Research Article

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von Willebrand factor and procoagulant imbalance predict outcome in patients with cirrhosis and thrombocytopenia

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Background & Aims: Several lines of evidence suggest that the hemostatic disorders of cirrhosis may have a significant clinical impact. We investigated the independent predictive value of components of the hemostatic system on the occurrence of ascites, variceal bleeding (VB), and survival.

Methods: One hundred and two patients with thrombocytopenia (Child-Pugh class A/B/C: 34/34/34) were enrolled. Platelet counts, factors (F) II, V, VII, and VIII, antithrombin, protein C (PC), FVIII-to-PC ratio as an index of procoagulant imbalance, von Willebrand factor antigen (vWF-Ag), and model for end-stage liver disease (MELD) were evaluated. Two multivariate analyses were performed: one excluding (model 1) and one including MELD (model 2).

Results: Higher vWF-Ag levels and FVIII-to-PC ratios were the most prominent hemostatic disorders in patients with cirrhosis. Increased levels of vWF-Ag and FVIII, and higher FVIII-to-PC ratios independently predicted the presence of ascites and varices at baseline. Independent predictors of ascites and VB during follow-up were vWF-Ag (model 1/2: $p = 0.001/p = 0.009$ and $p = 0.008/p = 0.01$, respectively) and FVIII-to-PC ratio (model 1/2: $p = 0.003/p = 0.02$ and $p = 0.01/p = 0.03$, respectively). vWF-Ag (model 1/2: $p = 0.007/p = 0.002$), FVIII-to-PC ratio (model 1/2: $p = 0.001/p = 0.01$), and MELD ($p = 0.02$) independently predicted mortality. Patient groups with significantly higher probability of new-onset ascites, VB, and mortality were identified by certain cut-offs of vWF-Ag (213%, 466%, and 321%, respectively) and FVIII-to-PC ratio (1.99, 3.29, and 2.36, respectively). vWF-Ag and FVIII-to-PC ratio equaled MELD in mortality prediction.

Conclusions: Advanced cirrhosis is characterized by increased thrombotic potential. vWF-Ag and FVIII-to-PC ratio independently predict new-onset ascites, VB, and mortality. Targeting hypercoagulability could improve the outcome of patients with cirrhosis.

Lay summary: Higher von Willebrand factor antigen (vWF-Ag) levels and factor VIII-to-protein C (FVIII-to-PC) ratio are the prominent hemostatic disorders in patients with cirrhosis. vWF-Ag and FVIII-to-PC ratio independently predict new-onset ascites, variceal bleeding, and mortality in these patients.

Introduction

Low platelet counts and decreased levels of most procoagulant factors have been recognized for many years as the main hemostatic abnormalities in patients with cirrhosis. Depressed synthesis of extrinsic pathway factors, particularly factor VII, accounts for prolongation of prothrombin time in cirrhosis. The combination of thrombocytopenia with a prolonged prothrombin time or its derivative, the international normalized ratio, has long been suggestive of a hypocoagulable state, which predisposes patients with cirrhosis to increased bleeding risk [1]. However, the coagulation disorder as measured by routine laboratory tests does not appear to fully reflect the underlying hemostatic changes [2]. Indeed, abnormal coagulation tests in cirrhosis neither are predictive of bleeding complications [3] nor protect from venous thromboembolism [4–6]. Further, recent data documented that international normalized ratio is not itself a significant predictor of decompensation and mortality in patients with cirrhosis [7,8] despite the fact that it is included in common prognostic indices (Child-Pugh, model for end-stage liver disease [MELD]) [9,10].

Laboratory experiments performed in the past decade have demonstrated that the hemostatic system in patients with cirrhosis may not actually be in a hypocoagulable state. First, defects in platelet number are accompanied by substantially elevated levels of the platelet adhesive protein von Willebrand factor (vWF) [11,12]. The elevated levels of vWF in cirrhosis may be a consequence of endothelial perturbation taking into account that vWF antigen (vWF-Ag) is released by activated endothelial cells

Keywords: von Willebrand factor antigen; Procoagulant imbalance; Cirrhosis; Decompensation; Mortality.

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Abbreviations: MELD, Model for End-stage Liver Disease; vWF-Ag, von Willebrand factor antigen; PC, protein C; AT, antithrombin; FVIII, factor VIII; PH, portal hypertension; VB, variceal bleeding; AUC, area under the curve.

