von Willebrand Factor
A Marker of Endothelial Damage?

Pier Mannuccio Mannucci

Von Willebrand factor (vWF), a large glycoprotein encoded by a gene on chromosome 12, is synthesized by vascular endothelial cells and circulates in human plasma at concentrations of 10 µg/mL. In plasma, vWF forms a noncovalent complex with coagulation factor VIII, the protein encoded by a gene on the X chromosome that is deficient or defective in hemophilia A. This molecular complex is essential for normal survival of factor VIII, which is stabilized in the circulation, potentiated in its cofactor activity in clot formation, and protected from proteolytic inactivation. The other important function of vWF in physiological hemostasis is in the formation of platelet plugs at sites of endothelial damage, in which the protein binds to the exposed subendothelium and forms a bridge between this surface and platelets. These functions are facilitated by the peculiar structure of vWF, which is arranged in multimers of increasing size up to 2×10^5 Da built up from a subunit of 2.5×10^4 Da, and by its exposure on the platelet membrane to the glycoprotein complexes Ib/IX/V and IIb/IIIa, which function as receptors for vWF. The importance of vWF in hemostasis is further supported by the fact that inherited deficiencies or dysfunctions of this protein cause a bleeding disorder called von Willebrand disease, relatively frequent in humans and animals.

vWF-mediated platelet adhesion to the injured endothelium is the first step in thrombus formation. That vWF plays a role in thrombosis is also supported by the demonstration that the largest multimeric forms of the glycoprotein aggregate platelets in vitro under conditions of high shear stress, such as those occurring in stenotic arteries. Recently, interest in vWF has extended beyond the pathophysiological roles listed above, since a few prospective clinical studies have demonstrated that in individuals with cardiovascular disease, high plasma levels of vWF predict the subsequent occurrence of major clinical events such as death and myocardial infarction. It is often believed that the value of vWF in the prediction of cardiovascular events is related to the fact that plasma levels of the protein signal the extent of damage in the vascular endothelium. This review will analyze whether this belief is supported by the available evidence.

Cell Biology of vWF

vWF is synthesized as a precursor called pro-vWF. After processing in the endoplasmic reticulum and the Golgi apparatus of the endothelial cell, the precursor undergoes multimerization and is cleaved into 2 products, the mature protein and a propeptide of 97 kDa. vWF is also synthesized by megakaryocytes and is contained in platelets, which make 15% of the circulating protein in blood. The platelet factor encoded by a gene on chromosome 12, is synthesized by vascular endothelial cells and circulates in human plasma at concentrations of 10 µg/mL. In plasma, vWF forms a noncovalent complex with coagulation factor VIII, the protein encoded by a gene on the X chromosome that is deficient or defective in hemophilia A. This molecular complex is essential for normal survival of factor VIII, which is stabilized in the circulation, potentiated in its cofactor activity in clot formation, and protected from proteolytic inactivation. The other important function of vWF in physiological hemostasis is in the formation of platelet plugs at sites of endothelial damage, in which the protein binds to the exposed subendothelium and forms a bridge between this surface and platelets. These functions are facilitated by the peculiar structure of vWF, which is arranged in multimers of increasing size up to 2×10^5 Da built up from a subunit of 2.5×10^4 Da, and by its exposure on the platelet membrane to the glycoprotein complexes Ib/IX/V and IIb/IIIa, which function as receptors for vWF. The importance of vWF in hemostasis is further supported by the fact that inherited deficiencies or dysfunctions of this protein cause a bleeding disorder called von Willebrand disease, relatively frequent in humans and animals.

