Elevated Levels of von Willebrand Factor in Cirrhosis Support Platelet Adhesion Despite Reduced Functional Capacity

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Cirrhosis of the liver is frequently accompanied by complex alterations in the hemostatic system, resulting in a bleeding tendency. Although many hemostatic changes in liver disease promote bleeding, compensatory mechanisms also are found, including high levels of the platelet adhesive protein von Willebrand Factor (VWF). However, conflicting reports on the functional properties of VWF in cirrhosis have appeared in literature. We have measured a panel of VWF parameters in plasma from patients with cirrhosis of varying severity and causes. Furthermore, we assessed the contribution of VWF to platelet adhesion, by measuring the ability of plasma from patients with cirrhosis to support adhesion of normal or patient platelets under flow conditions. VWF antigen levels were strongly increased in patients with cirrhosis. In contrast, the relative collagen binding activity, as well as the relative ristocetin cofactor activity, was significantly lower in patients as compared with controls, indicating loss of function. Accordingly, patients had a reduced fraction of high-molecular-weight VWF multimers. Both strongly elevated and reduced activity and antigen levels of the VWF cleaving protease ADAMTS13 were found in individual patients. Adhesion of either normal or patient platelets to a collagen surface was substantially increased when these platelets were resuspended in plasma of patients with cirrhosis, as compared with control plasma. In conclusion, highly elevated levels of VWF in patients with cirrhosis contribute to the induction of primary hemostasis despite reduced functional properties of the molecule. This phenomenon might compensate for defects in platelet number and function in patients with cirrhosis. (HEPATOLOGY 2006;44:53-61.)

Cirrhosis of the liver is often accompanied by extensive alterations in the hemostatic system. A decreased platelet count, impaired platelet function, a decreased thrombin generating capacity, defective fibrin formation due to dysfibrinogenemia, and defects in the fibrinolytic system may all be encountered in these patients (reviewed in Lisman et al.[1]). The net effect of these hemostatic changes is a bleeding tendency, which may be particularly manifested during invasive procedures.

Although many of the hemostatic changes found in patients with cirrhosis result in a reduced hemostatic capacity, compensatory mechanisms are also found. For example, the reduction in thrombin generating capacity caused by reduced levels of procoagulant proteins is, in part, compensated by the concomitant reduction of the natural anticoagulants.[1] In fact, tissue factor–induced thrombin generation, which is substantially depressed in a prothrombin-like assay, is completely normalized on addition of thrombomodulin, which allows activation of the anticoagulant protein C system.[2] Similarly, the reduction in profibrinolytic proteins may (in part) be compensated for by reduction of antifibrinolytics.[3,4]

A mild to moderate thrombocytopenia and a poorly defined thrombocytopathia are often present in patients

Abbreviations: VWF, von Willebrand factor; ADAMTS13, a disintegrin-like and metalloprotease with thrombospondin type 1 motifs type 13; VWF:Ag (von Willebrand factor antigen levels), VWF:Rco (von Willebrand factor ristocetin cofactor activity); MELD, model for end-stage liver disease; DIC, disseminated intravascular coagulation.

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with cirrhosis, but their clinical relevance is unclear. A possible compensatory mechanism for these platelet abnormalities is the presence of abnormally high plasma levels of von Willebrand factor (VWF).[5] VWF is a large, multimeric protein with a crucial role in primary hemostasis, as evidenced by the severe bleeding tendency associated with complete VWF deficiency.[6] Conversely, elevated levels of VWF are associated with (arterial) thrombosis.[7] Platelet–VWF interaction is the first step in platelet adhesion. After vessel wall damage, VWF from the circulation binds to exposed collagen fibers in the subendothelium. Once bound to collagen, VWF is able to interact with platelet glycoprotein Ib. Through transient VWF–glycoprotein Ib interactions, the platelet slows down, allowing subsequent stable interaction of the platelet with collagen through the collagen receptors $\alpha 2\beta 1$ and glycoprotein VI.

The elevated levels of VWF in cirrhosis may be a consequence of endothelial perturbation, possibly caused by bacterial infection.[5] Another possible mechanism of elevated VWF in cirrhosis is induction of synthesis of VWF in the liver with cirrhosis itself,[8] or reduced liver-mediated clearance. Although it is established that VWF antigen levels are increased in patients with cirrhosis, relatively little is known on the functional capacity of the protein in these patients. Conflicting reports on the capacity of VWF to bind platelets (as measured by ristocetin- or botrocetin-induced platelet aggregation) have appeared in literature, and patient numbers in these studies were small.[5,9,10] An important qualitative aspect of VWF is its multimeric composition. The high-molecular-weight multimers are more potent in supporting hemostasis, as they have a higher affinity for both glycoprotein Ib and collagen. The multimeric composition of VWF is partly regulated by the VWF cleaving protease ADAMTS13. Reduced levels of ADAMTS13 have been found in patients with liver disease, suggesting that elevated amounts of high-molecular-weight multimers may circulate in these patients.[11] However, the multimeric structure has been reported as either normal[5] or reduced,[9] which may indicate that VWF proteolysis in liver disease is also accomplished by other proteases such as plasmin or elastase.[12]

In this study, qualitative and quantitative parameters of VWF were measured in a large group of patients with cirrhosis of varying severity and causes. Parameters examined were VWF antigen levels, VWF ristocetin cofactor activity, which is a measure for the ability of VWF to interact with platelet glycoprotein Ib, the VWF collagen-binding activity, the VWF cleaving protease ADAMTS13 antigen and activity, the multimeric structure of VWF, and the levels of the propeptide of VWF. In addition, the ability of plasma from patients with cirrhosis to support adhesion of normal or patient platelets under flow conditions was examined. The combined results of these assays provide more insight to what extent elevated VWF levels in these patients contribute to induction of hemostasis.