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[13]. The induction of vWF-Ag synthesis in the cirrhotic liver is another possibility as vWF immunostaining is negative in the sinusoidal endothelial cells in the normal liver [14] but positive in cirrhotic liver tissues [15,16], presumably due to capillarization of the hepatic sinusoids [17]. Second, the prolongation of the coagulation times reflects the activity of only a portion of procoagulant factors and does not consider the concomitant decrease in anticoagulant factors [3]. It is now well-established that, despite the decreased levels of most coagulation factors, *in vitro* thrombin generation is maintained in patients with cirrhosis in the presence of thrombomodulin [18] or Protac® [19,20]. Even more counterintuitive is the increasing evidence that plasma from patients with cirrhosis may possess a procoagulant *vs.* anticoagulant imbalance, detected by thrombin generation tests, which appears to be related to two combined abnormalities: reduced levels of the most powerful naturally occurring anticoagulants antithrombin (AT) and protein C (PC) and increased levels of the most powerful procoagulant factor VIII (FVIII) [21]. Anticoagulant proteins decrease with liver disease severity due to impaired synthesis [1] while the considerable increase in FVIII levels in advanced cirrhosis has been attributed to decreased clearance from the circulation [22]. Since activated factor FVIII is inhibited *in vivo* by activated PC, the ratio of FVIII-to-PC is currently considered as an index of procoagulant imbalance [21].

From a clinical point of view, we have recently demonstrated a significant association of increased levels of thrombin-antithrombin complexes, as a marker of thrombotic potential in cirrhosis, with portal hypertension (PH)-related events, such as new-onset ascites and variceal bleeding (VB), and survival [7]. In addition, it was recently reported that prophylaxis with enoxaparin in patients with advanced cirrhosis reduced the risk of decompensation and improved survival without bleeding complications [8]. Further, vWF-Ag levels were shown to correlate with the degree of PH, as measured by hepatic venous pressure gradient, and predicted survival free of PH-related events and liver transplantation [23,24]. On the other hand, no data exist on the association of procoagulant imbalance with certain clinical endpoints in patients with advanced cirrhosis.

The aims of the present study were to investigate the impact and independent predictive value of major components of the hemostatic profile of cirrhosis, including platelet count, factors related to pro-and anticoagulant activity, FVIII-to-PC ratio, and vWF-Ag on the occurrence of major decompensating events, such as ascites and VB, and survival in patients with cirrhosis.

Patients and methods

Patients

One hundred and two adult patients with cirrhosis and thrombocytopenia defined by platelet count $<150 \times 10^9$/L [25] referred to the outpatient clinics between September 2010 and May 2013 were recruited after approval of the institutional review board and informed consent. Liver cirrhosis was proven either histologically or by unequivocal clinical and radiological findings. Severity of cirrhosis was evaluated by Pugh's modification of the Child classification [9] and MELD [10]. The patients were prospectively enrolled for each class of Child-Pugh until an equal distribution of severity of liver disease was attained. Criteria for exclusion at the time of blood sampling were: a) previously or ongoing use of therapy known to interfere with blood coagulation and platelet function (including concentrates of coagulation factors and fresh frozen plasma), b) known hemostatic disorders other than cirrhosis, c) portal, splenomesenteric or peripheral vein thrombosis by ultrasound evaluation and angio-computed tomography, d) portal hypertensive bleeding for at least 3 months before enrollment, e) ongoing bacterial infection, f) hepatocellular carcinoma or other intrahepatic or extrahepatic malignancy, g) abstinence from alcohol for less than 6 months, h) diabetes mellitus, i) hypertension, k) serum creatinine levels >1.5 mg/dl. All our patients abstained form alcohol throughout their follow-up and treated with indicated prophylactic as well therapeutic regimens. Patients were followed prospectively at least every 3 months at the outpatient clinic. PH-related events, such as ascites and varices at baseline, development of new-onset ascites and first episode of VB in patients with no history of VB during follow-up, portal vein thrombosis, hepatocellular carcinoma, liver transplantation, and death were recorded. As portal vein thrombosis is *per se* associated with development of ascites [26] and VB [6], only cases of new-onset ascites and VB not related to this complication were evaluated. Primary prophylaxis for VB in patients with varices on entry included propranolol, endoscopic band ligation or combination of the two. For survival analysis, the censoring was performed not only for death but also for hepatocellular carcinoma diagnosis and transplantation.

Methods

Blood collection and plasma preparation

Fasting whole blood samples (15 ml) were drawn from each patient at enrollment by clear venipuncture and collected in vacuum tubes (Becton and Dickinson, Meylan, France) containing 109 mmol/L trisodium citrate as anticoagulant in the proportion of 1:9 parts of anticoagulant/blood. Platelet-poor plasma was prepared by double centrifugation at 2000 g for 10 min. Plasma was aliquoted in plastic tubes, snap-frozen and stored at −80 °C to allow batch analysis in a blinded fashion. All measurements were performed on the same day and after all patients had been enrolled.