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TABLE 1. Triggers of In Vitro Secretion of vWF by Cultured Endothelial Cells

<table>
<thead>
<tr>
<th>Mediators of Hemostasis</th>
<th>Mediators of Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin</td>
<td>Histamine</td>
</tr>
<tr>
<td>Fibrin</td>
<td>Complement components C5a and C5b-9</td>
</tr>
<tr>
<td>Plasmin</td>
<td></td>
</tr>
<tr>
<td>Adenine nucleotides</td>
<td>Leukotrienes</td>
</tr>
<tr>
<td></td>
<td>Superoxide anions</td>
</tr>
<tr>
<td></td>
<td>Endotoxin</td>
</tr>
<tr>
<td></td>
<td>Interleukin 1*</td>
</tr>
<tr>
<td></td>
<td>Tumor necrosis factor*</td>
</tr>
</tbody>
</table>

*These cytokines do not directly secrete vWF, but they do enhance the activity of thrombin.16

TABLE 2. Conditions Associated With the Increase of Plasma vWF in Humans

<table>
<thead>
<tr>
<th>Rapid, Short-Term Increase</th>
<th>Slow, Long-Term Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection of Epinephrine</td>
<td>Liver cirrhosis</td>
</tr>
<tr>
<td>Desmopressin</td>
<td>Postoperative period</td>
</tr>
<tr>
<td>Muscular exercise</td>
<td>Malignancy</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>CNS stimulation</td>
<td>Renal failure</td>
</tr>
<tr>
<td>Venous occlusion</td>
<td>Acute coronary syndromes</td>
</tr>
<tr>
<td></td>
<td>Diabetes</td>
</tr>
<tr>
<td></td>
<td>Hemolytic anemias</td>
</tr>
</tbody>
</table>

CNS indicates central nervous system. For more details and references, see Reference 19.

Epinephrine or desmopressin and after strenuous muscular exercise, the increase of vWF is so rapid and transient that increased synthesis is unlikely to account for it, and release from storage organelles in the endothelial cells is a more plausible mechanism. Epinephrine is the mediator of the exercise-induced increase, because the vWF response can be blocked by the administration of β-receptor antagonists.19 Venous occlusion is another procedure accompanied by a short-term increase of vWF in plasma. Whether this increase is due to endothelial secretion in plasma or to hemococoncentration induced by venous stasis is not well established. Among these procedures, the infusion of desmopressin is the most suitable and convenient experimental model that can be used to investigate secretion of vWF in vivo, being devoid of significant side effects and being suitable for an accurate evaluation of poststimulus changes of vWF in plasma.16

In a large group of varied clinical conditions, listed in Table 2, there is a long-term and sustained increase of the protein in plasma.19 This is probably due to heightened synthesis, although a mechanism of decreased clearance cannot be ruled out. The increase in vWF is usually accompanied by increases of other plasma glycoproteins, most typically fibrinogen and other acute-phase reactants.19 This suggests that the long-term changes in plasma levels of vWF might simply be the expression of acute-phase reactions to various stimuli, such as inflammation, tissue necrosis and repair, and neoplastic growth.20 Acute coronary syndromes are the prototype of the clinical conditions associated with this pattern of increase of vWF,21–23 peaking 3 to 4 days after an infarction and concomitantly with acute myocardial necrosis and diminishing in the postinfarction period as healing progresses.

vWF: A Marker of Endothelial Damage?

Boneu et al8 were the first to propose the measurement of plasma vWF as an index of endothelial damage in vascular disease. Their hypothesis was based on the observation that patients with ischemic limb disease or septicemia had vWF levels raised in proportion with the extent of vascular involvement.8 Subsequently, several investigators found that vWF was high in an array of clinical situations, all characterized by vascular damage with denudation of the endothelium and exposure of the subendothelium (for instance, acute respiratory failure, acute and chronic renal insufficiency, hypertension, diabetic nephropathy, and vasculitis).21–29 To interpret these findings, it has been postulated that in patients with large- and small-vessel disease, the membranes of damaged endothelial cells would “leak” vWF, leading to an increase of the plasma levels of this protein. Clinicians became attracted by this simple approach to the evaluation of the degree of endothelial damage in vascular disease, because it reminded them of the usefulness and widespread adoption in clinical practice of the measurement of serum enzymes to evaluate the degree of hepatic and cardiac cell damage.

