Factor VIII/protein C ratio independently predicts liver-related events but does not indicate a hypercoagulable state in ACLD

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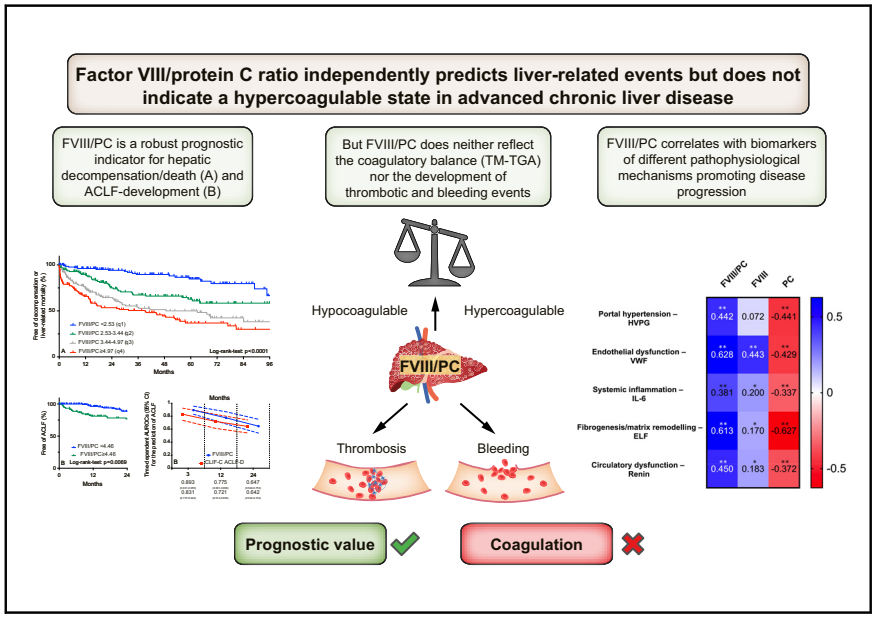
Graphical abstract



Highlights

- Factor VIII/protein C (FVIII/PC) ratio has prognostic value in advanced chronic liver disease.
- This may be explained by the link between FVIII/PC and pathophysiological mechanisms promoting advanced chronic liver disease.
- FVIII/PC was not associated with the development of bleeding or thrombotic events.
- Thus, the prognostic value of FVIII/PC should not be mistaken as evidence that hypercoagulability is a driver of disease progression.

Authors

Bernhard Scheiner, Lorenz Balcar, Rosa Johanna Nussbaumer, …, Thomas Reiberger, Ton Lisman, Mattias Mandorfer

Correspondence

j.a.lisman@umcg.nl (T. Lisman).

Lay summary

A balanced coagulation system is essential for preventing bleeding episodes and blood clot formation (thrombosis). Blood of patients with advanced liver disease may have increased coagulation potential, possibly promoting the worsening of liver disease via thrombosis in the blood vessels of the liver. The ratio between the results of 2 blood tests (procoagulant factor VIII to anticoagulant protein C) has been suggested to reflect these increases in coagulation potential. Our study demonstrates, on the one hand, that this ratio is a versatile predictor of the development of complications of cirrhosis, yet on the other hand, that it is unrelated to coagulation.

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Research Article
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Factor VIII/protein C ratio independently predicts liver-related events but does not indicate a hypercoagulable state in ACLD

Bernhard Scheiner[1,2,†], Lorenz Balcar[1,2,†], Rosa Johanna Nussbaumer[1], Johanna Weinzierl[1], Rafael Paternostro[1,2], Benedikt Simbrunner[1,2], Lukas Hartl[1,2], Mathias Jachs[1,2], David Bauer[1,2], Albert Friedrich Stättermayer[1,2], Georg Semmler[1,2], Matthias Pinter[1], Cihan Ay[3,4], Peter Quehenberger[5], Michael Trauner[1], Thomas Reiberger[1,2], Ton Lisman[6,*], Mattias Mandorfer[1,2]

[1]*Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria;* [2]*Vienna Hepatic Hemodynamic Lab, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria;* [3]*Clinical Division of Haematology and Haemostaseology, Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria;* [4]*I. M. Sechenov First Moscow State Medical University, Moscow, Russia;* [5]*Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria;* [6]*Surgical Research Laboratory and Section of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*

Background & Aims: It has been suggested that the ratio of procoagulant factor VIII to anticoagulant protein C (FVIII/PC) reflects the hemostatic equilibrium. Moreover, FVIII/PC predicted decompensation/death in a small study not accounting for portal hypertension severity. We investigated (i) the prognostic value of FVIII/PC (outcome-cohort) and (ii) whether FVIII/PC reflects the hypercoagulable state (assessed by thrombomodulin-modified thrombin generation assay [TM-TGA]) or the risk of bleeding/thrombotic events in patients undergoing hepatic venous pressure gradient (HVPG) measurement during follow-up.

Methods: (i) The outcome-cohort comprised 576 patients with evidence of advanced chronic liver disease (liver stiffness measurement ≥10 kPa and/or HVPG ≥6 mmHg). (ii) TM-TGA-cohort patients (n = 142) were recruited from the prospective VIenna CIrrhosis Study (VICIS: NCT03267615).

Results: (i) FVIII/PC significantly increased across clinical stages (p <0.001) as well as HVPG (p <0.001) and MELD score (p <0.001) strata and remained independently associated with decompensation/liver-related death (adjusted hazard ratio 1.06; 95% CI 1.01–1.11; p = 0.013), even after multivariable adjustment. It was also associated with acute-on-chronic liver failure (ACLF) development (adjusted hazard ratio 1.10; 95% CI 1.02-1.19; p = 0.015) in patients with decompensated cirrhosis. (ii) FVIII/PC showed a weak positive correlation with endogenous thrombin potential (Spearman's ρ = 0.255; p = 0.002), but this association disappeared after adjusting for the severity of liver disease. FVIII/PC was not associated with the development of bleeding (p = 0.272) or thrombotic events (p = 0.269). However, FVIII/PC correlated with biomarkers of different pathophysiological mechanisms that promote liver disease progression.

Conclusion: FVIII/PC provides prognostic information regarding hepatic decompensation/death and ACLF, independently of established prognostic indicators. However, this is not evidence that hypercoagulability drives disease progression, as the correlation between FVIII/PC and thrombin generation is confounded by liver disease severity and FVIII/PC was not associated with thrombosis. Therefore, FVIII/PC does not reflect coagulation and results from previous studies on FVIII/PC require re-interpretation.

Clinical trial number: NCT03267615 (in part).

Lay summary: A balanced coagulation system is essential for preventing bleeding episodes and blood clot formation (thrombosis). Blood of patients with advanced liver disease may have increased coagulation potential, possibly promoting the worsening of liver disease via thrombosis in the blood vessels of the liver. The ratio between the results of 2 blood tests (procoagulant factor VIII to anticoagulant protein C) has been suggested to reflect these increases in coagulation potential. Our study demonstrates, on the one hand, that this ratio is a versatile predictor of the development of complications of cirrhosis, yet on the other hand, that it is unrelated to coagulation.