Procoagulant factors

Factors II, V, VII, and VIII activities were measured using commercially available reagents on British comparative thromboplastin (BCT) (Siemens, Marburg, Germany) and the results were expressed as percentage of normal pooled plasma arbitrarily set at 100% of normal. FVIII activity was measured by a one stage assay using Dade®Actin® FSL activated partial thromboplastin time reagent (Siemens Healthcare Diagnostics Inc. Newark USA).

Anticoagulant factors

Antithrombin (AT) and protein C (PC) activities were detected using the Berichrom AT and PC kits, respectively (Siemens Healthcare Diagnostics Inc. Newark USA) and the results were expressed as percentage of normal pooled plasma arbitrarily set at 100% of normal.

Procoagulance imbalance index

The ratio of factor VIII (FVIII)-to-PC was taken as an index of the procoagulant imbalance (the greater the ratio the higher the procoagulant imbalance).

vWF-Ag assay

vWF-Ag levels were determined by a turbidimetric assay on a Siemens BCS XP system applying appropriate reagents. Normal ranges of vWF-Ag were 70–120%.

Other routine laboratory tests

Other laboratory examinations, including platelet count, international normalized ratio, serum creatinine, and liver function tests were done as part of routine patient care.

Statistical analysis

Statistical analyses were performed using the SPSS 19.0 statistical package (SPSS Inc., Chicago, IL). Continuous variables are expressed as means ± standard deviation and compared by the unpaired Student's *t* test. Univariate linear regression analysis was performed to evaluate the effect of liver disease severity on coagulation measurements and to identify a relation of coagulation measurements with PH-related events and survival. The following variables at baseline were considered for univariate analysis: platelet count, factors II, V, VII, and VIII, AT, PC, FVIII-to-PC ratio, vWF-Ag, and MELD score. Continuous variables showing significance in univariate analyses were used in a multivariate logistic regression analysis (forward stepwise method) to assess the independence of predictive factors. Two multivariate models were employed: one including (model 1) and one excluding (model 2) MELD score. Receiver operator characteristic curves were used to define the cut-off points for vWF-Ag and FVIII-to-PC ratio as predictors of new-onset ascites, VB, and death. The value with the best sensitivity and

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specificity in area under the curve (AUC) analysis (Youden's Index) was chosen for further analyses. Receiver operator characteristic curves were also created for the assessment of the predictive value of MELD score for mortality. AUCs were compared using Hanely and McNeil's approach. Cox's multivariate proportional hazard models were applied to identify risk factors for new-onset ascites, VB and death. The results of Cox's models are presented as the hazard ratio and 95% confidence intervals. A univariate analysis was first performed and variables significant in univariate analysis were evaluated in the multivariate models 1 and 2 (as described above). The cumulative probabilities for new-onset ascites, VB and survival were calculated using the Kaplan-Meier method and differences assessed by the log-rank test. All p values reported are two-sided, and p values <0.05 were considered statistically significant.

Results

Patient characteristics

One hundred and two (81 male and 21 female; mean age 56.6 ± 7 years; mean MELD score 12 ± 6) were studied. Cirrhosis was related to alcohol (n = 71), viral hepatitis (n = 18), other causes (n = 7) or was cryptogenic (n = 6). The mean follow-up time was 27.2 months (median time 28 months, range: 6–53 months). Fourteen patients (13.7%) developed portal vein thrombosis during follow-up. Three patients underwent liver transplantation (2.9%).

Coagulation parameters and liver disease severity

In univariate analysis, lower levels of factors II, V, and VII, AT, and PC, increased levels of FVIII and vWF-Ag, and higher FVIII-to-PC ratios were associated significantly with liver disease severity as measured according to Child-Pugh class (Table 1). In multivariate linear regression, only vWF-Ag ($p = 0.001$), FVIII-to-PC ratio ($p = 0.01$), and FVIII ($p = 0.04$) were independently related to liver disease severity.

Coagulation parameters and PH-related events at baseline

Fifty-eight (56.8%) and 55 (53.9%) patients had ascites and varices, respectively, at the time of enrollment. Independent associations with ascites were noted for vWF-Ag ($p = 0.001$), FVIII ($p = 0.003$), and FVIII-to-PC ratio ($p = 0.008$) in multivariate model 1; vWF-Ag ($p = 0.004$), FVIII-to-PC ratio ($p = 0.01$), and MELD score ($p = 0.02$) were independently associated with ascites in model 2 (Table 2). In multivariate models 1 and 2, the presence of varices at baseline was independently associated with vWF-Ag ($p = 0.002$ and $p = 0.009$, respectively), FVIII ($p = 0.01$ and $p = 0.02$, respectively), and FVIII/PC ratio ($p = 0.004$ and $p = 0.01$, respectively) (Table 2).

