inserted as such into the model. The regression coefficient of these variables indicates the difference between the categories. In univariate regression analysis with the systolic and diastolic blood pressures as independent variables, 12 individuals were excluded because they used antihypertensive drugs. Multivariate regression analysis was used to adjust for differences in exposure to the established risk factors (ie, age, smoking habits, waist-to-hip ratio, systolic blood pressure, total cholesterol, family history of cardiovascular disease, use of cholesterol-lowering drugs, and use of antihypertensive drugs) between the study groups. To examine whether there is an effect of clotting factor treatment on vessel wall thickness, we performed univariate and multivariate regression analysis among the vWD patients with mean annual vWF concentrate treatment as an independent variable.

Results

General characteristics and presence of established risk factors for atherosclerosis in patients and controls are shown in Table 1. Age and gender distribution and exposure to most of the risk factors were similar between both groups. A larger proportion of vWD patients used drugs to control blood pressure (difference 12%, 95% CI 1% to 24%). Hypertension (defined as either a systolic blood pressure \( \geq 140 \text{ mm Hg} \) or a diastolic blood pressure \( \geq 90 \text{ mm Hg} \) or use of antihypertensive drugs) was slightly more common in vWD patients (difference 10%, 95% CI –5% to 25%). Subdivision into grades of hypertension according to World Health Organization guidelines indicated that blood pressure among individuals with hypertension might be slightly higher in patients with severe vWD than in healthy controls (difference between patients and controls when moderate and severe hypertension are taken together 5%, 95% CI –2% to 13%). Lifetime treatment with vWF concentrate among the vWD patients ranged from 0 to 845 000 U (median 43 500 U). The mean annual treatment ranged from 0 to 18 667 U (median 1733 U).

A crude comparison of intima-media thickness between patients and healthy controls is shown in Figure 1 (carotid...
For both arteries, we found no difference between groups.

To examine the relation between vessel wall thickness in the carotid and femoral artery and the various risk factors, regression analysis was performed. First, univariate regression analysis was performed (Table 2). For both arteries, a clear relation was found between vessel wall thickness and most of the risk factors. We did not find an effect of gender (regression coefficient for carotid artery 0.002 mm, regression coefficient for femoral artery 0.06 mm) or of the presence of severe vWD (regression coefficient for carotid artery 0.005 mm, regression coefficient for femoral artery 0.022 mm) with regard to either artery. Furthermore, we found no effect of annual vWF concentrate treatment among vWD patients (regression coefficient for carotid artery 0.007 mm, regression coefficient for femoral artery –0.015 mm). Subsequently, we performed multivariate regression analysis to adjust for the minor differences in exposure to the established risk factors. Multivariate regression analysis revealed no difference in intima-media thickness in the carotid artery between patients and controls (regression coefficient 0.007 mm, 95% CI –0.022 to 0.036). For the femoral artery, however, a minor tendency for a thicker intima-media emerged among healthy controls compared with patients (regression coefficient 0.069 mm, 95% CI –0.056 to 0.19). Among vWD patients, we found no relation between annual vWF concentrate treatment (per 1000 U) and intima-media thickness (regression coefficient for carotid artery –0.002 mm, 95% CI –0.007 to 0.004; regression coefficient for femoral artery –0.005 mm, 95% CI –0.013 to 0.003).

To examine whether a complete deficiency of vWF protects against the development and growth of advanced atherosclerotic disease, we performed the same analysis for our 47 patients with Type 3 vWD.

