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Targeting von Willebrand factor in liver diseases: a novel therapeutic strategy?

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Abstract

Acute and chronic liver disease are associated with substantial alterations in the hemostatic system. Evidence from both experimental and clinical studies suggests that anticoagulants slow the progression of liver disease. Efficacy of those anticoagulant drugs is, in part, attributed to a reduction of microthrombi formation within the liver. Although anticoagulant drugs show promising results, bleeding risk associated with these drugs is an obvious drawback, particularly in patients with a complex coagulopathy driven by decreased liver function. Identifying therapies that reduce intrahepatic thrombosis with minimal bleeding risk would significantly advance the field. Among the hemostatic alterations observed in patients are substantially increased levels of the platelet-adhesive protein von Willebrand factor (VWF). In contrast, levels of ADAMTS-13, the enzyme that regulates VWF activity, are significantly reduced in patients with liver disease. Highly elevated VWF levels are proposed to accelerate intrahepatic thrombus formation and thus be a driver of disease progression. Strong clinical evidence suggesting a link between liver disease and changes in VWF is now being matched by emerging mechanistic data showing a detrimental role for VWF in the progression of liver disease. This review focuses on clinical and experimental evidence supporting a connection between VWF function and the progression of acute and chronic liver diseases. Furthermore, with the recent anticipated approval of several novel therapies targeting VWF, we discuss potential strategies and benefits of targeting VWF as an innovative therapy for patients with liver disease.

Keywords

von Willebrand factor; blood platelets; ADAMTS13 protein; fibrosis; liver failure

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Von Willebrand factor

Von Willebrand factor (VWF) is a large multimeric glycoprotein generally known for its key role in hemostasis. When activated upon vascular damage, VWF serves as an adhesion molecule for platelets thereby initiating platelet plug formation.[1] VWF is also the carrier protein of coagulation factor VIII (FVIII), protecting FVIII from premature proteolytic cleavage.[2] VWF is an acute phase reactant, and VWF plasma concentration increases in response to stress, exercise, and in many different inflammatory conditions.[3]

Von Willebrand factor synthesis and function

Synthesis of VWF is restricted to megakaryocytes and endothelial cells.[4] Part of the VWF synthesized by endothelial cells is secreted constitutively into the plasma, where it has a half-life of approximately 12 hours.[5] The remaining VWF is also stored in endothelial cell-specific organelles, called Weibel-Palade bodies (WPB).[6] In addition to endothelial cell-derived VWF, approximately 15% of total VWF is produced by megakaryocytes and stored in α-granules of platelets.[7] The highest molecular weight VWF multimers are stored in WPB and α-granules, and are released at sites of vascular damage in response to secretion stimuli like thrombin, stress, vasopressin or its synthetic analogue desmopressin (DDAVP).[6] VWF reactivity towards platelets depends on its multimeric size, with the high molecular weight (HMW) multimers being the most effective in supporting platelet adhesion.[4] VWF multimeric size is regulated by the enzyme ADAMTS13 (*A D*isintegrin *a*nd *M*etalloproteinase with *T*hrombospondin motifs), which proteolytically cleaves the large multimers into smaller, less active VWF multimers.[8] The ADAMTS13 cleavage site is located within the A2 domain of VWF and is only accessible after partial unfolding of VWF, which is most likely induced by the shear stress exerted on VWF after binding to the endothelial surface or exposed collagen.[9, 10] VWF size regulation is important for a normal hemostatic balance, as evidenced by severe thrombotic complications in patients with congenital or acquired ADAMTS13 deficiency.[11] Although best known for its role in hemostasis, several other novel biological functions for VWF have been identified in recent years. For example, VWF plays an important role in regulating angiogenesis and wound healing and modulates inflammatory responses.[12–15]

Von Willebrand factor clearance

The mechanisms involved in VWF clearance from the circulation remain poorly understood. VWF is primarily cleared in the liver. However, when taking the different sizes of organs into account, the spleen also efficiently clears VWF from the circulation although its absolute contribution remains limited.[16] The Ashwell-Morell receptor (also termed asialoglycoprotein receptor) on hepatocytes was the first VWF clearance receptor to be identified and is capable of binding desialylated VWF.[17] Recently it was shown that the macrophage galactose-type lectin (MGL) receptor also regulates VWF clearance in a sialic-acid dependent manner.[18] In addition, several other specific lectin receptors (including Sialic acid-binding Ig-like Lectin 5 (Siglec-5)[19] and C-type lectin domain family 4 member M (CLEC4M))[20] and scavenger receptors (including low-density lipoprotein receptor-related protein-1 (LRP-1)[21], scavenger receptor A1 (SR-A1)[22], scavenger

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receptor class A Member 5 (SCARA5)[23] and stabilin-2 (STAB2))[24] have been shown to participate in VWF clearance.

Hemostatic rebalance in patients with liver disease.

Since almost all hemostatic proteins and inhibitors are synthesized in the liver, substantial alterations in the hemostatic system are observed in patients with acute liver injury/failure and chronic liver disease.[25] Except for cholestatic liver diseases, which are generally associated with a more preserved hemostatic balance[26], most hemostatic changes seem to be similar across all etiologies.[27] The hemostatic changes in patients with liver disease are characterized by decreased plasma levels of all procoagulant factors, with the exception of FVIII levels, which are increased.[28, 29] This increase may be explained by increased extrahepatic FVIII synthesis in response to liver injury.[30, 31] In addition, FVIII is produced by sinusoidal endothelial cells, which retain their synthetic capacity for FVIII even when hepatocellular function is impaired.[30, 32] Elevated FVIII levels might also be a consequence of endothelial perturbation induced by endotoxemia, which is frequently observed in chronic liver disease.[33] Alternatively, the increased levels of VWF observed in patients might protect FVIII from hepatic clearance.[2]

Conventional coagulation tests such as the prothrombin time (PT) or activated partial thromboplastin time (aPTT) are frequently prolonged in patients with cirrhosis.[34] Consequently, cirrhosis was considered for a long time to be a bleeding disorder. However, the PT and aPTT are only sensitive for procoagulant proteins and do not take into account changes in anticoagulant factors. Global hemostasis tests, like the thrombin generation assay, which are sensitive for both the pro- and anticoagulant proteins, have shown a normal to even increased hemostatic potential of plasma from cirrhotic patients.[35, 36] Indeed, it was found that a reduction in procoagulant factors in patients with cirrhosis is paired with a decrease in plasma levels of anticoagulant factors such as protein C, protein S and antithrombin.[28, 29] Similar compensations have been observed for the fibrinolytic system, where decreased levels of anti-fibrinolytic factors such as α_2-antiplasmin and plasminogen activator inhibitor 1 (PAI-1) are accompanied by a decrease in levels of pro-fibrinolytic proteins such as plasminogen.[37]

