Prevention of bleeding and thrombosis in patients with liver disease

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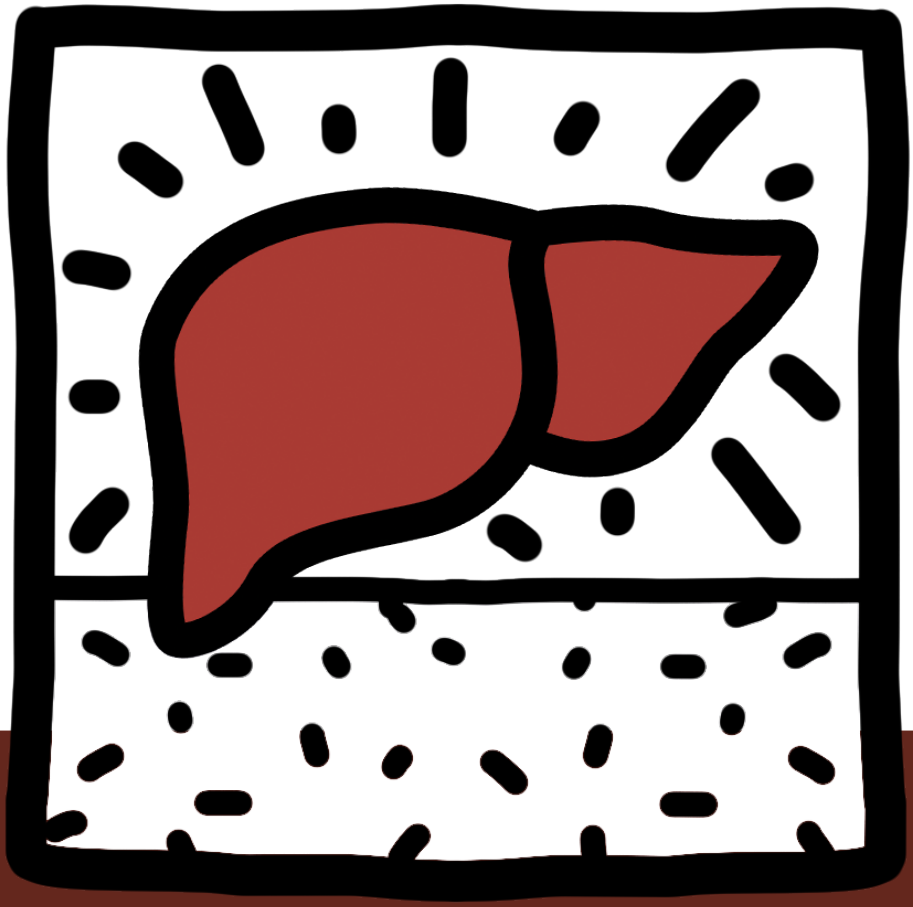

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Von Willebrand factor is an independent predictor of short-term mortality in acutely ill patients with cirrhosis

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Abstract

Background & aims: Levels of von Willebrand factor (VWF) are elevated in patients with cirrhosis, and correlate well with disease severity. In patients with decompensated cirrhosis (DC), plasma VWF is associated with mortality. The value of VWF in predicting short-term mortality risk in patients with acute-on-chronic liver failure (ACLF) is, however, unclear.

Methods: We included patients with DC (n=111) and ACLF (n=105). We measured VWF levels and correlated these with other laboratory parameters and prediction models for mortality. Also, we assessed the predictive value of VWF in prediction of 90 and 30 day mortality in patients with DC and ACLF, respectively, and compared this to the predictive value of clinically used prediction models. Finally, we determined the optimal cut-off value for VWF in patients with ACLF.

Results: Sixteen of 111 (14%) patients with DC and 35 of 105 (33%) with ACLF died within 90 days and 30 days, respectively. VWF was associated with mortality and correlated closely with other prediction models. In patients with ACLF, VWF levels had a discrimination for 30 day mortality comparable with these models and accurately identified ACLF patients with high 30 day mortality risk.

Conclusions: Levels of VWF associate closely with risk of mortality in patients with DC and ACLF, and may have predictive utility as a laboratory marker of prognosis. Further research is warranted to assess the additional value of VWF in prediction of mortality and associated complications in chronic liver failure syndromes.

Introduction

The endothelium functions not only as a barrier between blood and underlying tissues, but also regulates vascular tone, vascular homeostasis, hemostatic and inflammatory processes. In response to diverse physical and chemical stimuli, endothelial cells may produce vasoconstrictors and vasodilators, growth promoting and inhibiting factors, and pro- and antihemostatic factors[1]. Endothelial dysfunction is characterized by reduced vasodilatation and a pro-inflammatory and prothrombotic vascular state[2]. Endothelial dysfunction plays a pivotal role in the development of a many diseases including atherosclerosis[2,3], type 2 diabetes[4], sepsis[5], and various auto-immune diseases[6].