There are several problems, however, with this simple and attractive model. The most important is the poor specificity of the marker. First of all, vWF is an acute plasma reactant that can increase in plasma during clinical conditions not necessarily associated with endothelial cell damage. In addition,
plasma vWF is not endothelial cell specific because it might derive, if only in small part, from activated platelets. This may be an important confounder in clinical practice, because platelet activation is a consistent companion of endothelial damage and subendothelium exposure in vascular disease. Another problem is that vWF as a plasma marker of endothelial damage has been poorly validated in vivo by suitable animal experiments that have correlated the changes in plasma levels of the protein with the degree and extent of vascular damage induced. The bulk of the experimental evidence comes from in vitro experiments performed in human endothelial cells in culture. The in vitro environment is hardly a normal environment, because endothelial cells in culture are continuously responding to injury. Hence, any measured increase of vWF in plasma might not be the direct consequence of protein leakage from the damaged endothelial cells; it might also originate from secretion of the protein from viable endothelial cells perturbed in their steady state by pathological triggers (Table 1). Thus, plasma levels of vWF could not necessarily reflect the actual degree of endothelial cell damage and platelet activation but the stimulation and perturbation of viable endothelial cells.

On the whole, there is little theoretical or experimental evidence that the elevations of plasma levels of vWF observed in a variety of human diseases associated with pathological vascular states closely reflect the extent of vascular damage. It is more likely that these changes reflect a shift in the functions of the endothelium, leading to heightened secretion of the protein. Hence, it seems that the terms “endothelial perturbation” or “stimulation” are more adequate than the terms “endothelial damage” or “injury.”

Other Candidates as Markers of Endothelial Perturbation

Identifying a single marker for endothelial perturbation or stimulation is conceptually attractive for the clinician but quite simplistic if one considers the multitude of proteins synthesized and expressed by endothelial cells, which contribute to the multiple and often opposing functions of the normal endothelium in the regulation of hemostasis. Accordingly, several other proteins involved in hemostasis and synthesized by endothelial cells have been proposed as markers for endothelial perturbation and have been measured in patients with vascular disease. These proteins include tissue plasminogen activator, plasminogen activator inhibitor-1, thrombomodulin, and tissue factor. In general, the correlation between the levels of these proteins in various vascular diseases is poor, so that it would appear that they measure different properties of the vascular endothelium. In addition, the considerations of poor cellular specificity and lack of validation by in vivo experimental models mentioned for vWF are also applicable to these proteins. These limits, however, do not impinge on the predictive value of high plasma levels of some of them in cardiovascular disease, whatever their status may be as markers of endothelial damage. For instance, tissue plasminogen activator levels are good predictors of the risk of myocardial infarction and stroke.

Recently, the measurement of plasma vWF propeptide has been proposed as a more sensitive and early marker of endothelial cell perturbation. Measureable with specific immunoassays based on the use of monoclonal antibodies, this protein has some theoretical advantages over mature vWF. The propeptide does not adhere to the vascular subendothelium, so that any heightened secretion would be fully reflected by an increase in plasma levels. Importantly, the propeptide has a shorter plasma half-life (3 hours) than the mature protein (12 to 18 hours). Hence, it could be taken as an index of acute endothelial perturbation, whereas the measurement of mature vWF would provide information on the occurrence of sustained perturbation. These views are supported by the observation that in an animal model of acute thrombin and fibrin formation induced by the infusion of activated factor X, propeptide concentrations increased, whereas the concentrations of mature vWF remained substantially unchanged. Additional work should be done to establish whether or not the propeptide is a more reliable marker for endothelial perturbation than is mature vWF.

Concluding Remarks

At the moment, vascular biologists have not been able to provide clinicians with a reliable marker for endothelial cell damage in cardiovascular disease. The main problem with vWF as a candidate marker is its poor specificity, a problem shared by other soluble products of the endothelial cell that have been considered as noninvasive aids for diagnosis, for studying the extent of vascular involvement, and for monitoring the effect of treatment. Despite these caveats, it does appears that high vWF levels help to predict cardiovascular events, although the marker is not powerfully predictive in the individual at risk. The subject dealt with in this article has been previously reviewed.

References

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