Coagulation parameters and occurrence of ascites during follow-up

Sixteen patients without ascites at baseline (36.3%) developed ascites during follow-up. Cox regression analysis model 1 showed that vWF-Ag ($p = 0.001$) and FVIII-to-PC ratio ($p = 0.003$) were associated independently with a higher risk of new-onset ascites; vWF-Ag ($p = 0.009$) and FVIII-to-PC ratio ($p = 0.02$) were also the most important predictive factors for developing ascites in multivariate model 2 (Table 3). The actuarial probability of developing ascites was significantly higher for patients with vWF levels more than a cut-off value of 213% (sensitivity: 84.7% and specificity: 75.8% for prediction of new-onset ascites) (Fig. 1A) and for those with FVIII-to-PC ratios higher than 1.99 (sensitivity: 82.8% and specificity: 70.5% for prediction of new-onset ascites) (Fig. 1B).

Coagulation parameters and occurrence of VB during follow-up

VB occurred in 15 (27.2%) of 55 patients who had varices at the time of inclusion in the study. In multivariate Cox regression analysis models 1 and 2, vWF-Ag ($p = 0.008$ and $p = 0.01$, respectively) and FVIII-to-PC ratio ($p = 0.01$ and $p = 0.03$, respectively) were the only independent factors related to VB (Table 3). Kaplan-Meier curve analysis revealed a significantly higher probability of VB for patients with vWF-Ag levels above a cut-off of 466% (sensitivity: 87.2% and specificity: 76.5% for prediction of VB) (Fig. 1C) and for those with FVIII-to-PC ratios higher than 3.29 (sensitivity: 83.2% and specificity: 74.9% for prediction of VB) (Fig. 1D).

Coagulation parameters and survival

Overall, 35 (34.3%) patients died during follow-up. Causes of death were sepsis (11 patients), progressive liver failure (14 patients), hepatorenal syndrome (4 patients), VB (2 patients), and hepatocellular carcinoma (4 patients). vWF-Ag ($p = 0.007$) and FVIII-to-PC ratio ($p = 0.001$) in Cox regression analysis model 1, and vWF-Ag ($p = 0.002$), FVIII-to-PC ratio ($p = 0.01$), and MELD

Table 1. Coagulation parameters according to Child-Pugh classification.

	Child-Pugh class			*p* value*	*p* value**
	A (n = 34)	B (n = 34)	C (n = 34)		
Platelet count (x10⁹/L)	101 ± 33	90 ± 38	78 ± 19	0.07	
Factor II (%)	71.1 ± 40.2	55.8 ± 28.3	39.1 ± 21.3	<0.001	0.2
Factor V (%)	79.8 ± 31.9	58.5 ± 22.6	50.8 ± 24.1	<0.001	0.5
Factor VII (%)	74.9 ± 24.4	57.8 ± 22.7	43.7 ± 15.9	<0.001	0.1
Factor VIII (%)	140 ± 30.8	156 ± 33.3	190 ± 26.2	<0.001	0.04
Antithrombin (%)	76 ± 23.6	61.1 ± 16.5	40.9 ± 12.8	0.005	0.07
Protein C (%)	66.1 ± 23.2	47.8 ± 22.7	36.9 ± 15.4	0.005	0.08
Factor VIII-to-protein C ratio	1.98 ± 0.78	3.44 ± 1.17	4.47 ± 1.48	<0.001	0.01
Von Willebrand factor-antigen (%)	194 ± 49.3	325 ± 119	468 ± 117	<0.001	0.001

*Univariate analysis; **multivariate analysis. The values are expressed as mean ± standard deviation.

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Table 2. Coagulation parameters and MELD score according to the presence of ascites.

