Role of von Willebrand factor in venous thromboembolic disease

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ABSTRACT

Objective: Evolving evidence of the shared risk factors and pathogenic mechanisms in arterial and venous thrombosis questions of the strict vascular dichotomy of arterial vs venous. The connection between arterial and venous thrombosis has been highlighted by common underlying inflammatory processes, a concept known as thromboinflammatory disease. Using this relationship, we can apply knowledge from arterial disease to better understand and potentially mitigate venous disease. A protein that has been extensively studied in atherothrombotic disease and inflammation is von Willebrand factor (VWF). Because many predisposing and provoking factors of venous thromboembolism (VTE) have been shown to directly modulate VWF levels, it is, perhaps, not surprising that VWF has been highlighted by several recent association studies of patients with VTE.

Methods: In the present narrative review, we investigated more deeply the effects of VWF in venous disease by synthesizing the data from clinical studies of deep vein thrombosis of the limbs, pulmonary embolism, portal and cerebral vein thrombosis, and the complications of thrombosis, including post-thrombotic syndrome, venous insufficiency, and chronic thromboembolic pulmonary hypertension. We have also discussed the findings from preclinical studies to highlight novel VWF biochemistry in thrombosis and therapeutics.

Results: Across the spectrum of venous thromboembolic disease, we consistently observed that elevated VWF levels conferred an increased risk of VTE and long-term venous complications. We have highlighted important findings from VWF molecular research and have proposed mechanisms by which VWF participates in venous disease. Emerging evidence from preclinical studies might reveal novel targets for thromboinflammatory disease, including specific VWF pathophysiology. Furthermore, we have highlighted the utility of measuring VWF to prognosticate and risk stratify for VTE and its complications.

Conclusions: As the prevalence of inflammatory processes, such as aging, obesity, and diabetes increases in our population, it is critical to understand the evolving role of VWF in venous disease to guide clinical decisions and therapeutics. (JVS—Vascular Science 2022;3:17-29.)

Keywords: Thromboinflammatory; Venous disease; Venous thromboembolism; von Willebrand factor

INTRODUCTION TO PATHOPHYSIOLOGY OF THROMBOINFLAMMATORY DISEASE

Thrombotic cardiovascular disease, a pathologic classification that includes myocardial infarction, ischemic stroke, peripheral vascular disease, and venous thromboembolism (VTE), is the leading cause of mortality worldwide and a major contributor to the global burden of disease.[1] The pathogenesis and sequela of thrombosis are intimately associated with inflammation, resulting in the concept of thromboinflammatory disease.[2] Numerous inflammatory processes such as inflammatory bowel disease, aging, and cancer are all significant risk factors for both arterial and venous thrombosis.[3–5] Moreover, study of the pathophysiology of thrombus formation and resolution has revealed intimate links between the immune and hemostatic systems.

The concept of thromboinflammatory disease lessens the dichotomy between the classic concept of arterial and venous thrombosis. It has been suggested that arterial and venous thrombotic disease arise from a common source of predisposing and provoking factors.[4] The identification of shared, underlying inflammatory processes facilitates the application of knowledge from arterial disease to better understand and potentially mitigate venous disease. The increased prevalence of inflammatory factors such as obesity, diabetes, cancer, and older age in our patient populations could be a confounder that has blurred arterial and venous disease pathogenesis. This is highlighted by the increased incidence of atherosclerosis in patients with unprovoked VTE compared with matched controls.[6] Similarly, in a series

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of autopsy reviews, an increased prevalence of VTE was found in those with confirmed arterial thrombotic events.[7] Moreover, merging traditional treatments of VTE and atherothrombosis have proved beneficial in the peripheral arterial disease population and might lead to novel combination therapies for venous disease.[8]

Accepting that a common thromboinflammatory pathophysiology might exist for most vascular disease, we can investigate the characterized mediators of atherothrombosis in the context of VTE. von Willebrand factor (VWF) is a multimeric glycoprotein best known for its roles in platelet adhesion for primary hemostasis and the protection of circulating coagulation factor VIII (FVIII) from proteolytic cleavage. Numerous studies have investigated VWF's role in platelet capture under shear stress and response to inflammatory stimuli in acute coronary syndromes and ischemic stroke.[9] Now, we are unearthing its role in venous disease.

INTRODUCTION TO VWF IN THROMBOINFLAMMATORY DISEASE

Inflammation modulates VWF synthesis and release. VWF synthesis occurs exclusively in endothelial cells and megakaryocytes and requires careful post-translational modification through multimerization and glycosylation for appropriate structure and function.[10] This glycosylation includes modification by fucosyltransferases, which transfer a fucose to the glycan core (H antigen).[11] The H antigen can be subsequently modified by A or B glycosyltransferases, depending in the ABO blood group of the individual, to add more complex carbohydrate structures.[11] ABO glycosylation modifies VWF clearance and, therefore, circulating plasma levels (with a reported 25% decrease in type O individuals).[12] This characteristic of VWF processing is important to recognize, because VWF represents the best understood connection between non-O blood types and thrombotic cardiovascular disease.[13]

VWF is stored in endothelial Weibel-Palade bodies (WPBs) and platelet α-granules, which can be rapidly released in response to a hemostatic, noxious, or proinflammatory insult.[10] VWF has earned the definition of an "acute phase reactant," a class of biomarkers that indicates the onset of inflammation and is, therefore, clinically useful for understanding disease pathogenesis and intensity.[14] Inflammation-associated molecules, including cytokines, complement, and damage- or pathogen-associated molecular patterns, are especially potent stimuli for VWF synthesis and release. Also, endothelial cells constitutively secrete VWF to maintain a circulating plasma pool of VWF and stock the subendothelium.[15] VWF synthesis is influenced by vessel and endothelial cell type, hypoxia, shear stress, and inflammatory milieu (including the microbiome) through transcriptional activation and suppression and microRNAs.[16] Nearly every studied inflammatory disease has been associated with an increase in VWF levels (Table I).[3,12,17–39] The VWF concentration can also be lowered by treatment of these disease states, including lifestyle modifications such as weight loss and smoking cessation or antihypertensive and statin therapies.[40–42]