Introduction

As a consequence of abnormal routine coagulation tests and thrombocytopenia, cirrhosis has long been considered an acquired bleeding disorder.[1] Indeed, the liver plays a central role in coagulation and plasma levels of most procoagulant factors are significantly reduced in patients with advanced chronic liver disease (ACLD). However, these changes are balanced by decreased levels of anticoagulant proteins[2] and highly elevated levels of von Willebrand factor (VWF).[3] Based on the results of thrombomodulin-modified thrombin generation assays (TM-

Keywords: Coagulation; hypercoagulability; cirrhosis; portal hypertension; decompensation.
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* Corresponding author. Address: Surgical Research Laboratory and Section of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, BA44, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Tel.: +31-50-3619028, fax: +31-50-3632796.
E-mail address: j.a.lisman@umcg.nl (T. Lisman).
† Shared co-first authorship.

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TGAs), patients with cirrhosis are nowadays considered to have fully competent coagulation system, which commonly turns into a hypercoagulable state with disease progression.[4] It has been proposed that an overactive coagulation system drives disease progression in patients with cirrhosis. Indeed, microthrombi in the hepatic vein branches may trigger collagen formation by activated hepatic stellate cells,[5] possibly by inducing a so-called congestive escalator.[5] According to this vascular hypothesis of liver disease progression, congestion due to vascular occlusions leads to an intrahepatic compartment syndrome causing additional vascular lesions and parenchymal extinction. As a result, a hypercoagulable state may promote disease progression and thereby increase the development of liver-related events in patients who have already progressed to ACLD.[4]

As TM-TGAs require considerable expertise and are not broadly available, simple laboratory parameters have been suggested as surrogate markers for the hypercoagulable status. In this context, the ratio of procoagulant factor VIII to PC (FVIII/PC) has been shown to correlate with *ex vivo* thrombin generation.[6] Additionally, the imbalance of FVIII/PC has even been suggested as a major reason for hypercoagulability in ACLD[7] and predicted hepatic decompensation and death in a small study that did not account for the severity of portal hypertension (PH).[8] However, others have questioned the utility of FVIII/PC as a marker of hypercoagulability, as this ratio does not consider the complex changes in pro- and anticoagulant proteins in patients with ACLD.[9,10] Indeed, to date, no study has directly correlated FVIII/PC with TM-TGA results in a large thoroughly characterized cohort.

Therefore, we (i) investigated the prognostic value of FVIII/PC after adjusting for other relevant variables including the hepatic venous pressure gradient (HVPG), (ii) evaluated whether FVIII/PC reflects the coagulatory balance and the risk of bleeding or thrombotic events in large cohorts of patients undergoing HVPG measurement, and (iii) investigated other pathophysiological mechanisms correlated with FVIII/PC which may explain its prognostic implications.

Patients and methods

Study design and patients

We performed a retrospective, single-center, cohort study in patients with evidence suggestive/indicative of ACLD who were undergoing HVPG measurement at the Vienna Hepatic Hemodynamic Lab between 09/2003 and 12/2020 (outcome-cohort: FVIII/PC cohort). Inclusion criteria were (i) liver stiffness measurement (LSM) ≥10 kPa and/or HVPG ≥6 mmHg, (ii) valid HVPG measurement, and (iii) availability of factor VIII (FVIII) and protein C (PC). Patients were excluded if any of the following criteria were present: patients with a history of orthotopic liver transplantation, any active malignancy, presence of portal vein thrombosis (PVT), current anticoagulation and/or anti-platelet therapy, evidence of bacterial infection, or missing information on important laboratory parameters and/or clinical follow-up. Data from patients in this cohort were published previously with different research questions.[11–18]

In addition, we assessed biomarkers and performed TM-TGA in an overlapping cohort of patients (n = 142) from the prospective VIenna CIrrhosis Study (VICIS; NCT03267615;TM-TGA-cohort) who were recruited between 02/2017 and 08/2020.

Finally, we also included a control cohort comprising 122 individuals with healthy livers.[19,20] Detailed results on the healthy cohort are displayed in Table S1.

Clinical stages of ACLD and definition of ACLF

Patients were classified according to recently defined prognostic/clinical stages (CS). The definition was adapted from D'Amico *et al.*[21] and detailed characteristics are shown in the supplementary materials and methods. Acute-on-chronic liver failure (ACLF) was defined according to the EF-CLIF criteria.[22]

HVPG measurement

HVPG measurements were performed in adherence to a standard operating procedure.[23] A detailed description of the procedure can be found in the supplementary materials and methods.

Measurement of key coagulation parameters

Routine laboratory tests and the determination of FVIII and PC as well as biomarkers (VWF, lipopolysaccharide binding protein [LBP], procalcitonin [PCT], interleukin 6 [IL-6], enhanced liver fibrosis [ELF] test, mean arterial pressure, copeptin, renin, and bile acids [BAs]) were performed by the ISO-certified Department of Laboratory Medicine of the Medical University of Vienna using commercially available methods that are applied in clinical routine and blood samples obtained via a central venous line (*i.e.*, the side port of the catheter introducer sheath) at the time of HVPG measurement. A detailed description of FVIII and PC measurements can be found in the supplementary materials and methods.

Blood samples for TM-TGA measurement were also drawn from a central venous line at the time of HVPG measurement and TM-TGA measurements were performed at the Surgical Research Laboratory, University Medical Center Groningen, The Netherlands using a fluorometric method as previously described.[2,24,25]

Bleeding and thrombotic events

Detailed information regarding the definition and ascertainment of bleeding and thrombotic events is provided in the supplementary materials and methods.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 27 (IBM, New York, NY, USA), R 3.4.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria) or GraphPad Prism 8 (GraphPad Software, CA, USA). Categorical variables were reported as absolute (n) and relative frequencies (%), whereas continuous variables as mean ± SD or median (IQR) as appropriate. Student's *t* test was used for group comparisons of normally distributed variables and Mann-Whitney *U* test for non-normally distributed variables. Group comparisons of categorical variables were performed using either Chi-squared or Fisher's exact test. Simple and multiple linear regression analyses were applied to evaluate factors associated with FVIII/PC as well as with the individual variables FVIII and PC. A detailed description of the rationale for variable selection for multivariable models is shown in the supplementary materials and methods.

Patients were censored 6 months after the last visit at a Vienna hospital association institution with the rationale that patients may miss 1 routine three-monthly follow-up appointment but must be considered lost-to-follow-up once the patient misses the second appointment. Follow-up time was calculated as the time from HVPG measurement to the date of liver transplantation, death, or last follow-up at one of the hospitals of the Vienna hospital association. Uni- and multivariable Cox

regression analyses were performed to evaluate parameters independently associated with the events of interest. In a first step, we included all parameters potentially associated with hepatic decompensation or liver-related death into univariable Cox regression models. Parameters with a p value <0.1 in univariable analysis were further included into 2 separate multivariable models (rationale for variable selection provided in the supplementary materials and methods). The relationship between FVIII/PC and hepatic decompensation/liver-related death was assessed using restricted cubic spline analysis.[26] For the graphical demonstration of the function, the reference was set at the median FVIII/PC level of the outcome-cohort. We also performed uni- and multivariable Cox regression analyses to evaluate parameters associated with ACLF development in patients with dACLD. Patients with ACLF at baseline were not considered for these analyses. Time-dependent area under the receiver-operating characteristic curve (AUROC) analyses were performed and the R-package 'timeROC' was used to compare the prognostic performances for hepatic decompensation or liver-related death (United Network for Organ Sharing model for end-stage liver disease (2016) score [UNOS MELD (2016)-score], HVPG, and FVIII/PC) and ACLF (chronic liver failure-consortium acute-on-chronic liver failure-development [CLIF-C ACLF-D-score][27] and FVIII/PC) over time. Time-dependent event rates were obtained by the Kaplan-Meier method and groups were compared by applying the log-rank test.