Ascites	No (n = 44)	Yes (n = 58)	*p* value*	*p* value**	*p* value***
Platelet count (x10^9/L)	95 ± 32	87 ± 26	0.1		
Factor II (%)	70.9 ± 39.3	43.1 ± 21.1	<0.001	0.1	0.3
Factor V (%)	75.3 ± 31.6	53.6 ± 22.9	0.001	0.1	0.9
Factor VII (%)	69.2 ± 18.9	48.6 ± 22	0.001	0.1	0.3
Factor VIII (%)	144 ± 34.2	176 ± 31.7	<0.001	0.003	0.4
Antithrombin (%)	67.1 ± 21.4	51.1 ± 15.8	0.01	0.3	0.5
Protein C (%)	57.5 ± 32.5	43.1 ± 22.2	0.03	0.1	0.2
Factor VIII-to-protein C ratio	2.48 ± 0.85	4.13 ± 1.48	<0.001	0.008	0.01
von Willebrand factor antigen (%)	221 ± 82.9	413 ± 138	<0.001	0.001	0.004
MELD score	9 ± 4	14 ± 5	<0.001		0.02
Varices	No (n = 47)	Yes (n = 55)			
Platelet count (x10^9/L)	91 ± 36	81 ± 22	0.09		
Factor II (%)	63.1 ± 36.2	48.3 ± 29.3	0.05		
Factor V (%)	71.2 ± 29.5	55.8 ± 26.9	0.01	0.5	0.8
Factor VII (%)	67.7 ± 21.1	50.5 ± 17.2	0.01	0.4	0.5
Factor VIII (%)	142 ± 31.3	180 ± 31.1	0.001	0.01	0.02
Antithrombin (%)	65.1 ± 26.6	46.8 ± 17.5	0.009	0.1	0.1
Protein C (%)	60.6 ± 27.9	39.3 ± 15.6	0.01	0.2	0.3
Factor VIII-to-protein C ratio	2.35 ± 1.18	4.58 ± 1.37	<0.001	0.004	0.01
von Willebrand factor antigen (%)	239 ± 98.8	411 ± 143	<0.001	0.002	0.009
MELD score	9 ± 3	14 ± 6	<0.001		0.05

*Univariate analysis; **multivariate analysis not including MELD; ***multivariate analysis including MELD. The values are expressed as mean ± standard deviation.
MELD, model for end-stage liver disease.

Table 3. Univariate and multivariate analysis of the baseline risk factors for new-onset ascites and variceal bleeding.

	Univariate analysis		Multivariate analysis*		Multivariate analysis**	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
New-onset ascites						
Platelet count	0.936 (0.902-0.986)	0.1				
Factor II	0.978 (0.958-0.999)	0.03	0.996 (0.970-1.023)	0.7		
Factor V	0.984 (0.961-1.007)	0.1				
Factor VII	0.962 (0.943-1002)	0.07				
Factor VIII	1.012 (0.995-1028)	0.1				
Antithrombin	0.963 (0.941-1.014)	0.04	0.975 (0.926-1022)	0.2	1009 (0.971-1034)	0.4
Protein C	0.972 (0.935-0.992)	0.1				
Factor VIII-to-protein C ratio	5.080 (2.073-12.450)	<0.001	2.944 (1.095-7.914)	0.003	2.256 (1014-6.349)	0.02
vWF-Ag (%)	1.012 (1.007-1.018)	<0.001	1.008 (1.001-1.015)	0.001	1.007 (1.001-1.014)	0.009
MELD score	1.123 (0.923-1.366)	0.01			1.103 (0.885-1.376)	0.3
Variceal bleeding						
Platelet count	0.973 (0.952-1001)	0.09				
Factor II	0.985 (0.961-1.010)	0.2				
Factor V	0.994 (0.970-1.018)	0.6				
Factor VII	0.985 (0.970-1006)	0.09				
Factor VIII	1.028 (1.007-1.050)	0.009	1.011 (0.982-1.041)	0.4	1.015 (0.988-1.043)	0.2
Antithrombin	0.998 (0.975-1.021)	0.02	0.985 (0.963-1015)	0.1	0.973 (0.955-1006)	0.2
Protein C	0.955 (0.921-0.989)	0.08				
Factor VIII-to-protein C ratio	2.192 (1.470-3.269)	<0.001	1.575 (0.990-2.504)	0.01	1.281 (0.944-1.747)	0.03
vWF-Ag	1.011 (1.005-1.016)	<0.001	1.008 (1002-1014)	0.008	1.006 (1.001-1.010)	0.01
MELD score	1.105 (0.969-1.260)	0.01			1.122 (0.912-1.380)	0.2

*MELD included; **MELD not included.
HR, hazard ratio; CI, confidence interval; vWF-Ag, von Willebrand factor antigen; MELD, model for end-stage liver disease.