Increasing evidence also suggests a role of endothelial dysfunction in the pathophysiology of cirrhosis. An example of this is the progress in the understanding of the development of portal hypertension. Portal hypertension is a frequently observed complication of cirrhosis, and is the result of increased hepatic resistance to portal blood flow. Although the mechanical impact of the altered architecture of the cirrhotic liver is a major cause of this increased resistance[7], decreased levels of nitric oxide – a potent vasodilator produced by the endothelium – contribute substantially to the increased portal resistance[7]. Indeed, studies have shown a marked decrease in production of nitric oxide in the cirrhotic liver[8,9]. Similarly, other markers that reflect activation of the endothelium – such as soluble Vascular-Cell Adhesion Molecule-1 (sVCAM-1) and soluble Intercellular Adhesion Molecule-1 (sICAM-1) – are increased in patients with cirrhosis[10,11]. Another endothelium derived protein that serves as a marker for endothelial dysfunction is the glycoprotein von Willebrand factor (VWF), a multimeric protein that plays a central role in primary hemostasis by binding circulating platelets to the site of endothelial injury[12].

Levels of VWF are indeed elevated in patients with cirrhosis and correlate with pressure in the portal circulation. VWF levels increase proportionally to liver disease severity, with levels observed at 300% above healthy controls in stable cirrhosis, 360% in acutely decompensated cirrhosis and 700% in acute-on-chronic liver failure[13]. In line with the concept of endothelial dysfunction as a driver of portal hypertension, recent studies have shown close association between VWF and hepatic venous pressure gradient and the occurrence of portal hypertension-related events[14,15]. Interestingly, VWF levels predict not only portal hypertension-related events but also complications such as bacterial translocation, inflammation and even mortality in patients with decompensated cirrhosis (DC)[14–19].

These findings suggest that VWF might serve as a new, readily available non-invasive marker for clinical outcome in patients with cirrhosis, that could improve current prediction models[17,18]. However the majority of published studies have been performed in patients with stable or decompensated disease. Little is known about the predictive value of VWF in patients with acute-on-chronic liver failure (ACLF) – an entity characterized by acute deterioration of cirrhosis and multiple organ failure, with a consequent short-term mortality rate of 50-90%[20]. Although one recent single center study has found an association between VWF and in-hospital mortality in patients with ACLF[21], the sample size in this study was limited and thus further research on this topic is warranted. In this study, we therefore aimed to investigate the predictive value of VWF in a larger cohort of patients with DC and patients with ACLF.

Materials and methods

Patients

From November 2013 to August 2021, adult patients with established cirrhosis admitted to King's College Hospital London (UK) and Hospital Clínic Barcelona (Spain) were prospectively included in this study and followed up for 90 days (in case of DC) or 30 days (in case of ACLF). The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the National Research Ethics Service

(NRES) Committee London, Westminster (12/LO/1417), the Health Research Authority and Health Care and Research Wales (19/WA/0168), and the Medical Ethical Committee Hospital Clínic Barcelona (2017/0948). Informed consent or assent was obtained in writing from participants or their consultees in case of mental incapacity. Exclusion criteria for this study were acute liver failure, known hereditary coagulation disorders, pregnancy, HIV positivity, extrahepatic malignancy, hepatocellular carcinoma outside the Milan criteria, and orthotopic liver transplant before or during the follow up period. Cirrhosis was defined by either biopsy or radiology (FibroScan F4 and/or radiologic features suggestive of cirrhosis). Decompensation of cirrhosis at the time of sampling was defined by presence of at least one decompensation event, i.e. ascites or spontaneous bacterial peritonitis, variceal bleeding, or hepatic encephalopathy[22]. ACLF at the time of sampling was defined and graded in concordance with criteria reported in the CANONIC study[23]. Suspected infection was identified based on clinician assessment of physiological changes suggestive of an infectious process, and the combination of administration of antibiotics (oral or parenteral) and/or positive body fluid cultures (blood, urine, cerebrospinal fluid, etc.)[24]. Healthy controls (n=41) were included to establish reference values for the various laboratory tests employed. Exclusion criteria for healthy controls were a body mass index <18 or >28 kg/m2, history of thrombosis or liver disease, pregnancy or actively breastfeeding, untreated medical conditions, chronic medical conditions requiring regular primary or secondary care review, or current use of anticoagulants, platelet function inhibitors, or oral contraceptives.

Data collection

Baseline data on patient demographics, (transplant free) survival, full blood count, biochemistry and illness severity scores were obtained from patient records. Specifically, the illness severity scores of interest were the Model for End-stage Liver Disease (MELD), Child-Pugh Score, CLIF Consortium Organ Failure (CLIF-C OF) score[23], CLIF Consortium Acute Decompensation (CLIF-C AD) score[25], CLIF C Acute-on-Chronic Liver Failure (CLIF-C ACLF) score[26] and the Sequential Organ Failure Assessment (SOFA).

Blood samples

Blood samples were collected in sodium citrate-containing vacutainer tubes (3.2%) from an arterial line, central venous catheter, or by standard peripheral venous phlebotomy within the first 2 days of admission, or, in case of ACLF patients, within the first 2 days after development of ACLF. The citrated blood was processed to platelet-poor plasma within 2 hours of collection by double centrifugation at 2000g and 10,000g, respectively, for 10 minutes at 18°C. Plasma was stored at -80°C until use for analyses.

Hemostatic assays

INR and platelet levels were measured in context of clinical care in the local clinical laboratories of King's College Hospital London and Hospital Clínic Barcelona. VWF levels were assessed with an in-house enzyme-linked immunosorbent assay (ELISA) using commercially available polyclonal antibodies against VWF, as described previously[27]. Levels of VWF in pooled normal plasma were set at 100%, and values obtained in test plasma were expressed as a percentage of pooled normal plasma. Thrombomodulin-modified thrombin generation assays (TGA) were performed in platelet-poor plasma with the fluorimetric method described by Hemker *et al.*, Calibrated Automated Thrombography. Coagulation was activated using commercially available reagents containing recombinant tissue factor (final concentration: 5 PM), phospholipids (final concentration: 4 μM), in the presence of soluble thrombomodulin (the concentration of which is not revealed by the manufacturer). Reagents were purchased from Thrombinoscope BV, Maastricht, The Netherlands, and thrombin generation experiments were executed following protocols provided by Thrombinoscope. Fibrinolysis was assessed by a plasma-based clot lysis time as described previously[28].