Patients and Methods

Patients. Fifty-four patients with biopsy-proven cirrhosis of various causes—alcohol abuse (n = 16), viral hepatitis (n = 19), autoimmune hepatitis (n = 2), primary biliary cirrhosis (n = 4), cryptogenic cirrhosis (n = 8), and others (n = 5)—were included in this study. The patients were classified according to Pugh's modification of the Child classification.[13] Nineteen patients with Child A cirrhosis, 17 patients with Child B cirrhosis, and 18 patients with Child C cirrhosis were studied. In addition, five patients with acute liver failure were studied. Most patients were hospitalized for a short period as part of a liver transplant screening program in a single institution (Erasmus MC, Rotterdam, The Netherlands) at the time of inclusion. None of the patients received transfusion of platelets or plasma, and they did not use aspirin or other non-steroidal anti-inflammatory drugs in the 2 weeks before the blood draw. One patient in the Child A and three in the Child C group were on antibiotics at the time of the blood draw. One patient in the Child C group was admitted to the hospital with sepsis, and one was admitted for bacterial peritonitis. One patient in the Child C group had diabetes mellitus and hepatorenal syndrome.

A group of 40 healthy volunteers from our laboratory was used to establish reference values for all assays used. Pooled normal plasma was obtained by combining plasma from these healthy volunteers. In the platelet adhesion experiments, pooled cirrhosis plasma was used. For this, plasma from four patients with Child's A, four patients with Child's B, and four patients with Child's C cirrhosis was combined. For the platelet adhesion experiments, we recruited three additional patients with cirrhosis (all three had Child's C cirrhosis of alcoholic origin) and three healthy volunteers.

Blood samples were obtained by venipuncture from the antecubital vein into 3.2% sodium citrate (9:1, v/v). To obtain platelet-poor plasma, samples were centrifuged twice at 2,000*g* for 15 minutes, after which the samples were stored at −70°C until use.

Assays. VWF antigen (VWF:Ag) levels were determined using the STA Liatest from Roche (Almere, The Netherlands) in the Behring Coagulation System (BCS, Dade Behring, Marburg, Germany). VWF ristocetin cofactor activity (VWF:RCo) was determined using the BC von Willebrand reagent (Dade Behring) on the BCS ap-

paratus. VWF:Ag and VWF:RCo levels in pooled normal plasma were set at 100%.

VWF collagen binding activity was determined with an in-house assay. In short, collagen type III (Sigma, St. Louis, MO) was dissolved in 50 mmol/L acetic acid and dialyzed against phosphate-buffered saline to obtain fibrillar collagen. ELISA plates were coated with collagen (10 μg/well), and blocked with phosphate-buffered saline containing 3% bovine serum albumin and 0.1% Tween-20. Plasma samples were diluted according to their VWF:Ag content. Two dilutions, corresponding to 5 and 2.5% of the amount of VWF present in pooled normal plasma were prepared and added to the wells. Bound VWF was visualized using a horseradish peroxidase–conjugated polyclonal antibody against VWF (DAKO, Glostrup, Denmark). The collagen binding activity of pooled normal plasma was set at 100%, and the values obtained in patient samples were expressed as a percentage of pooled normal plasma.

The VWF cleaving protease ADAMTS13 activity was measured using a rapid functional assay that determines the digestion of VWF by ADAMTS13, based on the method described by Gerritsen et al.[14] The activity of ADAMTS13 in pooled normal plasma was arbitrarily defined as 1 U/mL. The values of ADAMTS13 in tested plasma samples are read from a calibration curve achieved by incubating the VWF substrate with dilutions of a normal plasma pool. The upper detection limit was 3 U/mL.

ADAMTS13 antigen levels were measured by a commercially available ELISA according to the instructions of the manufacturer (American Diagnostica, Stamford, CT).

VWF multimer analysis was performed by sodium dodecyl sulfate agarose gel electrophoresis followed by Western blotting according to Brosstad et al.[15] The blots were scanned and analyzed by densitometric analysis (ImageQuant 5.2., Molecular Dynamics, Sunnyvale, CA). The first five bands were considered as low-molecular-weight multimers, whereas the other bands were designated as high-molecular-weight multimers.

VWF propeptide levels were determined by ELISA using a polyclonal antibody raised against recombinant VWF propeptide that was purified as described.[16] The catching and detecting antibody were the same, except that the detection antibody was conjugated with horseradish peroxidase. The propeptide level measured in pooled normal plasma was set at 100%.

Platelet Adhesion Experiments. The ability of plasma from patients with cirrhosis to support platelet adhesion was studied under flow conditions in a reconstituted blood model. Red cells were isolated from whole blood from healthy volunteers who had blood group O as described previously.[17] Platelets were isolated from three patients with cirrhosis and three controls according to previously published methods.[17] After the final washing step, platelets from patients or controls were resuspended in pooled normal plasma or in pooled cirrhosis plasma. The platelets were mixed with red cells to obtain reconstituted blood with a platelet count of 200,000/μL and a hematocrit of 40%. In selected experiments, reconstituted blood with a reduced platelet count (25.000 or 100.000 platelets/μL) and a hematocrit of 40% was prepared. The reconstituted blood was perfused over a collagen type III–coated surface using a single-pass perfusion chamber[18] at a shear rate of 1,600s^{-1}. Platelet adhesion under these conditions is completely dependent on VWF. After 2 minutes of perfusion, the coverslips were stained with May-Grünwald and Giemsa as described previously.[19] Platelet adhesion was evaluated using computer-assisted analysis with OPTIMAS 6.0 software (Dutch Vision Systems [DVS], Breda, The Netherlands) and was expressed as the percentage of the surface covered with platelets.