VWF function in hemostasis. Extracellular VWF interacts with exposed subendothelial collagens during hemostatic insults, which is critical to anchor the innately globular form of VWF and permit fluid shear forces to elongate VWF.[43] This force exposes the VWF A1 domain binding site for platelet glycoprotein Ibα (GPIbα).[44] Engagement of GPIbα by VWF mediates platelet activation and α-granule release and exposes a second VWF receptor on the platelet membrane (integrin $\alpha_{IIb}\beta_3$).[43] Integrin $\alpha_{IIb}\beta_3$ is similarly a critical receptor for fibrin, aiding in stable clot formation as the simultaneously active coagulation cascade builds fibrin polymers. Of additional hemostatic significance, ~95% to 98% of circulating FVIII is complexed with VWF, and, in the absence of VWF, FVIII undergoes rapid proteolytic degradation and clearance.[45]

VWF is released from endothelial cells and platelets in an ultra-large (UL) form, and UL-VWF demonstrates enhanced procoagulant function, because it more readily binds to platelets than does VWF composed of fewer monomers. The regulation of VWF size in hemostasis is essential because high-molecular-weight VWF is needed to support efficient primary hemostasis. In contrast, UL-VWF has thrombotic consequences if it is not rapidly modified by partial proteolysis. A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), is a circulating enzyme responsible for cleavage of UL-VWF under shear stress, and VWF is its only known substrate.[46]

The half-life of VWF varies within a population, with one study demonstrating a range of 4 to 26 hours, and therapeutic VWF concentrates have demonstrated a half-life of ~16 hours.[47] VWF clearance studies have shown that this is a semiselective process that is mediated by several receptors on macrophages, hepatocytes, and sinusoidal endothelial cells. Receptors that have been shown to influence VWF levels or its half-life include low-density lipoprotein receptor-related protein, asialoglycoprotein receptor (also known as the Ashwell-Morrell receptor), sialic acid-binding immunoglobulin-like lectin 5, macrophage scavenger receptor A1, and macrophage galactose-type lectin, C-type lectin domain family 4 member M, scavenger receptor class A member 5, stabilin-2, and likely others.[48] The VWF lifecycle is summarized in Fig 1.

Developing VWF roles in thromboinflammatory disease. Although VWF—collagen, VWF—platelet, and VWF—FVIII interactions have been validated in human pathophysiology, novel VWF interactions with endothelial cells, leukocytes, erythrocytes, angiogenic proteins, and tumor cells are beginning to emerge in vitro and in

Table I. VWF levels and VTE risk in inflammatory disease

Inflammatory disease	VWF level	Risk of VTE
Classic contributors to vascular disease		
Diabetes	1.71-fold increase[27]	OR 1.3-1.4[33]
Aging	<20 years vs >55 years associated with 1.56-fold increase[12]	<35 years vs >70 years associated with HR ≈10[34]
Obesity	1.26-fold increase[35]	OR 2.33[33]
Vasculitis	Giant cell arteritis associated with 2.24-fold increase[36]	HR 2.26-3.94, depending on vasculitis subtype[37]
Emerging contributors to vascular disease		
Chronic kidney disease	1.21- to 1.77-fold increase (stage 3-5)[38]	OR 1.43[39]
Malignancy	1.48- to 2.31-fold increase (varying with tumor type and stage)[17]	HR 4-7, depending on cancer type and stage, with a 15% chance finding of occult cancer in those with an unprovoked VTE event[18]
Bacteremia	Endotoxemia associated with ≤5-fold increase[19]	OR 1.9 (community-acquired bacteremia)[20]
COVID-19; SARS-CoV-2	2- to 6-fold increase (varying with severity)[21]	Increased prevalence (20%-30% in hospitalized patients)[22]
Cirrhosis	3.8- to 7.6-fold increase (Child class A-C)[23]	OR 1.7[24]
Inflammatory bowel disease	1.3- to 1.7-fold increase (depending on active vs inactive disease)[25]	OR 2.0[3]
COPD	1.25- to 1.8-fold increase (depending on stable vs acute exacerbation)[26]	HR 1.6 in severe COPD[28]
Obstructive sleep apnea	1.5-fold increase[29]	OR 2-4[30]
Connective tissue disease	1.7-fold increase in rheumatoid arthritis[31]	OR 2.23 in rheumatoid arthritis[32]

COPD, Chronic obstructive pulmonary disease; *COVID-19*, coronavirus disease 2019; *HR*, hazard ratio; *OR*, odds ratio; *SARS-CoV-2*, severe acute respiratory syndrome coronavirus 2; *VTE*, venous thromboembolism; *VWF*, von Willebrand factor.

animal models. The extracellular VWF functions are summarized in Fig 2.

Circulating and acutely released VWF can interact with the endothelial surface through P-selectin, integrin $\alpha_v\beta_3$, and the complex endothelial glycocalyx.[49–51] This might explain key VTE pathophysiology by which thrombosis frequently occurs on an intact endothelial layer rather than after atherosclerotic plaque rupture exposing the subendothelium. Recent evidence has highlighted the association of leukocytes and erythrocytes with venous thrombotic disease.[52,53] In in vitro flow systems, VWF has been shown to bind to activated polymorphonuclear cells and monocytes, and animal models have shown that VWF is integral to leukocyte-dependent inflammation.[54–56] Moreover, support has been increasing that neutrophil-extracellular traps (NETs) play a key role in VTE. VWF can bind neutrophils and several NET constituents, including DNA and histones, and add to the prothrombotic capacity of NETs.[57,58] Erythrocytes have also demonstrated adhesion to VWF in vitro.[59] At present, it is unclear under which microenvironment conditions and how mechanistically the endothelium, VWF, and platelets coordinate leukocyte and erythrocyte recruitment in vivo. This is likely to be a productive avenue for future research.