Statistical analyses

Statistical analyses were performed using GraphPad Prism v9 and SPSS Statistics 28 (IBM). Data are expressed as medians (with interquartile ranges) or numbers (with percentages) as appropriate. Continuous variables were analyzed using the Mann-Whitney U test or Kruskal-Wallis test, as appropriate, and categorical variables were analyzed using the Chi-squared test. Univariate and multivariate binary logistic regression analysis with odds ratio was performed to ascertain predictors of mortality. Spearman's correlation coefficient was used to assess the association between continuous variables and VWF. Receiver operating characteristic (ROC) curve analysis was used to test the accuracy of continuous variables independently associated with prognosis. Pairwise analyses of the area under the ROC (AUROC) using the Z-test were performed to compare the predictive value of different prognostic scoring models. The best cut-off level of VWF to predict mortality was selected using Youden's J statistic. The discriminative ability of this cut-off to predict prognosis was analyzed by Kaplan-Meier curves and compared with the log-rank test. Statistical significance was established at $p<0.05$.

Results

Patient characteristics

In total, we included 111 consecutive patients with DC and 105 with ACLF. Of these patients, a total of 16 (14%) patients with DC died within 90 days and 34 (32%) patients with ACLF died within 30 days of inclusion. Precipitants of ACLF included infection (n=33), bleeding (n=19), alcohol intake (n=15), other specific causes (including trauma, invasive procedures, drugs, or nephropathy, n=10), or a combination of the causes above (n=20). In 8 patients, the cause of progression to ACLF remained unknown. Levels of VWF did not differ significantly between different causes of ACLF (data not shown). Of 105 patients with ACLF, 32 (30%) had ACLF grade 1, 23 (22%) had ACLF grade 2, and 50 (48%) had ACLF grade 3. Circulatory failure occurred in 48 (46%), respiratory failure in 31 (30%), renal failure in 80 (76%), hepatic failure in 51 (49%), brain failure in 39 (37%) and coagulation failure in 25 (24%) of patients with ACLF. Demographic and clinical data obtained on admission are shown in **Table 1**, laboratory data on admission in **Table 2**.

Table 1. Demographic and clinical data of the study population on admission.

	DC		P value	ACLF		P value
Variables	**Alive at 90d (n=95)**	**Dead at 90d (n=16)**		**Alive at 30d (n=71)**	**Dead at 30d (n=34)**	
Age (y)	54 [44-62]	63 [56-69]	**0.005**	56 [46-62]	55 [45-61]	0.753
Female (%)	35 (36.8%)	5 (31.3%)	0.666	21 (29.6%)	12 (35.3%)	0.555
Aetiology of liver disease *ALD* *MAFLD* *Cholestatic* *Viral* *Other*	 53 (55.8%) 15 (15.8%) 9 (9.5%) 1 (1.1%) 17 (17.9%)	 10 (62.5%) 3 (18.8%) 1 (6.3%) 0 (0%) 2 (12.5%)	0.946	 47 (66.2%) 10 (14.1%) 3 (4.2%) 7 (9.9%) 4 (5.6%)	 21 (61.8%) 5 (14.7%) 1 (2.9%) 3 (8.8%) 4 (11.8%)	0.855
MELD score	19 [15-22]	22 [18-24]	0.117	29 [25-35]	39 [32-40]	**<0.001**
Child-Pugh Score	9 [8-10]	10 [8-11]	0.101	10 [8-12]	12 [11-13]	**<0.001**
Ascites *None* *Slight* *Moderate*	 36 (37.9%) 30 (31.6%) 29 (30.5%)	 0 (0%) 6 (37.5%) 10 (62.5%)	**0.006**	 21 (29.6%) 22 (31.0%) 28 (39.4%)	 5 (14.7%) 10 (29.4%) 19 (55.9%)	0.177
HE *None* *Grade 1-2* *Grade 3-4*	 68 (71.6%) 26 (27.4%) 1 (1.1%)	 12 (75.0%) 4 (25.0%) 0 (0%)	0.896	 38 (53.5%) 22 (31.0%) 11 (15.5%)	 9 (26.5%) 13 (38.2%) 12 (35.3%)	**0.016**
Suspected infection	45 (47.4%)	8 (50%)	0.905	62 (87.3%)	32 (94.1%)	0.287
Hepatocellular carcinoma *No* *Yes*	 91 (95.8%) 4 (4.2%)	 16 (100%) 0 (0%)	>0.999	 69 (97.2%) 2 (2.8%)	 32 (94.1%) 2 (5.9%)	0.594
TIPS in situ *No* *Yes*	 93 (97.9%) 2 (2.1%)	 15 (93.7%) 1 (6.3%)	0.376	 65 (91.5%) 6 (8.5%)	 30 (88.2%) 4 (11.8%)	0.724
Portal hypertensive bleeding* *No* *Yes*	 85 (89.5%) 10 (10.5%)	 13 (81.2% 3 (18.8%)	0.397	 50 (70.4%) 21 (29.6%)	 22 (64.7%) 12 (35.3%)	0.654
Non-portal hypertensive bleeding* *No* *Yes*	 85 (89.5%) 10 (10.5%)	 14 (87.5%) 2 (12.5%)	0.683	 62 (87.3%) 9 (12.7%)	 27 (79.4%) 7 (20.6%)	0.385
Thrombosis* *No* *Yes*	 86 (90.5%) 9 (9.5%)	 14 (87.5%) 2 (12.5%)	0.658	 66 (93.0%) 5 (7.0%)	 31 (91.2%) 3 (8.8%	0.712
CLIF-C AD score	48 [43-54]	57 [54-62]	**<0.001**	61 [54-66]	71 [63-76]	**<0.001**
CLIF-C ACLF score	n.a.	n.a.		50 [41-59]	66 [60-71]	**<0.001**
CLIF-OF score	n.a.	n.a.		10 [8-12]	14 [13-16]	**<0.001**
ACLF grade Grade 1 Grade 2 Grade 3	n.a.	n.a.		 31 (43.7%) 18 (25.4%) 22 (31.0%)	 1 (2.9%) 5 (14.7%) 28 (82.4%)	**<0.001**
SOFA score	n.a.	n.a.		8 [6-11]	13 [10-17]	**<0.001**
Mechanical ventilation (yes)	n.a.	n.a.		22 (31.0%)	16 (47.1%)	0.109

