

## Von Willebrand Factor in Cardiovascular Disease Focus on Acute Coronary Syndromes

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**Abstract**—von Willebrand factor (VWF) plays a pivotal role in platelet adhesion and aggregation at sites of high shear rates (eg, in coronary arteries that have stenotic or ruptured atherosclerotic plaque lesions). Numerous studies have investigated the relationship between VWF plasma levels and thromboembolic cardiovascular events. In contrast to the rather weak association in the general population, in patients with preexisting vascular disease, VWF is significantly predictive for adverse cardiac events, including death. Likewise, VWF typically rises during the course of acute coronary syndrome, and the extent of this VWF release is an independent predictor of adverse clinical outcome in these patients. Various lines of evidence indicate that VWF is not only a marker but also actually an important effector in the pathogenesis of myocardial infarction. This central role of VWF in thrombogenesis has made it a promising target for research into new antiplatelet therapies that specifically inhibit VWF. This review focuses on the role of VWF in acute coronary syndrome and further outlines the relevance of therapeutic interventions targeting VWF for acute coronary syndrome patients. (*Circulation*. 2008;117:1449-1459.)

**Key Words:** coronary disease ■ myocardial infarction ■ platelets ■ thrombosis ■ von Willebrand factor

For >150 years, it has been known that alterations in blood flow, vascular wall, and blood components, the so-called Virchow's triad,<sup>1</sup> may progressively lead to thrombus formation. Yet, a more complete understanding of the complex interactions among the vascular endothelium, platelet adhesion, activation, aggregation, and clotting factor activation involved in this process is still emerging from contemporary research.

Under physiological conditions, the vascular endothelium produces many substances that contribute importantly to hemostasis, fibrinolysis, and regulation of vessel tone and permeability. One such substance is the multimeric glycoprotein von Willebrand factor (VWF), which is produced almost exclusively by endothelial cells.<sup>2</sup> Plasma levels of VWF are raised in different states of endothelial damage and have therefore been proposed as useful markers of endothelial dysfunction.<sup>3</sup> Along this line, blockade of nitric oxide enhances the stimulated release of VWF in humans.<sup>4,5</sup> Furthermore, VWF plays a crucial role in platelet adhesion and aggregation under high shear conditions.<sup>6</sup> Finally, VWF supports the third component of Virchow's triad, clotting factor activation, by acting as a carrier protein and stabilizer for factor VIII.<sup>7</sup>

Almost all acute coronary syndromes (ACS) result from thrombus formation in preexisting coronary atherosclerosis. As a result of plaque rupture and exposure of prothrombotic subendothelial matrix, local thrombus formation occurs, which subsequently leads to coronary artery occlusion and

acute myocardial infarction (AMI). The presence of VWF has been shown to play a pivotal role in platelet aggregation at sites of high shear, eg, at the sites of lesions in the coronary arteries.<sup>8</sup> Accordingly, VWF is a well-characterized marker of cardiovascular risk, and VWF plasma levels are increased in patients with ACS.<sup>9</sup> Considering the central role of VWF in thrombogenesis, therapies that specifically inhibit VWF are of particular interest as potential new antiplatelet drugs. This review focuses on the role of VWF in ACS and further outlines the relevance of potential anti-VWF interventions in the context of recent preclinical research.

### Von Willebrand Factor

VWF is a multimeric protein encoded on the short arm of chromosome 12. The VWF gene encodes a 250-kDa protein that forms the basic monomer.<sup>10</sup> The mature molecule is composed of 50 to 100 monomers and can reach an ultimate size of up to 20 MDa. Each VWF subunit has binding sites for factor VIII, platelet glycoprotein Ib (GPIb), GPIIb/IIIa, heparin, and collagen, some of which are dependent on the shear-induced conformational change.<sup>11,12</sup>

Endothelial cells produce VWF almost exclusively. Although the presence of VWF in megakaryocytes has been proved, crossover transplantation models argue against a significant physiological mechanism of VWF release from megakaryocytes.<sup>13</sup> Accordingly, VWF can be found in platelet  $\alpha$  granules without any exchange with plasma VWF in vitro or in vivo.<sup>14</sup>