Statistical Analysis. Statistical analysis was performed using the GraphPad InStat software package (GraphPad, San Diego, CA). Differences in VWF parameters were examined by standard one-way analysis of variance (ANOVA) using the Tukey post-test, except for differences in VWF propeptide, ADAMTS13 activity and antigen levels, and VWF multimer analysis, which were assayed using the Kruskal Wallis ANOVA with Dunn's post-test because in these measurements standard deviations were substantially different between groups, and values were not normally distributed. P values less than .05 were considered statistically significant.

Results

VWF:Ag Levels Are Substantially Elevated in Cirrhosis and Acute Liver Failure. As shown in Fig. 1A, VWF:Ag levels were strongly elevated in plasma from patients with Child A (380% [165-980]; median [range]), Child B (500% [130-1455]), and Child C (760% [385-1855]) cirrhosis compared with the reference group in which the median VWF:Ag level was 107% [38-180]) ($P < .001$ for mild, moderate, and severe cirrhosis compared with control). In the five patients with acute liver failure, median VWF:Ag level was 790% [650-890] ($P < .01$ compared with control). When patients were classified according to the model for end-stage liver disease (MELD) score, we also observed a strong correlation between VWF:Ag levels and severity of the disease as assessed by the MELD score ($r = 0.448$, $P < .001$). For calculation of the MELD score, we used the modified

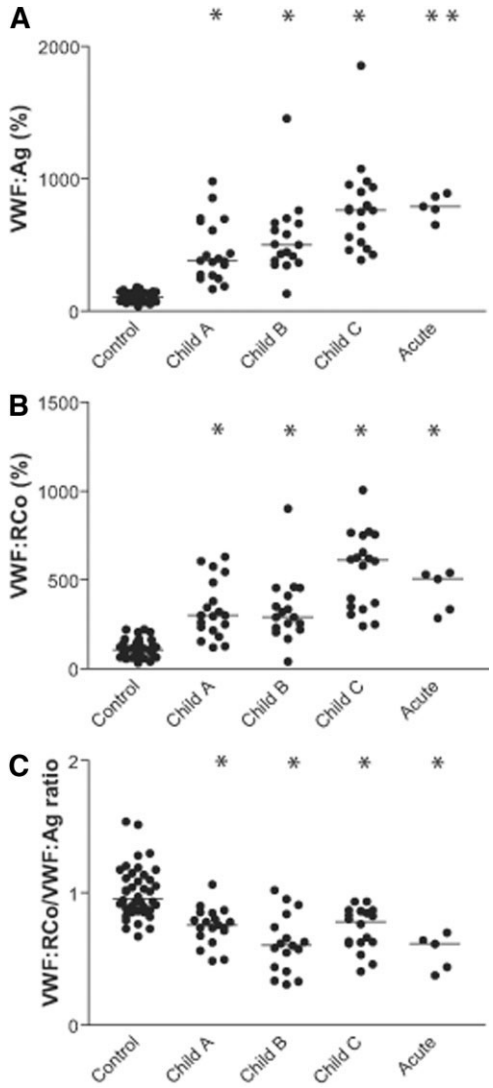

Fig. 1. VWF:Ag levels (A), VWF:RCo levels (B), and VWF:RCo/VWF:Ag ratio (C) in patients with Child A, B, and C cirrhosis and in patients with acute liver failure compared with VWF parameters as measured in healthy controls. VWF:Ag and VWF:RCo levels are expressed as a percentage of pooled normal plasma. Horizontal lines represent medians. *$P < .001$, **$P < .01$. VWF, von Willebrand factor.

MELD score as employed by UNOS for organ allocation: MELD = [0.957 × $\log_e$ (creatinine) + 0.378 × $\log_e$(bilirubin) + 1.12 × $\log_e$ (international normalized ratio) + 0.64] × 10 (available at: http://www.unos.org/resources). In accordance, mean MELD scores paralleled the Child classification. The MELD score was 10 [6-18] (median [range]) in the Child A group, 13 [7-23] in the Child B group, 18 [13-36] in the Child C group, and 28 [22-36] in the acute liver failure group.

Substantially Elevated VWF:RCo Levels, But Depressed VWF:RCo/VWF:Ag Ratio in Cirrhosis and Acute Liver Failure. As shown in Fig. 1B, VWF:RCo levels were found substantially elevated in patients with Child A (300% [121-630]; median [range]), Child B (290% [40-900]), and Child C (610% [240-1005]) cirrhosis compared with the reference group in which the median VWF:RCo level was 105% [33-222]) ($P < .001$ for mild, moderate, and severe cirrhosis compared with control). In the five patients with acute liver failure, median VWF:RCo level was 505% [285-540] ($P < .001$ compared with control). However, the VWF:RCo levels are not elevated to the same extent as compared with the antigen levels. In other words, although the amount of VWF is substantially elevated, it appears less functional with respect to glycoprotein Ib binding. This is demonstrated by a significantly depressed VWF:RCo/VWF:Ag ratio in all groups: Child A (0.75 [0.48-1.064]; median [range]), Child B (0.60 [0.31-1.02]), and Child C (0.78 [0.41-0.93]) cirrhosis compared with the reference group in which the median ratio was 0.96 [0.67-1.538]) ($P < .001$ for mild, moderate, and severe cirrhosis compared with control, see Fig. 1C). In the five patients with acute liver failure, median VWF:RCo/VWF:Ag ratio was 0.61 [0.38-0.70] ($P < .001$ compared with control). The functionality of VWF was reduced to the same extent in all patients, as shown by a strong correlation between VWF:RCo and VWF:Ag levels ($r = 0.812$ vs. $r = 0.852$ in the control group) ($P < .0001$).