Spearman's correlation and linear regression analyses were conducted to investigate potential associations between FVIII/PC and TM-TGA results as well as biomarkers. A heatmap plot was used for graphical illustration of associations between FVIII/PC and biomarkers. The level of significance was set at a 2-sided p value <0.05.

Ethics

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee (EK1446/2018 and EK1262/2017). The requirement of written informed consent for the retrospective outcome-cohort was waived by the ethics committee. All patients included in the prospective TM-TGA-cohort (*i.e.*, VICIS-study) provided written informed consent for study participation.

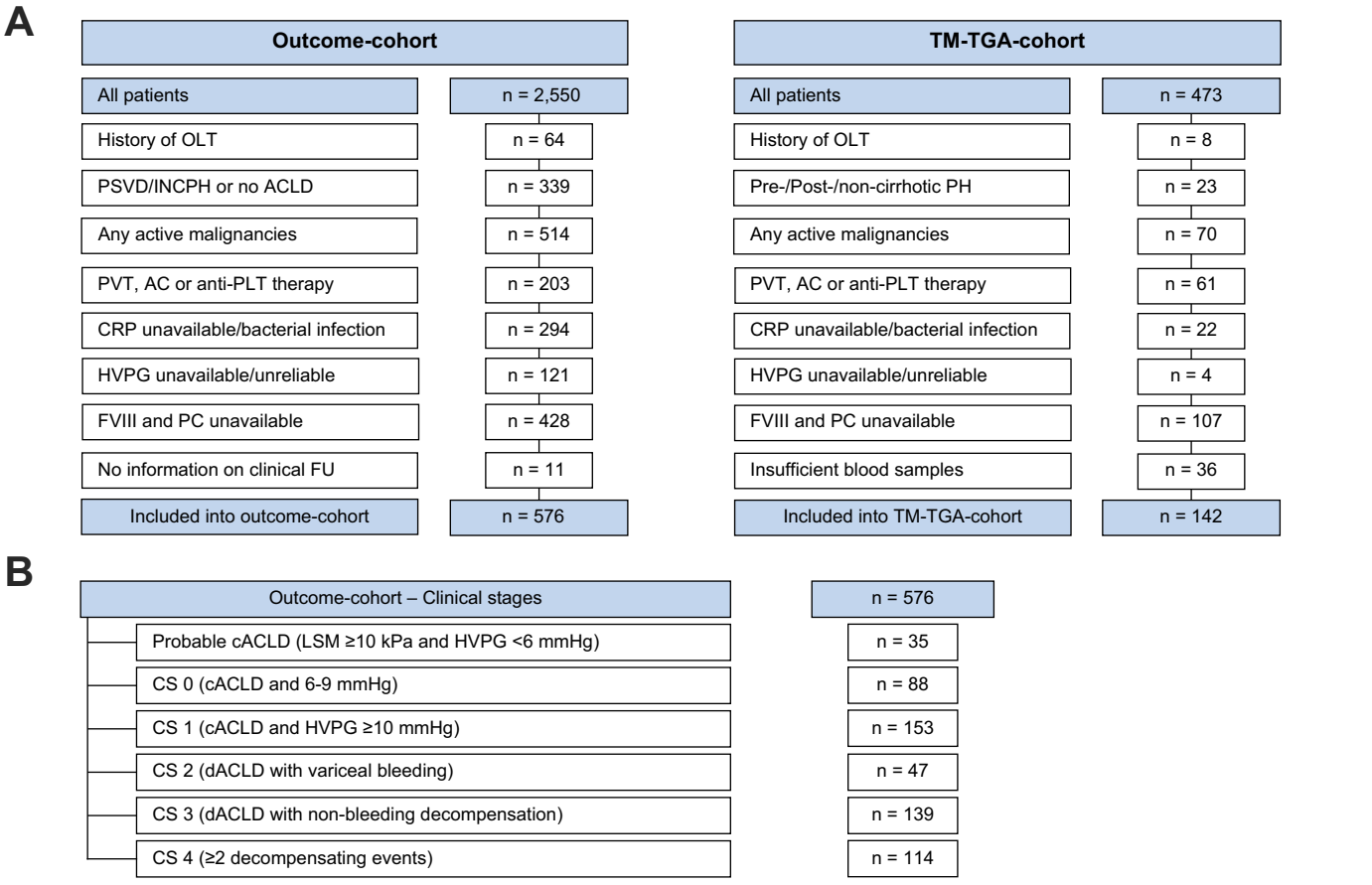


Fig. 1. Patient flowchart and number of patients within different CS in the outcome-cohort. (A) Patient flowchart showing the application of in- and exclusion criteria for the outcome-cohort and the TM-TGA-cohort. (B) Number of patients within different CS in the outcome-cohort. AC, anticoagulant; (c/d) ACLD, (compensated/decompensated) advanced chronic liver disease; CRP, C-reactive protein; CS, clinical stage; FU, follow-up; FVIII, factor VIII; HVPG, hepatic venous pressure gradient; INCPH, idiopathic non-cirrhotic portal hypertension; OLT, orthotopic liver transplantation; PC, protein C; PH, portal hypertension; PLT, platelet; PSVD, porto-sinusoidal vascular disease; PVT, portal vein thrombosis.

Results

Patient selection and characteristics of the outcome-cohort and comparison with the healthy cohort

Overall, 2,550 individual patients underwent HVPG measurement at the Vienna General Hospital during the study period and were considered for inclusion in the outcome-cohort. After applying the in- and exclusion criteria, 576 patients were included in the outcome-cohort (Fig. 1). At baseline, 276 (48%) patients were compensated, while 300 (52%) had already experienced hepatic decompensation, and 15 (3%) presented with ACLF at study inclusion. The number of patients within different CS is shown in the supplementary results. Mean HVPG was 16±7 mmHg, and mean UNOS MELD (2016)-score was 12±5 points.

Median FVIII/PC was 3.4 (IQR 2.5–5.0) in the outcome-cohort compared to 1.3 (IQR 1.0–1.5) in the healthy cohort (p <0.001). More detailed information on patient characteristics is displayed in Tables S1 and S2.

FVIII/PC increases with liver disease severity in the outcome-cohort

As shown in Fig. 2 and Table S3, FVIII/PC consistently increased with liver disease/PH severity.

Univariable and multivariable analyses of factors associated with FVIII/PC in the outcome-cohort

Results of the uni- and multivariable analysis (Tables S6, S7, S8) are provided in the supplementary materials and methods.