Compared to cirrhotic patients, coagulation alterations observed in patients with acute liver injury/failure (ALI/ALF) are more extensive, with levels of liver-derived coagulation proteins becoming as low as 1–10% of normal.[38, 39] ALI/ALF patients display a prolonged PT, reflected as an increase in the international normalized ratio (INR), as a component of criteria used to define ALI/ALF.[40] Although plasma levels of both pro-and anticoagulant factors are substantially reduced in patients with ALI/ALF[41, 42], thrombin generation appears to be normal to even increased in patients compared to healthy individuals.[38, 39] In addition, minimal effects on hemostasis were observed in ALI/ALF patients using thromboelastography (TEG)[43], a viscoelastic hemostatic assay that measures the dynamics and physical properties of clot formation in whole blood, despite an elevated INR.[43] In contrast to chronic liver disease, ALI/ALF is characterized by a profound hypofibrinolytic status, which is likely related to substantially elevated plasma levels of PAI-1 and low plasminogen levels.[44]

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The overall effect of these hemostatic changes and related compensatory mechanisms is a rebalanced hemostatic system in patients with acute and chronic liver disease. However, patients show clear hypo- and hypercoagulable features (including decreased clot formation and stability but enhanced thrombin generation) which may contribute to either bleeding or thrombotic episodes when the delicate hemostatic rebalance is disrupted.[25]

Changes in primary hemostasis in patients with liver disease.

As outlined above, multiple changes in coagulation produce a rebalanced hemostatic system in patients with liver disease. In addition, compensatory mechanisms are observed for primary hemostasis in both acute and chronic liver disease. Thrombocytopenia is frequently observed in liver disease patients and correlates with disease severity.[45–47] The underlying mechanism for the observed thrombocytopenia is not completely understood, but most likely includes reduced platelet production, increased splenic platelet sequestration, and/or increased platelet consumption/clearance.[48] Decreased production of the liver-derived hormone thrombopoietin, which regulates platelet production, appears to be major contributor to thrombocytopenia in patients with cirrhosis.[49] In contrast, thrombopoietin levels in ALI/ALF patients appear to be normal to increased, and do not correlate with the degree of thrombocytopenia[50], suggesting other mechanisms are responsible for the low platelet count observed in ALI/ALF patients. Platelet function defects in liver disease patients have been observed, including prolonged bleeding time and decreased agonist-induced platelet aggregation.[51, 52] However, more recent studies using flow cytometry, platelet count-adjusted aggregometry and flow-based methods have shown conflicting results and both decreased, normal and increased platelet function have been observed.[53–58] Regardless of the functional status of platelets, the thrombocytopenia in liver disease patients seems to be compensated for by mechanisms involving increased plasma levels of VWF, as platelet adhesion and aggregation is better supported by plasma of patients with liver disease.[44, 47, 59]

Changes in the VWF-ADAMTS13 axis

VWF antigen levels are substantially elevated (~5-10-fold increase) in both acute[44, 59–62] and chronic liver disease patients.[59, 63] Plasma VWF activity, assessed by either its ability to bind to the GPIb receptor or to collagen, is also increased in patients, although not to the same extent as protein levels.[64] This partially reduced functionality results in a reduced VWF:Act/VWF:Ag ratio in patients with liver disease compared to healthy individuals. The relative decrease in VWF activity suggests a qualitative alteration of the VWF protein. Indeed several studies found that the relative portion of the most active VWF multimers (the HMW multimers) were reduced in plasma of patients with acute and chronic liver disease.[44, 47, 59, 63, 65, 66] However, in a proportion of patients the presence of ultra-large VWF multimers has been observed, almost all in patients with advanced liver disease.[47, 61, 63, 66, 67]

Interestingly, whereas VWF levels are highly elevated in patients, ADAMTS13 antigen and activity in plasma are significantly reduced (~3-5 fold), particularly in patients with advanced liver disease or that manifest complications (e.g. portal vein thrombosis, portal

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hypertension, or ascites).[44, 47, 61, 62, 66–69] ADAMTS13 antigen and activity are decreased to a similar extent in patients with cirrhosis, as the ratio of ADAMTS13 activity and antigen is comparable to healthy controls.[67] As ADAMTS13 is produced by hepatic stellate cells[70, 71], which play a key role in initiation and progression of liver fibrosis, the observed decrease might be related to impaired ADAMTS13 synthesis as the quiescent hepatic stellate cells transdifferentiate to a myofibroblast-like phenotype. Interestingly, in ALI/ALF patients the ADAMTS13 activity/antigen ratio is substantially reduced, indicating that ADAMTS13 activity decreases by mechanisms independent of reduced ADAMTS13 protein levels.[44] Interestingly, there is not always a definitive connection between reduced ADAMTS13 functionality and increased VWF multimer size. In fact, some studies indicate that liver disease patients show a reduction rather than an increase in the HMW VWF multimers.[44, 59, 65] This disconnect may not be surprising, as HMW VWF multimers may be actively consumed and incorporated into platelet-rich microthrombi in the liver, or other tissues. It is also possible that VWF proteolysis may be mediated by ADAMTS13-independent mechanisms in the setting of liver disease.[59] Indeed, Federici et al. observed novel VWF fragments in cirrhotic patients, including plasmin-generated fragments.[65] However, a study by Ferro et al. showed no evidence for novel VWF proteolytic fragments.[72] Furthermore, in an experimental model of diet-induced liver steatosis a compensatory role of plasmin could not be demonstrated.[73] It thus remains unclear whether ADAMTS13-independent proteolysis is occurring in patients with liver disease.

Unbalanced VWF/ADAMTS13 axis as a contributor to liver disease progression?

Many studies have questioned whether changes in VWF and ADAMTS13 in liver disease patients are connected to outcome or the development of liver disease complications (see Table 1). Increased VWF and decreased ADAMTS13 levels are associated with disease severity and related to poor outcome (i.e. needing liver transplant or death). Furthermore, the balance between VWF and ADAMTS13 seems to especially shift when stable cirrhosis decompensates or when complications such as portal hypertension occur, suggesting VWF and ADAMTS13 might contribute to disease progression (see Table 1). High VWF levels were associated with advanced liver disease and complications of liver disease such as esophageal varices (abnormal, enlarged veins in the esophagus), ascites (peritoneal fluid excess), and portal hypertension (Table 1).[74–78] Furthermore, studies have shown that VWF is an independent predictor of hepatic decompensation (i.e. acute deterioration of liver function), hepatocellular carcinoma (HCC) development, and mortality in patients with cirrhosis.[68] Correspondingly, severely decreased ADAMTS13 levels are observed in patients with advanced chronic liver disease compared to patients with mild liver disease.[63] In addition, survival was lowest in cirrhotic patients with severe to moderate ADAMTS13 deficiency, and ADAMTS13 activity was significantly lower in patients with hepatocellular carcinoma than in those without liver cancer.[66] The association between unbalanced VWF/ADAMTS13 and progression of ALI is less well studied (see Table 2). However, two small cohorts showed that low ADAMTS13 activity levels were associated with poor outcome in ALI/ALF patients.[44, 61] Recent analysis of a large cohort of