VWF has also been proposed to act as a negative regulator of angiogenesis through WPB physiology with angiogenic proteins and the potential for modification of vascular endothelial growth factor signaling.[60,61] VWF interaction with integrin $\alpha_v\beta_3$ can also modify vascular permeability and smooth muscle cell proliferation and, together with its angiogenic functions, can affect wound healing.[62,63] VWF has been implicated in tumor metastasis through its ability to bind a variety of tumor cells in vitro and its conspicuous localization in murine tumors.[64] Considering the essential role of VWF in endothelial cell physiology and its large size and electrostatic domain structure, it is not surprising that VWF has been implicated in many processes beyond hemostasis.

VWF in thrombosis. VWF levels range in the healthy population from 0.5 to 2.0 IU/mL (range, 50%-200%), with low VWF levels associated with bleeding and elevated VWF levels with thrombosis. Circulating VWF levels are influenced by its synthesis, basal and

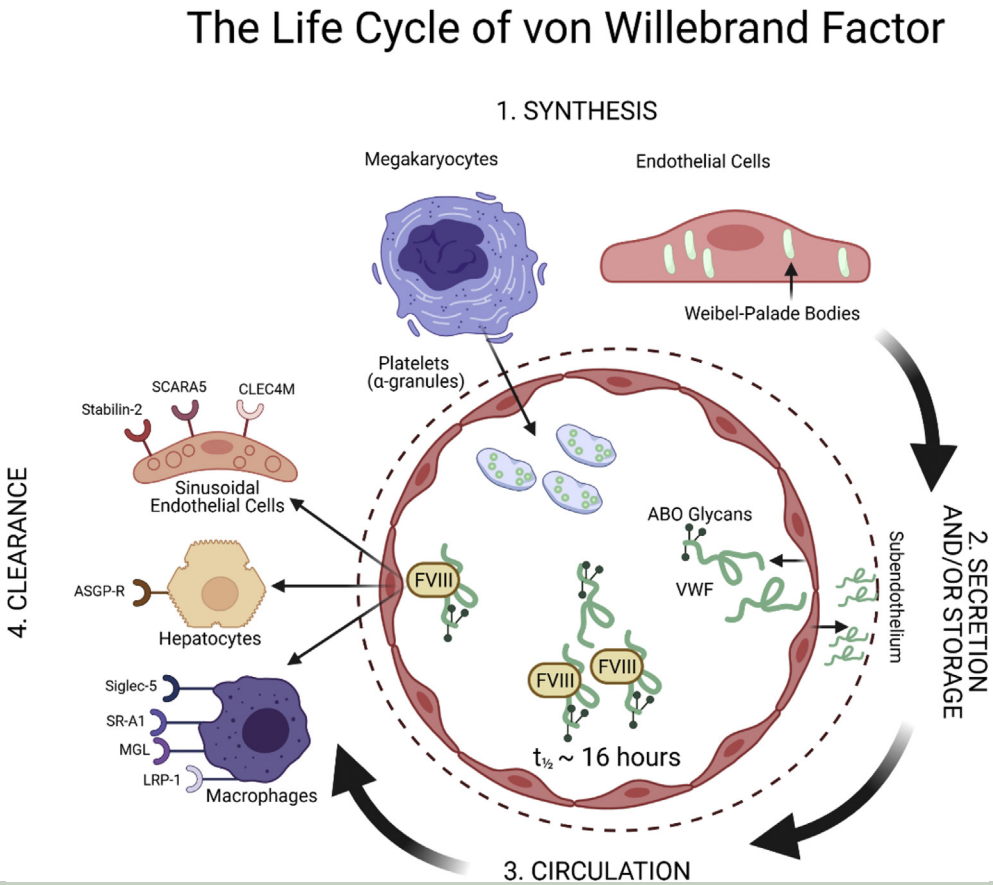

Fig 1. The lifecycle of von Willebrand factor (VWF). *1,* VWF is synthesized by endothelial cells and megakaryocytes. *2,* It is stored in platelet α-granules and Weibel-Palade bodies (WPBs) to undergo stimulated or basal secretion, contributing to both subendothelial and circulating VWF levels. *3,* VWF circulates with or without factor VIII (FVIII) and has an estimated half-life of 16 hours, with a high degree of interindividual variability. *4,* VWF clearance is mediated by sinusoidal endothelial cells, macrophages, and hepatocytes, with contributions from an array of receptors. ABO glycans present on VWF modify its clearance. *ASGP-R,* Asialoglycoprotein receptor; *CLEC4M,* C-type lectin domain family 4 member M; *LRP-1,* low-density lipoprotein receptor-related protein; *MGL,* macrophage galactose-type lectin; *SCARA5,* scavenger receptor class A member 5; *Siglec-5,* sialic-acid binding immunoglobulin-like lectin 5; *SR-A1,* scavenger receptor A1.

stimulated secretion, and clearance. Similarly, VWF function is influenced by the source of release (endothelial vs platelet), ADAMTS13 degradation, shear stress, and mutations and variants that affect the interaction between VWF and its many binding partners.