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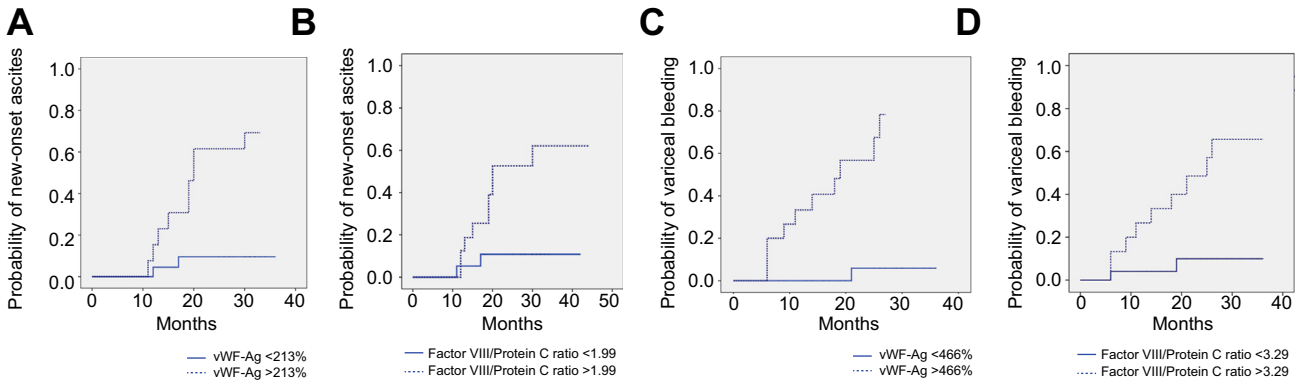

Fig. 1. Kaplan-Meier curves showing actuarial probabilities. Actuarial probabilities of (A) developing new-onset ascites in patients according to a vWF-Ag cut-off value of 213% ($p = 0.001$), (B) developing new-onset ascites according to a factor VIII-to-protein C ratio cut-off value of 1.99 ($p = 0.005$), (C) developing the first episode of variceal bleeding according to a vWF-Ag cut-off value of 466% ($p < 0.001$), (D) developing the first episode of variceal bleeding according to a factor VIII-to-protein C ratio cut-off value of 3.29 ($p = 0.001$).

Table 4. Univariate and multivariate analysis of the baseline risk factors for survival.

	Univariate analysis		Multivariate analysis*		Multivariate analysis**	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Platelet count	0.952 (0.927-0.985)	0.1				
Factor II	0.985 (0.971-1.000)	0.04	1.015 (0.994-1.036)	0.1	1.009 (0.980-1.038)	0.5
Factor V	0.980 (0.962-0.997)	0.02	0.977 (0.950-1.005)	0.1	1.004 (0.973-1.036)	0.8
Factor VII	0.990 (0.972-1.006)	0.01	0.988 (0.970-1.002)	0.01	1.002 (0.979-1.017)	0.2
Factor VIII	1.026 (1.014-1.039)	<0.001	1.023 (1.004-1.041)	0.07	1.018 (0.999-1.037)	0.09
Antithrombin	0.975 (0.962-1.002)	0.005	0.988 (0.968-1.024)	0.4	0.990 (0.966-1.031)	0.6
Protein C	0.978 (0.963-0.992)	0.03	0.994 (0.981-1.006)	0.1	1.013 (0.996-1.031)	0.3
Factor VIII-to-protein C ratio	1.677 (1.343-2.095)	<0.001	1.720 (1.387-2.593)	0.001	1.702 (1.405-2.412)	0.01
vWF-Ag	1.007 (1.004-1.010)	<0.001	1.055 (1.001-1.008)	0.007	1.006 (1.002-1.010)	0.002
MELD score	1.295 (1.187-1.432)	<0.001			1.361 (1.174-1.579)	0.02

*MELD included; **MELD not included.
HR, hazard ratio; CI, confidence interval; vWF-Ag, von Willebrand factor antigen; MELD, model for end-stage liver disease.

score ($p = 0.02$) in model 2, were associated independently with mortality (Table 4).

Receiver operator characteristic curve analysis showed that vWF-Ag and FVIII-to-PC ratio (AUC = 0.802 [95% confidence interval: 0.699–0.904] and AUC = 0.782 [95% confidence interval: 0.672–0.893], respectively) equaled MELD score (AUC = 0.753 [95% confidence interval: 0.640–0.866]) in predicting mortality ($p = 0.12$ and $p = 0.14$, respectively) (Fig. 2). Kaplan-Meier curve analysis revealed a significantly higher 3-year mortality rate in patients with vWF-Ag levels above a cut-off of 392% (sensitivity: 78.6% and specificity: 70% for prediction of survival) (Fig. 3A) and for those with FVIII-to-PC ratios higher than 2.92 (sensitivity: 71.4% and specificity: 58% for prediction of VB) (Fig. 3B).

Discussion

The potential contribution of hemostatic disorders to certain clinical aspects of cirrhosis is currently a topic of increasing interest [27,28]. Apart from thrombocytopenia and prolonged global coagulation tests due to deficiency of most procoagulant factors, the hemostatic profile of a patient with cirrhosis includes a concomitant decrease in plasma levels of major natural anticoagulant proteins PC and AT, and increased plasma levels of FVIII and vWF-Ag [3]. In addition, patients with cirrhosis demonstrate a procoagulant imbalance, which has been attributed to increased FVIII combined with decreased PC activity [21]. FVIII is the most powerful limiting factor in the amplification of thrombin generation [29] while PC is one of the most important anticoagulant factors that downregulates thrombin generation through inhibition of FVIII [30]. Considering the dynamic interplay of the hemostatic changes in cirrhosis, it seems plausible that any conclusions on the relation of rebalanced hemostasis with the progression of liver disease and clinical outcome in patients with cirrhosis cannot be drawn by simply measuring individual components of either portion of the hemostatic system but through evaluation of the balance of pro- and anticoagulant forces.