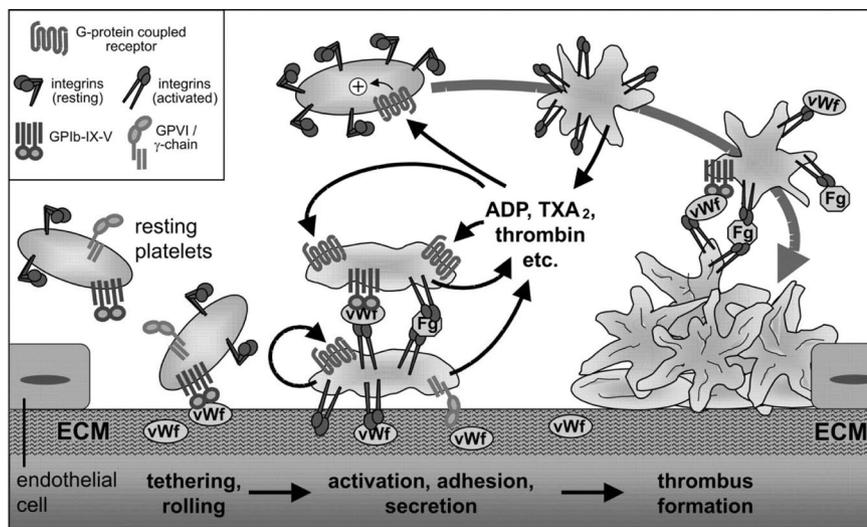
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**Figure.** The function of VWF in platelet activation at sites of vascular injury. Subendothelial vWf and GPIb mediate the initial contact of platelets with the extracellular matrix at sites of vascular injury. Plasma VWF, activated under conditions of high shear rates, binds to GPIb-IX-V, which further amplifies platelet activation, enabling VWF binding to activated integrins, GPIIb/IIIa. The platelet-GPIIb/IIIa-VWF interactions play a leading role in platelet aggregation and thrombus formation, especially under high shear conditions. ECM indicates extracellular matrix; Fg, fibrinogen. Reprinted with permission from Offermanns S. Activation of platelet function through G protein-coupled receptors. *Circ Res.* 2006;99:1293–1304; copyright © 2006, the American Heart Association.

### Plasma and Subendothelial VWF

VWF can be produced and released by endothelial cells by a variety of stimuli *in vitro* and *in vivo* such as hypoxia,<sup>15</sup> inflammatory cytokines,<sup>16</sup> thrombin,<sup>17</sup> leukocyte elastase,<sup>18</sup> histamine,<sup>4</sup> endotoxin,<sup>19</sup> exercise,<sup>20</sup> adrenaline,<sup>21</sup> and especially vasopressin.<sup>22</sup> Indeed, patients with von Willebrand disease are effectively treated with desmopressin, the synthetic analog of vasopressin, to raise their VWF levels.<sup>23</sup>

Both the constitutive and regulated pathways of secretion deposit VWF in subendothelium. Normal subendothelium contains varying amounts of VWF, depending on the anatomic region. Experimental evidence suggests that VWF constitutively present in the vessel wall can mediate platelet adhesion, but subendothelial VWF by itself is insufficient for optimal primary hemostasis.<sup>24,25</sup> That VWF may not be present in the subendothelium of arterioles and arterial capillaries,<sup>26,27</sup> the smallest vessels that participate actively in hemostasis, is relevant.<sup>28</sup> Thus, plasma VWF also is necessary during the initial platelet response to vascular injury. The Figure shows the various functions of VWF for platelet activation at sites of vascular injury.

### Pathophysiological Role of VWF in Thrombus Formation

VWF has 3 important functions in the process of thrombus formation. First, binding of plasma VWF to platelets is critical for the normal platelet adhesion and aggregation that occurs at high shear rates. Binding to platelets requires initial VWF activation, leading to a structural change so that the A1 domain can bind to the platelet receptor GPIb-IX-V complex on the platelet surface.<sup>29,30</sup> This binding occurs even on nonactivated platelets.<sup>31</sup> VWF is activated by binding to subendothelial structures exposed after endothelial damage<sup>32</sup> or under high shear stresses present in small arterioles and atherosclerotic arteries (shear rates >1000/s).<sup>12</sup> Additionally, binding of VWF to platelet GPIb seems to generate procoagulant platelet-derived microparticles, which further enhance thrombus formation.<sup>33</sup>

A second platelet receptor for VWF, GPIIb/IIIa, does not bind VWF unless the platelets are activated, which can be

elicited by a variety of stimuli, including activation induced by the GPIb-IX-V VWF complex.<sup>34</sup> The platelet-GPIIb/IIIa-VWF interactions appear to contribute to the final, irreversible binding of platelets to the subendothelium and play a leading role in platelet aggregation, especially under high shear conditions.<sup>8</sup> However, platelet aggregation in an experimental model seemed to be independent of GPIIb/IIIa under extremely elevated shear rates (>10 000/s) and solely dependent on VWF binding to GPIb.<sup>35</sup>

Second, plasma VWF binds to several types of collagen, most importantly collagen type IV, in the subendothelial connective tissue.<sup>36</sup> Collagen binding appears to induce a conformational change within the factor VIII-binding motif of VWF that lowers the affinity for factor VIII. Consequently, released factor VIII may locally support fibrin clot formation.<sup>37</sup> This appears to be of particular importance when vasculature is injured and becomes denuded, as occurs in ACS.