Thrombosis is a dynamic interplay between pro- and anticoagulant proteins, platelets, endothelial cells, erythrocytes, leukocytes, immune mediators, and fibrinolytic pathways to create a net hypercoagulable state. For simplicity, these factors can be categorized into (1) inherited predisposition, (2) acquired predisposition, and (3) provoking mechanisms. A review by Anderson and Weitz[65] has discussed these factors in the context of a "thrombosis threshold," such that the system reaches a tipping point and pathologic thrombus formation ensues. As stated, VWF levels and function are influenced by many of the same factors associated with the risk of thrombosis (Table II).

VWF IN VENOUS THROMBOEMBOLIC DISEASE

Clinical association. Although VWF has been thought of as a contributor primarily to arterial thrombosis through its seeming dependency on shear stress and platelet-binding capabilities, a smaller body of studies has described VWF levels in VTE. Some of the first evidence that VWF was involved in venous thrombogenicity dates to 1995. Two vascular surgeons (Cho and Ouriel[66]) at the University of Rochester used an ex vivo flow system to assess for thrombus formation on a vein luminal surface. These experiments showed an abundance of VWF in the venous endothelium and subendothelium and, when inhibited by a polyclonal antibody, significantly impaired thrombus formation.[66] In 1995, Koster et al[67] first described the increasing risk of VTE associated with elevated VWF and FVIII levels in a population-based patient-control study of 301 patients. Similarly, in 2019, Rietveld et al[68] reported a Dutch retrospective

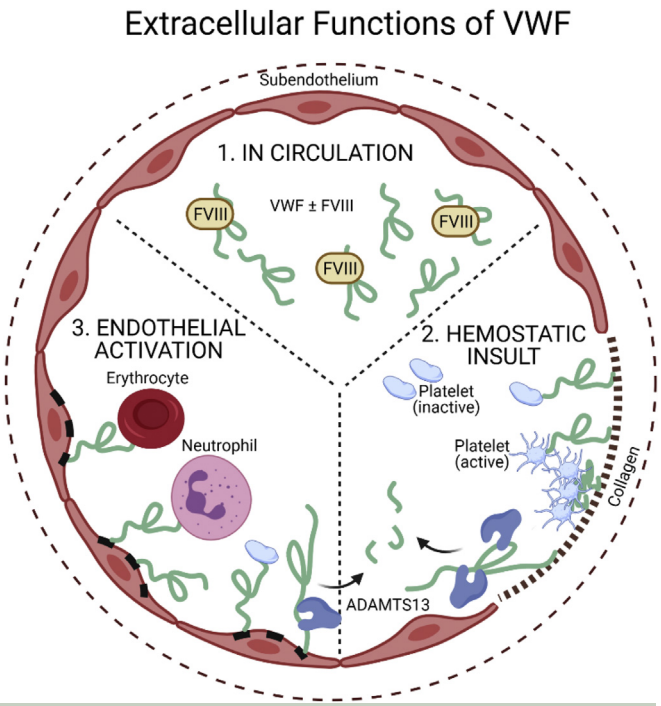

Fig 2. The extracellular functions of von Willebrand factor (VWF). *1,* VWF carries factor VIII (FVIII) in circulation and protects FVIII from proteolytic degradation. *2,* VWF binds to subendothelial collagen during hemostatic insult to capture platelets and enable platelet plug formation. Ultra-large (UL) VWF is cleaved by A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) under conditions of shear stress to regulate its thrombotic potential. *3,* Stimulated VWF release via many potential noxious stimuli allows for VWF to capture platelets and, possibly, leukocytes and erythrocytes on an intact endothelial surface. ADAMTS13 might also regulate VWF when anchored to the endothelium.

case-control study (2377 cases; 2940 controls; all aged <70 years). They demonstrated that VWF and FVIII had the strongest association with VTE risk compared with thrombin, FVII, FIX, FX, and FXI.[68] This association was strengthened by the findings from a prospective study reported in 2021, which showed a dose-dependent association between the VWF concentration and the future risk of incident VTE (414 cases; 843 matched controls) in a Norwegian population.[69]

As previously stated, the ABO blood type contributes significantly to the genetic determination of VWF levels (~30% from association studies of twin siblings). The association of the ABO blood group with VTE predates that of VWF, beginning in 1969, when a white, female population from the United States, Sweden, and the United Kingdom with deep vein thrombosis (DVT) and/or pulmonary embolism (PE) were retrospectively examined for blood type, contraceptive use, and pregnancy. A non-O blood type was associated with a 1.6 to 3.3 relative risk of VTE, depending on the cohort.[70] Since then, several studies have demonstrated the same association. A 2012 meta-analysis

Table II. Known VTE risk factors that also influence VWF concentration and function

Risk factor
Predisposing
Inherited
Race; ABO blood group
Acquired
Age; increased BMI; smoking; oral contraceptive pills; hormone replacement therapy; chronic inflammatory disease[a]
Provoking
Trauma; surgery; pregnancy; cancer; infection; acute exacerbation of chronic disease[a]

BMI, Body mass index; *VTE,* venous thromboembolism; *VWF,* von Willebrand factor.
[a]A list of inflammatory disease states is provided in Table I.

and a prospective study of a cohort with known cardiovascular risk factors highlighted a non-O blood type as the most common risk factor for VTE (odds ratio [OR], 2.09; and OR, 1.64, respectively).[71,72] Congruently, the ABO blood type and VWF levels also modify the risk of VTE in families with hereditary thrombophilias and could add to their overall VTE risk assessment.[73,74]