In line with previous observations [7,8,11–13,16,18,19,21], all evaluated hemostatic parameters in the present study were associated significantly with increasing severity of cirrhosis in univariate analysis. However, vWF-Ag, FVIII, and FVIII-to-PC ratio were the only parameters to be independently related to liver disease severity, which reinforces the existence of a hypercoagulable profile in patients with advanced cirrhosis.

Previous studies have shown a significant association of higher levels of vWF-Ag with the presence of ascites [24] and esophageal varices [16] in patients with cirrhosis. Our study further

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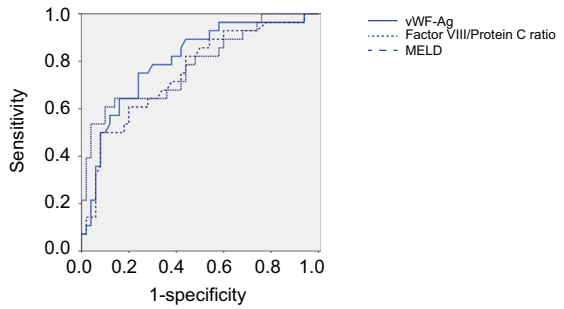

Fig. 2. Kaplan-Meier curves showing actuarial probabilities of survival in the total cohort. According to (A) a vWF-Ag cut-off value of 321% (p <0.001), (B) a factor VIII-to-protein C ratio cut-off value of 3.29 (p <0.001).

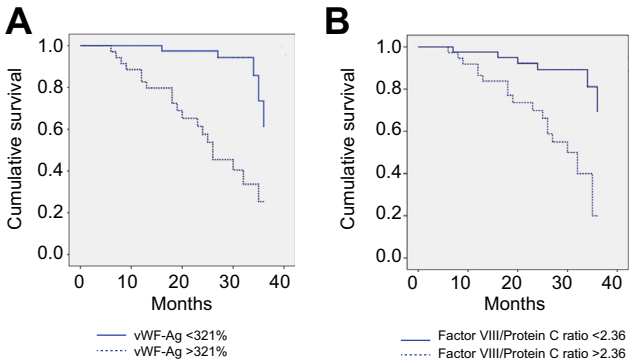

Fig. 3. Comparison of receiver operator characteristic curves for vWF-Ag, factor VIII-to-protein C ratio, and MELD in prediction of mortality. vWF-Ag *vs.* MELD, $p = 0.12$; VIII-to-protein C ratio *vs.* MELD, $p = 0.14$.

demonstrated the independent association of vWF-Ag, FVIII, and FVIII-to-PC ratio with the above PH-related complications at baseline. Most interestingly, higher vWF-Ag levels and FVIII-to-PC ratio independently predicted the development of ascites and VB during follow-up. Moreover, vWF-Ag cut-offs at 213% and 466%, and FVIII-to-PC ratio cut-offs at 1.99 and 3.99, clearly identified patient populations with a highly different probability of development of new-onset ascites and VB, respectively. Notably, neither platelet counts nor reduced levels of procoagulant factors were associated with increased risk of VB, which argues against an association of the perceived hypocoagulability in cirrhosis with bleeding outcome. vWF-Ag and FVIII-to-PC ratio were also major predictive factors of poor outcome in the present cohort of patients with cirrhosis. In particular, vWF-Ag levels >392% and FVIII-to-PC ratio >2.92 identified risk groups of shortened survival. With respect to vWF-Ag, similar findings have been reported by other investigators [23,24].

The clinical consequences of cirrhosis including the development of varices, the occurrence of major decompensating events, such as ascites and VB, and mortality are foremost related to severity of PH [31]. In this point of view, our results suggest that vWF-Ag, FVIII, and FVIII-to-PC ratio could be noninvasive predictors of PH-related complications and clinical outcome in patients with cirrhosis. Indeed, vWF-Ag levels in plasma [23,24] and cirrhotic liver tissue [16] have been significantly correlated with invasively measured portal pressure. Of interest, the vWF-Ag cut-off of 213% for new-onset ascites in our study was close to 241% reported by Ferlitsch *et al.* [24], by using the same method for vWF-Ag plasma level measurement, to represent the optimal cut-off to discriminate between the presence and absence of clinically significant PH (hepatic venous pressure gradient $\geqslant$10 mmHg), which is associated with a higher risk of ascites development [32]. The higher vWF-Ag levels and FVIII-to-PC ratios observed in the patients who subsequently developed VB in our study probably reflect the higher degree of portal pressure required for the occurrence of this complication. The cut-off value for mortality reported by Ferlitsch *et al.* was 315% [24], which was also similar to that observed in the present cohort (321%).