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676 ALI/ALF patients showed that non-transplant-free patients (i.e. patients undergoing liver transplantation or who died within 21 days of admission) had higher VWF:Ag levels and lower ADAMTS13 activity than transplant-free survivors and severity of disease was associated with higher VWF levels.[78] Further evidence supporting a detrimental role for VWF/ADAMTS13 unbalance is the reported increase in VWF to ADAMTS13 ratio in patients with liver disease, wherein this ratio correlated with disease severity and poor outcome.[47, 66, 68]

Although there is a clear association between elevated VWF levels, low ADAMTS13 activity and liver disease severity/outcome, the mechanism behind this association is not fully understood. A frequent complication in patients with liver disease is the development of portal vein thrombosis (PVT)[79], and thrombosis can be a major complicating factor in chronic liver disease.[80] The prevalence of PVT appears to increase with disease severity, reaching 15-25% in patients awaiting liver transplantation.[81] Interestingly, increased VWF levels and low ADAMTS13 levels are associated with PVT in patients with liver disease[82], and microthrombi were found in one or more organs in a majority of patients with liver disease upon autopsy.[83] Moreover, evidence from both experimental studies[84–88] and one randomized trial[89] suggests that anticoagulants slow the progression of liver disease. While efficacy of these drugs have been, in part, attributed to a reduction of thrombus formation within the liver, direct evidence for this mechanism is lacking and multiple additional mechanisms have been proposed.[25] The marked unbalance between VWF and ADAMTS13 may lead to platelet hyperaggregability. Indeed, the Lisman group showed elevated VWF-dependent platelet adhesion and aggregation in plasma of ALI/ALF patients as well as chronic liver disease patients, compared to plasma from healthy controls.[44, 59] It has therefore been postulated that insufficient regulation of VWF activity could drive disease progression by facilitating hepatic platelet-induced microthrombi formation. In this theory, called ‘parenchymal extinction’, tissue ischemia caused by these platelet-rich microthrombi is held responsible for the effects on disease progression.[48, 90]

Role of VWF and ADAMTS13 in the pathogenesis of experimental chronic liver disease.

Observations in animal models of acute and chronic liver disease have provided mechanistic explanations for the observed connection between VWF and ADAMTS13 levels and outcome in liver disease patients. In this section we cover the mechanistic roles for both VWF and ADAMTS13 in liver disease examined in a variety of experimental models of chronic liver disease.

Role of VWF and ADAMTS13 in animal models of non-alcoholic fatty liver disease (NAFLD)

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver diseases ranging from simple steatosis through steatohepatitis to liver fibrosis. The prevalence of NAFLD in industrialized countries ranges from 35-60 % and is set to become the major cause of chronic liver disease in many parts of the world.[91] Non-alcoholic steatohepatitis (NASH) is the more severe form of NAFLD and is present in more than one-third of the NAFLD cases. NASH can eventually progress into and hepatocellular carcinoma (HCC).

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Although the pathogenesis of NASH is not fully elucidated, the “multiple-hit” mechanism is widely accepted as the pathogenesis, involving inflammation, oxidative stress, and insulin resistance. The basis of animal models used to study the impact of the hemostatic system on liver disease is extensively reviewed elsewhere.[92] Although animal models do not yet fully recapitulate all features of this complex human disease, multiple experimental models recapitulate important features of chronic liver disease including steatosis, steatohepatitis, and liver fibrosis as hepatic consequences of obesity. Experimental NAFLD/NASH in rodents is often induced by feeding mice of a high-fat diet (HFD). Mice fed an HFD for 10-12 develop features of the human disease such as obesity, hyperlipidemia, insulin resistance and hepatic steatosis.[93] However, much longer feeding (i.e. up to a year) is needed to induce inflammation and fibrosis of the liver and the results depend on rodent species, strain, and composition and content of the fat in the diet.[93]

The methionine and choline deficient (MCD) diet is widely used as an experimental model of non-obese NASH. Deficiencies of both methionine and choline will result in reduced secretion of very-low-density lipoprotein (VLDL) and consequently reduced triglyceride clearance and hepatic lipid accumulation. Steatohepatitis develops after approximately 3-4 weeks and approximately 10 weeks of feeding produces liver fibrosis, inflammation and elevated liver enzymes.[93] Although the MCD model replicates some important histological phenotypes typical of human NASH (such as liver injury and steatohepatitis), the model does not replicate the NAFLD-related metabolic syndrome. The main disadvantage is that the metabolic profile in mice on an MCD diet is the opposite of that seen in humans with NAFLD. For example, mice on an MCD diet typically lose weight, do not develop hyperlipidemia or hypertriglyceridemia and do not present with insulin resistance.[93]

Hepatic platelet accumulation appears to be a conspicuous feature of multiple animal models of NASH, with the transition of simple steatosis to NASH linked to the appearance of platelets in the liver.[94] Compared with mice fed a control diet, hepatic platelet accumulation increased in mice that were fed a choline-deficient high fat diet (CD-HFD) for 6 months, and platelets seem to contribute to disease progression through glycoprotein Ibα (GPIbα),[94] the VWF receptor on platelets. Specifically, treatment with an antibody against GPIbα as well as genetic dysfunction of GPIbα reduced NASH and HCC development.[94] VWF deficiency did not affect liver pathology in mice fed a CD-HFD for 6 months, although the impact of VWF deficiency on hepatic platelet accumulation was not evaluated in this model.[94] In addition, VWF plasma levels were not evaluated in wild-type mice fed a CD-HFD, and thus it remains unknown whether this animal model is associated with increased VWF plasma levels. Prior studies have shown that inducing NASH with the MCD diet does not affect plasma VWF levels, highlighting difficulties in translating findings in animal models to humans.[95] Interestingly, Yang et al.[96] found that VWF antigen levels increased about 4-fold in mice fed a HFD (containing 60% kcal from fat) for 12 weeks. In this model, $VWF^{-/-}$ mice developed less hepatic steatosis and showed reduced systemic and hepatic inflammation upon HFD challenge.[96] Notably, while seemingly contrasting results, it should be noted that the mechanisms and severity of liver pathology in these models are different. Overall, while there is ample evidence to suggest that platelets participate in the pathogenesis of NAFLD/NASH, the precise role of VWF in this

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process requires further study, with a particular need to determine which animal model best recapitulates changes in VWF observed in patients with NAFLD/NASH.

The role of ADAMTS13 in the progression of NAFLD has also been evaluated in different experimental models. Plasma ADAMTS13 antigen and activity levels increased in mice fed a HFD (42% kcal from fat) compared with mice fed a control diet.[97] VWF-positive microthrombi were increased in livers of ADAMTS13$^{-/-}$ mice on a HFD compared to wild-type mice.[73] Furthermore, lower platelet counts and a higher prevalence of ultra-large VWF multimers were observed in HFD-fed ADAMTS13$^{-/-}$ mice.[73] Notably, ADAMTS13 deficiency did not affect HFD-induced liver steatosis. In contrast, plasma ADAMTS13 activity was similar in wild-type mice fed a MCD diet compared with control-fed mice.[95] ADAMTS13 deficiency did not affect plasma VWF levels in MCD diet-fed mice, nor did it affect steatosis, liver injury, and lipid metabolism.[95]Although these studies suggest ADAMTS13 is not particularly critical in the pathogenesis of NAFLD, there are important caveats to be considered. None of the diet models studied to date fully recapitulates the complex changes in VWF/ADAMTS13 that accompany development of liver disease in humans. However, despite the shortcomings of these models, the results so far do not indicate a major effect of complete ADAMTS13 deficiency in experimental models of NAFLD/NASH.