Several large genome-wide association studies reported in the past decade have demonstrated an association between single nucleotide variants at the *VWF* locus and genes that modify VWF plasma levels and DVT and/or PE.[75,76] Two-sample Mendelian randomization analyses were used to test the causal role of VWF plasma levels on the risk of VTE in the INVENT (international network of venous thromboembolism clinical research networks) consortium (7507 VTE case subjects and 52,632 control subjects; European ancestry).[76] These analyses demonstrated a causal OR estimate of 2.28 (95% confidence interval [CI], 2.18-2.38) for VWF and VTE.[76] Although genome-wide association study-type analyses can be valuable for associating common genetic variations with phenotypic traits, they cannot adequately identify rare population variants that can exhibit a large effect size. To understand rare variants in VTE, Desch et al[77] used whole exome sequencing of 393 patients with unprovoked VTE (with 6114 control patients) to identify genes with an excess frequency of damaging variants in patients with VTE. They found that 7.8% of VTE cases and 2.4% of controls had had a rare damaging variant in *STAB2* (encodes the VWF clearance receptor stabilin-2; OR, 3.37; $P = 2.70E-7$). Furthermore, the investigators analyzed a separate healthy cohort of 1162 individuals and found elevated VWF:antigen (Ag) levels in 38 samples with rare damaging *STAB2* variants. These data are suggestive of impaired VWF clearance and support the role of impaired sinusoidal endothelial clearance of VWF as a mechanism promoting venous thrombosis.

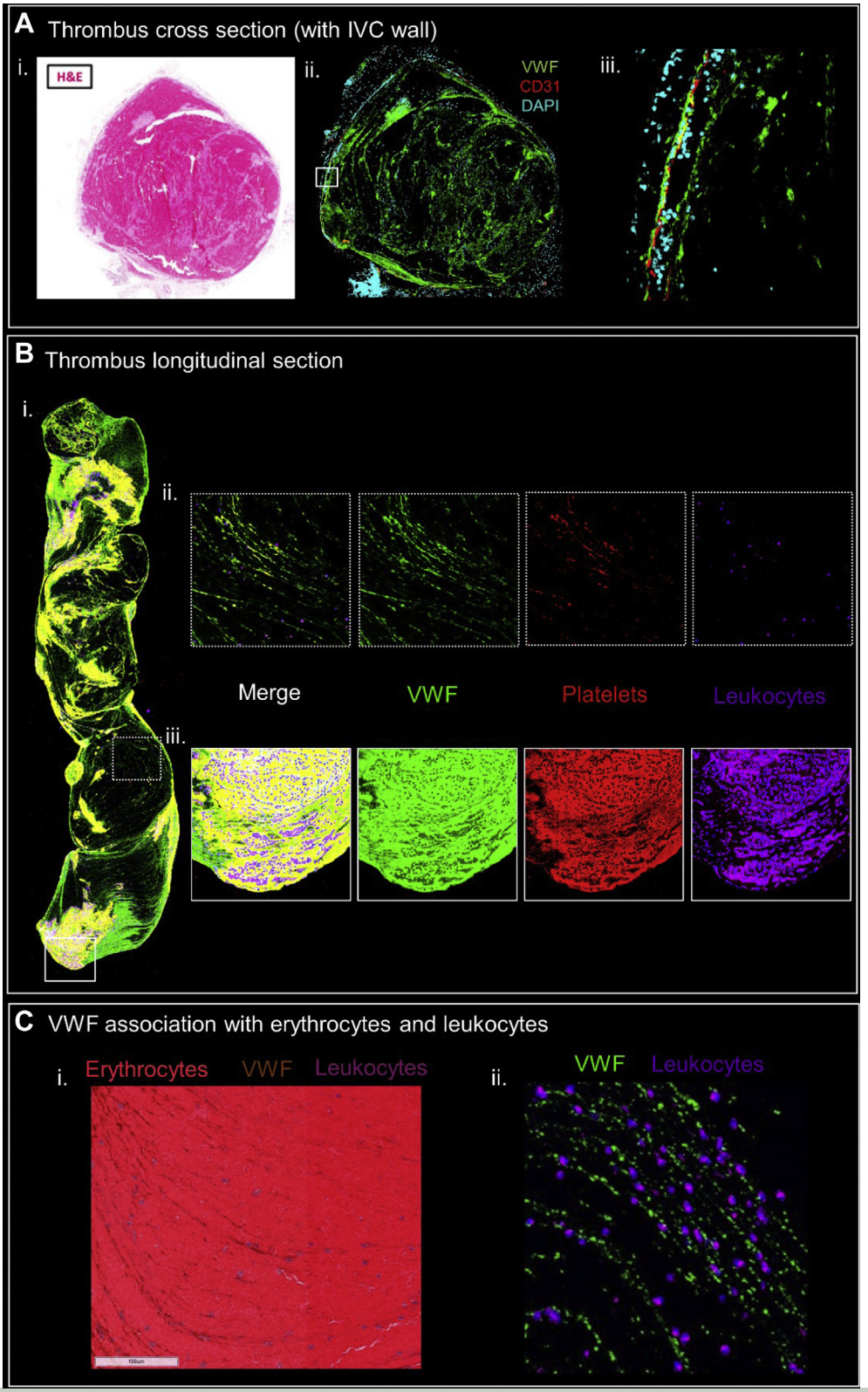

Fig 3. Association of von Willebrand factor (VWF) with thrombus constituents in murine venous thrombi. Using a murine model of deep vein thrombosis (DVT; the inferior vena cava [IVC] stenosis model), the thrombus with IVC wall was dissected and cross-sectioned. **A.i,** A laminar pattern of red and white thrombus was demonstrated with hematoxylin and eosin (*H&E*) staining. **A.ii,** Immunofluorescent staining showing VWF (*green*) colocalized (*yellow*) with the endothelium (CD31; *red*) and in close proximity to recruited leukocytes (DAPI; *blue*). **A.iii,** Magnified view of inset in **A.ii**. Longitudinal thrombus sections (IVC wall removed; **B.i**) showing an abundance of VWF (*green*) and its association with platelets (CD41; *red*) and leukocytes (CD45 and DAPI; *purple*; **B.ii,B.iii**). **C.i,** An isolated red thrombus image was DAB-stained with a VWF antibody to show VWF (*brown*) localization with erythrocytes (*red*). **C.ii,** VWF lamination in lines of Zahn are decorated with leukocytes (*purple*).