Accumulating data highlights the important role of vWF-Ag [33] and FVIII [34] in the pathogenesis of thrombosis. In the setting of cirrhosis, this is clinically supported by studies reporting that liver disease not only does not protect against deep venous thrombosis [4,5] but may confer increased susceptibility for in-hospital thromboembolic events [35,36]. It can be hypothesised that the combination of elevated vWF-Ag levels due to local sinusoidal endothelial activation and procoagulant imbalance in patients with advanced cirrhosis predisposes to progressive occlusion of portal microvasculature by platelet-induced microthrombi despite the presence of thrombocytopenia [11,12]. The intrasinusoidal prothrombotic state in patients with cirrhosis could be potentiated by the reduced activity of the liver produced protease ADAMTS13 [37], which cleaves vWF-Ag into smaller less thrombogenic forms [38]. Microvascular thrombosis of portal vein branches due to highly elevated levels of vWF-Ag have been implicated in the pathogenesis of non-cirrhotic intrahepatic PH [39] and hepatic necrosis in acute liver failure [40]. Intrahepatic microthrombi have also been demonstrated in patients with cirrhosis [41] and could intensify PH thus accounting for the prognostic significance of vWF-Ag and FVIII-to-PC ratio in our study. Indeed, it was recently reported that prophylactic enoxaparin *vs.* no therapy administered for 48 weeks in 70 patients with advanced cirrhosis was associated by significantly less decompensating events and improved survival in the enoxaparin group [8]. In addition, portal microthrombi may accelerate liver fibrogenesis due to local tissue ischemia [42]. In fact, progression of fibrosis to cirrhosis in patients with chronic liver diseases has been strongly associated with increased expression of FVIII [43], early PC deficiency [43,44], and increasing FVIII-to-PC ratio [45].

Upregulation of coagulation within the cirrhotic liver could also link bacterial translocation-mediated inflammation and PH [46,47]. Endotoxemia has been strongly correlated with plasma vWF-Ag levels in patients with cirrhosis with endotoxin causing a dose-dependent release of vWF-Ag from activated endothelial cells [13,48]. Inflammatory cytokines have also been shown to stimulate the release of vWF-Ag from endothelial cells [48,49], suppress ADAMTS13 synthesis in hepatic stellate cells [50], and downregulate hepatic synthesis of PC [51]. Notably, the beneficial effects of enoxaparin on the occurrence of decompensation in the study by Villa *et al.* [8] were associated with a concomitant decrease in bacterial translocation-related inflammatory response, possibly due to improvement of PH-related intestinal microthromboses, which in turn could ameliorate the ongoing prothrombotic state in the portal circulation.

The predicting performance of vWF-Ag and FVIII-to-PC ratio in new-onset ascites and VB in the present study was higher than MELD score, which indicates that hypercoagulability might be a more determinant factor than liver disease severity for the

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occurrence PH-related complications. On the other hand, vWF-Ag and FVIII-to-PC ratio predicted mortality equally to MELD score suggesting that the inclusion of these hemostatic parameters to MELD score might improve its predictive value.

The main limitation of the present study was the absence of objective evidence about the association of vWF-Ag and FVIII-to-PC ratio with increased intrahepatic thrombotic potential, despite their strong correlation with PH-related events. In addition, no data were available regarding thrombin generation or changes in portal hemodynamics during the study period.

In conclusion, the presence of thrombogenic mechanisms operative within the cirrhotic liver might be an underlying mechanism for the progression of PH and adverse clinical outcome in patients with cirrhosis. vWF-Ag and FVIII-to-PC ratio could become valuable markers for the prediction of major PH-related events and mortality in these patients, which could have a profound effect on their clinical management by allowing further risk stratification. Finally, our findings warrant the design of clinical trials assessing whether antithrombotic therapy could improve the clinical outcome of patients with cirrhosis.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors' contributions

Georgios N. Kalambokis: study design, analysis and interpretation of the data, statistical analysis, and drafting of the manuscript, Aikaterini Oikonomou: acquisition of data, Leonidas Christou and Epameinondas V. Tsianos: critical revision of the manuscript for important intellectual content, Nikolaos I. Kolaitis: technical and material support, Gerasimos Baltayiannis: study concept and supervision.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2016.06.002.

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