Vasopressors required (yes)	n.a.	n.a.		23 (32.4%)	24 (70.6%)	**<0.001**
RRT (yes)	n.a.	n.a.		25 (35.2%)	22 (67.7%)	**0.004**
GCS	15 [15-15]	15 [15-15]	0.991	15 [10-15]	12 [4-15]	0.104

* During hospital admission

Abbreviations: DC, decompensated cirrhosis; ACLF, acute-on-chronic liver failure; ALD, alcoholic liver disease; MAFLD, metabolic dysfunction associated fatty liver disease; MELD, model for end-stage liver disease; TIPS, transjugular intrahepatic portosystemic shunt; CLIF-C, CLIF Consortium; OF, organ failure; SOFA, sequential organ failure assessment; RRT, renal replacement therapy; GCS, Glasgow coma scale.

Table 2. Laboratory data of the study population on admission.

	DC		P value	ACLF		P value
Variables	**Alive at 90d (n=95)**	**Dead at 90d (n=16)**		**Alive at 30d (n=71)**	**Dead at 30d (n=34)**	
Haematology						
Haemoglobin (g/L)	108 [93-121]	99 [86-114]	0.238	83 [77-95]	88 [79-101]	0.241
Platelet count (x10^9/L)	91 [62-150]	99 [83-180]	0.302	94 [59-125]	66 [38-86]	**0.002**
WBC count (x10^9/L)	4.89 [3.61-6.87]	5.96 [5.09-9.30]	**0.031**	8.66 [4.06-13.50]	11.80 [8.00-20.81]	**0.008**
Biochemistry						
Sodium (mmol/L)	136 [132-139]	133 [129-135]	**0.013**	137 [133-140]	136 [132-137]	0.222
Urea (mmol/L)	5.0 [3.4-7.0]	8.3 [4.7-10.5]	**0.010**	9.0 [5.0-13.6]	10.9 [7.4-16.6]	0.129
Creatinine (umol/L)	64 [53-87]	85 [69-106]	**0.017**	164 [77-256]	185 [106-232]	0.547
Bilirubin (umol/L)	49 [31-96]	54 [25-152]	0.791	70 [39-244]	368 [234-513]	**<0.001**
Gamma glutamyl transaminase (IU/L)	89 [54-186]	83 [32-136]	0.276	91 [41-185]	43 [27-76]	**0.007**
Alkaline phosphatase (IU/L)	145 [97-201]	125 [95-172]	0.298	120 [76-168]	106 [73-133]	0.240
Aspartate transaminase (IU/L)	61 [43-93]	53 [33-64]	0.079	64 [44-105]	103 [73-153]	**0.006**
Albumin (g/L)	30 [26-34]	31 [25-39]	0.762	29 [26-34]	29 [25-33]	0.410
Coagulation						
INR	1.47 [1.30-1.72]	1.56 [1.40-2.02]	0.180	1.72 [1.40-2.05]	2.44 [2.14-2.85]	**<0.001**
Fibrinogen (g/L)	2.7 [2.1-3.2]	3.4 [1.8-4.1]	0.271	2.4 [1.6-3.3]	1.5 [0.9-2.0]	**<0.001**
VWF (%)	451 [330-624]	665 [442-933]	**0.022**	572 [378-794]	871 [573-1043]	**<0.001**

Abbreviations: DC, decompensated cirrhosis; ACLF, acute-on-chronic liver failure; WBC, white blood cell; INR, international normalized ratio; VWF, von Willebrand factor.