Third, VWF plays a role in fibrin clot formation by acting as a carrier protein for factor VIII. Without VWF, the half-life of factor VIII is shortened 10- to 20-fold because of proteolytic inactivation by activated protein C and its cofactor protein S.<sup>38</sup> VWF can bind factor VIII only when factor VIII has not been cleaved by thrombin.<sup>39</sup> The clinical importance of factor VIII for thrombin generation is illustrated by the fact that its deficiency leads to the severe clinical pattern of hemophilia A. Factor VIII is further decreased in von Willebrand disease. Low levels of factor VIII result in defective fibrin clot formation and a reduction in primary platelet plug formation in these patients.

Another example of the importance of VWF in human thrombotic disease is illustrated by ADAMTS-13. This metalloproteinase enzymatically converts ultralarge VWF to smaller forms normally seen in the circulation by cleaving the A2 domain.<sup>40</sup> Because these larger forms are most active in platelet aggregation, failure to cleave large multimers promotes thrombosis.<sup>41</sup> Indeed, patients with ADAMTS-13 deficiency have an increased thrombosis risk.<sup>42</sup> Furthermore, the association of thrombotic thrombocytopenic purpura with antibodies against or congenital deficiency of ADAMTS-13

illustrates the physiological importance of this cleaving protease.<sup>43</sup>

### Laboratory Identification of VWF

Many different VWF-dependent laboratory assays have been developed to correctly diagnose and classify von Willebrand disease.<sup>44</sup> If VWF is investigated as a risk factor for cardiovascular disease, plasma VWF antigen and plasma VWF activity, commonly assayed as ristocetin cofactor activity, are applied in most cases.

Plasma VWF antigen usually is identified by ELISA on microtiter plates. Automated methods using latex beads coated with antibodies to VWF and a turbidimetric end point also have been introduced.

VWF activity is commonly assayed as ristocetin cofactor activity, which tests the ability of VWF to agglutinate platelets by binding to its primary receptor, platelet GPIb, in the presence of the antibiotic ristocetin that binds to both VWF and GPIb and causes agglutination of platelets.<sup>45</sup> Although the ristocetin cofactor test has been difficult to standardize (interassay and interlaboratory variability), it is automated, is still the gold standard method for VWF activity testing, and has some capacity to preferentially recognize the larger VWF multimers.

The binding of plasma VWF to collagen-coated plates has been introduced as an alternative, albeit more time-consuming, functional assay.<sup>46</sup> Furthermore, the platelet function analyzer (Platelet Function Analyzer 100) closure time is highly dependent on VWF function and therefore is used as a highly sensitive screening tool for von Willebrand disease.<sup>47</sup> Finally, multimer analysis can be performed particularly in von Willebrand disease and thrombotic thrombocytopenic purpura patients.<sup>48</sup>

### VWF as a Prognostic Marker for Coronary Artery Disease in Initially Healthy Subjects

Elevated plasma levels of VWF are associated with established cardiovascular risk factors such as age,<sup>49</sup> smoking,<sup>50</sup> cholesterol,<sup>51</sup> diabetes mellitus,<sup>52</sup> and hypertension.<sup>53</sup> Moreover, raised levels of VWF are predictive of stroke and vascular events among patients with atrial fibrillation.<sup>54</sup> Correspondingly, many studies have investigated the association between VWF levels and the development of cardiovascular disease in a prospective manner.

In the large Atherosclerosis Risk in Communities (ARIC) study, the relative risk for coronary artery disease (CAD) was significantly, but only slightly, elevated (relative risk,  $\approx 1.3$ ) in the highest VWF tertile. However, adjustment for conventional CAD risk factors, especially diabetes mellitus, eliminated this association.<sup>55</sup> Data from 2 Swedish cohorts showed a more pronounced association between VWF and CAD risk (relative risk  $> 2.0$  for the highest versus the lowest quartile). However, this association also lost significance after adjustment for coexisting risk factors.<sup>56</sup> Accordingly, several other studies in initially healthy subjects described a rather weak association between VWF levels and CAD risk that did not always reach statistical significance.<sup>57–59</sup>

More encouragingly, a recent nested case-control study, the Prospective Epidemiological Study of Myocardial Infarc-

tion (PRIME), showed a 3-fold increased risk for severe CAD (fatal or nonfatal MI) in individuals with plasma VWF levels in the highest quartile compared with those in the lowest quartile. The difference persisted even after adjustment for multiple markers of inflammation.<sup>60</sup> In addition, Whincup et al<sup>61</sup> demonstrated a significant increase in CAD risk for the highest VWF tertile that persisted even after adjustment for conventional risk factors. They further conducted a meta-analysis of all relevant population-based prospective studies, which additionally confirmed this relationship.