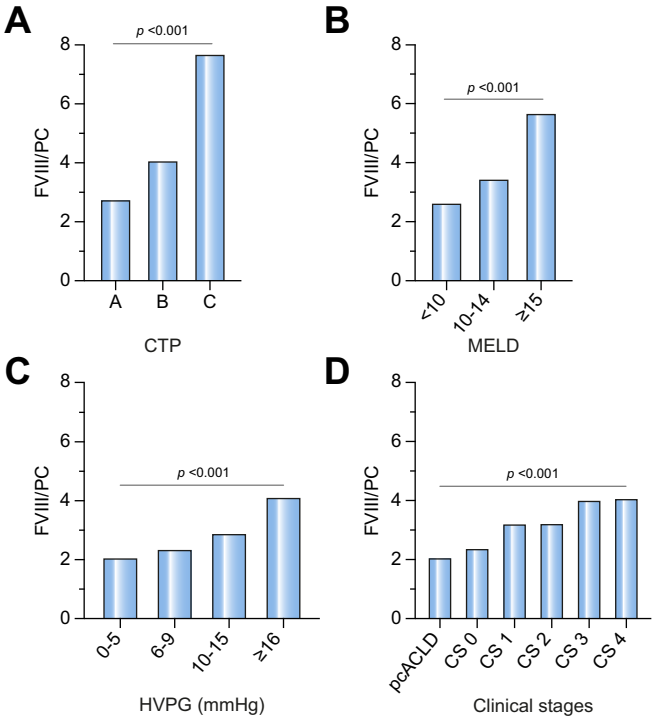


Fig. 2. Comparison of FVIII/PC to disease severity in the outcome-cohort. Comparison according to (A) Child-Pugh, (B) UNOS MELD (2016)-score and (C) HVPG strata as well as (D) clinical stages in the outcome-cohort. CS, clinical stage; FVIII, factor VIII; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease; PC, protein C; UNOS MELD (2016) score, United Network for Organ Sharing model for end-stage liver disease (2016) score.

Impact of FVIII/PC on hepatic decompensation/liver-related death in the outcome-cohort

Median follow-up in the outcome-cohort was 31.8 (IQR 12.0–60.3) months. FVIII/PC not only increased with liver disease severity in cross-sectional analyses but was also longitudinally associated with hepatic decompensation or liver-related death (hazard ratio [HR] 1.11; 95% CI 1.08–1.13; p <0.001). Importantly, using restricted cubic spline analysis, we found that the risk for hepatic decompensation/liver-related death increased almost linearly with increasing FVIII/PC (Fig. S1). Its independent prognostic value was confirmed in 2 multivariable models (model 1: adjusted HR [aHR] 1.06; 95% CI 1.01–1.11; p = 0.013; model 2: aHR 1.08; 95% CI 1.04–1.13; p <0.001), which were adjusted for age, etiology of liver disease, HVPG, and C-reactive protein (CRP) as well as Child-Pugh score, sodium, and serum creatinine levels in model 1 and UNOS MELD (2016)-score, CS and serum albumin levels in model 2 (Table 1). Next, we evaluated the prognostic performance of FVIII/PC for hepatic decompensation/liver-related death and compared it to UNOS MELD (2016)-score and HVPG in time-dependent AUROC analyses for the following time points: 3, 12, 24, 36, 48, and 60 months. Importantly, time-dependent AUROC of FVIII/PC for hepatic decompensation/liver-related death was comparable to those of UNOS MELD (2016)-score at all tested time points as well as to those of HVPG at most time points (Fig. 3). Stratifying the outcome-cohort according to FVIII/PC quartiles (q1: <2.53, q2: 2.53-3.44, q3: 3.44-4.97, q4: ≥4.97) identified patient groups with a distinct prognosis (log-rank p <0.0001; Fig. 4). While the probability of remaining free of hepatic decompensation/liver-related death at 1 year was high in q1 (96.1%) and q2 (88.8%) patients, it significantly decreased in patients with a higher FVIII/PC (q3: 73.9%, q4: 65.8%). These differences were even more pronounced at 5 years (q1: 84.7%, q2: 62.8%, q3: 50.0%, q4: 36.8%; log-rank p <0.0001).

Finally, we aimed at identifying patients with dACLD and a low UNOS MELD (2016)-score (*i.e.*, <15 points), who are at increased risk for further hepatic decompensation/liver-related death, in order to demonstrate the prognostic ability of FVIII/PC in this specific clinical context. In this subgroup, a FVIII/PC above the Youden's index-optimized cut-off for hepatic decompensation/liver-related death (≥3.32) identified patients with a particularly poor prognosis. Rates of further hepatic decompensation/liver-related death were 40% at 1 year and 53% at 2 years in patients with a FVIII/PC ≥3.32 (49.7%), compared to 15% at 1 year and 29% at 2 years in those with a FVIII/PC <3.32 (50.3%; p = 0.0047; Fig. S2).

Impact of FVIII/PC on ACLF development in the outcome-cohort

Among patients with dACLD without ACLF at baseline, 74 (13.2%) patients developed ACLF during follow-up. We performed a univariable Cox regression analysis in patients with dACLD and found that FVIII/PC was predictive of the development of ACLF (HR 1.13; 95% CI 1.05-1.21; p <0.001): this may be explained by its associations with pathophysiological mechanisms predisposing to further decompensation and ACLF development (as demonstrated later). Importantly, FVIII/PC conferred prognostic information regarding ACLF development (aHR 1.10; 95% CI 1.02-1.19; p = 0.015), independently of the CLIF-C ACLF-D-score (Table S9). Time-dependent AUROC values were comparable between FVIII/PC and CLIF-C ACLF-D-score

Table 1. Uni- and multivariable Cox regression analyses of factors associated with hepatic decompensation/liver-related death in the outcome-cohort.

