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ORIGINAL ARTICLE

Plasma von Willebrand factor levels predict in-hospital survival in patients with acute-on-chronic liver failure

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Abstract
Background and Aims Circulating levels of von Willebrand factor (vWF) predict mortality in patients with cirrhosis. We hypothesized that systemic inflammation in acute-on-chronic liver failure (ACLF) will stimulate endothelium, increase vWF levels, and promote platelet microthrombi causing organ failure.
Methods In this prospective study, we correlated plasma vWF levels with organ failure, liver disease severity, sepsis, and systemic inflammatory response syndrome (SIRS) and also analyzed if vWF levels predicted in-hospital composite poor outcome (i.e. death/discharged in terminal condition/liver transplantation) in consecutive ACLF patients.
Results Twenty-one of the 50 ACLF patients studied had composite poor outcome. ACLF patients had markedly elevated vWF antigen and activity (sevenfold and fivefold median increase, respectively) on days 1 and 3. Median ratio of vWF to a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13 (ADAMTS13) activity on day 1 was significantly higher in ACLF patients (11.2) compared to 20 compensated cirrhosis patients (3.3) and healthy volunteers (0.9). On day 1, area under ROC curve (AUROC) to predict composite poor outcome of hospital stay for ACLF patients for vWF antigen, vWF activity, and model for end-stage liver disease (MELD) score were 0.63, 0.68, and 0.74, respectively. vWF activity correlated better with liver disease severity (MELD score, ACLF grade) and organ failure (Sequential Organ Failure Assessment [SOFA] score) than vWF antigen; in contrast, neither vWF antigen nor activity correlated with platelet count, sepsis, or SIRS.
Conclusions vWF levels are markedly elevated, correlate with organ failure, and predict in-hospital survival in ACLF patients. This data provides a mechanistic basis for postulating that vWF-reducing treatments such as plasma exchange may benefit ACLF patients.

Keywords ADAMTS13 · Endothelial activation · Von Willebrand factor

Introduction

Acute-on-chronic liver failure (ACLF) is associated with high (50 % to 90 %) short- and medium-term mortalities [1]. Increasing grade of ACLF (i.e. increasing numbers of organs failing) [2] is a strong predictor of short-term mortality [3]. The high mortality in ACLF is mainly attributed to unregulated systemic inflammation, causing (both hepatic and extrahepatic) organ failure [2, 4]. Presence of systemic inflammatory response syndrome (SIRS) predicts development of ACLF in patients with alcoholic liver disease [5].

In critically ill patients, SIRS contributes to disseminated intravascular coagulation, development of microvascular thrombosis (varying from 20 % to 100 % depending on the organ studied), and consequent multiorgan failure [6]. Von Willebrand factor (vWF), released from activated endothelium in very high molecular weight forms, is an adhesive protein to which platelets stick. In patients with

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sepsis, development of organ failure and systemic inflammation is linked to imbalance of vWF–ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13), high vWF levels and low levels of ADAMTS13 (a vWF-cleaving protease) [7]. A typical example is complicated malaria, where in a thrombocytopenic patient, vWF–ADAMTS13 imbalance may be the pathogenic mechanism linking inflammation to microvascular occlusion [8]. vWF levels also predict 28-day mortality in ICU patients with SIRS [9].

Similar vWF–ADAMTS13 imbalance occurs in cirrhosis, acute liver failure, and when systemic inflammation is superimposed on cirrhosis. In patients with cirrhosis (of varied etiology, including viral and alcohol), vWF levels correlate with hepatic fibrosis [10], hepatic vein pressure gradient [10–12], and predict survival over next 2–3 years [11, 12]. In acute liver failure, vWF–ADAMTS13 imbalance predicts survival [13]. vWF levels predict survival in patients with systemic inflammation superimposed on cirrhosis [14]. In addition, vWF–ADAMTS13 imbalance is noted in acute alcoholic hepatitis [15].

Although there is an increase in vWF levels in patients with cirrhosis, there may not be a commensurate increase in the vWF activity. This, hitherto unexplained phenomenon, may be one of the body's mechanisms to limit propensity of widespread thrombotic events in these patients [13]. ADAMTS13 regulates vWF multimer fraction, limiting the biological activity of vWF, and a decrease in ADAMTS13 concentration and activity may exacerbate the imbalance. Role of other vWF cleaving proteases in health and disease has not been well studied [13].