However, these studies are dwarfed by the very large Reykjavik study with nearly 20 000 subjects, of whom almost 2500 developed major CAD after a median follow-up of 17.5 years. This study reported an odds ratio of only 1.23 for the highest versus the lowest quartile of VWF, which decreased even further to nonsignificant values after adjustment for CAD risk factors.<sup>62</sup>

Taken together, these data indicate that plasma VWF levels are at best a weak independent predictor of future CAD in initially healthy subjects.<sup>63</sup> However, the fact that the association between VWF and CAD risk disappears after adjustment for conventional risk factors, in particular diabetes mellitus, does not exclude the possibility that these factors exert their deleterious effect via VWF increase; rather, it may simply reflect the high degree of correlation among them.

### VWF and ACS

ACS is caused by rupture of atherosclerotic plaques, exposure of subendothelial procoagulant factors, and subsequent thrombus formation leading to myocardial ischemia. VWF is fundamentally involved in this process. It supports platelet adhesion to the subendothelial matrix of injured vessel walls, enhances platelet aggregation, and promotes fibrin clot formation. Correspondingly, shear-induced platelet aggregation is markedly enhanced as a result of increased VWF concentrations in plasma from patients with AMI compared with control subjects.<sup>64,65</sup> In addition, the shear rate threshold required to induce measurable VWF binding to platelets is significantly reduced in plasma from patients with AMI.<sup>66</sup> Furthermore, detection of VWF in fresh, human coronary thrombi suggests a causative role of VWF in platelet thrombus growth.<sup>67,68</sup> The distinctive role of VWF is evidenced by elevated VWF levels and VWF activity in the context of ACS. The unweighted mean  $\pm$  SD of published VWF data<sup>64,69–82</sup> shows that patients with AMI ( $196 \pm 39\%$ ;  $n=877$ ) have markedly increased VWF values compared with unstable angina pectoris ( $156 \pm 41\%$ ;  $n=345$ ) and CAD ( $140 \pm 30\%$ ;  $n=300$ ) patients, as well as healthy control subjects ( $110 \pm 17\%$ ;  $n=394$ ).

The association between VWF levels and the prospective incidence of MI in vascular disease patients is well established. The large European Concerted Action on Thrombosis and Disabilities (ECAT) study showed that VWF is an independent predictor of recurrent MI in patients with angina pectoris. The relative risk for the recurrence of cardiovascular events was 1.85 between the extreme quintiles, even after adjustment for cardiovascular risk factors.<sup>83</sup> These results are corroborated by numerous studies that also described an association between VWF levels and reinfarction and/or

**Table 1. Studies on the Prognostic Value of VWF in Patients With Coronary Heart Disease**

Study Population	Outcome Parameters	Subjects With Events/ Subjects Under Observation	Results	Follow-Up, y	Reference
MI patients	Death	68/272	Mean±SD VWF Ag levels: 204±113 (death) vs 145±85 (survival); $P<0.001$	1	71
MI survivors	Reinfarction and death from all causes	59/123	$R=0.20$ for any event; $P<0.001$	4.9	84
MI survivors and stable AP patients	Atherothrombotic events (fatal/nonfatal MI, cardiac death, TIA, minor stroke, acute peripheral ischemia, peripheral bypass occlusion)	31/417	SRE=1.7–1.8; $P=0.004$ – $0.026$	2	85
AP patients	MI and sudden cardiac death	106/2960	Relative risk=1.85 between extreme quintiles; $P=0.05$	2	83
MI patients	Reinfarction	86/2246	Odds ratio=2.32 (95% CI, 1.33–4.02) vs age-matched controls without reinfarction	≈3	86
ACS patients	MI, revascularization, hospitalization because of angina, cardiac death	58/208	Hazards ratio=6.3 (95% CI, 2.1–18.3) between extreme quartiles; $P<0.001$	2.3	87

Ag indicates antigen; R, Regression coefficient, Cox regression model; AP, angina pectoris; TIA, transient ischemic attack; SRE, standardized regression effect, univariate analysis; and CI, confidence interval.