In addition to the epidemiologic and genetic associations of VWF with DVT and/or PE, VWF can be directly visualized in iliofemoral thrombus or PE samples removed after autopsy, thrombectomy, or venous excision.[59,78,79] Collectively, these studies have shown an abundance of VWF in association with platelets, fibrin, erythrocytes, and NET-producing leukocytes.

Data from animal models. The development of novel, pathophysiologically relevant animal models of venous thrombosis has significantly enhanced our knowledge of VTE. The inferior vena cava (IVC) stenosis model induces DVT formation within a period of 6 to 48 hours through slowing of blood flow (and limited initial endothelial disruption) within the IVC, similar to the stasis mechanism by which human DVT forms around the valves in the deep veins of the limbs.[80] Furthermore, the thrombi produced in the stenosis model recapitulate the salient morphologic features of human thrombi, demonstrating a laminar structure with distinct white and red thrombus regions.[80,81]

Using the murine model of IVC stenosis, we recently showed that VWF deficiency through germline knockout or systemic antibody infusion was protective against DVT development in obese mice,[82] in line with previous studies of healthy mice by Brill et al.[83] We also examined the colocalization of VWF with multiple thrombus constituents in murine thrombi and found a predilection of VWF for white thrombus areas, including the growing tail and lines of Zahn. VWF appears to scaffold erythrocytes and recruit platelets and/or leukocytes (Fig 3).

VWF IN SPECIAL CASES OF VTE DISEASE

VTE can also occur within the upper limb, cerebral, retinal, renal, or splanchnic venous systems. However, most of population-based evidence that has been previously discussed for VWF in venous disease has come from lower extremity DVT and PE cohorts. Thus, we have reviewed the evidence of VWF in rare and distinct VTE circumstances.

Upper limb DVT. Non—catheter- or intraluminal device-associated upper limb DVT is rare outside of malignancy. VWF (1.22-fold) and FVIII levels were significantly elevated in a population of 107 patients with upper extremity DVT without malignancy. When analyzing a subpopulation of patients with VWF levels greater than the 90th percentile, the OR of upper limb DVT was 4.0, similar to that of FVIII (OR, 4.2), and only fibrinogen (OR, 2.9) approached this level of risk compared with all other measured prothrombotic factors.[84] Cancer-associated VTE, including upper limb DVT, has been discussed further in a separate section.

Portal vein thrombosis. Cirrhosis is associated with an increase in circulating VWF:Ag concentrations, and VWF levels have correlated with disease severity via the Child-Pugh score.[23] VWF is not synthesized by hepatocytes. However, its cleaving metalloprotease, ADAMTS13, is synthesized by hepatic stellate cells and can be impaired by liver synthetic dysfunction. Decreased ADAMTS13 levels and ADAMTS13 activity have been associated with portal vein thrombosis development in cirrhosis, presumably through an overabundance of UL-VWF, which possesses increased platelet-binding capacity.[85,86] Noncirrhotic portal vein thrombosis has also been associated with increases in VWF and FVIII.[87]

The clinical indicators of portal hypertension (ie, varices, hepatic decompensation, elevated hepatic venous pressure gradient) and inflammation (C-reactive protein levels) have been associated with increased VWF:Ag in patients with advanced liver disease.[88] Moreover, a pronounced local increase in VWF/FVIII occurs in the presence of decompensated cirrhosis, as evidenced by blood samples taken from the portal vein during transjugular intrahepatic portosystemic shunt procedures in 20 individuals.[89] Mechanistically, the VWF levels correlated with the lipopolysaccharide levels, a biomarker reflecting gut permeability and a potent stimulator of WPB exocytosis. In splanchnic vein thrombosis, a 1998 study of simultaneous kidney and pancreas transplants in 30 uremic patients with type 1 diabetes showed a significant association between VWF levels and pancreatic vein thrombosis (6 of 30 patients).[90] This finding was unique to VWF. The investigators studied other prothrombotic parameters, including fibrinogen, thrombin, prothrombin time, proteins C and S, and plasminogen activator inhibitor, and demonstrated no association with these factors.[90]

Cerebral and retinal venous thrombosis. Small case-control studies have linked elevated VWF and FVIII levels to cerebral sinus and venous thrombosis (CSVT). In a French cohort of 16 CSVT cases (13 females and 3 males) and 64 controls, the VWF levels were 1.52-fold higher in those with CSVT ($P = .01$).[91] Estrogen increases VWF synthesis, and 10 CSVT cases were associated with either oral contraceptive pill use or hormonal replacement therapy.[91] A single case report also highlighted the VWF levels of 275% and FVIII of 183% in a 30-year old woman with Cushing syndrome and CSVT, without significant perturbation of other prothrombotic proteins.[92] Glucocorticoids have similarly been shown to increase VWF production.

In central retinal vein thrombosis, Murray et al[93] showed elevated VWF levels in 53% of patients (mean, 232%; $P = .0002$). However, they could not predict between ischemic and nonischemic subgroups of disease.[93] Subsequently, a study of 63 patients with central retinal vein thrombosis showed no association with VWF or FVIII levels.[94] More research is required to clarify this association.