Role of VWF in experimental liver fibrosis

Carbon tetrachloride (CCl_4) is one of the most widely used hepatic toxins for experimental induction of liver fibrosis in rodents.[93] Chronic administration of CCl_4 (twice weekly for at least 4 weeks) to mice produces persistent hepatocellular injury and eventually liver fibrosis as evidenced by hepatic collagen deposition. Plasma VWF levels were increased in wild-type mice after chronic CCl_4 challenge[98], and VWF$^{-/-}$ mice had significantly reduced liver fibrosis (assessed by collagen deposition) after chronic CCl_4 challenge compared with wild-type mice.[99] These studies provide experimental evidence in support of the association between VWF levels and disease progression in patients with hepatic fibrosis (Table 1). The mechanism whereby VWF contributes to experimental liver fibrosis induced by CCl_4 is not completely understood. It is likely that this mechanism involves platelets, as both clinical and experimental studies have suggested a link between platelet (function) and progression of chronic liver disease (reviewed elsewhere[48]). Although experimental and clinical evidence is emerging, the precise contribution of VWF to chronic liver disease, including its connection to platelets, deserves further study.

Role of VWF in acute liver injury and repair.

Overdose with the over-the-counter drug acetaminophen (APAP/Paracetamol) is the leading cause of ALI/ALF in the Unites States and other Western countries[100]. Experimental APAP overdose in mice produces a dose-dependent hepatotoxicity that resembles observations in ALF caused by acetaminophen overdose in humans.[101] APAP overdose is associated with a rapid and persistent thrombocytopenia in both mice and humans,[46, 102] and in mice the thrombocytopenia is paralleled by platelet accumulation in the injured liver.[102] Experimental evidence indicates that platelets contribute to the progression of

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APAP-induced liver injury. Miyakawa et al.[102] showed that antibody-mediated platelet depletion in mice significantly attenuated APAP-induced liver damage. The mechanisms whereby platelets contribute to progression of APAP-induced acute liver injury are not entirely understood. The foremost hypothesis is that the formation of hepatic platelet-rich microthrombi disrupt blood flow in liver sinusoids, leading to tissue ischemia and cellular necrosis.

Clinical data has suggested that VWF might contribute to liver injury after APAP overdose (see Table 2). Recent studies suggest a mechanism for the clinical association between VWF levels and poor outcome in ALI/ALF patients.[78, 99] Specifically, VWF appears to inhibit repair of the APAP-injured liver. Mice challenged with a hepatotoxic dose of APAP demonstrated increased plasma VWF levels as observed in patients with ALI/ALF, and VWF-platelet aggregate deposition was evident in the APAP-injured liver.[99] Interestingly, whereas VWF deficiency had no effect on initial platelet accumulation or peak liver injury (i.e., 24 hours after APAP challenge), hepatic platelet accumulation was dramatically reduced during liver repair (e.g. 48 hours and 72 hours) in $VWF^{-/-}$ mice. This reduction in hepatic platelet accumulation in $VWF^{-/-}$ mice was accompanied by faster repair of the injured liver. Moreover, antibody-mediated inhibition of VWF or administration of a small molecule antagonist of platelet integrin $\alpha_{IIb}\beta_3$ significantly reduced hepatic platelet accumulation and accelerated liver repair in mice with established APAP hepatotoxicity.[99]

These studies suggest a novel mechanism whereby VWF inhibits repair of the APAP-injured liver by promoting hepatic platelet accumulation. Because recent studies continue to link VWF to poor outcome in patients with ALI/ALF[78], targeting VWF in acute liver injury might provide a novel therapeutic approach to improve repair of the APAP-injured liver.

Targeting VWF as a therapeutic strategy in liver disease

Experimental studies and one clinical trial suggest that anticoagulation is a promising therapeutic strategy in the management of acute and chronic liver disease.[84, 86, 89, 103–106] Although anticoagulants show promising results, the risk of bleeding is a major drawback. This is especially true in patients with a complex coagulopathy caused by hepatic impairment.[107] Vitamin K antagonists (VKA) have major drawbacks, as therapy requires monitoring by the INR, which is frequently already prolonged in patients with liver disease.[108] LMWH seems an attractive alternative, and does not necessitate monitoring. However, there is little information available regarding the pharmacodynamics of LMWH in patients with liver disease[109], and increased volume of distribution makes it difficult to determine the optimal dose of LMWH.[110] Additionally, monitoring by anti-Xa assays is inaccurate and cannot be used to guide therapy in liver disease patients.[111] Although there is increasing experience with LMWH in patients with liver disease, clinical experience with direct oral anticoagulants (DOACs) is lacking and in vitro data suggests that the optimal dosage for DOACs may be different in patients with liver disease compared with healthy individuals.[112]Antiplatelet drugs showed promising results in animal models[94, 113], however, drawbacks include resistance to antiplatelet drugs in patients, escalated bleeding risk, high inter-individual variability and in the case of aspirin, a mechanism of action

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involving irreversible inhibition.[107] Moreover, liver disease is a contraindication for many antiplatelet drugs due to increased risk of gastrointestinal bleedings.[107]

The limitations of currently used anticoagulants raise an urgent need for the development of novel drugs capable of neutralizing thrombus formation but with improved efficacy and safety. Another challenge for the treatment of chronic liver disease is that effective therapy is likely to require life-long administration to maintain its efficacy. As with all antithrombotic therapy, risk of bleeding is a limiting factor for clinical use. Notably, clinical testing of compounds targeting VWF in animal models and healthy individuals points towards a profoundly lower bleeding risk with equal or higher antithrombotic efficacy as compared to the established drugs.[114] Given the clinical and experimental evidence suggesting a role for VWF in acute and chronic liver disease, it is conceivable that VWF-targeted therapies could be applied in patients with acute and chronic liver disease[115], but to date these remain untested. In the following sections we cover several potential VWF-targeted strategies and discuss pros and cons of their use in liver disease patients, with a focus on acute liver injury.

N-acetyl cysteine in APAP overdose

Administration of N-acetylcysteine (NAC) has been used since the late 1970s as the primary therapy for acute liver injury after APAP overdose. Although NAC likely reduces liver damage through multiple mechanisms, its efficacy is greatest when administered quickly[116], as it supports the synthesis of glutathione. Glutathione conjugation is critical to detoxify N-acetyl-p-benzo-quinone imine (NAPQI), the primary toxic APAP metabolite produced in excess when APAP metabolic pathways are overwhelmed.[101]

Interestingly, NAC has been shown to affect hemostatic activity both in vitro and in vivo by reducing platelet-VWF string formation and VWF multimeric size.[117] It is possible that regulation of VWF multimer size is a component of the mechanism whereby NAC benefits ALI/ALF patients, even beyond its effects on APAP hepatotoxicity.[118] Indeed, it has been suggested that the reduction in plasma HMW VWF multimers in ALI/ALF patients is a consequence of NAC treatment.[44] However, animal studies have shown a similar decrease of HMW VWF multimers in plasma after APAP overdose[99], indicating that NAC treatment might not be responsible for the VWF size reduction. However, this does not exclude a potential beneficial effect of NAC in reducing hepatic VWF-platelet thrombi formation or accelerated removal of these hepatic microthrombi. In fact, a recent study showed that NAC has a direct thrombolytic effect on VWF-rich platelet thrombi in three experimental models of acute ischemic stroke.[119] Interestingly, some experimental[120, 121] and clinical evidence[122] point towards hepatoprotective effects of NAC in chronic liver disease as well. Whether the potential anti-fibrotic effects of NAC are mediated in part via its role in VWF size regulation or thrombolytic potential remains to be determined.