We hypothesized that systemic inflammation in ACLF will further activate the endothelium and elevate plasma vWF levels, increase platelet microthombi formation in affected organs, reduce organ perfusion, and potentiate organ failure.

The prognostic significance of vWF levels in ACLF has not been studied. The objectives of this study were to document plasma vWF levels and correlate these with organ failure, liver disease severity, sepsis, and SIRS in ACLF patients; to analyze plasma vWF levels as predictors of in-hospital survival in ACLF patients; and to compare plasma vWF–ADAMTS13 balance in ACLF patients, in compensated cirrhosis patients who did not have ACLF, and in healthy volunteers.

Methods

From October 2014 to March 2015, consecutive adult patients with presentation as ACLF (as per Asia-Pacific Association for the Study of the Liver (APASL) definition) [16] admitted in our department were prospectively recruited for this study after obtaining their informed consent.

Patients with hepatocellular carcinoma or portal vein thrombosis and those unwilling to participate were excluded from this study. Pregnant women and children were also excluded.

All patients underwent routine clinical examination and laboratory assessment. Underlying chronic liver disease was diagnosed based on clinical, biochemical, radiological, and/or histological features. Assessment for causes of acute hepatic insult and of underlying chronic liver disease (e.g. alcohol, hepatotropic viruses, use of hepatotoxic drugs, and autoimmune hepatitis) was done.

Assays for plasma von Willebrand factor

As per pre-specified study protocol, all study patients underwent assays for plasma vWF antigen and activity on days 1 and 3 of hospital stay. For vWF assays, blood was collected using 0.109-M citrate anticoagulant and centrifuged at 2500*g* for 15 min at 4 °C. The separated plasma were aliquoted and stored frozen at −80 °C until assay.

vWF antigen was measured using an ELISA Kit (quantitative ELISA) as per manufacturer's instructions (Zymutest vWF catalog no. RK 030 A Hyphen BioMed, France). Collagen-binding activity of vWF was measured by a similar method (Zymutest vWF:CBA catalog no. RK 038 A Hyphen BioMed, France) using microwells coated with fibrillar collagen types I and III [17]. A second-order polynomial standard curve was used to obtain vWF antigen level as well as activity. In patients with high vWF levels, samples were pre-diluted (1 in 10) for the assays. Normal values for both plasma vWF antigen and activity were 50 % to 160 %. Specific vWF activity was calculated as ratio of vWF activity:vWF antigen.

Assay for plasma ADAMTS13 activity

ADAMTS13 activity was estimated on day 1 in all ACLF study patients on citrated platelet-poor plasma by an in-house collagen-binding assay as previously described [18]. Normal range of ADAMTS13 activity was 55 % to 160 %.

Plasma vWF–ADAMTS13 balance in ACLF, compensated cirrhosis, and healthy volunteers

We compared vWF–ADAMTS13 balance in ACLF patients, patients with hepatitis B/C-related compensated cirrhosis (none were in ACLF as per APASL criteria [16]), and in healthy volunteers. vWF:ADAMTS13 ratio was calculated by dividing day 1 vWF activity by day 1 ADAMTS13 activity.

Assessment of organ failure, liver disease severity, sepsis, and SIRS in ACLF patients

Sequential Organ Failure Assessment (SOFA) score [19] (to assess organ failure), model for end-stage liver disease (MELD) score, and ACLF grade (as per European Association for the Study of the Liver–Chronic Liver Failure (EASL-CLIF) consortium definition) (to assess liver disease severity) were calculated on day 1 of hospital stay. Presence of SIRS [20] and sepsis [21] was assessed as per standard definition.

Incidence of new onset acute kidney injury and/or hepatic encephalopathy was documented on a daily basis during hospitalization.

Follow up and study outcome parameters in ACLF patients All ACLF patients received standard medical treatment (no patient was treated with plasma exchange or N-acetyl cysteine) and were followed up daily. Study outcome (in-hospital transplant-free survival) was classified as discharge from hospital alive (in stable condition) or composite poor outcome (death/discharge in terminal condition/liver transplant).