mortality risk. For example, Haines et al<sup>71</sup> showed that patients with MI who died within 1 year had higher VWF levels than did those who survived. Similarly, high concentrations of VWF have been shown to be independently associated with both reinfarction and mortality in MI survivors <70 years of age.<sup>84</sup> Likewise, the prospective Italian Progetto Lombardo Atero-Trombosi (PLAT) study in patients with preexisting ischemic vascular disease demonstrated that in MI survivors a 1-SD rise in VWF levels was associated with a 70% increase in event rates. Furthermore, this trial showed that VWF was most significantly related to atherothrombotic events in the angina pectoris group.<sup>85</sup> Consistent with these results is the finding in the large population-based Stockholm Heart Epidemiology Program (SHEEP) study that higher VWF concentrations ( $\geq 75$  percentile) proved to be a very good predictor of AMI recurrence, with a crude odds ratio for reinfarction of 2.3.<sup>86</sup> Table 1 summarizes the key information of published studies on the prognostic value of VWF in patients with CAD.<sup>71,83–87</sup>

Taking all these results together, it appears that although the association between VWF levels and coronary events in the general, apparently healthy, population is weak, this association becomes much stronger in patients with preexisting vascular disease, particularly MI survivors.

Because VWF is normally proteolytically degraded by the enzyme ADAMTS13, it is natural to consider the possibility that ADAMTS13 activity is itself abnormal in the setting of ischemic cardiovascular disease. However, the exact role of this VWF cleaving protease in the context of ACS remains unclear. On the one hand, ADAMTS-13 levels were shown to be reduced in patients with AMI.<sup>88</sup> On the other hand, a recently published case-control study demonstrated an increased MI risk in patients with higher ADAMTS-13 levels.<sup>89</sup> This discrepancy remains unexplained and merits further investigation.

VWF levels show a typical time course during an acute cardiovascular event. In the setting of ST-elevation MI (STEMI), VWF levels become elevated at 24 hours and peak at 48 to 72 hours before returning to baseline at around day 14.<sup>76</sup> The extent of this rise in VWF also has prognostic value. Patients with AMI have increased VWF levels compared with patients with unstable angina pectoris.<sup>79,90</sup> Moreover, the extent of the VWF increase is an independent predictor of short-term adverse clinical outcome in patients with ACS (Table 2).<sup>91–94</sup>

For instance, the extent of VWF release (ie, the difference between baseline and 24-hour VWF values during the index event), not only is associated with the incidence of acute heart failure but also is significantly correlated with 30-day mortality in patients with STEMI.<sup>93</sup> In addition, an Enoxaparin and TNK-tPA With or Without GPIIb/IIIa Inhibitor as Reperfusion Strategy in STEMI (ENTIRE) Thrombolysis in Myocardial Infarction (TIMI) 23 substudy found a significant association between the VWF rise and the incidence of death or MI at 30 days in STEMI patients treated with fibrinolysis; the  $\geq 75$ th percentile showed an event rate of 11.2% compared with 4.1% below the 75th percentile.<sup>94</sup> Likewise, a subgroup analysis of the Efficacy and Safety of Subcutaneous Enoxaparin in Non-Q-Wave Coronary Events (ESSENCE) trial demonstrated that the early rise of VWF is an independent predictor of adverse clinical outcome at days 14 and 30 in patients with unstable angina pectoris or non-Q-wave MI.<sup>91</sup>

The combined evaluation of VWF and troponin I in this patient group provides information on the long-term prognosis. High VWF and high troponin I are significantly associated with an increase in the composite end point of death, AMI, recurrent angina, and revascularization at the 1-year follow-up.<sup>95</sup>

Interestingly, administration of either enoxaparin or pegylated-hirudin is able to blunt the VWF rise in patients

**Table 2. Studies on the Short-Term Predictive Value of VWF Release in Patients With Acute STEMI and UAP**

Study Population	Outcome Parameters	Subjects Under Observation, n	Results	Reference
UAP or non-Q-wave MI	Death, recurrent angina, and revascularization at day 14	68	$\Delta$ VWF (H48-H0) 75±15% (point) vs 34±11 (no point); <i>P</i> =0.02	91
UAP	Death, recurrent angina, and revascularization at day 30	154	$\Delta$ VWF (H48-H0) 73±7% (point) vs 7±14 (no point); <i>P</i> =0.004	92
STEMI patients	Death at day 30	153	$\Delta$ VWF (H24-H0) 65.8±20.0% (point) vs 10.0±5.1% (no point) (mean±SEM); <i>P</i> =0.004	93
STEMI patients	Death or MI at day 30	314	$\Delta$ VWF (H48/72-H0), ≥75th percentile vs <75th percentile; subjects with point: 11.2 vs 4.1%; <i>P</i> =0.027	94