Supplementation with ADAMTS13

The importance of ADAMTS13 for normal hemostatic function is evidenced by patients who suffer from thrombotic thrombocytopenic purpura (TTP) due to a congenital or acquired ADAMTS13 deficiency. TTP is characterized by the presence of VWF- and

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platelet-rich microthrombi in the microvasculature of various organs because of inadequate ADAMTS13 proteolysis.[123] The observed prothrombotic phenotype in liver disease patients as well as experimental evidence suggests that a local TTP-like mechanism could also occur in the liver microvasculature (e.g. HMW VWF multimers driving platelet-rich microthrombi formation). Standard treatment for TTP is administration of ADAMTS13 by either infusion of fresh frozen plasma (FFP) or plasmapheresis with FFP in the case of acquired TTP in order to remove inhibitory ADAMTS13 antibodies from the circulation.[123] Transfusion with FFP seems an unlikely therapeutic approach to increase ADAMTS13 activity as it is associated with poor outcome in ALF patients, most likely because transfusion would increase VWF and other procoagulant factors.[124] As patients with both acute and chronic liver disease have reduced ADAMTS13 activity, plasma exchange could be a potential therapeutic option to not only replenish ADAMTS13, but also decrease VWF and hepatotoxins from the circulation. Limited data is available on the efficacy and safety of plasma exchange with FFP in liver disease patients. To date, only one randomized, controlled clinical trial has been performed and showed potential improvement in transplant-free survival in ALF patients with high volume plasmapheresis (HVP).[125] However, the benefits of HVP were relatively small, showing approximately a 10% improvement compared to standard care. Another disadvantage is that large volumes of FFP were required (8-12 liters of FFP per day for a total of 3 consecutive days), and the duration of each treatment is approximately 9 hours, making HVP a burdensome treatment option.

Recombinant ADAMTS13 (rADAMTS13) therapy may provide a promising therapeutic opportunity. In a mouse model of congenital TTP, rADAMTS13 therapy has been shown to decrease the incidence and severity of TTP biomarkers.[126] Prophylactic and therapeutic treatment with rADAMTS13 effectively reduced brain microvascular thrombosis and dissolved thrombi in vivo in a mouse model of congenital TTP.[127] Results from a phase 1 study in congenital TTP patients provided the first evidence of rADAMTS13 administration in reducing VWF multimer size in humans.[128] A phase 3 trial investigating the safety and efficacy of rADAMTS13 in the prevention and treatment of thrombotic episodes in TTP patients is expected to complete in 2023 (Clinical trial number NCT03393975). Although still at the stage of animal research, several studies have shown efficacy of rADAMTS13 in managing ischemic stroke through regulating VWF-mediated immunothrombosis. De Meyer et al. found that administration of rADAMTS13 reduced myocardial infarct size in mice.[129] More recently, Denorme et al.[130] demonstrated that in a mouse model of acute ischemic stroke, occlusive VWF-rich thrombi induced by localized injury were rapidly resolved by administration of rADAMTS13.[130] Importantly, administration of rADAMTS13 did not increase the risk of intracranial bleeding in murine stroke models.[130]

One possible caveat for the use of rADAMTS13 in liver disease is that its efficacy could be reduced by the presence of auto-inhibitors of ADAMTS13 has been documented in a subset of liver disease patients, specifically in patients with severe ADAMTS13 deficiency.[61, 66, 67] However, in some cases rADAMTS13 can override anti-ADAMTS13 inhibitory antibodies, as shown previously in a rat model of acquired TTP, resulting in restoration of ADAMTS13 activity and degradation of HWM VWF multimers.[131] Similar results were obtained when plasma from acquired TTP patients was supplemented with rADAMTS13.

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[132] A phase 2 clinical trial evaluating the efficacy or rADAMTS13 in the treatment of acquired TTP is currently recruiting patients (NCT03922308). Results from this trial are important to determine whether observations in experimental settings extend to patients.

Inhibitors of von Willebrand factor

The VWF-platelet interaction is an important step in thrombus formation and has been implicated in a variety of thrombotic diseases. Blocking the interaction between VWF and platelets reduces adhesion of platelets to the endothelium and subsequent VWF-mediated platelet activation. Blocking the VWF-platelet interaction thus seems an attractive target to reduce VWF function in liver disease. Because of the limitations of currently used antiplatelet drugs, including increased risk of bleeding, a variety of new strategies targeting VWF function have emerged that reduce thrombus formation without major effects on hemostasis.

Several pharmacologic strategies targeting the VWF-platelet interaction have been developed in the past decade, with each showing antithrombotic effects with minimal increase in the risk of bleeding (see table 2). Caplacizumab (initially known as ALX-0081) is a humanized nanobody targeting an epitope in the VWF A1 domain that blocks the interaction with platelet GPIbα to prevent VWF-platelet microthrombosis.[133] Caplacizumab has shown marked efficacy in treating TTP and its complications in clinical trials.[134] This nanobody was approved by the European Medicines Agency in 2018 and by the US FDA in 2019 for the treatment of acquired TTP. Most VWF inhibitors have yet to enter clinical evaluation for efficacy and safety, although many have shown promising results in preclinical studies (see Table 3). Besides Caplacizumab, the aptamer ARC1779 and the monoclonal antibody AJW200 also reached clinical evaluation. ARC1779 has been developed for use in patients with acute coronary syndrome and showed inhibition of VWF function without causing bleeding events in healthy controls.[135] Development of ARC1779 has been hindered by slow enrollment in multiple clinical trials (NCT00742612, NCT00726544). However, despite the reduction in sample size, ARC1779 was able to reduce cerebral embolism after carotid endarterectomy (CEA)[136], a procedure used to remove plaques from carotid arteries. AJW200 is the humanized version of the murine monoclonal antibody AJvW-2, directed against the A1 domain of VWF. To date, only one Phase 1 study in healthy volunteers has been completed. However, results showed that AJW200 produced a dose-dependent inhibition of VWF activity without prolonging bleeding time.[137]

As the field progresses towards inhibition of VWF in the context of liver disease, there is both opportunity and challenge in the use of VWF-targeting compounds for studies in experimental settings of liver disease. For example, most VWF inhibitors (including Caplacizumab) do not target murine VWF, limiting the use of these inhibitors for proof-of-concept or mechanistic studies in standard mouse models. At least one novel nanobody can recognize an epitope within the VWF A1 domain, in both humans and mice, thereby blocking the VWF-GPIbα interaction.[15] In addition, novel mice have also been developed in which the VWF-GPIbα interaction has been humanized, allowing the use of otherwise human VWF specific tools to be applied in mouse models.[138] Another need in the field

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is for additional tools to target other specific VWF-integrin interactions. For example, experimental evidence suggests that the interaction between GPIIbIIIa and VWF may also contribute to the formation of hepatic microthrombi.[99] If developed, VWF-targeted inhibitors that prevent the GPIIbIIIa-VWF interaction would be very useful for mechanistic studies. As new tools become available and these challenges are addressed, it seems plausible that mechanistic studies may provide further proof-of-concept in support of targeting VWF function in liver disease.