Reduced Collagen Binding Capacity of VWF in Patients With Cirrhosis and Acute Liver Failure. As shown in Fig. 2, VWF collagen-binding activity decreased with increasing severity of the disease in patients with cirrhosis. VWF collagen-binding activity was 95% [80-113] (median [range]) in Child A, 83% [42-98] in Child B, and 78 [61-90] in Child C cirrhosis, whereas in the reference group in the median collagen-binding activity was 98% [0.87-107] ($P < .001$ for moderate and severe

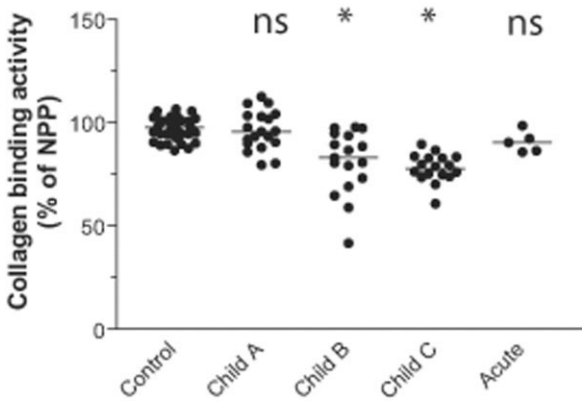

Fig. 2. VWF collagen-binding capacity in patients with cirrhosis of varying severity and in patients with acute liver failure as compared with the activity in plasma from healthy controls. The collagen-binding capacity was measured at equal antigen levels of VWF. The collagen-binding capacity of pooled normal plasma was set at 100%. Horizontal lines represent medians. *$P < .001$. VWF, von Willebrand factor.

cirrhosis compared with control, $P > .05$ for mild cirrhosis compared with control). In the five patients with acute liver failure, the VWF collagen-binding activity was slightly decreased (90% [86-99]), but this difference did not reach statistical significance.

ADAMTS13 Activity and Antigen Levels Are Highly Variable in Patients With Liver Disease. ADAMTS13 activity levels were determined using the collagen-binding assay as described by Gerritsen et al.[14] Compared with controls, ADAMTS13 levels in patients with cirrhosis showed a high variability, and both substantially elevated and substantially depressed ADAMTS13 levels were found in all patient groups, as shown in Fig. 3A. Some patients had ADAMTS13 activity levels exceeding 3 U/mL on repeated testing, even after a further predilution of the sample before the assay. Mean ADAMTS13 levels in Child A and Child B cirrhosis were elevated compared with controls, but this difference did not reach statistical significance. Because ADAMTS13 activity levels were previously reported to be decreased in patients with cirrhosis using the same assay used here,[11] we decided to measure ADAMTS13 antigen levels as well. ADAMTS13 antigen levels also showed a high variability in the patient samples compared with the variation observed in control samples, as shown in Fig. 3B. ADAMTS13 antigen levels were significantly elevated in Child B ($P < .05$) cirrhosis, and in patients with acute liver failure ($P < .05$) compared with controls, whereas the mean ADAMTS13 antigen levels found in Child A and Child C cirrhosis were not significantly different from the levels in the control group. The activity and antigen levels showed a weak, but statistically significant, correlation ($r = 0.39$, $P = .0029$). Both ADAMTS13 activity and antigen levels were negatively correlated with the VWF:RCo/VWF:Ag ratio ($r = 0.29$, $P = .29$ for activity, $r = 0.61$, $P < .0001$ for antigen levels).