UAP indicates unstable angina pectoris;  $\Delta$ VWF, changes in VWF compared with baseline (H0).

with unstable angina pectoris compared with unfractionated heparin or dalteparin and is associated with a more favorable clinical outcome.<sup>92</sup> Similarly, use of enoxaparin after fibrinolytic therapy reduces the VWF rise in patients with STEMI and is associated with a reduction in death or subsequent MI at the 30-day follow-up.<sup>94</sup> However, 2 other studies could not confirm the beneficial effects of enoxaparin compared with dalteparin and unfractionated heparin in patients with ACS.<sup>96,97</sup>

Recent studies provide evidence that not only VWF but also platelet plug formation under high shear rates, as measured by a platelet function analyzer (Platelet Function Analyzer 100), is predictive of the degree of myocardial necrosis, myocardial blood flow, and future events in patients with ACS.<sup>79,87,98</sup> Treatment of ACS was predominantly by percutaneous coronary intervention (PCI) in these studies. Because PCI nearly universally restores epicardial flow, one can hypothesize that the predictive value of excessive VWF-dependent platelet function is attributable to impaired microcirculatory perfusion in the heart, not to epicardial flow. This notion is supported by the correlation between platelet hyperfunction and impaired myocardial muscle perfusion as measured by the degree of ST-segment resolution and myocardial blush grade.<sup>98</sup>

**VWF and PCI**

PCI has become the reperfusion therapy of choice in patients with either STEMI or high-risk non-STEMI AMI because it has been demonstrated to improve clinical outcome.<sup>99</sup> However, a significant proportion of patients in whom Thrombolysis in Myocardial Infarction grade 3 flow is achieved with primary PCI for STEMI still show abnormal myocardial perfusion, which in turn results in increased infarct size, reduced left ventricular ejection fraction, and reduced survival.<sup>100-102</sup> In the context of this problem of impaired microvascular circulation and myocardial perfusion after otherwise successful PCI, it may be relevant that PCI and stent implantation are themselves associated with endothelial damage and concurrent VWF release,<sup>103</sup> especially in the case of multiple coronary stenting, which induces a rapid increase in VWF expression in the coronary circulation.<sup>104</sup> It is possible therefore that this mechanically induced increase in VWF

may exacerbate the prothrombotic state of the ACS patient and inadvertently contribute to the impaired myocardial microcirculation remaining after PCI despite restored normal epicardial flow. Consistent with this hypothesis is the observation that stenting with drug-eluting devices is associated with a reduced inflammatory response and diminished VWF antigen levels in the coronary circulation.<sup>105</sup> This may at least in part be causative for the observed reduction in periprocedural troponin elevation in patients receiving drug-eluting stents compared with bare metal stents in the Randomized Trial to Evaluate the Relative Protection Against Post-PCI Microvascular Dysfunction and Post-PCI Ischemia Among Anti-Platelet and Anti-Thrombotic Agents—Thrombolysis in Myocardial Infarction-30 trial (PROTECT-TIMI 30).<sup>106</sup>

**VWF and Thrombolysis**

Thrombolytic agents have an established role in the management of patients with AMI. Given that VWF is a key factor in coronary thrombus formation, interaction of the thrombolytic agents with VWF seems to be crucial. Several trials have studied the regulation of VWF in relation to thrombolytic therapy.

Successful thrombolysis, as measured by a patent infarct-related artery, in patients with evolving MI limits the rise in VWF levels.<sup>107</sup> Moreover, the change in plasma VWF during the first 24 hours after thrombolysis appears to identify successful coronary recanalization and in this regard compares favorably with established markers of reperfusion. Patients with a patent infarct-related artery showed a highly significant fall in VWF as compared to those with an occluded artery.<sup>108</sup> Consistently, a protracted elevation in VWF levels has been observed in patients with occluded infarct-related artery for whom rescue PCI was required.<sup>109</sup>

Although the clinical benefits of thrombolytic therapy in the management of STEMI are well established, concerns have been raised that further myocardial damage via endothelial activation may occur after reperfusion therapy. Indeed, thrombolysis in AMI patients leads to some degree of endothelial cell damage as evidenced by a slight increase in VWF levels peaking between 3 (streptokinase) and 72 (recombinant tissue plasminogen activator) hours after lysis.<sup>75,110</sup> Furthermore, VWF undergoes degradation during

thrombolytic therapy in AMI patients. The degree of degradation depends on the type and dose of thrombolytic agent (being greater for streptokinase than for recombinant tissue plasminogen activator or urokinase). In contrast to the increase in VWF protein levels, a relative decrease in VWF ristocetin cofactor activity and high-molecular-weight multimers of VWF was observed after thrombolysis.<sup>110</sup> VWF degradation has been speculated to be a potential causative factor for bleeding complications in treated patients.