Statistical analysis Continuous variables were expressed as mean with standard deviation or median with range. Discrete variables were expressed as numbers and percentage. Continuous variables were compared by Mann-Whitney *U* test/Wilcoxon sign rank and discrete variables by chi-squared test or Fischer's exact test as relevant. Bivariate correlation was assessed by Spearman's correlation coefficient. Multivariate logistic regression was done to assess independent factors affecting survival in patients with ACLF. Univariate ordinal regression was done to analyze the ability of variables to predict baseline grade of ACLF (as per EASL-CLIF grading). Receiver operating characteristic (ROC) curve was used to assess sensitivity and specificity of plasma vWF as predictor of composite poor outcome. Assuming a 30 % mortality rate in patients admitted to hospital with ACLF (audit estimates), we calculated that a sample size of 50 patients would allow us to evaluate role of vWF as a predictor, independent of MELD score, of in-hospital mortality. SPSS version 15 was used for statistical analysis, and a two-sided *p*-value of <0.05 was considered as significant.

The study was approved by the institutional review board and ethics committee.

Results

Of the 50 ACLF patients studied, 29 (58 %) were discharged in a stable condition while 21 (42 %) had composite poor outcome—10 died (hospital stay 7, 2–12 days; median, range), 9 were discharged in terminal condition (hospital stay 6, 2–11 days), and 2 underwent liver transplantation (hospital stay prior to transplant 8, 5–11 days).

Baseline demographics and relevant laboratory data are depicted in Table 1. All ACLF study patients were in Child's class C at admission. Alcohol was the most common cause of chronic damage and of acute insult in ACLF patients. Other causes of chronic liver damage were hepatitis B [5], cryptogenic [3], autoimmune [2], and non-alcoholic fatty liver disease [1]. Other causes of acute insult were cryptogenic [6], hepatitis E [3], autoimmune flare [2], hepatitis B [1], and swine flu [1].

With increasing ACLF grade on day 1 of hospital stay, MELD score as well as the proportion of patients with composite poor outcome increased (Table 2). New onset renal failure/encephalopathy were more common in ACLF patients with composite poor outcome ($n = 7$) when compared to patients who were discharged in a stable state ($n = 2$; p-value 0.025).

Plasma vWF level on day 1 of hospital stay (Fig. 1)

Day 1 plasma vWF antigen (725 %, 212 % to 1347 %; median, range) and activity (534 %, 97 % to 1157 %) were elevated in 50 ACLF patients studied. Day 1 plasma vWF antigen was higher in patients with composite poor outcome (742 %, 264 % to 1347 %) when compared to patients discharged in stable condition (699 %, 212 % to 1249 %; p-value 0.135). Day 1 plasma vWF activity was also significantly higher in patients with composite poor outcome (632 %, 119 % to 1157 %) when compared to patients discharged in stable condition (490 %, 97 % to 986 %; p-value 0.025).

Plasma vWF level on day 3 of hospital stay

Day 3 plasma vWF antigen and activity remained elevated (712 %, 279 % to 1411 %; median range and 475 %, 100 % to 1304 %, respectively) in 42 ACLF patients studied. There was no difference in plasma vWF antigen (746 %, 326 % to 1157 % vs. 689 %, 279 % to 1411 %; p-value 0.6) and activity (457 %, 244 % to 1334 % vs. 497 %, 100 % to 1304 %; p-value 0.916) in patients with composite poor outcome compared to those discharged in stable condition.

The interval change in plasma vWF antigen and activity over 3 days (days 1 to 3 of hospital stay) was not significantly different in patients with composite poor outcome as compared to patients discharged in stable condition.

Table 1 Baseline characteristics in acute-on-chronic liver failure patients

Parameter	All ACLF patients (n = 50)	Discharged alive (n = 29)	Composite poor outcome (n = 21)	p-value[a]
Age (years)	43.5 (28–64)	43 (28–64)	40 (30–58)	0.7
Sex (M:F)	45:5	26:3	19:2	1
Etiology of chronic liver disease (alcohol:hepatitis B:others)	39:5:7	22:4:3	17:1:3	–
Acute insult (alcohol:viral:others)	37:5:8	21:3:5	16:2:3	–
Serum bilirubin (mg/dL)	17.3 (5–37)	11.6 (5–33.4)	21.7 (5.1–37)	0.1
Serum creatinine (mg/dL)	1.4 (1–6)	1 (1–6)	2.2 (1–6)	0.005
Prothrombin time (INR)	2 (1.5–10)	1.9 (1.5–4.5)	2.2 (1.5–10)	0.293
Platelet counts ($\times 10^3/\mu L$)	93 (30–353)	92 (30–240)	96 (30–353)	1
MELD score	29 (17–49)	26 (17–49)	35 (22–47)	0.002
SOFA score	7 (4–14)	6 (4–14)	8 (4–14)	0.007
ACLF grading (grade 0:1:2:3)	16:13:13:8	15:8:3:3	1:5:10:5	0.001
SIRS, n (%)	41 (82 %)	24 (83 %)	17 (81 %)	1.000
Sepsis, n (%)	20 (40 %)	15[b] (52 %)	5[c] (24 %)	0.08
Duration of hospital stay (days)	6 (2–28)	7 (3–28)	5 (2–12)	0.08