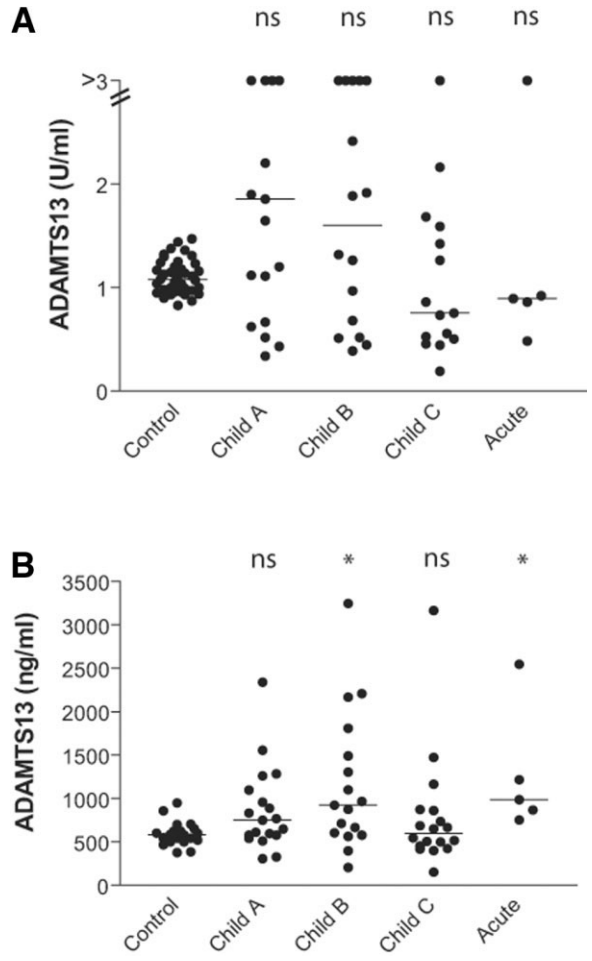

Fig. 3. ADAMTS13 activity (A) and antigen (B) levels in patients with cirrhosis of varying severity and in patients with acute liver failure as compared with the levels measured in plasma from healthy controls. ADAMTS13 activity levels were calibrated to pooled normal plasma, in which the activity was set at 1 U/mL. ADAMTS13 antigen levels were calibrated using recombinant ADAMTS13 according to the instructions of the manufacturer. Horizontal lines represent medians. *$P < .05$; NS, not significant.

Reduced High-Molecular-Weight VWF Multimers in Plasma From Patients With Liver Disease. We determined the multimeric pattern of VWF in a subset of the patients (12 with Child A, 10 with Child B, 8 with Child C, and 3 with acute liver failure). Consistent with the collagen-binding data, we observed a significantly decreased proportion of high-molecular-weight multimers in patients with Child A (63% [32-74]; median [range]), Child B (66% [56-72]), and Child C (58% [52-77]) cirrhosis, compared with the amount of high-molecular-weight multimers in the controls (73% [61-81]) ($P < .01$ for mild and severe cirrhosis, and acute liver failure compared with control, $P < .05$ for moderate cirrhosis compared with control) (Fig. 4). In the five patients with acute liver failure, a decreased proportion of high-molecular-weight multimers was also observed (60% [33-61], $P < .01$). The fraction of high-molecular-weight multimers was negatively correlated with the ADAMTS13 activity ($r = -0.55$, $P < .001$), and with ADAMTS13 antigen levels, which almost reached statistical significance ($r = -0.34$, $P = .051$). Also, a correlation between the fraction of high-molecular-weight multimers and VWF:RCo/VWF:Ag ratio was observed ($r = 0.38$, $P = .03$).

Plasma From Patients With Cirrhosis Supports VWF-Dependent Platelet Adhesion Better Than Normal Plasma. The ability of pooled cirrhosis plasma (VWF:Ag 402%, VWF:RCo 301%) cirrhosis to support adhesion of normal platelets or platelets from patients with cirrhosis was compared with that of pooled normal plasma (VWF:Ag and VWF:RCo 100%, per definition). The pooled plasmas were added to isolated red cells and platelets from three healthy volunteers or three patients

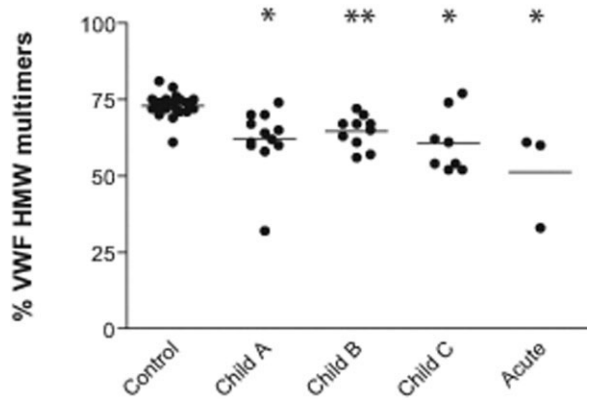

Fig. 4. The proportion of high-molecular-weight (HMW) VWF multimers in patients with cirrhosis of varying severity and in patients with acute liver failure as compared with the proportion of high-molecular-weight multimers in plasma from healthy controls. The proportion of high-molecular-weight multimers was estimated from densitometric analysis of Western blots of agarose gels. The first five visible bands were considered to represent the low-molecular-weight multimers. Horizontal lines represent medians. $*P < .01$; $**P < .05$.

with cirrhosis and perfused over collagen type III for 2 minutes. As shown in Fig. 5A, the amount of platelets isolated from healthy controls or patients adhering to the surface was significantly higher when platelets were resuspended in pooled plasma from patients with cirrhosis as compared with resuspension of the platelets in normal plasma. Also, when normal or patient platelets were resuspended in pooled plasma from patients with cirrhosis, the aggregates were substantially larger as compared with the aggregate size obtained when platelets were resuspended in pooled normal plasma (Fig. 5B). The adhesion of platelets isolated from patients with cirrhosis was similar to that of the adhesion of platelets isolated from healthy controls under these conditions of standardized platelet count and hematocrit.

When normal platelets were resuspended in either normal plasma or plasma from patients with cirrhosis under conditions representing thrombocytopenia (25,000 and 100,000 platelets/μL, we also observed a significant elevation in platelet deposition when plasma from patients with cirrhosis was used (data not shown).

VWF Propeptide Levels. As shown in Fig. 6, VWF propeptide levels were substantially elevated in Child A (488% [234-2942]; median [range]), Child B (711% [261-2190]), and Child C (735% [221-5129]) cirrhosis, whereas in the reference group the median VWF propeptide level was 89% [30-237]) ($P < .001$ for mild, moderate, and severe cirrhosis compared with control). Also in the patients with acute liver disease, elevated propeptide levels were found (1,139% [803-1,594], $P < .001$).