Summary

Acute and chronic liver diseases are frequently accompanied by complex alterations in the hemostatic system due to impaired synthesis and/or catabolism of hemostatic proteins. Although many hemostatic changes promote bleeding, compensatory mechanisms including high VWF levels are also present. While VWF levels are substantially elevated in both acute and chronic liver disease, ADAMTS13 levels are severely reduced. This VWF/ADAMTS13 unbalance correlates with disease severity and poor outcome in both the acute and chronic liver disease setting. Although it has been postulated that inadequate VWF regulation might contribute to liver disease progression via facilitation of platelet-rich microthrombi within the liver, direct mechanistic connections were lacking. However, recent studies using experimental models of acute and chronic liver injury have provided more insight into the role of VWF and ADAMTS13, suggesting that the unbalance between VWF and ADAMTS13 levels in patients with liver disease are mechanistically linked to progression of disease (summarized in figure 1). Increasing evidence from experimental liver disease models suggest that antithrombotic treatment slows down the progression of disease. Although antithrombotic drugs show promising results in animal models and humans, bleeding risk limits broad application of these drugs in patients with decreased liver function. Clinical testing of compounds targeting VWF in animal models and healthy individuals points towards a profoundly lower bleeding risk with equal or higher efficacy as compared to the established antiplatelet drugs. Given the potential roles of VWF in intrahepatic thrombus formation and PVT, VWF seems a promising target to reduce the progression of acute and chronic liver disease.

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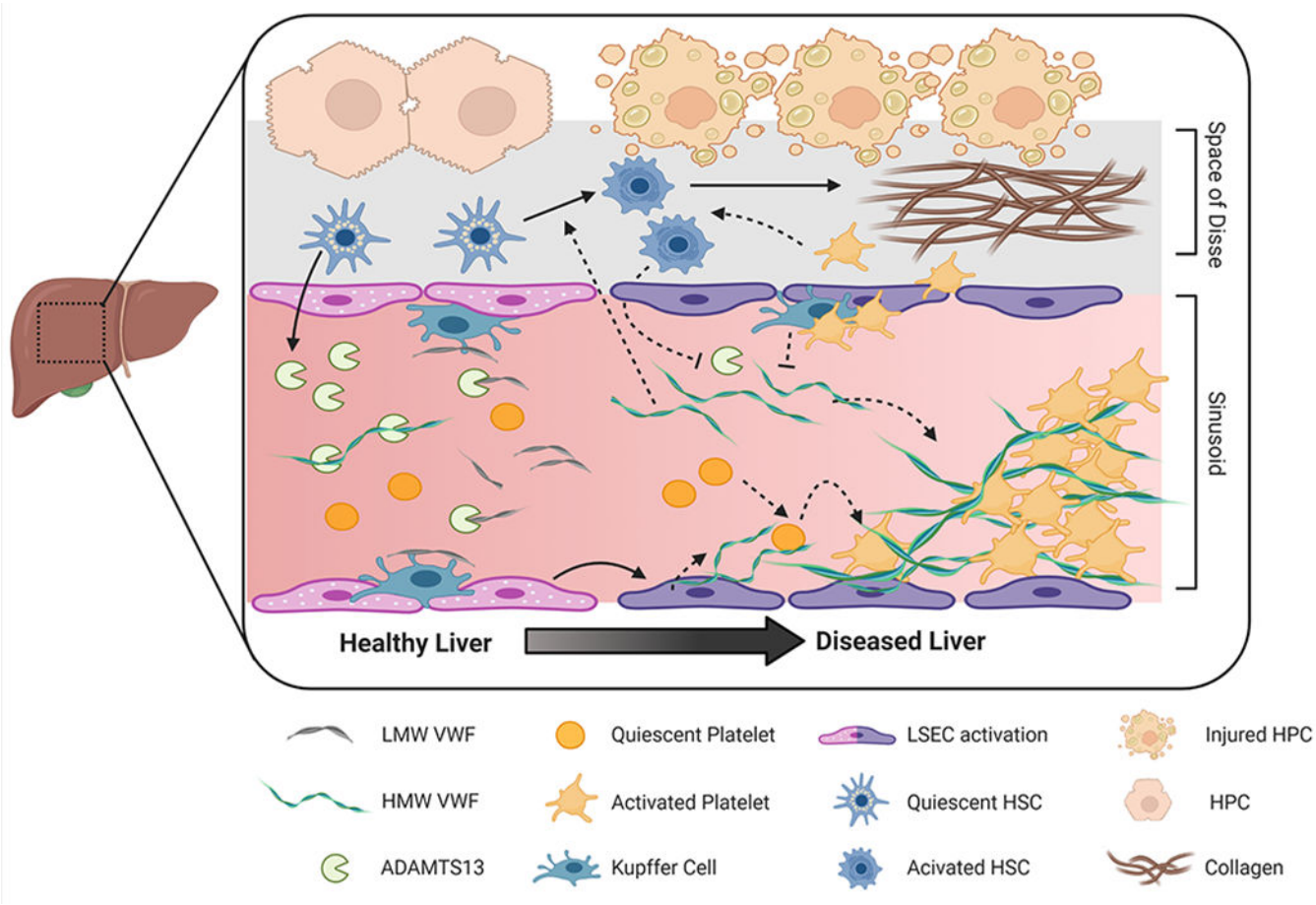

Figure 1. Proposed mechanisms whereby the VWF/ADAMTS13 axis contributes to liver disease.

The liver plays a key role in regulating plasma VWF levels. Hepatic stellate cells release ADAMTS13 into the microcirculation where it regulates plasma VWF multimeric size. VWF is also cleared by liver resident macrophages (i.e., Kupffer cells) and hepatocytes. Acute and chronic liver damage impact VWF regulation. For example, loss of endothelial fenestration impairs fluid exchange between hepatocytes and the sinusoidal blood. Plasma ADAMTS13 levels may be altered by disease-dependent changes in expression, secretion, or consumption. Similarly, increased endothelial secretion of VWF combined with impaired clearance by the diseased liver elevates VWF plasma levels. There is also evidence that HMW VWF multimers can be actively consumed and incorporated into platelet-rich microthrombi in the injured liver. Downstream pathologic effects of these microthrombi include disruption of blood flow, exacerbation of tissue injury and delayed liver repair. In addition, VWF and platelets may contribute to hepatic fibrosis by mechanisms dependent or independent of micro-thrombi by amplifying activation of hepatic stellate cells, the primary cell type responsible for exaggerated collagen deposition in chronic liver diseases. LMW, Low-molecular weight; HMW, High molecular weight, LSEC, liver sinusoidal endothelial cell; HSC, hepatic stellate cell; HPC, hepatocyte. Figure created with BioRender.com

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Table 1.