Cancer-associated VTE. Malignancy carries a hazard ratio (HR) of 4 to 7 for VTE, depending on the population and tumor characteristics.[18] Also, a 15% risk exists of an occult cancer underlying an unprovoked VTE event. In a

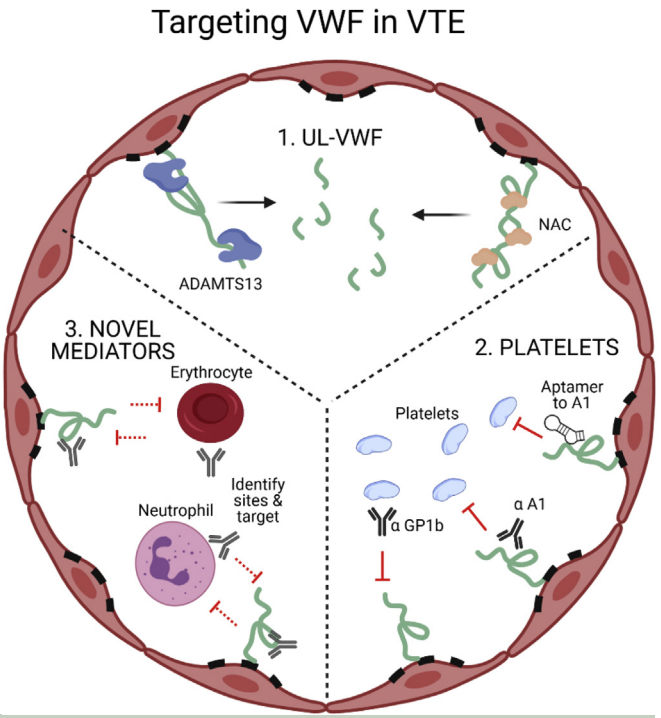

Fig 4. Potential for von Willebrand factor (VWF)-directed therapeutics in venous thromboembolism (VTE). A reduction in VWF-related prothrombotic activity might be achieved through *1,* disruption of ultra-large (UL)-VWF multimers via A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13)-mediated cleavage or N-acetylcysteine (*NAC*) reduction; *2,* impairment of VWF–platelet interactions by targeting binding sites on VWF or platelets with antibodies and/or aptamers; and *3,* identify sites important for VWF–leukocyte and VWF–erythrocyte interactions as novel mechanisms to target thrombosis but preserve hemostasis.

case-control study, the VWF levels were elevated in cancer-associated VTE patients compared with those without a VTE event (1.35-fold) and correspondingly increased with progression of the disease stage.[95] Furthermore, a prospective cohort study of 795 Austrian patients with various tumor types showed a doubling of VWF:Ag, resulting in an increased HR of 1.56 for VTE on multivariate analysis. In contrast, no association was shown between ADAMTS13 activity and VTE.[96] A recent meta-analysis reviewing 609 cases demonstrated that an O blood type is protective against VTE in children with cancer (OR, 0.56).[97] Similarly, the odds of VTE in those with glioblastoma, pancreatic cancer, and prostate cancer were increased in those with a non-O vs O blood type, similar to the ABO effect in the cancer-free population.[98–100]

von Willebrand disease and VTE. The inherited quantitative and/or qualitative deficiency of VWF results in the bleeding disorder known as von Willebrand disease (VWD), and the bleeding phenotype correlates with VWF levels and functional impairment. VWD has a prevalence in the general population of 0.6% to 1.3%, making it the most common inherited bleeding disorder.[101] Although limited high-quality evidence has been reported, a 2015 review of reported studies described a total of 33 VTE events in VWD patients, synthesizing information from 14 reports (primarily case reports) dating from 1981 to 2012.[102] Girolami et al[102] also reviewed 486 of their own VWD patient files (from 1972 to 2010 in Padua, Italy) and found no reports of VTE. The reported patients with VTE were primarily receiving VWF replacement therapy (26 of 33), had also undergone surgery (5 of 33), or had evidence of congenital thrombophilia or impaired fibrinolysis (5 of 33). Therefore, it appears that VWF deficiency is protective against VTE, which has also been demonstrated in small animal models.[83]

VWF IN VTE COMPLICATIONS AND OTHER ASPECTS OF CHRONIC VENOUS DISEASE

Recurrence. Predicting the risk of VTE recurrence is important for anticoagulation decisions and prognosis. In a prospective cohort study, the patients were followed up from their first VTE episode, and 343 of the 2242 patients enrolled had developed recurrent thrombosis.[103] FVIII activity and VWF:Ag were measured from plasma at 3 months after the cessation of the anticoagulation course. The recurrence rates increased in parallel with elevated VWF and FVIII levels, and VWF >200% was associated with a HR of ≥3.7 and FVIII >200% was associated with a HR of ≥3.4.[103] These results are in line with a study that demonstrated a relative risk of recurrence of 1.08 for each 10% increase in FVIII.[104] Furthermore, 106 patients with a first presentation of PE were followed up prospectively, and a B blood type was associated with a 2.7-fold increased risk of VTE recurrence.[105] Similar results were found in a large prospective study of Swedish blood donors, showing 4468 recurrent VTE events, with an approximate relative risk of 1.45 for non-O blood types.[13]

Post-thrombotic syndrome and venous insufficiency. Post-thrombotic syndrome (PTS) is a complication affecting ≤50% of patients with DVT, in whom persistent venous obstruction, valvular reflux, and chronic inflammation lead to impaired muscle perfusion and tissue compromise.[106] Clinically silent DVT is thought to precede peripheral venous disease.[107] In a matched study of 308 patients (primarily identified by trophic changes of the skin, deep venous functional disease detected by duplex ultrasound, and symptoms, including aching and edema) and 346 controls, VWF levels >110% were associated with an OR of 1.7 (95% CI, 1.1-2.5) of peripheral venous disease.[108] Furthermore, a non-O blood type was associated with impairment of recanalization after DVT (OR, 3.71; $P < .01$) and a higher risk of PTS (HR, 1.53; $P = .028$), both in single-center, Italian populations.[109,110]

Venous ulcers are a common manifestation of chronic venous insufficiency, and punch biopsies obtained from eight patients with venous ulcers demonstrated increased capillary VWF staining, which was also associated with more advanced disease compared with controls.[111] In addition, obesity is an independent risk factor for PTS and obese patients also have an increased expression of VWF, supporting the possible mechanistic association of these two variables.