	Univariable		Model 1 (incl. Child-Pugh score, sodium, and creatinine)		Model 2 (incl. MELD, CS, and albumin)	
Patient characteristics	**HR (95% CI)**	***p* value**	**aHR (95% CI)**	***p* value**	**aHR (95% CI)**	***p* value**
Age, year	1.02 (1.01-1.04)	**<0.001**	1.02 (1.01-1.03)	**0.002**	1.03 (1.01-1.04)	**<0.001**
Etiology						
ALD	1		1		1	
Viral	0.26 (0.18-0.36)	**<0.001**	0.69 (0.47-1.02)	0.060	0.77 (0.52-1.14)	0.190
NAFLD	0.62 (0.40-0.98)	**0.041**	1.41 (0.88-2.25)	0.152	1.30 (0.80-2.11)	0.295
Other	0.49 (0.32-0.77)	**0.002**	1.10 (0.69-1.77)	0.684	1.11 (0.70-1.78)	0.654
HVPG, mmHg	1.13 (1.10-1.16)	**<0.001**	1.07 (1.05-1.10)	**<0.001**	1.05 (1.02-1.08)	**0.003**
UNOS MELD (2016) score, point	1.13 (1.10-1.15)	**<0.001**	-	-	1.01 (0.97-1.05)	0.637
Child-Pugh score						
A	1		1		-	-
B	5.43 (3.86-7.63)	**<0.001**	2.29 (1.54-3.40)	**<0.001**	-	-
C	9.32 (6.13-14.15)	**<0.001**	3.14 (1.81-5.46)	**<0.001**	-	-
CS						
Probable cACLD/CS 0	1		-		1	
CS 1	5.08 (1.79-14.47)	**0.002**	-	-	3.00 (1.02-8.83)	**0.046**
CS 2	18.69 (6.23-56.06)	**<0.001**	-	-	7.78 (2.46-24.58)	**<0.001**
CS 3	27.05 (9.85-74.33)	**<0.001**	-	-	9.59 (3.24-28.38)	**<0.001**
CS 4	33.26 (12.14-91.15)	**<0.001**	-	-	12.06 (4.07-35.79)	**<0.001**
Sodium, mmol × L^{-1}	0.87 (0.84-0.89)	**<0.001**	0.93 (0.90-0.97)	**<0.001**	-	-
Creatinine, mg × dl^{-1}	1.51 (1.22-1.87)	**<0.001**	1.54 (1.15-2.07)	**0.004**	-	-
Albumin, g × L^{-1}	0.89 (0.87-0.92)	**<0.001**	-	-	0.98 (0.95-1.02)	0.275
CRP, mg × L^{-1}	2.53 (2.10-3.05)	**<0.001**	1.44 (1.13-1.84)	**0.004**	1.49 (1.16-1.92)	**0.002**
FVIII/PC	1.11 (1.08-1.13)	**<0.001**	1.06 (1.01-1.11)	**0.013**	1.08 (1.04-1.13)	**<0.001**

P values in bold designate values <0.05. ALD, alcohol-related liver disease; cACLD, compensated advanced chronic liver disease; CRP, C-reactive protein; CS, clinical stage; FVIII, factor VIII; (a)HR, adjusted hazard ratio; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease; NAFLD, non-alcoholic fatty liver disease; PC, protein C.

(Fig. S3). Indeed, stratifying patients with dACLD according to a Youden's index-optimized cut-off for ACLF development (≥4.46) discriminated between patients at low *vs*. high risk of developing ACLF during follow-up (Fig. 5).

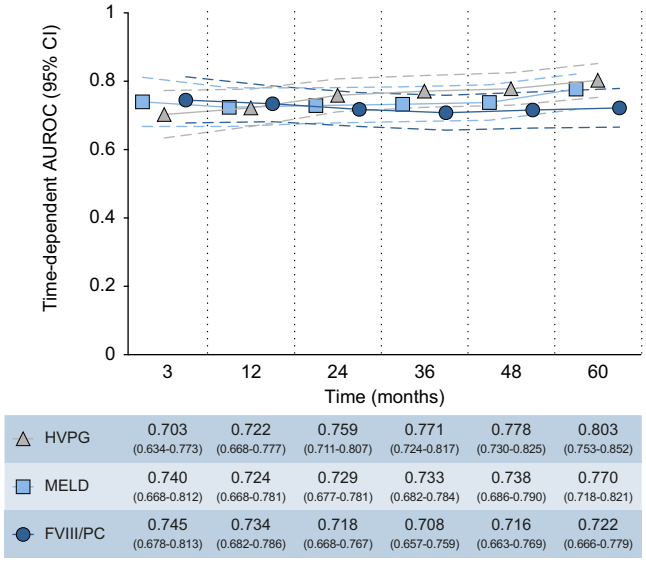


Fig. 3. Time-dependent AUROC analyses assessing the performance of UNOS MELD (2016), HVPG, and FVIII/PC for prognostication of hepatic decompensation/liver-related death in the outcome-cohort. AUROC, area under the receiver-operating characteristic curve; FVIII, factor VIII; HVPG, hepatic venous pressure gradient; PC, protein C; UNOS MELD (2016) score, United Network for Organ Sharing model for end-stage liver disease (2016) score.

Associations between FVIII/PC and biomarkers in the TM-TGA cohort

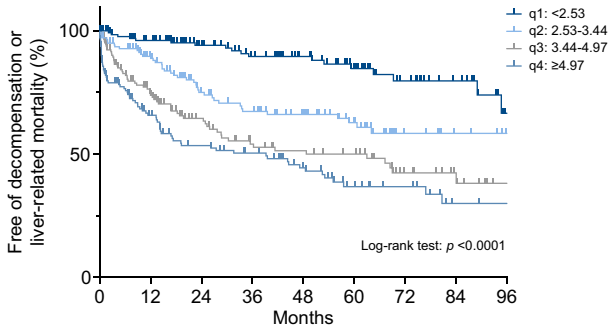
Next, we evaluated the correlation of FVIII/PC as well as FVIII and PC individually with the severity of liver disease (UNOS MELD [2016]-score and HVPG), markers of endothelial dysfunction (VWF), markers of bacterial translocation (LBP) and systemic inflammation (CRP, PCT, and IL-6) as well as liver fibrogenesis/matrix remodeling (ELF test), markers of hyperdynamic circulation/systemic hemodynamic impairment (mean arterial pressure, copeptin, renin, and serum sodium), and serum BA levels.

Importantly, FVIII and PC did not correlate with each other (Spearman's ρ = -0.003; p = 0.972), indicating that both parameters reflect distinct pathophysiological processes.

As demonstrated in Fig. 6, FVIII/PC showed moderate to strong correlations with UNOS MELD (2016)-score (ρ = 0.500; p <0.001), HVPG (ρ = 0.442; p <0.001), VWF (ρ = 0.628; p <0.001), serum sodium (ρ = -0.487; p <0.001), and renin (ρ = 0.450; p <0.001), as well as associations with systemic inflammation: CRP (ρ = 0.230; p = 0.006), IL-6 (ρ = 0.381; p <0.001), and PCT (ρ = 0.257; p = 0.002). Finally, it also strongly correlated with the ELF test as an indicator of fibrogenesis/matrix remodeling (ρ = 0.613; p <0.001) and BAs (ρ = 0.664; p <0.001). Further results on the correlations of FVIII and PC individually as well as correlations of FVIII/PC with these biomarkers among compensated and decompensated patients are reported in the supplementary materials and methods (Fig. S4).