Discussion

In this study, we have performed a comprehensive study on VWF in a large group of patients with cirrhosis of varying severity and causes. Highly elevated levels of VWF were found, which were strongly related to the severity of the disease. However, the functional capacity of the VWF decreased with increasing severity of the disease as shown by a reduction in VWF:RCo/VWF:Ag ratio and reduced collagen binding capacity. Despite the sup-

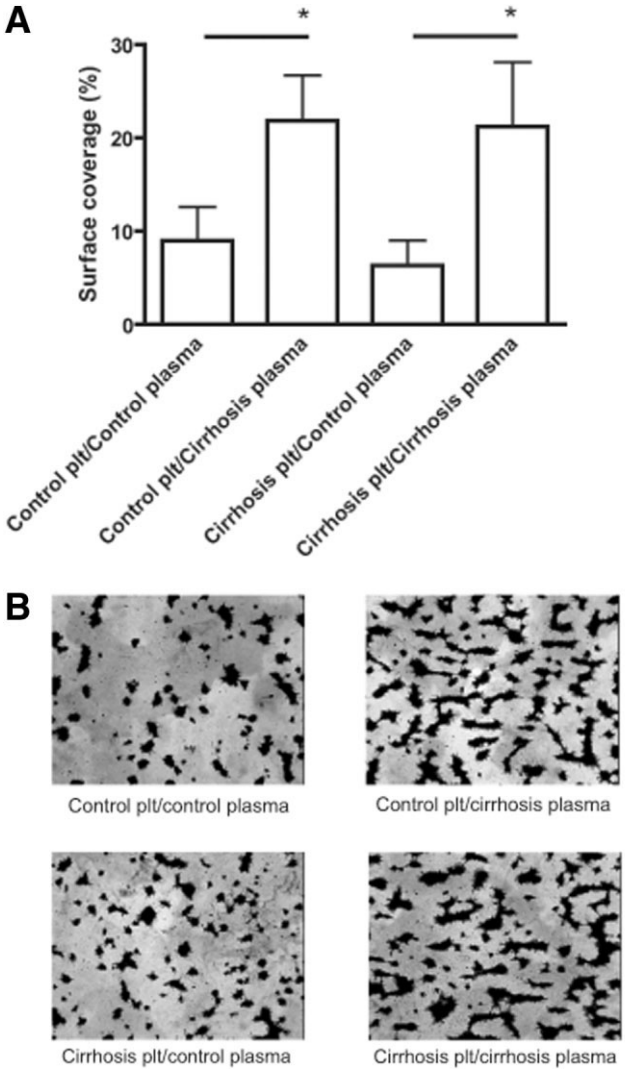

Fig. 5. Plasma from patients with cirrhosis better supports platelet adhesion than normal plasma. (A) Pooled plasma from patients with cirrhosis or pooled normal plasma was mixed with red cells and platelets isolated either from healthy volunteers or from patients with cirrhosis and perfused over a collagen-coated coverslip for 2 minutes. After May-Grünwald staining, surface coverage was determined (shown are results from experiments with three different patients and three different controls performed in triplicate; error bars indicate standard error of mean). $*P < .01$. (B) Morphological appearance of the platelet thrombi on a collagen surface with reconstituted blood with patient or control plasma or platelets as indicated. Shown are representative examples of the experiment presented in panel A. Original magnification is $\times$400.

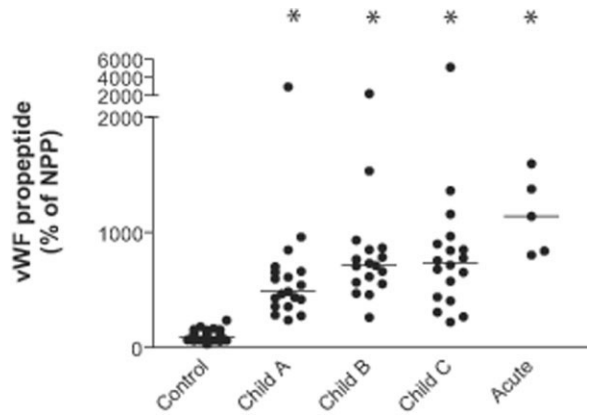

Fig. 6. VWF propeptide levels in patients with cirrhosis of varying severity and in patients with acute liver failure as compared with the levels in healthy controls. The VWF propeptide level in pooled normal plasma was set at 100%. Horizontal lines represent medians. $*P <$ .001. VWF, von Willebrand factor.

pressed binding capacity to both glycoprotein Ib and collagen, the highly elevated VWF levels in plasma from patients with cirrhosis resulted in a substantially elevated platelet deposition to collagen in a VWF-dependent, flow-driven platelet adhesion assay. This indicates that the quantitative increase in VWF in cirrhosis overrules the qualitative defects, and that the elevated levels of VWF might in part compensate for the qualitative and quantitative platelet defects found in these patients. The increased adhesion induced by plasma from patients with cirrhosis was observed with both normal and patient platelets, and at normal and thrombocytopenic platelet counts.

Surprisingly, platelet deposition under conditions of standardized platelet count and hematocrit were similar when normal or patient platelets were used, suggesting that platelets in cirrhosis are functionally normal. This is in contrast with experiments describing intrinsic platelet defects in cirrhosis (reviewed in Lisman et al.[1]). However, most of these experiments were performed under static conditions.