Plasma VWF levels are substantially elevated in acutely ill patients with cirrhosis, with a marked increase in patients with ACLF grade 3

Plasma VWF levels were similarly elevated in patients with DC (n=111), ACLF grade 1 (n=32) and ACLF grade 2 (n=23) (**Figure 1**). In patients with ACLF grade 3 (n=50), VWF levels were nearly twofold higher compared to patients with DC or ACLF grade 1-2. Of note, there was no difference in VWF levels in patients with 3 organ failures (n=21) compared to patients with 4 to 6 organ failures (n=29, data not shown). A total of 29 out of 216 patients had plasma VWF levels higher than 1000% (9/111 of patients with DC, 4/32 of patients with ACLF grade 1, 2/23 of patients with ACLF grade 2, and 14/50 of patients with ACLF grade 3). Levels of VWF did not differ between patients with or without infection (p=0.667 in DC, p=0.086 in ACLF), with or without hepatocellular carcinoma (p=0.589 in DC, p=0.179 in ACLF) or with or without transjugular intrahepatic portosystemic shunt (p=0.135 in DC, p=0.774 in ACLF).

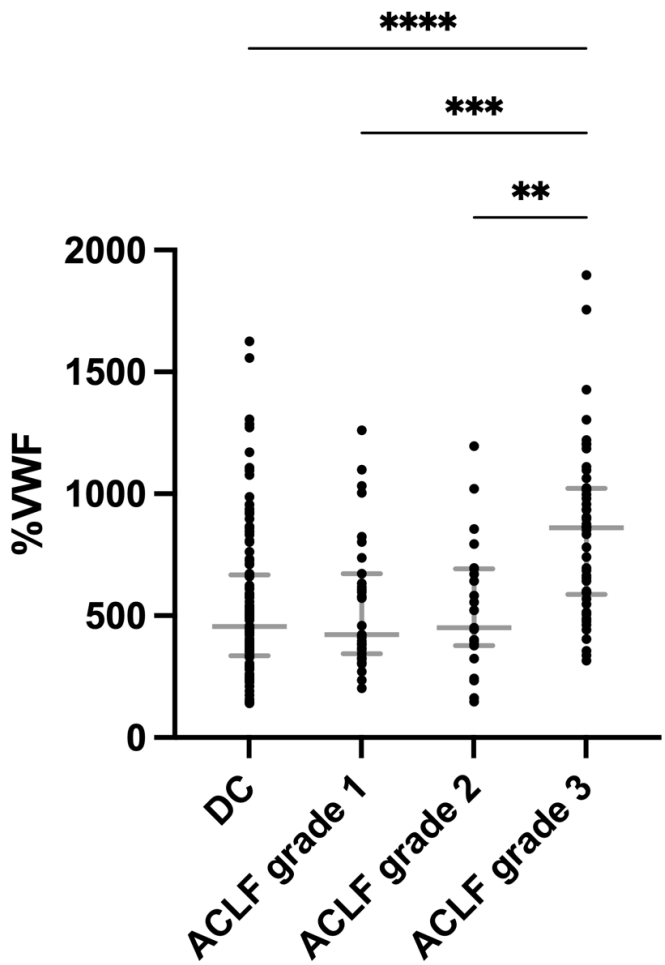

Figure 1. Plasma von Willebrand factor on admission. Horizontal lines indicate medians, error lines indicate interquartile range. **** P<0.0001, *** p<0.001, ** p<0.01. Abbreviations: VWF, von Willebrand factor; DC, decompensated cirrhosis; ACLF, acute-on-chronic liver failure.

VWF is a laboratory marker for mortality in DC and ACLF

Plasma levels of VWF were substantially elevated in patients that died within 90 or 30 days compared to survivors , regardless of severity of disease (**Figure 2**). In univariable analysis, patients with DC who had lower serum sodium, higher serum urea or higher plasma VWF were found to have higher risk of 90-day mortality (**Table 3**). Due to a limited amount of cases (n=16), multivariable analysis was not performed.

In patients with ACLF, lower platelet counts, plasma fibrinogen and serum GGT, and higher WBC counts, total serum bilirubin, INR and VWF were associated with increased risk of 30-day mortality in univariable analysis (**Table 4**). In multivariable analysis of laboratory markers, only total serum bilirubin, fibrinogen levels and VWF were independently associated with 30-day mortality.

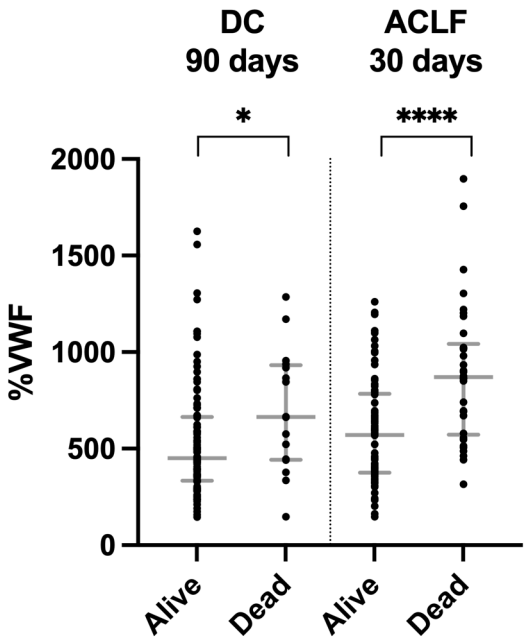

Figure 2. Plasma VWF in patients with decompensated cirrhosis that died or did not die within 90 days, and in patients with acute-on-chronic liver failure that died or did not die within 30 days. Horizontal lines indicate medians, error lines indicate interquartile range. ****P<0.0001, *p<0.05. Abbreviations: VWF, von Willebrand factor; DC, decompensated cirrhosis; ACLF, acute-on-chronic liver failure.