Chronic thromboembolic pulmonary hypertension. Chronic thromboembolic pulmonary hypertension (CTEPH) results from the failure of thrombus resolution in the pulmonary arteries in ~3% of cases after acute PE.[112] In an impressive study of 208 British patients with CTEPH, the VWF levels were significantly elevated in those CTEPH (167%) and patients with chronic thromboembolic disease (170%) without pulmonary hypertension compared with patients with a prior PE without complications (92%) or idiopathic pulmonary arterial hypertension (116%).[113] The ADAMTS13 antigen concentration followed an inverse pattern and was found at significantly lower concentrations in those with CTEPH and patients with chronic thromboembolic disease. The combination of low ADAMTS13 and high VWF:Ag levels had a synergistic effect on the odds of CTEPH (OR, 14.5; 95% CI, 5.33-47.4; $P < .001$) compared with healthy controls. Other studies have supported these conclusions, demonstrating similar elevations in VWF and FVIII levels in those with CTEPH and an overrepresentation of non-O blood types.[114,115] In addition, 22 of the 208 patients with CTEPH had undergone pulmonary endarterectomy and showed no improvement in the VWF—ADAMTS13 axis, suggesting its role in the pathogenesis and that it was not simply a consequence of the accrued thrombus material.[113]

VWF-DIRECTED THERAPEUTICS

Although advances in antithrombotic pharmaceutical agents have resulted in more specific targeting of the coagulation cascade, platelet receptors and fibrinolysis, major bleeding, and breakthrough thrombosis still present significant clinical challenges. Patients with inflammatory comorbidities, such as cancer and infection, have a substantial risk of treatment failure and can require elevated antithrombotic and/or antiplatelet doses that can increase the incidence of bleeding.[116,117] Therefore, it might be beneficial to identify and target key drivers of thromboinflammation, including VWF, to address the foundational elements of this pathophysiology.

Current therapies. A single VWF-directed therapy has been approved for use for patients with acquired thrombotic thrombocytopenic purpura (TTP), a rare, but serious, microvascular thrombotic disease resulting from autoimmune ADAMTS13 deficiency.[118] Caplacizumab is a bivalent nanobody directed against the VWF A1 domain to prevent VWF—platelet interactions and microvascular thrombosis.[119] Moreover, caplacizumab is efficacious in reducing the risk of thromboembolic events and TTP-related mortality in patients with acquired ADAMTS13 deficiency.[119] Although these findings are is critically important for patients with TTP, the applicability of this therapy to other, more prevalent, thromboinflammatory diseases is unclear. Additionally, bleeding-related adverse events were reported in 46 patients (65%) of the caplacizumab group and 35 patients (48%) of the placebo group, highlighting a common peril with the use of antithrombotic agents.

We recently reported data from a mouse model of obesity-associated DVT using a similar anti-VWF A1 domain nanobody that demonstrated a significant reduction in thrombus burden, suggesting that VWF inhibition might be beneficial in those with VTE.[82] Aymé et al[56] showed that although this same anti-VWF A1 domain nanobody abrogates VWF-dependent leukocyte recruitment and vascular leakage in two murine models of inflammation, it results in a dose-dependent prolongation of the bleeding time. The hemostatic balance is the crux of all current antithrombotic therapies, and more research is needed to identify improved molecular targets and better select patients who might achieve benefit from therapy.

Directions for development. Preclinical studies are applying VWF-targeted therapies (including antibodies and/or nanobodies, aptamers, recombinant GPIbα fragments, and ADAMTS13) with success to animal models of stroke, myocardial infarction with reperfusion injury, and VTE.[120,121] A VWF-directed aptamer demonstrated efficacy in thrombus prevention and recanalization in a baboon model of DVT.[122] Moreover, the thiol-reducing agent, N-acetylcysteine, has been shown to chemically disrupt VWF multimers to reduce the procoagulant activity of VWF, promote thrombolysis in murine arterial thrombi, and prevent murine pulmonary thrombosis.[123–125] N-acetylcysteine is frequently used as a mucolytic and to treat acetaminophen toxicity in patients and, therefore, might be a relevant clinically approved agent to explore for targeting VWF in VTE. Furthermore, additional research into VWF—leukocyte and VWF—erythrocyte interactions is necessary because little evidence is available of their necessity for physiologic hemostasis but could be of high importance in treating VTE. The potential strategies for VWF-directed therapeutic agents in VTE are shown in Fig 4.

CONCLUSIONS

In the present review, we have summarized the current knowledge of VWF in venous thrombosis, highlighting the increasing body of data demonstrating an association of VWF with diverse VTE disease states and sequela. It can be hypothesized from the preclinical studies and VWF

biochemistry that the mechanism linking VWF to VTE could involve its determination of the FVIII concentration, platelet adhesion and/or aggregation, and, potentially, leukocyte and erythrocyte interactions. Although elevated VWF levels confer an increased risk of VTE and long-term complications of venous disease, few therapies are available to interfere with VWF-dependent thromboinflammatory pathophysiology. Moreover, VWF single nucleotide polymorphisms, plasma levels, or surrogate measures such as the ABO blood type might be useful supplements to the current tools in risk stratification, prognostication, and determining the anticoagulation duration, as genetic and biochemical risk score analyses continue to develop in the era of personalized medicine.[103] The increased prevalence of chronic inflammatory diseases such as obesity and diabetes in vascular surgery patients further emphasizes the need for targeted thromboinflammatory therapeutics.

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AUTHOR CONTRIBUTIONS

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