TM-TGA results in the TM-TGA-cohort

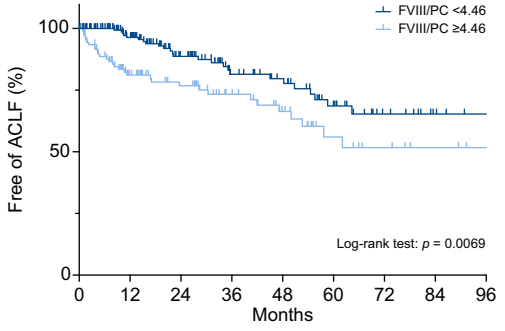
Detailed in- and exclusion criteria as well as patient characteristics of the **TM-TGA-cohort** are shown in Fig. 1 as well as the supplementary materials and methods. Endogenous thrombin potential (ETP) measured by TM-TGA increased with severity of



N° at risk									
FVIII/PC q1	144	114	91	77	62	46	30	19	8
FVIII/PC q2	144	109	73	61	50	35	17	11	7
FVIII/PC q3	144	89	60	43	38	34	18	11	4
FVIII/PC q4	144	74	55	47	35	20	15	7	5

Fig. 4. Probability of remaining free of hepatic decompensation/liver-related death according to FVIII/PC quartiles in the outcome-cohort. FVIII, factor VIII; PC, protein C.

liver disease (p = 0.007) and PH (p = 0.010) and was significantly higher in patients with dACLD (p = 0.026; Table S10). Peak thrombin generation statistically significantly increased with PH severity (p = 0.037) and was also significantly higher in patients with dACLD (p = 0.039). In contrast, we observed no conclusive associations between peak thrombin generation and liver disease severity (Child-Pugh and UNOS MELD [2016]-scores). Interestingly, FVIII/PC showed a weak positive correlation with ETP (Spearman's ρ = 0.255; p = 0.002) and peak thrombin generation (ρ = 0.187; p = 0.027). However, this association disappeared after adjusting for the severity of underlying liver disease, as assessed by Child-Pugh score (model 1): p = 0.332 for ETP and p = 0.167 for peak thrombin generation. Similar results were obtained when adjusting for UNOS MELD (2016) (model 2; p = 0.410 for ETP and p = 0.377 for peak thrombin generation) and HVPG (model 3: p = 0.641 for ETP and p = 0.344 for peak thrombin generation). Detailed results are displayed in Table 2 and 3.



N° at risk									
FVIII/PC <4.46	174	129	79	51	42	27	14	7	4
FVIII/PC ≥4.46	115	67	52	37	25	14	10	7	5

Fig. 5. Kaplan-Meier analysis stratifying patients with dACLD from the outcome-cohort according to FVIII/PC <4.46 *vs.* ≥4.46 (Youden's index-optimized cut-off for ACLF development). ACLF, acute-on-chronic liver failure; dACLD, decompensated advanced chronic liver disease; FVIII, factor VIII; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease; PC, protein C.

Thrombotic and bleeding events during follow-up

Major bleeding events were observed in 44 (7.6%) patients and bleeding episodes were attributed to PH in 35 (6.3%) patients. In the TM-TGA-cohort, major bleeding events occurred in 5 (3.5%) and portal hypertensive bleeding episodes in 4 (2.8%). Thrombotic events were diagnosed in 50 (8.7%) patients in the outcome-cohort and 9 (6.3%) patients in the TM-TGA-cohort (Table S11).

FVIII/PC was neither associated with the incidence of major bleeding nor with the occurrence of thrombotic events during follow-up in the outcome-cohort. Furthermore, TM-TGA results were not associated with the occurrence of these events during follow-up in the TM-TGA-cohort (median follow-up: 10.6 months [IQR 2.7–24.3]; Table S12).

Discussion

Changes in hemostatic parameters are commonly observed in patients with cirrhosis[28] and predict clinical outcomes.[8,29] Kalambokis and colleagues reported that VWF and FVIII levels as well as FVIII/PC were independently linked to the presence of ascites and varices at baseline.[8] This is not surprising, since plasma activity/levels of FVIII and the platelet-adhesive protein VWF (which stabilizes FVIII in the circulation) increase with more advanced liver dysfunction in patients with cirrhosis.[16,30,31] In contrast to FVIII, PC levels are reduced in patients with ACLD and more severe liver disease due to impaired hepatic synthetic function.[32] As a result, and in line with the study by Kalambokis and colleagues,[8] we observed steady increases of the FVIII/PC with disease severity. Moreover, FVIII/PC was also independently associated with hepatic injury (as assessed by aspartate aminotransferase): this may be explained by the association between FVIII and aspartate aminotransferase that has also been observed in a previous study by our group which was based on a partly overlapping patient cohort.[16] Interestingly, FVIII/PC was also associated with components of the metabolic syndrome, *i.e.* arterial hypertension and low HDL levels. However, in patients with ACLD, HDL may also be interpreted as a marker of systemic inflammation.[33]

The prognostic importance of VWF levels for longitudinal outcome prediction has been studied extensively in patients with cirrhosis.[14,34,35] Moreover, PC was independently associated with 3-month mortality in a recent retrospective study in patients with cirrhosis.[36] However, the prognostic importance of FVIII/PC in ACLD has not been sufficiently studied in large, thoroughly characterized patient cohorts. In one study, the prognostic accuracy of FVIII/PC was equal to UNOS MELD (2016)-score for the prediction of mortality, and FVIII/PC cut-offs for the prediction of new-onset ascites, variceal bleeding, and death were introduced.[8] However, this study was performed in a rather small cohort of only 102 patients and did not adjust for CS, PH severity, and systemic inflammation. While Child-Pugh and UNOS MELD (2016)-scores are useful to quantify liver dysfunction,[37,38] CS may better reflect the patients' clinical situation.[39] Moreover, PH and systemic inflammation are the main mechanisms promoting first/further hepatic decompensation and ACLF development.[40,41] Accordingly, this study was unable to evaluate whether the observed association between FVIII/PC and clinical outcomes was confounded by these well-established determinants of disease progression/prognostic factors. In our cohort comprising almost 600 patients, we were able to confirm the prognostic importance of FVIII/PC even in different fully

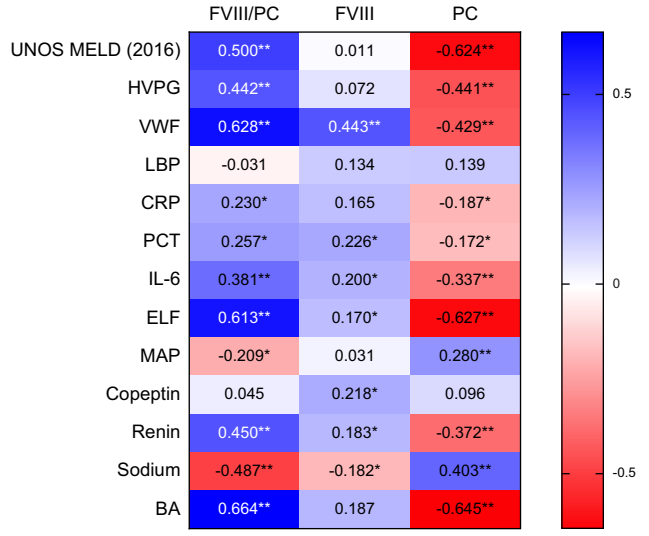


Fig. 6. Correlations of FVIII/PC, FVIII, and PC with key biomarkers in the TM-TGA-cohort. *Indicates *p* values <0.05, whereas **denotes *p* values <0.001. BA, bile acid; CRP, C-reactive protein; ELF, enhanced liver fibrosis; FVIII, factor VIII; HVPG, hepatic venous pressure gradient; LBP, lipopolysaccharide binding protein; MAP, mean arterial pressure; MELD, model for end-stage liver disease; PC, protein C; PCT, procalcitonin; TM-TGA, thrombomodulin-modified thrombin generation assay; UNOS MELD (2016) score, United Network for Organ Sharing model for end-stage liver disease (2016) score; VWF, von Willebrand factor.

adjusted models, considering not only liver disease and PH severity, but also CS and even systemic inflammation. Importantly, in our outcome-cohort, time-dependent AUROC of FVIII/PC – a parameter that can be easily calculated – for hepatic decompensation/liver-related death were as high as those for UNOS MELD (2016)-score at all evaluated time points. Additionally, it was almost as high as those of HVPG, which is possibly the strongest prognostic factor in patients with ACLD, but can only be obtained by invasive, resource-intensive hemodynamic assessments, which require considerable expertise and are not broadly available.[23] Moreover, FVIII/PC quartiles showed a good ability to discriminate between patients with a favorable, intermediate, or a poor prognosis.