Clinical studies investigating changes in VWF and ADAMTS13 parameters in chronic liver diseases.

Cohort	VWF				ADAMTS13			VWF: ADAMTS13	Other	Ref
	Ag	Act	Ratio	HMW Multimers	Ag	Act	Ratio	Ratio		
Decompensated Cirrhosis (n=10)	↑	↑	↓	↓	NA				Heightened VWF proteolysis. Evidence of plasmin-cleaved VWF fragments	[65]
Cirrhosis Child A,B,C Cholestasis excluded (n=32)	↑ in Child B & C	↑ in Child B & C	NA	≡	NA				Normal proteolysis, no evidence of novel VWF fragments	[72]
Cirrhosis with PH, mixed etiology, Child-Pugh score <9 or ≥9 (n=27)	↑ with disease severity	NA							VWF:Ag higher in patients with ascites than without.	[74]
Decompensated Cirrhosis (n=42)	↑	↑	↓	NA	NA	↓	NA			[69]
Liver disease patients who underwent liver resection, mixed etiology (biliary disease &viral hepatitis) (n= 19)	↑	NA							Correlation between VWF levels and FVIII levels. Hepatic VWF mRNA increased in cirrhotic patients. More VWF staining in liver of patients with cirrhosis.	[139]
Cirrhosis (n=54) and ALF (n=5), mixed etiologies, Child A,B,C	↑ with disease severity, highest in ALF	↑ with disease severity, highest in ALF	↓ with disease severity, lowest in ALF	↓	Variable, not different	Variable, not different	NA		Increased platelet adhesion over collagen surface. VWFpp ↑ with disease severity	[59]
Cirrhosis Child C alcohol-induced (n=3), Child B viral hepatitis (n=1)	NA								Decreased platelet adhesion over collagen and fibrinogen coated surface, but normal when corrected for lower platelet count.	[56]
Cirrhosis Child A,B,C (n=90)	↑ with disease severity	NA	NA	Shift from LMW to normal to UL-VWF as liver function deteriorated	↓ with disease severity	↓ with disease severity	≡	NA	PVT often observed in advanced disease patients. IgG autoantibodies present in severe ADAMTS13 deficiency.	[67]

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Cohort	VWF				ADAMTS13			VWF: ADAMTS13	Other	Ref
	Ag	Act	Ratio	HMW Multimers	Ag	Act	Ratio	Ratio		
Cirrhosis, mixed etiology (n=109) and chronic hepatitis (n=33) Child A,B,C	↑ with disease severity	↑ with disease severity	↓ with disease severity	Variable. 53% had normal, 30% had loss of HMW, 16% had UL-VWF	↓ with disease severity	↓ with disease severity	NA	↑ with disease severity	Patients with UL-VWF had lowest ADAMTS13 levels. ADAMST13 was lowest when complicated with HCC	[47]
Cirrhosis (Cholestasis excl) with PH (n=42)	↑ correlated with Child-Pugh score and MELD score	NA							Positive correlation between VWF levels and portal pressure. VWF levels predict poor outcome (death, portal hypertension-related complications or transplant)	[75]
NCIPH (n=18), other chronic liver disease (n=25)	Not different	↓ in NCIPH	NA	Variable	↓ in NCIPH	↓ in NCIPH	↓ in NCIPH	NA		[140]
Cirrhosis with PH (n=211) or without PH (n= 75) and compensated (n=189) or decompensated (n=97)	↑ higher in decompensated vs compensated, higher in PH patient than without PH. Higher in patients who died.	NA							↑ VWF associated with varices and ascites. Correlation between VWF and portal pressure. VWF predicted mortality in compensated and decompensated patients	[76]
Cirrhosis, mixed etiology (n=108) Child A,B,C	↑ with disease severity	↑ with disease severity	↓ with disease severity	Variable, degraded and normal in Child B, majority normal in Child C but UL-VWF found in 24%	NA	↓ with disease severity	NA	↑ with disease severity	Inhibitor against ADAMTS13 in 83% of patients. Survival lowest in patients with severe to moderate ADAMST13 deficiency. Child A with HCC had a significantly lower ADAMTS13:AC than those without HCC	[66]
Chronic Hepatitis C (n=294)	↑ with stage of fibrosis	NA							VWF level predicts advanced liver fibrosis and cirrhosis	[141]
Stable Cirrhosis with (n=31) or without (n=114) HPS, mixed etiology Child A,B,C	↑ in HPS patients and in patients with complications (ascites, encephalopathy, GI bleeding, transplant/death)	NA							Association between high VWF and HPS, VWF predictor for presence of HPS	[142]

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Cohort	VWF				ADAMTS13			VWF: ADAMTS13	Other	Ref
	Ag	Act	Ratio	HMW Multimers	Ag	Act	Ratio	Ratio		
NCIPH (n=29), Cryptogenic Liver disease (n=22)	↑ compared to healthy controls, but lower than cryptogenic liver disease				↓ in NCIPH	↓ in NCIPH		↑ compared to healthy controls, no difference between disease groups		[143]
Cirrhosis on the transplant waiting list (n=72), mixed etiologies Child A,B,C	↑ with disease severity	↑ with disease severity	↓	NA	NA	↓	NA	NA	Association between VWF and closure time (PFA), VWF higher in thrombocytopenic patients with normal PFA	[144]
Early cirrhosis (n=25), or advanced cirrhosis with (n=24) or without (n=31) infection/SIRS, mixed etiology	↑ with disease severity	↑ with disease severity	≡	≡	↓ in advanced cirrhosis with infection/ SIRS compared to early cirrhosis (Child A)	↓ in advanced cirrhosis with infection/SIRS compared to early cirrhosis (Child A)	NA	NA	↓ ADAMTS13 and ↑ VWF correlated inflammation markers and organ dysfunction. ↑ VWF in non-survivors, no association with PVT. VWF and ADAMTS13 predict transplant-free survival	[77]
Cirrhosis, Hepatitis B (n=60), with or without CSPH, SPH, or EV	↑, higher in patients with CSPH and SPH, and EV patients than without	NA							↑ VWF staining in livers of cirrhotic patients, VWF staining correlated with VWF:Ag and PH. VWF is predictor of CSPH and SPH diagnosis, and for presence and degree of EV.	[145]
Cirrhosis with (n=24) or without (n=60) PVT, mixed etiologies	↑ in PVT patients vs no PVT but not significant	↑ in PVT patients vs no PVT	↑ in PVT patients vs no PVT but not significant	≡	NA	↓ in PVT patients vs no PVT	NA	NA	↑ VWF and ↓ ADAMTS13 was associated with PVT	[82]
ACLF (n=50), mixed etiology and Compensated cirrhosis (n=20), Viral Hepatitis	↑ higher in ACLF than Compensated	↑ higher in ACLF than Compensated	↓ lower in ACLF	NA	NA	↓ lower in ACLF than compensated patients	NA	↑ in ACLF	VWF higher in patients with poor outcome. Correlated with organ failure. VWF/ADAMTS13 ratio ↑ in patients with poor outcome	[68]
Cirrhosis with thrombocytopenia (n=102), Child A,B,C	↑ with disease severity	NA							VWF ↑ in patients with ascites or varices. VWF independently predicted	[146]