Table 1. General Characteristics of Patients With Type 3 VWD and Controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>VWD (n=47)</th>
<th>Healthy Controls (n=84)</th>
<th>95% CI of Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>35</td>
<td>37</td>
<td>–6 to 3</td>
</tr>
<tr>
<td>Smoking, pack-years</td>
<td>9.3</td>
<td>8.3</td>
<td>–0.4 to 1.2*</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.85</td>
<td>0.85</td>
<td>–0.04 to 0.04</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>123.2</td>
<td>118.0</td>
<td>–0.01 to 0.08*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>79.0</td>
<td>74.7</td>
<td>–0.002 to 0.1*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.6</td>
<td>5.0</td>
<td>–0.8 to 0.02</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>20 (43)</td>
<td>32 (39)</td>
<td>–13 to 22%</td>
</tr>
<tr>
<td>Positive family history, n (%)</td>
<td>5 (11)</td>
<td>12 (15)</td>
<td>–15 to 8%</td>
</tr>
<tr>
<td>Use of cholesterol-lowering drugs, n (%)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>–2 to 6%</td>
</tr>
<tr>
<td>Use of antihypertension drugs, n (%)</td>
<td>8 (17)</td>
<td>4 (5)</td>
<td>1 to 24%</td>
</tr>
<tr>
<td>Hypertension (overall), n (%)</td>
<td>13 (28)</td>
<td>15 (18)</td>
<td>–5 to 25%</td>
</tr>
<tr>
<td>Hypertension (mild), n (%)</td>
<td>2 (4)</td>
<td>10 (12)</td>
<td>–17 to 1%</td>
</tr>
<tr>
<td>Hypertension (moderate and severe), n (%)</td>
<td>3 (6)</td>
<td>1 (1)</td>
<td>–2 to 13%</td>
</tr>
</tbody>
</table>

Overall hypertension was defined as either a systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg or use of antihypertension drugs. Mild hypertension was defined as either a systolic blood pressure between 140 and 159 mm Hg or a diastolic blood pressure between 90 and 99 mm Hg (individuals using antihypertension drugs were excluded). Moderate and severe hypertension was defined as either a systolic blood pressure ≥160 mm Hg or a diastolic blood pressure ≥100 mm Hg (individuals using antihypertension drugs were excluded).

*After logarithmic transformation.

Figure 1. Comparison of mean intima-media thickness in carotid artery between patients with severe vWD and healthy controls.

Figure 2. Comparison of mean intima-media thickness in femoral artery between patients with severe vWD and healthy controls.
lesions, we compared the presence of atherosclerotic plaques in the carotid and femoral arteries between study groups. A similar proportion of vWD patients and controls had 1 or more visible atherosclerotic plaques in the measurement areas of the carotid and femoral arteries (19% and 17%, respectively). Subsequently, the thickness of the thickest plaque in every measurement area was summed, which resulted in a plaque score that ranged from 0 to 11.3 mm. No adjusted difference in plaque score between the 2 groups was found (difference \(-0.22 \text{ mm}, 95\% \text{ CI} -0.69 \text{ to} 0.26\)). Furthermore, we found no effect of annual vWF concentrate treatment (per 1000 U) on plaque score among vWD patients (regression coefficient \(-0.017 \text{ mm}, 95\% \text{ CI} -0.107 \text{ to} 0.072\)).

### Discussion

In the present study, we examined whether a hereditary complete deficiency of vWF results in fewer atherosclerotic vessel wall changes. Patients with type 3 vWD have no measurable vWF levels in plasma, platelets, and endothelial cells and therefore form the ideal group to study the pathogenic role of vWF in human atherogenesis. The results show that individuals with a complete deficiency are not protected against the development of early and advanced atherosclerotic lesions.

We measured intima-media thickness in the carotid and femoral artery by B-mode ultrasonography as an indicator of generalized and coronary atherosclerosis. Histological studies have shown that quantification of the intima-media thickness in the far wall of arteries by ultrasonography corresponds well with the true histological thickness. The results of the present study and numerous other studies showed clear relationships between ultrasonographically determined intima-media thickness in the carotid and femoral artery and exposure to the established risk factors of atherosclerosis, such as arterial hypertension and hypercholesterolemia, even in young individuals. Furthermore, it has been shown that intima-media thickness of the carotid and femoral artery is a good indicator of atherosclerotic lesions in the coronary and peripheral arteries. In a recent study, we have shown that the technique we have used is reliable and accurate to determine intima-media thickness in the carotid and femoral artery. One might wonder, however, whether intima-media thickness in relatively young individuals represents a true stage in the development of atherosclerosis. Atherosclerotic plaques (present in nearly 20% of the individuals) were therefore also quantified and included in the analysis.