Table 3. Laboratory values and their association with 90 day mortality in the DC cohort, in univariable analyses.

	Univariable analysis		
Variables	**Odds ratio**	**95% CI**	**P value**
WBC count (x10^9/L)	1.133	0.999-1.291	0.051
Sodium (mmol/L)	0.877	0.784-0.980	**0.021**
Urea (mmol/L)	1.157	1.035-1.293	**0.010**
Creatinine (umol/L)	1.007	0.995-1.020	0.260
VWF (%)	1.002	1.000-1.003	**0.041**

Abbreviations: DC, decompensated cirrhosis; 95% CI, 95% confidence interval; WBC, white blood cell; VWF, von Willebrand factor.

Table 4. Laboratory values and their association with 30-day mortality in the ACLF cohort, in univariable and multivariable analyses.

	Univariable analysis			Multivariable analysis		
Variables	**Odds ratio**	**95% CI**	**P value**	**Odds ratio**	**95% CI**	**P value**
Platelet count (x10^9/L)	0.985	0.974-0.995	**0.005**	0.987	0.973-1.001	0.063
WBC count (x10^9/L)	1.059	1.005-1.11	**0.033**	1.045	0.947-1.154	0.384
Bilirubin (umol/L)	1.006	1.003-1.008	**<0.001**	1.004	1.001-1.008	**0.024**
Aspartate transaminase (IU/L)	1.002	0.999-1.004	0.241			
INR	2.194	1.240-3.881	**0.007**	0.888	0.491-1.607	0.694
Fibrinogen (g/L)	0.353	0.197-0.631	**<0.001**	0.406	0.180-0.912	**0.029**
VWF (%)	1.003	1.002-1.005	**<0.001**	1.002	1.000-1.004	**0.026**

Abbreviations: ACLF, acute-on-chronic liver failure; 95% CI, 95% confidence interval; WBC, white blood cell; INR, international normalized ratio; VWF, von Willebrand factor.

VWF correlates well with clinically used prediction models and independently associates with outcome
VWF correlated with other laboratory markers associated with mortality, including WBC and sodium in DC, and total bilirubin, WBC, and INR in ACLF (**Supplementary table 1**). Of note, in both cohorts no correlation was found between VWF and platelet count or fibrinogen levels.

In both DC and ACLF cohorts, plasma VWF correlated well with MELD scores, Child Pugh scores, and CLIF-C-AD or CLIF-C-ACLF scores, respectively (**Supplementary table 1**). In patients with ACLF, VWF was associated with circulatory (OR 1.001, 95%CI 1.000-1.003, p=0.019), respiratory (OR 1.002, 95%CI 1.000-1.003, p=0.015) and hepatic failure (OR 1.003, 95%CI 1.002-1.005, p<0.001), but not with renal (p=0.621), brain (p=0.209) and coagulation (p=0.089) failure.

In patients with ACLF, VWF similarly associated with outcome as MELD scores, Child Pugh scores, and CLIF-C-ACLF scores (**Figure 3**). The AUROC of VWF was 0.76 (95%CI 0.67-0.86), compared to AUROCs of 0.78 (95%CI 0.69-0.88) for the MELD score, 0.76 (95%CI 0.66-0.85) for the Child Pugh score and 0.82 (95%CI 0.73-0.90) for the CLIF-C-ACLF score. In pairwise analysis of these models, AUROCs were not statistically different (data not shown).

In separate pairwise multivariable analyses, both VWF and MELD score (OR 1.002, 95%CI 1.001-1.004, p=0.008 and OR 1.126, 95%CI 1.036-1.223, p=0.005, respectively), VWF and Child Pugh score (OR 1.002, 95%CI 1.000-1.004, p=0.012 and OR 1.576, 95%CI 1.158-2.145, p=0.004, respectively), and VWF and CLIF-C-ACLF score (OR 1.002, 95%CI 1.000-1.004, p=0.030 and OR 1.098, 95%CI 1.042-1.156, p<0.001, respectively) were associated with 30-day mortality in patients with ACLF.

To assess the potential utility of VWF as an adjunct marker in combination with clinically used mortality prediction models, we assessed association between the MELD-Sodium-VWF score as proposed by Györi et al[17]. First, we calculated the MELD-Sodium-VWF score using the formula ((1.127*MELD Score)+(1.002×vWF-Ag))×5.5. The AUROC of this new model was 0.849 (95%CI 0.777-0.920) compared to the AUROC of 0.78 (95%CI 0.69-0.88) for the regular MELD score. However, in pairwise comparison of these models, the AUROCs were not statistically different (data not shown).