In addition, FVIII/PC identified patients with dACLD and a low UNOS MELD (2016)-score (*i.e.*, <15 points) who were at increased risk for further hepatic decompensation/liver-related death. Disease-modifying therapies may be particularly beneficial in these patients, if indicated in the individual clinical context; moreover, timely evaluation for liver transplantation may be considered. The ability of FVIII/PC to improve the predictive ability of MELD on the waiting list deserves further study. However, prognostication/organ allocation on the waiting list for liver transplantation are particularly sensitive topics and our cohort was not ideal for such investigations due to the inclusion of patients without an indication or with contraindications for liver transplantation – accordingly, we abstained from conducting analyses on MELD score improvement.

FVIII/PC was independently associated with the development of ACLF, and its predictive power was equal to that of the CLIF-C ACLF-D-score, the most capable ACLF prediction score which was derived from the PREDICT-study.[27] Of note, the CLIF-C ACLF-D-score was developed in another clinical context (from patients hospitalized for acute decompensation), and thus, its applicability may be limited in our study, indicating that this is not necessarily a fair comparison. We hypothesize that FVIII/PC reflects the risk of (hepatic decompensation/liver-related death and) ACLF development due to its associations with biomarkers of pathophysiological hallmarks of ACLD/pathophysiological mechanisms promoting ACLD progression, as investigated in detail in the TM-TGA-cohort. Indeed, FVIII/PC was not only associated with PH severity,[42] but also with systemic inflammation,[11–13,35] liver fibrogenesis/matrix remodeling,[43] BA levels,[44] and hyperdynamic circulation/systemic hemodynamic impairment.[45] These are important drivers of disease progression in ACLD, *i.e.*, the transition from cACLD to dACLD as well as the development of further decompensation, ACLF, and death.[11–13,35]

Additionally, FVIII/PC has been suggested as a simple marker of the hemostatic equilibrium (which cannot be investigated by routine coagulation tests) as it correlates with the *ex vivo* thrombin generation using a simplified approach (*i.e.*, the Protac®-induced coagulation inhibition – PICI%[6,46]). TM-TGA may be considered the best test to assess the plasmatic coagulation system/hemostatic balance in patients with ACLD.[4] However, even though these assays are well-studied in patients with cirrhosis, they are performed under experimental conditions and have limitations that are inherent to *ex vivo* coagulation tests. Finally, there is still no broad consensus on the amount of TM that needs to be added *in vitro*.[1] Nevertheless, from a clinical perspective, assessing the current hemostatic situation in a patient with cirrhosis would be important, as the rebalanced hemostatic equilibrium may easily tip, thereby eventually contributing to bleeding or thrombosis.[47] Indeed, Kalambokis and colleagues speculated that FVIII/PC identifies patients with a procoagulant imbalance for whom anticoagulation may be particularly beneficial.[8] The authors substantiated their hypothesis by demonstrating an association between higher FVIII/PC values and the incidence of PVT.[29] However, other studies did not confirm this association[48] and some concerns regarding the biological plausibility of ratios such as FVIII/PC have been raised.[10] Therefore, we evaluated the correlation of FVIII/PC and ETP/peak thrombin generation by TM-TGA in the TM-TGA-cohort which comprised 142 patients with ACLD and comparable characteristics. While FVIII/PC showed a positive correlation with both parameters in unadjusted analysis, these associations disappeared after adjusting for the severity of underlying liver disease (Child-Pugh or UNOS MELD [2016]-scores) as well as PH (HVPG) in multivariable regression analysis. Therefore, FVIII/PC reflects the severity of underlying liver disease, rather than being an indicator of procoagulant imbalance on an individual patient level.

Finally, direct clinical endpoints were assessed to substantiate this claim. FVIII/PC was associated with neither thrombotic nor major bleeding events. Not unexpectedly, TGA results were also

Table 2. Correlations between FVIII/PC and TM-TGA parameters.

Unadjusted correlations	ρ	*p* value
FVIII/PC – ETP	0.255	**0.002**
FVIII/PC - peak thrombin generation	0.187	**0.027**
FVIII/PC – velocity index	0.163	0.055
FVIII/PC – lag time	−0.081	0.346

P values in bold designate values <0.05. ETP, endogenous thrombin potential; FVIII, factor VIII; PC, protein C; TM-TGA, thrombomodulin-modified thrombin generation assay.

Table 3. Multiple linear regression analyses evaluating the association between FVIII/PC and TM-TGA parameters, while adjusting for Child-Pugh score (model 1), UNOS MELD (2016)-score (model 2), or PH severity (model 3) in the TM-TGA-cohort.

	ETP		Peak thrombin generation		Velocity index		Lag time	
Adjusted correlations	**B**	***p* value**	**B**	***p* value**	**B**	***p* value**	**B**	***p* value**
Model 1								
FVIII/PC	−7.103	0.332	−1.870	0.167	−1.331	0.134	0.025	0.094
Child-Pugh score, point	28.424	0.210	5.243	0.210	4.575	0.096	−0.100	0.028
Model 2								
FVIII/PC	−6.215	0.410	−1.233	0.377	−0.867	0.345	0.011	0.480
UNOS MELD (2016) score, point	8.479	0.342	0.628	0.702	0.729	0.501	−0.009	0.623
Model 3								
FVIII/PC	−2.782	0.641	−1.048	0.344	−0.570	0.436	0.008	0.523
HVPG, mmHg	11.819	**0.023**	1.840	0.055	1.006	0.113	−0.020	0.055

P values in bold designate values <0.05. ETP, endogenous thrombin potential; FVIII, factor VIII; PC, protein C; TM-TGA, thrombomodulin-modified thrombin generation assay; UNOS MELD (2016) score, United Network for Organ Sharing model for end-stage liver disease (2016) score.

not associated with the occurrence of thrombotic events and major bleeding episodes. The latter finding has repeatedly been reported in the literature, as spontaneous bleeding events in patients with cirrhosis are rare and do not seem to be related to the coagulatory balance, but rather occur due to PH or intercurrent conditions such as renal failure and infections.[49] Accordingly, the majority of bleeding episodes observed in our study were considered to be associated with PH. Similarly, procedure-related bleeding events are rare, and they are more likely attributed to local factors than coagulation.[49,50] A recent large prospective study evaluating factors associated with the development of PVT (the most common thrombotic event in patients with ACLD) did not observe any association between acquired or inherited hemostatic disorders (including FVIII/PC) and the development of PVT, after adjusting for liver disease severity and portal blood flow velocity.[51] For TM-TGA, we would like to abstain from drawing firm conclusions based on our data, as sample size/follow-up duration in the TM-TGA cohort were considerably shorter, which led to a very low number of events, and thus, insufficient statistical power. However, it seems implausible that any coagulation test performed at baseline can predict the incidence of bleeding or thrombotic events in the long-term, independently of the severity of underlying liver disease and related factors. Finally, it still remains to be established whether procoagulant imbalance (regardless of the method used to detect it) is a cause or a consequence of liver disease progression.[9] Our study cannot answer this question; however, it provides important evidence against the misuse/misinterpretation of FVIII/PC as a marker of the coagulatory balance in this context.