All continuous variables are expressed as median (range) and categorical variables as numbers (percentage)

MELD model for end-stage liver disease, *SOFA* Sequential Organ Failure Assessment, *ACLF* acute-on-chronic liver failure, *SIRS* systemic inflammatory response syndrome

[a] Comparing "discharged alive" vs. "composite poor outcome"

[b] Either culture positive from blood [4], ascitic fluid [3], urine [2] samples, and/or neutrophilic ascitic fluid suggesting spontaneous bacterial peritonitis [11]

[c] Either culture positive from blood [2], ascitic fluid [2], urine [2], and/or neutrophilic ascitic fluid suggesting spontaneous bacterial peritonitis [3]

Correlation of day 1 plasma vWF level with organ failure (SOFA score), liver disease severity (MELD score, ACLF grade), sepsis, and SIRS in ACLF patients

There was a moderate but significant positive correlation of day 1 SOFA score with day 1 vWF antigen ($\rho = 0.35$; p-value 0.02) and with day 1 vWF activity ($\rho = 0.4$; p-value 0.002). There was a moderate but significant positive correlation of day 1 MELD score with day 1 vWF antigen ($\rho = 0.25$; p-value 0.09) and also with day 1 vWF activity ($\rho = 0.31$; p-value 0.03). With increasing grade of ACLF on day 1, there was a trend to increase in day 1 vWF antigen and significant increase in day 1 vWF activity (Table 2).

Neither vWF antigen nor activity showed any correlation with baseline SIRS/sepsis nor with new onset kidney injury and hepatic encephalopathy (Table 3). Neither vWF antigen

Table 2 Liver disease severity (MELD score), plasma von Willebrand factor levels (on days 1 and 3 of hospital stay), and in-hospital survival in patients in different grades of acute-on-chronic liver failure

	ACLF grades				p-value[a]
	Grade 0 (n = 16)	Grade 1 (n = 13)	Grade 2 (n = 13)	Grade 3 (n = 8)	
Age (years)	41 (30–63)	49 (30–62)	41 (28–64)	40.5 (35–58)	0.75
MELD	23 (19–27)	29 (17–36)	37 (26–47)	41 (30–49)	<0.001
vWF antigen (day 1)	690 (264–1082)	700 (212–1347)	686 (515–1338)	986 (742–1344)	0.09
vWF antigen (day 3)	654 (301–1169)	640 (279–1411)	594 (334–998)	885 (702–1054)	0.5
vWF activity (day 1)	491 (97–949)	468 (116–835)	532 (221–1090)	882 (676–11,157)	0.02
vWF activity (day 3)	466 (100–1247)	447 (132–1218)	441 (277–1304)	766 (713–904)	0.16
Platelets ($\times 10^3/\mu L$)	99 (39–243)	71 (30–225)	121 (30–353)	94 (35–198)	0.9
Composite poor outcome n (%)	1 (6 %)	5 (38.5 %)	10 (76.9 %)	5 (62.5 %)	0.01

ACLF acute-on-chronic liver failure, *MELD* model for end-stage liver disease, *vWF* von Willebrand factor