### Is VWF Only a Marker or Also a Pathogenic Mediator?

As mentioned previously, VWF is prominently present at the sites of platelet accumulation in coronary artery thrombi.<sup>67,68</sup> These studies have elegantly shown the colocalization of VWF with platelet thrombi, tissue factor, and platelets with fibrin in fresh coronary thrombi of patients with MI. Furthermore, shear-induced platelet aggregation and VWF binding to platelets is markedly enhanced in plasma from patients with AMI compared with control plasma.<sup>64,65</sup> An antagonist of the VWF-GPIIb binding domain effectively abolished cyclic flow variations in stenotic, endothelium-injured coronary arteries.<sup>111</sup> Likewise, and as further outlined below, various antibodies targeting VWF inhibited platelet aggregation *in vitro* and reduced coronary artery thrombosis *in vivo* in animal models of disease.<sup>74,112</sup>

Interestingly, certain rare diseases provide compelling indirect evidence of a pathogenic role of VWF in AMI. First, presentation of acute thrombotic thrombocytopenic purpura, the hallmark of which is a pronounced rise in the level of circulating ultralarge von Willebrand factor, the most active multimers in a patient's plasma, is quite commonly associated with myocardial injury.<sup>113,113a</sup> This may lead to severe MI, cardiogenic shock, and cardiac arrest as described in several recently published case reports.<sup>114–116</sup> A causative role of VWF in such cases is further evidenced by the autopsy finding of large amounts of VWF within arteriolar microthrombi in areas of myocardial necrosis.<sup>117</sup> Second, Hoylaerts et al<sup>118</sup> reported a case of recurrent arterial thrombosis in a young woman linked to autoimmune antibodies enhancing VWF binding to platelets and inducing platelet activation. Conversely, arterial thrombosis appears to be very rare in patients with the forms of von Willebrand disease that arise from a qualitative or quantitative deficiency of VWF.<sup>119</sup>

In addition, an association between the genetic trait of the ABO blood group and MI has been recognized for a long time.<sup>57,120</sup> Interestingly, the O allele carriage not only is linked to a significant MI risk reduction but also is highly correlated with reduced VWF antigen levels.<sup>121</sup>

Another finding that supports an adverse association between VWF levels and cardiovascular events was described after desmopressin infusion. Various authors observed the uncommon side effect of MI in a temporal relationship to desmopressin infusion.<sup>122–125</sup> Because desmopressin is a potent secretagogue for VWF<sup>123</sup> and notably does not lead to coronary vasoconstriction, induction of a short-term prothrombotic state seems to be the underlying cause of this particular complication of desmopressin treatment. This also indicates that high concentrations of or rapid increases in

circulating plasma VWF may be sufficient to cause MI in certain patients.

These various lines of evidence support the hypothesis that VWF is not merely a prognostic marker for presence of CAD and for outcome in AMI but rather directly involved pathogenically as a causative agent.

### Interventions Targeting VWF

The established role of VWF in arterial thrombogenesis makes it a promising therapeutic target; indeed, VWF antagonists have been proposed as potentially advantageous, novel antiplatelet drugs.<sup>126</sup> Various approaches have been taken to block the interaction of VWF and the platelet GPIIb receptor and thereby to inhibit VWF function in thrombogenesis.

Heparins have been shown to significantly impair VWF-dependent platelet hemostatic mechanisms by binding to a site on the VWF molecule that overlaps its A1 domain responsible for GPIIb binding<sup>127,128</sup>; as discussed previously, heparin administration, in particular the low-molecular-weight heparin enoxaparin, is associated with a reduction in VWF release, recurrent MI, and death in the setting of acute MI.<sup>91,94</sup> Interestingly, subspecies of heparins can be developed with explicitly enhanced potency to inhibit VWF/platelet interactions.<sup>129</sup> Some *ex vivo* studies indicate that the GPIIb/IIIa inhibitors, mainly the monoclonal antibody c7E3 Fab (abciximab), suppress the VWF-mediated platelet activation. Infusion of abciximab in unstable angina pectoris patients leads to inhibition, albeit rather minor, of ristocetin-induced platelet aggregation.<sup>130</sup> Under conditions of high shear stress, the addition of abciximab to patients' blood reduces the extent of platelet aggregation to the collagen I surface mediated by platelet GPIIb interactions with VWF.<sup>131</sup> Interestingly, blockade of VWF binding to GPIIb/IIIa by eptifibatid is not as effective as abciximab under high shear conditions.<sup>132</sup> Simple platelet adhesion to collagen I/VWF is not affected by abciximab or eptifibatid in this model. Goto et al<sup>133</sup> showed that abciximab compared with tirofiban and eptifibatid inhibits the VWF-mediated platelet activation and procoagulant activities measured as phospholipid expression on platelets and microparticles, resulting in the shortening of the kaolin recalcification clotting time. This may be relevant in areas of arterial stenosis with rapid blood flow, particularly in patients with MI.<sup>65n</sup> However, it is not clear whether and to what extent the action of heparins or GPIIb/IIIa inhibitors on VWF signaling contributes to their overall beneficial effects.