Experimental animal studies in pigs with vWD showed a protection against aortic atherosclerosis in pigs with homozygous (severe) vWD, whereas no protection was found in pigs with heterozygous (mild) vWD. These findings are supported by the results of a recently published study in mice with a complete deficiency of vWF. Because vWF plays a role in primary and secondary hemostasis, protection against atherosclerosis could be explained by decreased adhesion and aggregation of platelets to endothelial and subendothelial structures and by decreased plasmatic coagulability. In a recent study, we found no substantial protection against atherosclerosis among individuals with hemophilia. That result suggests that the protection that was found in pigs and mice with severe vWD is caused by an impaired adhesion and aggregation of platelets to the vessel wall. Postmortem studies of individuals who had severe vWD, however, showed that patients with a complete deficiency of vWF developed advanced atherosclerotic lesions.

The results of the present study do not support the protection that was found in the pigs and mice with severe vWD and confirm previous autopsy findings in men. An explanation for this difference in results might be that the patients in the present study were treated with clotting factors during bleeding episodes, whereas the pigs and mice were never treated. Among the patients with severe vWD, however, we found no relation between treatment with clotting factors and atherosclerotic vessel wall changes. Furthermore, platelet and endothelial vWF are not modified by transfused concentrates. Another explanation might be the different arteries in which we have quantified the atherosclerotic

### TABLE 2. Univariate Regression Analysis of Intima-Media Thickness in Carotid and Femoral Artery

<table>
<thead>
<tr>
<th>Variables</th>
<th>Carotid Artery</th>
<th>Femoral Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (10 years)</td>
<td>0.075</td>
<td>0.19</td>
</tr>
<tr>
<td>Smoking (10 pack-years)</td>
<td>0.038</td>
<td>0.10</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.38</td>
<td>0.64</td>
</tr>
<tr>
<td>Systolic blood pressure (10 mm Hg)</td>
<td>0.026</td>
<td>0.64</td>
</tr>
<tr>
<td>Diastolic blood pressure (10 mm Hg)</td>
<td>0.029</td>
<td>0.006</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>0.029</td>
<td>0.080</td>
</tr>
<tr>
<td>Gender (males vs females)</td>
<td>0.002</td>
<td>-0.06</td>
</tr>
<tr>
<td>Positive family history</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.13</td>
<td>0.37</td>
</tr>
<tr>
<td>vWD vs controls</td>
<td>-0.005</td>
<td>0.022</td>
</tr>
<tr>
<td>Annual vWF concentrate treatment (1000 U)*</td>
<td>-0.007</td>
<td>-0.015</td>
</tr>
</tbody>
</table>

*Hypertension was defined as either a systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg or use of antihypertension drugs.

*Analysis among vWD patients only.
changes. In the pigs, protection against aortic atherosclerosis was found, whereas no protection was found in the coronary arteries. It is known that the pace of development of atherosclerosis is different for the various sites in the vessel tree, depending on some recognized factors, such as shear stress, and some as yet unknown factors. In the present study, we found advanced atherosclerotic lesions and found no differences between groups. Furthermore, we showed in a previous study that intima-media thickness measurements in the carotid and especially in the femoral artery are a good indicator of advanced atherosclerotic lesions in the coronary arteries. Because the major role of vWF is to promote thrombus formation in stenosed vessels, we can speculate that type 3 vWD patients, who lack vWF in plasma, platelet, and subendothelium, might be protected against arterial occlusion even in the presence of atherosclerotic lesions.

The data suggest a slightly higher prevalence and severity of hypertension among patients with severe vWD. In a study that was performed in patients with hemophilia, a higher prevalence of hypertension was found. The results of the present study are based on a relatively small number of subjects. Our results, however, warrant a larger study on the prevalence of hypertension in patients with vWD. The results of the present study show no protection against atherosclerosis in patients with type 3 vWD and suggest that vWF does not play a significant role in the origin of human atherosclerosis.

Acknowledgments

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References

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