Our study has several limitations: First of all, TM-TGAs were not assessed in all patients. However, severity of liver disease/PH, as expressed by UNOS MELD (2016) and Child-Pugh scores as well as HVPG, and FVIII/PC were comparable between the 2 cohorts and, therefore, we believe that results of the TM-TGA-cohort can be extrapolated to the outcome-cohort. Furthermore, a considerable number of patients had to be excluded due to missing FVIII/PC values, which is explained by the fact that laboratory workup in patients undergoing HVPG measurement changed over time. However, we compared baseline characteristics of patients excluded due to missing FVIII and/or PC to patients who were included and did not find any clinically meaningful differences (data not shown), suggesting that missing values are non-informative. Furthermore, due to the retrospective design of the study, we cannot exclude that some hepatic decompensation events may have been missed. However, we have thoroughly reviewed electronic health records of the Vienna hospital association and also nation-wide electronic health records (for the more recent years). In addition, we have also performed searches of the liver transplant database of our institution (*i.e.*, the only transplant center in eastern Austria) and examined the nation-wide death registry. Finally, our study largely relies on regression modelling, and we have to acknowledge its inherent limitations. Even though we attempted to avoid redundancy when selecting variables and additionally checked for multicollinearity in multiple linear regression models, we cannot exclude residual multicollinearity. Additionally, we were unable to consider several potentially important prognostic indicators (*e.g.*, control of the primary etiological factor and co-factors as well as sarcopenia/frailty), as they have not been recorded systematically. Nevertheless, patients included in our study were extensively characterized in terms of PH severity, prognostic scores, CS and routine laboratory parameters including markers of systemic inflammation – importantly, all of these aspects have been considered in our analyses.

In conclusion, FVIII/PC increases across the CS of ACLD as well as with severity of hepatic dysfunction and of PH. Even after fully adjusting for these and other established prognostic factors, FVIII/PC remained a robust prognostic indicator for hepatic decompensation or liver-related death and conferred CLIF-C ACLF-D-score-independent prognostic information towards ACLF development in patients with dACLD. This may be explained by the link between FVIII/PC and pathophysiological mechanisms promoting ACLD progression. Therefore, FVIII/PC should be further evaluated as a prognostic parameter in a prospective cohort of patients with ACLD. Importantly, the prognostic relevance of FVIII/PC should not be mistaken as evidence for the concept of procoagulant imbalance as a driver of disease progression, as the correlation between FVIII/PC and TM-TGA is only a result of confounding by liver disease severity and FVIII/PC was not predictive of thrombotic events. Thus, FVIII/PC does not reflect the hemostatic balance.

Abbreviations

AC, anticoagulation; ACLD, advanced chronic liver disease; ACLF, acute-on-chronic liver failure; aHR, adjusted hazard ratio; AUROC, area under the receiver-operating characteristic curve; BAs, bile acids; cACLD, compensated advanced chronic liver disease; CLIF-C ACLF-D, chronic liver failure-consortium acute-on-chronic liver failure-development score; CRP, C-reactive protein; CS, clinical stage; dACLD –decompensated advanced chronic liver disease; ELF, enhanced liver fibrosis, ETP, endogenous thrombin potential; FU, follow-up; FVIII, factor VIII; HE, hepatic encephalopathy; HIV, human immunodeficiency virus; HR, hazard ratio; HVPG, hepatic venous pressure gradient; IL-6, interleukin 6; IQR, interquartile range; LBP, lipopolysaccharide binding protein; LSM, liver stiffness measurement; PC, protein C; PCT, procalcitonin; PH, portal hypertension; PLT, platelet count; PVT, portal vein thrombosis; TM-TGA, thrombomodulin-modified thrombin generation assay; UNOS MELD (2016) score, United Network for Organ Sharing model for end-stage liver disease (2016) score; VWF, von Willebrand factor antigen; VWF, von Willebrand factor.

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Conflicts of interest

The authors have nothing to disclose regarding the work under consideration for publication. Conflicts of interests outside the submitted work: L.B., R.J.N., J.W., R.P., L.H., M.J., A.F.S., G.S., P.Q., and T.L. have nothing to disclose. Be.Sc. received travel support from AbbVie, Ipsen and Gilead. Be.Si. received travel support from AbbVie and Gilead. D.B. received travel support from AbbVie and Gilead and speaker fees from AbbVie. M.P. served as a speaker and/or consultant and/or advisory board member for Bayer, Bristol-Myers Squibb, Eisai, Ipsen, Lilly, MSD, and Roche and received travel support from Bayer and Bristol-Myers Squibb. C.A. received honoraria for lectures and advisory boards from Bayer, Boehringer-Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, and Pfizer. M.T. received grant support from Albireo, Alnylam, Cymabay, Falk, Gilead, Intercept, MSD, Takeda, and UltraGenyx, honoraria for consulting from Albireo, Boehringer-Ingelheim, BiomX, Falk, Genfit, Gilead, Intercept, Janssen, MSD, Novartis, Phenex, Regulus, and Shire, speaker fees from Bristol-Myers Squibb, Falk, Gilead, Intercept, and MSD, as well as travel support from AbbVie, Falk, Gilead, and Intercept. T.R. received grant support from AbbVie, Boehringer-Ingelheim, Gilead, Intercept, MSD, Myr Pharmaceuticals, Philips Healthcare, Pliant, Siemens, and W. L. Gore & Associates; speaking honoraria from AbbVie, Gilead, Gore, Intercept, Roche, and MSD; consulting/advisory board fees from AbbVie, Bayer, Boehringer-Ingelheim, Gilead, Intercept, MSD, and Siemens; and travel support from AbbVie, Boehringer-Ingelheim, Gilead, and Roche. M.M. served as a speaker and/or consultant and/or advisory board member for AbbVie, Bristol-Myers Squibb, Collective Acumen, Gilead, and W. L. Gore & Associates and received travel support from AbbVie, Bristol-Myers Squibb, and Gilead.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Conception and design of the study (Be.Sc., L.B., T.L., and M.M.) as well as acquisition (Be.Sc., L.B., R.J.N., J.W., R.P., Be.Si., L.H., M.J., B.D., A.F.S., G.S., M.P., C.A., P.Q., T.R., T.L., and M.M.), analysis (Be.Sc., L.B., G.S., and M.M.), and interpretation of data (all authors). Be.Sc., L.B., T.L., and M.M. drafted the manuscript, which was revised for important intellectual content and approved by all authors.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.12.038.

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Author names in bold designate shared co-first authorship

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