[a] Univariate ordinal regression

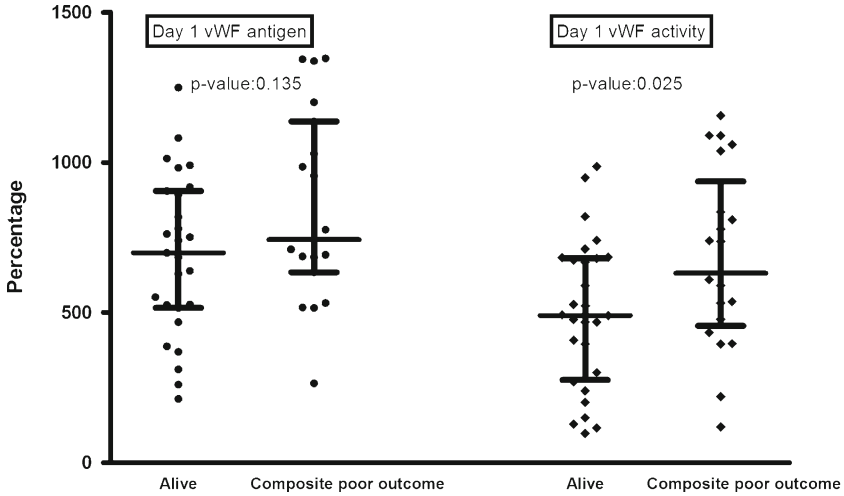

Fig. 1 Comparing day 1 plasma von Willebrand factor antigen and activity in acute-on-chronic liver failure patients who were discharged from hospital in stable state to those with composite poor outcome

nor activity on day 1 had any significant correlation with platelet counts nor with alcohol as an etiology of acute insult (data not shown).

vWF antigen showed excellent and statistically significant correlation with vWF activity in all ACLF patients ($\rho = 0.85$; p-value <0.001) and also in ACLF patients with SIRS ($\rho = 0.84$; p-value <0.001).

Plasma vWF level as predictor of in-hospital survival

On multivariate logistic regression analysis, adjusting for MELD score, day 1 vWF activity showed a trend towards prediction of composite poor outcome (adjusted hazard ratio 1.002; 95 % CI 1–1.005; p-value 0.1).

Area under ROC curve (AUROC) for day 1 plasma vWF antigen to predict composite poor outcome of hospital stay was 0.63 (95 % CI 0.47–0.8) and for vWF activity was 0.68 (95 % CI 0.52–0.84). AUROC for day 1 MELD score and SOFA score to predict composite poor outcome were 0.74 (95 % CI 0.6–0.89) and 0.72 (95 % CI 0.6–0.8), respectively. There was no statistical difference between AUROC of MELD score and vWF activity to predict composite poor outcome (p-value 0.4). For day 1 vWF activity, the optimal cutoff for predicting composite poor outcome, as disclosed by Youden index, was 712 % (sensitivity 48 % and specificity 86 %). Table 4 describes the sensitivity, specificity, predictive values, and likelihood ratios for various cutoffs of day 1 vWF activity in predicting in-hospital survival.

Correlation of day 1 plasma ADAMTS13 activity with in-hospital survival in ACLF patients

Day 1 ADAMTS13 activity was similar in ACLF patients with composite poor outcome (n=21, 42 %, 11 % to 120 %; median, range) and in ACLF patients who were discharged in stable condition (n=29, 58 %, 16 % to 125 %; p-value 0.11). There was a significantly higher day 1 vWF:ADAMTS13 activity ratio in ACLF patients with composite poor outcome (19, 1.4–96.4) as compared to patients who were discharged in stable state (8.5, 1.2–42.9; p-value 0.03).

Plasma vWF–ADAMTS13 balance in ACLF patients as compared to compensated cirrhosis patients and healthy volunteers

Figure 2 reflects the vWF antigen and activity in ACLF patients as compared to 20 patients with compensated cirrhosis (age 47 years, 23–64 years; male 14; hepatitis B-related 10 patients, hepatitis C-related 10 patients; Child's class A-9,

Table 3 Comparing day 1 plasma von Willebrand factor levels in 50 acute-on-chronic liver failure patients as per presence of sepsis and systemic inflammatory response syndrome at presentation and as per new onset renal failure or encephalopathy, which developed during hospital stay

	SIRS			Sepsis			New onset renal failure/encephalopathy		
	Yes (n = 41)	No (n = 9)	p-value	Yes (n = 20)	No (n = 30)	p-value	Yes (n = 9)	No (n = 41)	p-value
Day 1 vWF antigen	725 (212–1347)	789 (264–1338)	1	696 (260–1249)	779 (212–1347)	0.3	955 (369–1347)	711 (212–1344)	0.4
Day 1 vWF activity	589 (97–1157)	522 (119–1090)	0.8	510 (97–1411)	564 (119–1157)	0.25	736 (221–835)	532 (97–1157)	0.7

vWF von Willebrand factor, *SIRS* systemic inflammatory response syndrome

Table 4 Plasma von Willebrand factor activity on day 1 as a predictor of in-hospital composite poor outcome (death/transplant/discharged in terminal condition) in acute-on-chronic liver failure patients