The elevated VWF levels in cirrhosis possibly reflect endothelial damage, which has been suggested to be stimulated by endotoxemia (bacterial infection).[5] Another possibility is that the synthesis of VWF is increased, because it was recently shown that liver disease induces VWF expression in the liver.[8] Alternatively, VWF synthesis may be increased due to the substantially enhanced endothelial surface in patients with cirrhosis as a consequence of extensive collateral formation. Also, the increased levels of vasoconstrictors (notably vasopressin), which arise as a consequence of the hyperdynamic state in these patients, could be responsible for elevated release of VWF from the endothelium.[20] Finally, reduced VWF clearance may contribute to elevated levels, but no experimental evidence has been presented. We have measured levels of VWF propeptide in an attempt to investigate whether the elevated VWF levels represent acute endothelial damage, as found, for example, in patients with sepsis, or chronic endothelial perturbation such as is found in patients with diabetes.[21] Because the half-life of VWF propeptide is much shorter compared with that of VWF itself, only elevated levels of VWF and normal to slightly elevated levels of VWF propeptide are found in patients with chronic endothelial damage.[21] The highly elevated propeptide levels found in patients with cirrhosis in our study suggest acute endothelial damage, which may be compatible with the presence of (low-grade) disseminated intravascular coagulation (DIC) in these patients, although the presence of DIC in cirrhosis has been debated.[22] Conversely, the increased propeptide levels also may be explained by a reduced clearance of this molecule in patients with liver disease, or it may reflect persistent enhanced VWF synthesis by the diseased liver.[8] The hypothesis that patients with cirrhosis have continuously enhanced VWF release into the bloodstream is supported by analysis of the VWF propeptide/VWF:Ag ratio, which is slightly elevated in patients with Child's A and B cirrhosis, but not different from controls in patients with Child's C cirrhosis (data not shown). The VWF propeptide may have physiological relevance in the plasma environment in processes related to inflammation and cell adhesion (reviewed in Takagi et al.[23]). Whether the highly elevated VWF propeptide levels in patients with cirrhosis interfere with inflammatory or cell adhesion processes is unknown. The propeptide may bind to collagen, resulting in inhibition of collagen-induced platelet aggregation[24,25]; however, our platelet adhesion studies do not support these observations.

The clearly reduced VWF:RCo/VWF:Ag ratio, the reduced collagen binding activity, and the reduced proportion of circulating high-molecular-weight multimers measured in this large group of patients resolves the controversy of VWF functional capacity in patients with liver disease. Previously, both increased, and decreased functional VWF parameters were reported (reviewed in Lisman et al.[1]). From our study it has become clear that the functional capacity of VWF is reduced in patients with cirrhosis. This may be caused by increased proteolysis by VWF proteases, such as plasmin or elastase.[12] Recently a selective VWF cleaving protease has been discovered, which is important for cleavage of large, active VWF multimers into smaller less functional multimers. Surprisingly, we found a strong variability in ADAMTS13 levels in patients with cirrhosis. These results are in contrast with a previous report in which significantly reduced lev-

els were demonstrated in a group of patients with Child's C cirrhosis by Mannucci et al.[11] In our patient group, we find both elevated and reduced activity and antigen levels of ADAMTS13. However, the measurement of ADAMTS13 activity is difficult and shows a large coefficient of variation. A lot of controversies on ADAMTS13 activity measurement still exist, especially in cases with mild or moderately reduced levels.[26] This is also illustrated by the relatively poor correlation between the two assays used in this study. Moreover, although ADAMTS13 levels would be expected to be decreased in cirrhosis, as the principle site of synthesis is presumably the liver,[27] it also may be that either reduced clearance or release of ADAMTS13 from platelets[28] (as a result of platelet activation secondary to DIC) lead to the elevated levels observed in some patients in our study. Alternatively, it might be possible that ADAMTS13 synthesis is induced in liver disease, as ADAMTS13 is synthesized in hepatic stellate cells,[29] which are known to show enhanced protein synthesis in patients with liver cirrhosis.[30] Interestingly, ADAMTS13 antigen levels, and, to a lesser extent, ADAMTS13 activity levels, were negatively correlated with VWF:RCo/VWF:Ag ratio, indicating that the excess of ADAMTS13 in some patients results in excessive VWF proteolysis, resulting in impaired VWF activity. This is also supported by the negative correlation observed between ADAMTS13 levels and the proportion of high-molecular-weight VWF multimers. These data also suggest that in those patients with high levels of ADAMTS13, the reduced functional capacity of VWF is a direct consequence of excessive ADAMTS13 proteolysis.

In conclusion, highly elevated levels of VWF in patients with cirrhosis contribute to the induction of primary hemostasis despite reduced functional properties of the VWF molecule. This phenomenon might compensate for defects in platelet number and function, which are present in patients with cirrhosis.