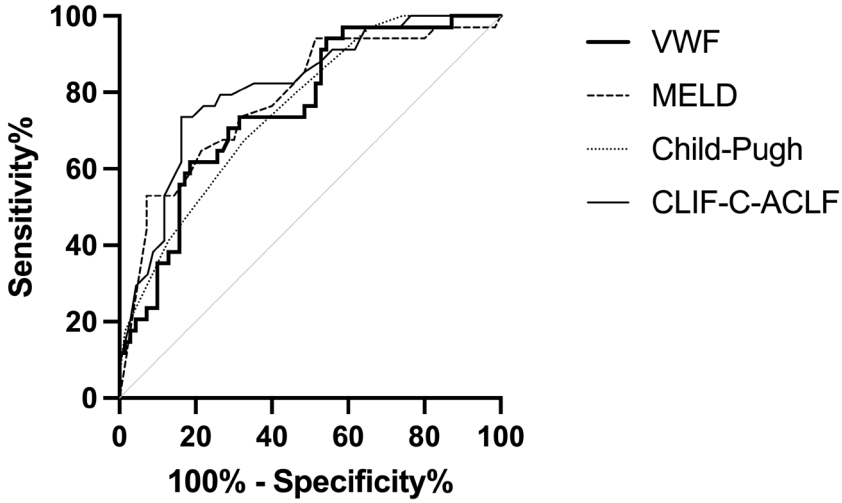

Figure 3. Receiver Operating Characteristic (ROC) curves of VWF and clinical scores predicting 30-day mortality in patients with acute-on-chronic liver failure. Abbreviations: VWF, von Willebrand factor; MELD, model for end-stage liver disease; CLIF-C-ACLF, CLIF Consortium acute-on-chronic liver failure score.

A cut-off of 842% VWF accurately identifies patients with a high 30-day mortality risk in patients with ACLF

Using Youden's J statistic, we classified patients with into high- and low-risk for 30-day mortality according to their VWF level. The optimal cut-off was identified at 842% (VWF842%), which achieved a sensitivity of 61.8% and a specificity of 84.4%. Patients with VWF>842% were 7 times more likely to die within 30 days (**Figure 4**) than patients with VWF<842%. In separate pairwise multivariable models including VWF842% and MELD score, VWF842% and Child Pugh Score, and VWF842% and CLIF-C-ACLF score, VWF842% was independently associated with outcome (OR 4.212, 95%CI 1.578-11.246, p=0.004; OR 4.097, 95%CI 1.542-10.885, p=0.005; OR 3.555, 95%CI 1.272-9.942, p=0.016).

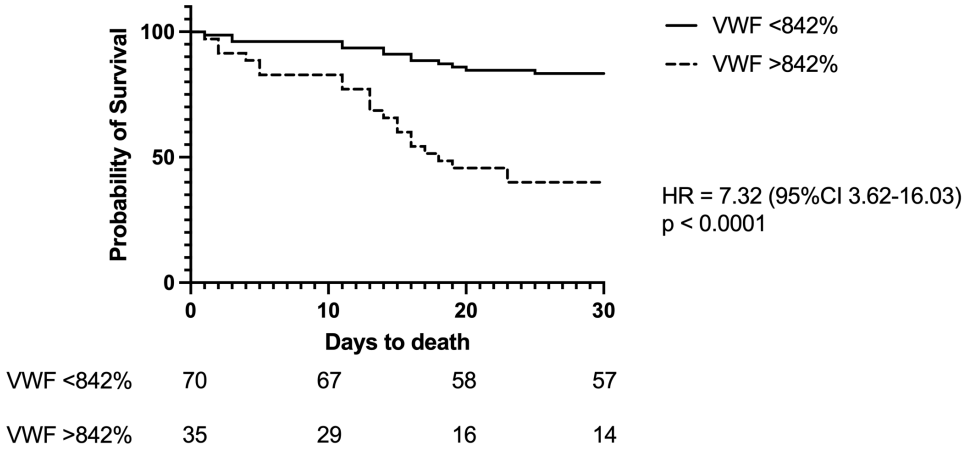

Figure 4. Kaplan-Meier's curve showing 30 day survival for patients with acute-on-chronic liver failure and a von Willebrand factor (VWF) cut-off value of 842%. Numbers below graph indicate numbers at risk.

Discussion

In this study, we have found that elevated levels of VWF associate with mortality in acutely ill hospitalised patients with cirrhosis. Although VWF levels of patients with DC were similar or slightly higher compared to those reported in previous studies[14–17], we demonstrated that VWF levels in the ACLF cohort – specifically in patients with grade 3 ACLF i.e. those with 3 or more organ failures – were substantially higher than those reported in DC patients. In some individuals with ACLF exceptionally high VWF levels were observed with some patients reaching levels of over 1500%. These patients often had higher MELD and CLIF-C scores, which further confirms the link between VWF and illness severity as previously demonstrated by our group and others[14,15,17,18,21,29]. Moreover, similar to other studies, these elevated VWF levels correlate closely with mortality [14,15,17,18,21]. We confirm and extend previous findings that VWF is not only a predictor of outcome in patients with DC, but also in those who are critically ill and requiring (multi-)organ support. In our ACLF cohort, VWF levels showed prognostic discrimination equivalent to standard clinically utilized prediction models, such as MELD, Child Pugh, and CLIF-C-ACLF scores. Further, multivariable analysis including these prediction models and VWF showed that VWF is independently associated with short term mortality in patients with ACLF. This suggests that VWF but may have additional predictive value in prediction of short term mortality.