More specific antagonists of the VWF-GPIIb interaction have been investigated, but none have yet achieved regulatory approval to be marketed as drugs. Recombinant peptide fragments of VWF can compete with native VWF for GPIIb binding and can interfere with platelet adhesion and aggregation *in vitro*.<sup>134</sup> Aurin tricarboxylic acid is a small molecule with affinity for a site within the A1 domain of VWF<sup>135</sup> that inhibits ristocetin-induced, VWF-mediated platelet aggregation.<sup>136</sup>

Most recently, high-molecular-weight antagonists have been designed to bind to the A1 domain of VWF and block its binding to the GPIIb receptor. Examples of this approach include monoclonal antibodies and drug-like oligonucleo-

tides,<sup>137</sup> or aptamers,<sup>138</sup> some of which are presently undergoing clinical testing. A murine monoclonal antibody directed against the A1 domain of human VWF, AJvW-2, specifically blocks the interaction between plasma VWF and platelet GPIIb. AJvW-2 significantly inhibited platelet aggregation and reduced coronary artery thrombosis, as well as thrombus deposition and neointimal formation, after balloon injury in different animal species.<sup>139–141</sup> In addition, ex vivo incubation of platelet-rich plasma from patients with ACS with AJvW-2 significantly inhibited shear-induced platelet aggregation as measured by a cone-and-plate viscometer.<sup>74</sup> Analogous results have been demonstrated with the related humanized monoclonal antibody AJW200 that likewise reacts with the A1 domain of human VWF.<sup>112,142</sup> Recently, the pegylated anti VWF aptamer ARC1779 has completed a phase I trial program,<sup>143</sup> and a phase II efficacy and safety trial of ARC1779 in patients with MI has been initiated (ClinicalTrials.gov ID: NCT00507338).

These encouraging findings further reinforce the importance of the role of the VWF-GPIIb interaction in coronary occlusion and support the possibility that therapeutic inhibition of this interaction may benefit patients with ACS.

### Conclusions

A large body of epidemiological evidence reviewed herein establishes a strong correlation between elevated VWF and the incidence and prognosis of ACS. A causal role is suggested for VWF in both epicardial coronary thrombus formation and the associated microcirculatory dysfunction in patients with ACS. Deficits in myocardial perfusion, which persist despite optimal use of PCI and currently available adjunctive drug therapies, occur quite commonly in ACS patients. Hence, VWF antagonism represents a novel, potentially valuable addition to the armamentarium of antithrombotic agents.

### Take-Home Points

VWF is a useful clinical marker of risk associated with atherosclerosis. Systemic levels of VWF are elevated in the setting of atherosclerotic cardiovascular disease in a graded manner proportionate to the risk of cardiovascular disease events. VWF serves as a prognostic index of future cardiovascular event risk in the general population, in asymptomatic patients with established CAD, and particularly in patients with ACS.

Pathophysiological evidence suggests that VWF is not only a marker but also a mediator of cardiovascular disease events. VWF is produced and released by vascular endothelial cells in response to a variety of stimuli associated with acute ischemic syndromes, including hypoxia, inflammatory cytokines, thrombin, and adrenaline. VWF plays important roles in the pathological process of arterial thrombus formation with respect to both platelet function and the coagulation cascade. VWF is critically important to the initiation of

platelet adhesion and subsequent aggregation occurring at the high shear rates found in the arterial circulation, and it promotes fibrin formation in response to arterial injury in a site-directed manner as a result of its function as a chaperone for factor VIII and ligand for subendothelial collagen.

None of the currently available therapeutic interventions for AMI such as PCI, GPIIb/IIIa antagonism, or thrombolysis specifically target VWF. Preclinical testing of experimental VWF antagonists has shown promise in various models of ischemia, and some of these are now entering early clinical trials.

### Disclosures

Dr Gilbert is an employee of Archemix Corp, which is engaged in clinical development of ARC1779, an investigational anti-VWF aptamer mentioned in the present review.

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