vWF activity	Composite poor outcome	Alive	Hazard ratio	Sensitivity	Specificity	NPV	PPV	NLR	PLR
>250 %	19	23	2.5 (0.5–13.7)	0.9 (0.8–1)	0.2 (0.1–0.3)	0.8 (0.4–1)	0.5 (0.4–0.5)	0.46 (0.1–2.3)	1.1 (0.9–1.3)
<250 %	2	6							
>500 %	15	13	3.1 (0.9–10.2)	0.7 (0.5–0.9)	0.5 (0.4–0.7)	0.7 (0.6–0.9)	0.5 (0.4–0.7)	0.5 (0.2–1.1)	1.6 (0.9–2.5)
<500 %	6	16							
>750 %	8	3	5.3 (1.2–23.5)	0.4 (0.2–0.5)	0.9 (0.8–1)	0.7 (0.6–0.7)	0.7 (0.4–0.9)	0.7 (0.5–1)	3.7 (1–16.6)
<750 %	13	26							
>1000 %	5	0	∞ (1.3–∞)	0.2 (0.1–0.2)	1 (0.9–1)	0.6 (0.6–0.6)	1 (0.5–0.9)	0.8 (0.8–1)	∞ (1.3–∞)
<1000 %	16	29							

NPV negative predictive value, *PPV* positive predictive value, *NLR* negative likelihood ratio, *PLR* positive likelihood ratio

B-5, C-5; MELD score 10, 7–24) and 19 healthy volunteers (age 33 years, 27–65 years; median, range; male 16).

vWF antigen in ACLF patients (725 %, 212 % to 1347 %; median, range) was significantly higher than in patients with hepatitis B/C-related cirrhosis (332 %, 81 % to 785 %) and in healthy volunteers (85 %, 46 % to 128 %; *p*-value <0.001).

Similarly, vWF activity was significantly higher in ACLF patients (534 %, 97 % to 1157 %) as compared to patients with hepatitis B/C-related cirrhosis (275 %, 80 % to 860 %) and healthy volunteers (89 %, 45 % to 130 %; *p*-value <0.001).

Specific vWF activity was significantly lower in ACLF patients (0.77, 0.15–1.5; median, range) than healthy volunteers (0.98, 0.62–1.9) and patients with compensated cirrhosis (0.9, 0.5–1.2; *p*-value <0.001).

Day 1 plasma ADAMTS13 activity was significantly lower in 50 ACLF patients (47.5 %, 11 % to 125 %; median, range) as compared to 19 healthy volunteers (98.5 %, 55 % to 122 %; *p*-value <0.001) and 20 patients with compensated cirrhosis (96.5 %, 27 % to 127 %; *p*-value <0.001).

Ratio of vWF activity:ADAMTS13 activity (on day 1) was significantly higher in ACLF patients (11.2, 1.2–96.4; median, range) as compared to patients with compensated cirrhosis (3.3, 0.7–28.1; *p*-value 0.01) and healthy volunteers 2.4–0.4, 0.9); *p*-value <0.001).

Discussion

We document markedly elevated plasma vWF antigen (sevenfold median increase) and activity (fivefold median increase) on days 1 and 3 in ACLF patients. vWF antigen and activity levels were twofold higher in ACLF patients when compared to compensated cirrhosis patients.

Of the different vWF assays done in ACLF patients, vWF activity on day 1 was the best predictor of composite poor outcome at the end of hospital stay, with vWF activity level of >1000 % having 100 % positive predictive value (Table 4). Similarly, vWF activity on day 1 correlated better with liver disease severity (MELD score, ACLF grade) and organ failure