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Cohort	VWF				ADAMTS13			VWF: ADAMTS13	Other	Ref
	Ag	Act	Ratio	HMW Multimers	Ag	Act	Ratio	Ratio		
									new-onset ascites, VB, and mortality	
Cirrhosis with (n=198) or without (n=38) CSPH, mixed etiology, Child A,B,C	↑ with disease severity, ↑ in CSPH vs no CSPH	NA							VWF predicts CSPH, ↑ Vitro score (VWF/ platelet ratio) with disease severity, correlates with CSPH, and PH manifestations (EV, ascites, decompensation).	[147]
Compensated (n=99) and AD (n=54) cirrhosis, mixed etiology	↑ highest in AD patients	↑ highest in AD patients	↓ in both groups	Presence of UL-VWF in 25% of patients, majority of them AD	↓ in AD patients	↓ in AD patients	NA	NA	↑ VWF and ↓ ADAMST13 in AD, possible markers and contributors to disease progression	[63]
Cirrhosis with CSPH (n=225)									VWF correlated with markers of bacterial translocation, inflammation and C-reactive protein independent of HVPG. VWF was independent predictor of VB, bacterial infections and transplant-free mortality.	[148]
Decompensated cirrhosis with (n=31) or without (n=33) PVT, mixed etiologies	NA				↓ Lowest in PVT group	NA				[149]
Cirrhosis (n=109), split by etiology, Child A and B	↑ across all etiologies	NA	NA	NA	NA	↑ in NASH, trend towards ↓ in cholestasis	NA	NA		[27]

NA, not assessed; PH, portal hypertension; VWFpp, VWF propeptide; PVT, portal vein thrombosis; HCC, hepatocellular carcinoma; AKI, acute kidney failure; HPS, hepatopulmonary syndrome; NCIPH, non-cirrhotic intrahepatic portal hypertension; PFA, platelet function analyzer; SIRS, systemic inflammatory response syndrome; CSPH, clinically significant portal hypertension; SPH, severe portal hypertension; EV, esophageal varices; ACLF, acute-on-chronic liver failure; VB, variceal bleeding; PH, portal hypertension; AD, acute decompensation; NASH, non-alcoholic steatohepatitis.

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Table 2.

Clinical studies investigating changes in VWF and ADAMTS13 parameters in acute liver injury/failure.

Cohort	VWF				ADAMTS13			VWF: ADAMTS13	Other	Ref
	Ag	Act	Ratio	HMW Multimers	Ag	Act	Ratio	Ratio		
ALI/ALF, mixed etiology (50% APAP) n=50	↑	↑	↓	↓	↓	↓	↓	NA	Increased VWF-dependent platelet adhesion and aggregation. Low ADAMTS13:Act associated with poor outcome	[44]
ALF, mixed etiology (45% APAP) with (n=10) or without (n=6) AKI	↑ in both groups, higher in patients with AKI	NA								[62]
AH (n= 27) and ALF (n=11)	↑ higher in ALF than AH	NA	NA	4 out 11 ALF had UL-VWF. None in AH	NA	↓ Lower in ALF than AH	NA	NA	ADAMTS13:Act higher in survivors than non-survivors. VWF:Ag lower in survivors. ADAMTS13 inhibitor in 67% of ALF patients and in 22% of AH.	[61]
ALF (n=40), unknown etiology	NA	↑	NA						↑ VWF deposition in liver	[64]
ALI/ALF (n=676), mixed etiology (46% APAP)	↑ highest in non-APAP	NA				↓ lowest in non-APAP	NA		↑ VWF levels, ↓ ADAMTS13 levels in patients with more severe ALI/ALF (presence of encephalopathy or SIRS). ↑VWF and lower ADAMTS13 in non-transplant-free survivors.	[78]

ALI/ALF, acute liver injury/failure; NA, not assessed; AKI, acute kidney failure; SIRS, systemic inflammatory response syndrome; AH, acute hepatitis; APAP, acetaminophen.

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Table 3.

Currently developed VWF inhibitors and their mechanisms of action.

VWF INHIBITOR		TARGET	MECHANISM	CLINICAL TRIAL	REF
AJVW-2	Murine anti-human VWF monoclonal antibody	Directed against VWF A1 domain	Blocks VWF- GPIbα- interaction	No	[150]
AJW200	IgG4 humanized version of AJvW-2	Directed against VWF A1 domain	Blocks VWF- GPIbα- interaction	Yes	[151]
ANFIBATIDE (AGKICETIN)	Snake venom-derived GPIb antagonist	Binds to GPIbα	Blocks VWF- GPIbα- interaction	No	[152]
ARC15105	Second-generation anti-VWF aptamer	Binds to VWF A1 domain	Blocks VWF- GPIbα- interaction	No	[153]
ARC1779 (ARC	Second-generation PEG-conjugated aptamer	Binds to activated VWF A1 domain	Blocks VWF- GPIbα- interaction	Yes	[154]
BT200	Third-generation anti-VWF aptamer	Binds to the A1 domain of VWF.	Blocks VWF- GPIbα- interaction	No	[155]
CAPLACIZUMAB/ CABLIVI (ALX-0081 OR ALX-0681)	Humanized anti-VWF bivalent nanobody	Binds to the A1 domain of VWF.	Blocks VWF- GPIbα- interaction	Yes	[134]
82D6A3	Monoclonal anti-VWF antibody	Binds to the VWF A3 domain.	Inhibits VWF-collagen type I and III interactions	No	[156]
H82D6A3	Humanized version of 82D6A3	Binds to the VWF A3 domain.	Inhibits VWF-collagen type I and III interactions	No	[157]
DTRI-031	Anti-VWF aptamer	Binds to VWF	Inhibits VWF-platelet interaction	No	[158]
H6B4-FAB	Fab-fragment of a humanized monoclonal antibody	Targets GPIbα.	Inhibits binding of VWF to GPIbα	No	[159]
GPG-290	Recombinant chimeric protein.	Contains gain-of-function GPIbα fragment that binds to VWF A1 domain	Inhibits binding of VWF to GPIbα	No	[160]
KB-VWF-006BI	Anti-VWF nanobody	Recognizes an epitope located within human and mouse VWF A1 domain	Blocks VWF- GPIbα- interaction	No	[15]
MA-16N7C2	Recombinant mouse antibody	Recognizes human GPIIbIIIa	Blocks VWF-GPIIbIIIa interaction	No	[161]
TAGX-0004	DNA aptamer	Recognizes the VWF A1 domain		No	[162]

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