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References

1. Lisman T, Leebeek FWG, de Groot PG. Haemostatic abnormalities in patients with liver disease. J Hepatol 2002;37:280-287.
2. Tripodi A, Salerno F, Chantarangkul V, Clerici M, Cazzaniga M, Primignani M, et al. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. HEPATOLOGY 2005;41: 553-558.
3. Lisman T, Leebeek FW, Mosnier LO, Bouma BN, Meijers JC, Janssen HL, et al. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. Gastroenterology 2001;121:131-139.
4. Leebeek FWG, Kluft C, Knot EAR, de Maat MPM, Wilson JHP. A shift in balance between profibrinolytic and antifibrinolytic factors causes enhanced fibrinolysis in cirrhosis. Gastroenterology 1991;101:1382-1390.
5. Ferro D, Quintarelli C, Lattuada A, Leo R, Alessandroni M, Mannucci PM, et al. High plasma levels of von Willebrand factor as a marker of endothelial perturbation in cirrhosis: relationship to endotoxemia. HEPATOLOGY 1996;23:1377-1383.
6. Sadler JE, Mannucci PM, Berntorp E, Bochkov N, Boulyjenkov V, Ginsburg D, et al. Impact, diagnosis and treatment of von Willebrand disease. Thromb Haemost 2000;84:160-174.
7. Reiner AP, Siscovick DS, Rosendaal FR. Hemostatic risk factors and arterial thrombotic disease. Thromb Haemost 2001;85:584-595.
8. Hollestelle MJ, Geertzen HG, Straatsburg IH, van Gulik TM, van Mourik JA. Factor VIII expression in liver disease. Thromb Haemost 2004;91:267-275.
9. Escolar G, Cases A, Vinas M, Pino M, Calls J, Cirera I, et al. Evaluation of acquired platelet dysfunctions in uremic and cirrhotic patients using the platelet function analyzer (PFA-100): influence of hematocrit elevation. Haematologica 1999;84:614-619.
10. Beer JH, Clerici N, Baillod P, von Felten A, Schlappritzi E, Buchi L. Quantitative and qualitative analysis of platelet GPIb and von Willebrand factor in liver cirrhosis. Thromb Haemost 1995;73:601-609.
11. Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Changes in health and disease of the metalloprotease that cleaves von Willebrand factor. Blood 2001;98:2730-2735.
12. Federici AB, Berkowitz SD, Lattuada A, Mannucci PM. Degradation of von Willebrand factor in patients with acquired clinical conditions in which there is heightened proteolysis. Blood 1993;81:720-725.
13. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60:646-649.
14. Gerritsen HE, Turecek PL, Schwarz HP, Lammle B, Furlan M. Assay of von Willebrand factor (VWF)-cleaving protease based on decreased collagen binding affinity of degraded VWF: a tool for the diagnosis of thrombotic thrombocytopenic purpura (TTP). Thromb Haemost 1999;82: 1386-1389.
15. Brosstad F, Kjonniksen I, Ronning B, Stormorken H. Visualization of von Willebrand factor multimers by enzyme-conjugated secondary antibodies. Thromb Haemost 1986;55:276-278.
16. Borchiellini A, Fijnvandraat K, ten Cate JW, Pajkrt D, van Deventer SJ, Pasterkamp G, et al. Quantitative analysis of von Willebrand factor propeptide release in vivo: effect of experimental endotoxemia and administration of 1-deamino-8-D-arginine vasopressin in humans. Blood 1996; 88:2951-2958.
17. Lisman T, Moschatsis S, Adelmeijer J, Nieuwenhuis HK, de Groot PG. Recombinant factor VIIa enhances deposition of platelets with congenital or acquired αIIbβ3 deficiency to endothelial cell matrix and collagen under conditions of flow via tissue factor-independent thrombin generation. Blood 2003;101:1864-1870.
18. Sixma JJ, de Groot PG, van Zanten H, Ijsseldijk M. A new perfusion chamber to detect platelet adhesion using a small volume of blood. Thromb Res 1998;92:S43-S46.
19. Zwaginga JJ, Sixma JJ, de Groot PG. Activation of endothelial cells induces platelet thrombus formation on their matrix. Arteriosclerosis 1990;10:49-61.
20. Moller S, Bendtsen F, Henriksen JH. Vasoactive substances in the circulatory dysfunction of cirrhosis. Scand J Clin Lab Invest 2001;61:421-422
21. van Mourik JA, Boertjes R, Huisveld IA, Fijnvandraat K, Pajkrt D, van Genderen PJ, et al. von Willebrand factor propeptide in vascular disorders: A tool to distinguish between acute and chronic endothelial cell perturbation. Blood 1999;94:179-185.
22. Ben Ari Z, Osman E, Hutton RA, Burroughs AK. Disseminated intravascular coagulation in liver cirrhosis: fact or fiction? Am J Gastroenterol 1999;94:2977-2982.

23. van Mourik JA, Romani de Witt. Von Willebrand factor propeptide in vascular disorders. Thromb Haemost 2001;86:164-171.
24. Takagi J, Kasahara K, Sekiya F, Inada Y, Saito Y. A collagen-binding glycoprotein from bovine platelets is identical to propolypeptide of von Willebrand factor. J Biol Chem 1989;264:10425-10430.
25. Takagi J, Sekiya F, Kasahara K, Inada Y, Saito Y. Inhibition of platelet-collagen interaction by propolypeptide of von Willebrand factor. J Biol Chem 1989;264:6017-6020.
26. Studt JD, Bohm M, Budde U, Girma JP, Varadi K, Lammle B. Measurement of von Willebrand factor-cleaving protease (ADAMTS-13) activity in plasma: a multicenter comparison of different assay methods. J Thromb Haemost 2003;1:1882-1887.
27. Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem 2001; 276: 41059-41063.
28. Suzuki M, Murata M, Matsubara Y, Uchida T, Ishihara H, Shibano T, et al. Detection of von Willebrand factor-cleaving protease (ADAMTS-13) in human platelets. Biochem Biophys Res Commun 2004;313: 212-216.
29. Uemura M, Tatsumi K, Matsumoto M, Fujimoto M, Matsuyama T, Ishikawa M, et al. Localization of ADAMTS13 to the stellate cells of human liver. Blood 2005;106:922-924.
30. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209-218.