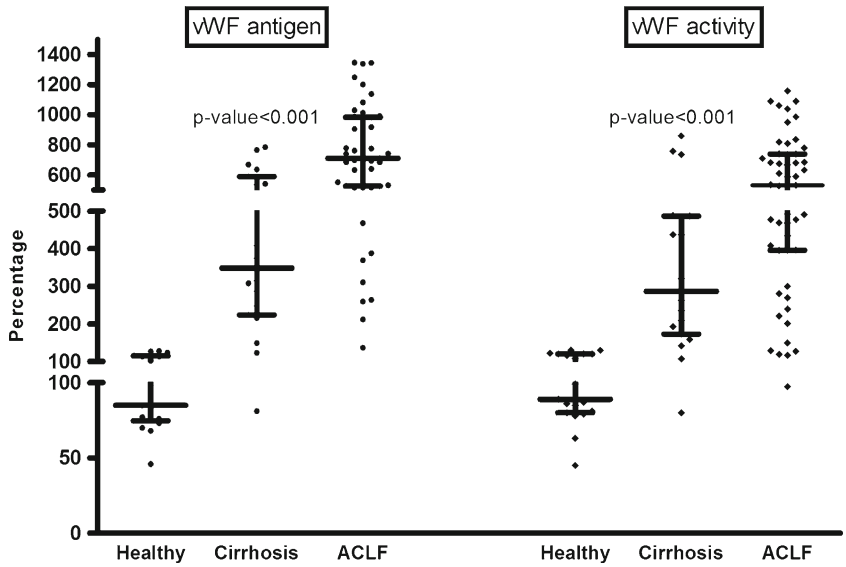

Fig. 2 Plasma von Willebrand factor antigen and activity in acute-on-chronic liver failure patients as compared to healthy volunteers and patients with hepatitis B/C-related compensated cirrhosis

(SOFA score) on day 1 than vWF antigen on day 1; in contrast, neither vWF antigen nor activity correlated with platelet count, sepsis, or SIRS on day 1 nor with new onset renal failure or hepatic encephalopathy during hospital stay.

Although all 50 ACLF patients studied had marked elevation of vWF levels, 9 (18 %) patients did not fulfill criteria for SIRS and 30 (60 %) did not have sepsis (Table 3). As APASL criteria for ACLF provides definition of ACLF and not disease severity classification, we used three different severity scores—MELD score, SOFA score, and EASL-CLIF grading to stratify patients. Our study protocol pre-specified composite poor outcome of hospital stay as death/discharged in terminal state/liver transplant. This was to account for terminally ill patients requesting discharge from hospital, which is a reality in many resource-constrained settings in developing countries. Duration of jaundice and of "liver failure" prior to admission were not documented.

Infusion of endotoxin to human volunteers induced systemic inflammation, thrombocytopenia, leukocytosis, high vWF, and low ADAMTS13 levels at 4 and 24 h and ultra-large VWF multimers after 4 h [22]. Similar vWF–ADAMTS13 imbalance occurred after desmopressin infusion in healthy volunteers [23]. Marked elevation of vWF levels in ACLF patients in our study probably reflects an "acute-on-chronic" pro-inflammatory milieu leading to endothelial activation by release of inflammatory mediators reported in ACLF [1]. As most patients with ACLF had SIRS at admission, we failed to demonstrate its correlation with raised vWF levels.

vWF–ADAMTS13 imbalance occurs in patients with advancing cirrhosis [24]; in contrast, this imbalance occurs in patients with noncirrhotic intrahepatic portal hypertension (NCIPH), who have well-preserved liver functions [25, 26], suggesting that vWF–ADAMTS13 imbalance may be a pathogenic mechanism of chronic portal microangiopathy which causes NCIPH [27]. As NCIPH is often labeled as "cryptogenic" cirrhosis, we did not include cryptogenic cirrhosis as a disease control.

In our study, etiology of ACLF patients (most patients had alcohol as etiology of liver disease) was different from patients with compensated cirrhosis (hepatitis B and C related). This is a limitation of our study, but previous studies (which included patients with alcohol liver disease) have otherwise shown an increase in vWF proportional to disease and portal hypertension severity irrespective of the etiology [11, 12]. We also did not observe the effect of etiology of acute insult (alcohol vs. others) on vWF levels in ACLF study patients.

Of the many vWF assays tested in patients with liver disease, vWF antigen level is the easiest and most commonly performed assay. N-acetyl cysteine used to treat acute liver failure can be a confounder in some vWF assays, as it reduces vWF multimer size and activity [28].

Increased vWF levels induce further platelet adhesion, despite reduced function of vWF molecule in patients with cirrhosis [29]. Specific vWF activity in the current study also shows reduced vWF function, despite increased vWF levels in compensated cirrhosis; this phenomenon is even more exaggerated in ACLF. This may be secondary to compensatory, but often unsuccessful, mechanisms trying to limit the harmful thrombogenic effects of increased vWF.

Exaggerated platelet aggregation secondary to ultra-large vWF multimers and microvascular occlusion could explain the prognostic significance of vWF in cirrhosis [11, 12, 14, 30], in acute liver injury [13], and in ACLF (current study). The degree of vWF elevation (Table 5) probably reflects degree of endothelial activation and tendency for microvascular occlusion (and hence failure) of the liver and other organs affected and probably determines the length of survival in these patients. Based on vWF levels, compared to endothelial "activation" in compensated cirrhosis patients, endothelial "hyperactivation" is seen in ACLF patients (Table 5).