The results of this study suggest that endothelial dysfunction is closely related to organ failure and mortality in patients with ACLF. We found that VWF is associated with some, but not all types of organ failures that may occur in patients with ACLF. We found a specific link between VWF and failure of organs as defined by the CLIF-C-ACLF score with particularly large amounts of endothelium, such as the lungs, the liver, and the circulatory vascular bed. Elevated levels of VWF are associated with dysfunction and failure of these organs, for example in patients with acute coronary disease[30], chronic obstructive pulmonary disease[31], acute liver failure[32], or COVID-19 infection[33]. In these same studies, VWF levels independently associate with mortality risk, which may indicate a role of the endothelium as a driver of disease progression and mortality. Indeed, prevention or treatment of endothelial dysfunction with (non-selective) beta-blockers not only improves endothelial function[34], but also may reduce mortality in patients with sepsis without underlying liver disease[35]. A similar effect has been suggested in patients with cirrhosis: a recent study by Jachs *et al.*[36]. suggests that non-selective beta-blocker therapy reduces the risk of decompensation in cirrhosis, the risk of progression to ACLF and even the risk of death. The exact role of endothelial dysfunction in the progression of chronic liver disease is still unclear, and specific pathogenic pathways poorly understood. Further research into this subject – and on the role of pharmacological treatment – is thus warranted.
Although VWF has been shown to be an independent marker of mortality in cirrhotic patients in addition to clinically used prediction models, there are currently no validated models that include VWF measurement. The MELD-Na-VWF model by Györi et al.[17] integrated VWF levels with the MELD score. Though in this report this novel score showed significant improvement in discrimination compared to the regular MELD, we were unable to reproduce this in our current study, in either DC or ACLF cohorts. Additional research is needed to confirm whether supplemental VWF measurements have additional clinical value in improving prognostic assessment scores, and if they do, to determine which of the current prediction models are most appropriate for inclusion and whether continuous VWF levels or cut-off values are preferable. The cut-off examined in our current study requires validation in an independent cohort, and alternative cut-off values (e.g. for patients with acute or chronic decompensation of cirrhosis and various grades of ACLF) should be explored. Importantly, future research is necessary to assess whether the potential prognostic benefits justify the costs of VWF measurements.

Whilst this is not the first report on the association between VWF and mortality in cirrhosis, it is the first that includes a large cohort of ACLF patients from two centres. Limitations of this study include its cross-sectional design, lack of stratification for specific drugs used at inclusion, the exclusion of patients that

underwent liver transplantation during the follow-up period, and the lack of a validation cohort. Firstly, as we only obtained clinical data upon admission, we were unable to define sepsis according to the latest Sepsis-3[37] guidelines (in which sepsis is defined by an increase in SOFA score of 2 points or more over time) in our cohort. Although we were able to gain an estimation of the rate of suspected infections by looking at antimicrobials use, we were unable to follow up sequential clinical data (such as SOFA scores). The cross-sectional design also meant that we did not measure VWF levels over time. However, previous data from our lab indicated that levels of VWF remain relatively stable over time in patients with ACLF, with similar levels on day 3, day 5 or 7, and day 10[38]. A longitudinal design might therefore not necessarily add to the analyses performed in the current study. Secondly, we did not stratify our cohorts based on drugs or clinical variables that may have influenced levels of VWF. A substantial percentage of our ACLF cohort received organ support using vasopressors at time of inclusion, which may have affected VWF levels and thus may have confounded our results. Due to the substantial heterogeneity of our cohorts in terms of vasopressor types, dosages, and duration of treatment, we were however unable to stratify our cohorts and take these effects into account. Larger follow-up studies would be needed to further address this issue. In particular, large multicenter studies would be needed to validate and extend our findings, and we therefore highly advocate for global collaboration of tertiary care hospitals specialized in care for acutely ill patients with liver disease.

In conclusion, we found that VWF levels were closely related to mortality risk in hospitalized patients with cirrhosis. In patients with ACLF, VWF levels showed prognostic discrimination comparable to prediction scores in widespread clinical use, such as the MELD, Child-Pugh and CLIF-C-ACLF scores. Although VWF appears to be an independent marker of mortality, it remains unclear when and how to use this marker, and what potential cut-off should be used. Further research is warranted to assess the additional value of VWF in prediction of mortality and the associated complications of chronic liver failure syndromes, and whether specific therapeutic interventions that favourably modulate endothelial dysfunction impact upon levels and positively influence clinical outcomes.

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Supplementary data

Supplementary table 1. Correlation between plasma VWF and other laboratory markers of mortality, and plasma VWF and clinical scores.

AD				ACLF			
Variable vs VWF	**Spearman R**	**95% CI**	**P value**	**Variable vs VWF**	**Spearman R**	**95% CI**	**P value**
Laboratory variables							
Sodium (mmol/L)	-0.24	-0.41- -0.05	**0.012**	Platelet count ($x10^9$/L)	0.02	-0.18-0.21	0.864
Urea (mmol/L)	-0.07	-0.27-0.13	0.498	WBC count ($x10^9$/L)	0.49	0.33-0.63	**<0.001**
				Bilirubin (umol/L)	0.47	0.30-0.61	**<0.001**
				INR	0.37	0.18-0.53	**<0.001**
				Fibrinogen (g/L)	-0.11	-0.31-0.09	0.262
				CLT (min)	0.32	0.13-0.49	**<0.001**
Clinically used prediction scores							
MELD score	0.27	0.09-0.44	**0.004**	MELD score	0.45	0.28-0.59	**<0.001**
Child-Pugh score	0.45	0.29-0.59	**<0.001**	Child-Pugh score	0.45	0.28-0.59	**<0.001**
CLIF-C-AD score	0.39	0.22-0.54	**<0.001**	CLIF-C-ACLF score	0.52	0.35-0.65	**<0.001**

Abbreviations: AD, acute decompensation; ACLF, acute-on-chronic liver failure; VWF, von Willebrand factor; 95%CI, 95% confidence interval; WBC, white blood cell; INR, international normalized ratio; CLT, clot lysis time; MELD, model for end-stage liver disease; CLIF-C, CLIF Consortium.