Table 5 Plasma von Willebrand factor levels as predictor of survival in different studies of patients with acute liver failure, acute-on-chronic liver failure, and cirrhosis

	ACLF (present study)	Cirrhosis [11]	Cirrhosis [12]		Cirrhosis ± systemic inflammation [14]	Acute liver failure [13]
n	50	42	286 189 (compensated)	97 (decompensated)	80	50
vWF antigen	725 % (212–1347) %	222 ± 17 %	264 % (194–345) %	394 % (303–505) %		547 % (242–1420) %
Follow up	8 (3–28) days	24 (1–24) months	33 (30–36) months		2 years	
Predicting outcome						
vWF antigen	AUC 0.63 (95 % CI 0.47–0.8)	AUC 0.74[a] (95 % CI 0.58–0.9)	AUC 0.71 (95 % CI 0.65–0.77)[a]		AUC 0.78[b] (95 % CI 0.66–0.91)	NS

AUC area under the curve, *NS* not significant, *ACLF* acute-on-chronic liver failure

[a] Predicting death/transplant/portal hypertension-related event

[b] Predicting transplant-free survival

While intrahepatic endothelial activation and microvascular occlusion may impede hepatic perfusion and contribute to liver failure and short-term mortality in acute liver failure, it leads to formation of focal parenchymal extinction lesions and confluent fibrosis in the liver [31] and probably contributes to medium-term mortality in cirrhosis. In ACLF, it is likely that both these processes are superimposed on each other.

In this study, we found significantly reduced ADAMTS13 activity (as measured by collagen-binding assay) in ACLF patients as compared to compensated cirrhosis and healthy controls. We did not estimate ADAMTS13 activity by fluorescence resonance transfer (FRET) to avoid confounding by raised serum bilirubin (present in all ACLF patients).

High vWF levels causing microvascular occlusion by exaggerated platelet adhesion onto activated endothelium causing organ failure typically occurs in thrombotic thrombocytopenic purpura and hemolytic uremic syndrome [32]. Plasma exchange with fresh frozen plasma replacement dramatically reduced mortality in these conditions [33]. Removal of large volumes of plasma during plasma exchange non-selectively reduces many plasma proteins including vWF [34, 35]. ADAMTS13 supplementation is provided by fresh frozen plasma infusion [36]. Apart from primary thrombotic microangiopathies [32, 33], plasma exchange may also be beneficial in other syndromes associated with thrombocytopenia and vWF–ADAMTS13 imbalance [8, 37–40].

ACLF (current study) and acute liver failure patients have thrombocytopenia associated with vWF–ADAMTS13 imbalance [13]. In hepatitis B-related ACLF, addition of plasma exchange improved short-term survival compared to treatment with nucleoside analogs alone [41–43]. High-volume plasma exchange reduced multiorgan dysfunction and improved transplant-free survival in acute liver failure patients [44]. vWF reduction (which interrupts excessive platelet adhesion, microvascular thrombosis, and multiorgan failure) may be one mechanism by which plasma exchange improves survival in ACLF and acute liver failure patients.

In ACLF and other thrombocytopenic conditions wherein vWF-rich activated endothelium entraps more platelets, it is logical to avoid platelet transfusions; when platelet transfusions are deemed necessary, it is better to transfuse fresh frozen plasma initially (which supplements ADAMTS13), followed by platelet transfusion [45, 46].

Studies exploring the role of vWF in progression of liver disease, by measuring vWF in hepatic venous blood and liver tissue, can be undertaken. Further studies of plasma vWF levels as a prognostic marker of short-term mortality in larger number of ACLF patients are needed. Effect of plasma exchange and other vWF-reducing treatments [47–49] needs to be studied in ACLF and in acute liver failure patients. In conclusion, ACLF is characterized by markedly raised plasma vWF levels. vWF levels correlate with organ failure, liver disease severity, and predict in-hospital survival in ACLF patients.

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Compliance with ethical standards

Conflict of interest KSP, AG, GJA, AR, KAB, IM, UZ, KGS, EE, and CEE declare that they have no conflict of interest.

Ethical approval The study was performed in a manner to conform with the Helsinki Declaration of 1975, as revised in 2000 and 2008 concerning human and animal rights, and the authors followed the policy concerning informed consent as shown on Springer.com.

Informed consent Informed consent was obtained from all individual participants included in the study.

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