Von-Willebrand factor as a predictor of three-month mortality in patients with liver cirrhosis compared to MELD score

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Abstract

Introduction and aim : **Endothelial dysfunction is involved in the pathogenesis of portal hypertension and in the progression of liver disease. As an indicator of endothelial dysfunction, von Willebrand factor (vWF-Ag) can be a useful mortality predictor in patients with liver cirrhosis. The aim of the study is to compare the predictive value of vWF-Ag with the predictive value of MELD score regarding the three-month mortality in patients with liver cirrhosis.**

Materials and methods : **In 70 patients with cirrhosis and portal hypertension we measured the vWF-Ag concentration and we followed the patients for 90 days. We registered all manifestations and complications of liver cirrhosis and the three-month mortality was the main end-point.**

Results : **We registered mean vWF-Ag of 341.9±155.8%, median 312%, IQR (214-410), vWF-Ag significantly correlated with MELD score (R=0.3713 ; p<0.05) and vWF-Ag median was higher in the uncensored compared to the median in the censored patients (p<0.0067). vWF-Ag and MELD score were significantly associated with three-month mortality, with no significant difference in the diagnostic performance between the two parameters [AUC=0.735, p=0.007 for vWF-Ag ; AUC=0.885, p=0.000 for MELD score], (Z=-1.473, p=0.1407).**

Conclusion : In patients with liver cirrhosis vWF-Ag is a relevant predictor of three-month mortality that equals the MELD score. (Acta gastroenterol. belg., **2019**, 82, **487-493**).

Key words *:* von-Willebrand factor, mortality, cirrhosis, endothelial dysfunction.

Introduction

The mortality prediction in patients with end-stage liver disease is a highly complex process that has a great importance mainly in terms of prioritizing the liver transplantation waiting lists. The currently used prognostic scores are characterized with certain weakness and they are not considered ideal mortality predictors. Model of end-stage liver disease (MELD) score is currently the most widely used indicator of the severity of liver disease and a valuable tree-month mortality predictor for patients with end-stage liver disease. The value of MELD score has been confirmed in a large number of patients, but still, in about 15-20% of patients MELD score is not able to predict the mortality accurately (1). Different outcomes in patients with similar risk profiles (2) clearly indicate that there are probably other factors involved in modifying the death risk in these patients (3). This raises the need of research for identifying new biological variables that would act as useful prognostic indicators in patients with liver cirrhosis.

Numerous studies emphasize the role of systemic inflammation and endothelial dysfunction (ED) as conditions that are directly involved in the liver disease pathogenesis and progression. By producing a large number of different mediators, the endothelial cell plays an extremely important role in the regulation of vasomotor tone, vascular homeostasis and inflammatory processes in the body (4,5). ED is defined as a state of impaired function of the endothelial cell due to chronic exposure to physical or chemical stimuli (4). ED in the liver circulation is caused by liver inflammation due to the primarily acting inflammatory agent, while the endotoxemia is the cause for ED in systemic circulation (6).

Von-Willebrand factor (vWF-Ag) is a large multimeric adhesive glycoprotein that plays a role in the primary hemostasis, in the process of fibrin clot formation, and acts as a factor VIII carrier prolonging its half-life for about five times (7-11). Since vWF-Ag is predominately released by the activated endothelial cell, it is considered an indicator of endothelial dysfunction (4). The role of ED in the pathogenesis of portal hypertension has been recently more widely recognized defining vWF-Ag as a valuable prognostic indicator. A study that analyzed few indicators of ED in patients with cirrhosis showed that there was a difference between the concentration in the hepatic and in the peripheral vein only for vWF-Ag. Also, vWF-Ag was the only indicator of ED confirmed to have been associated with clinical outcomes and mortality (12). Studies that evaluated the predictive value of vWF-Ag in patients with cirrhosis have demonstrated that vWF-Ag level correlates with portal hypertension and hepatic venous pressure gradient (12,13), with NO level (14) and endotoxemia (15), with hepatopulmonary syndrome (16) as well as with the stage of liver disease estimated by CTP and MELD score (12,14). Also vWF-Ag has been shown as an independent predictor of clinical events (12) and mortality that equals MELD score (13). The aim of this study was to compare the predictive value of vWF-Ag with the predictive value of MELD score in

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prediction of three-month mortality in patients with liver cirrhosis.

Materials and methods

Patients

In the study we enrolled patients with liver cirrhosis and clinically evident portal hypertension without other significant comorbidities. Some patients were enrolled during the hospitalization at our unit and some were enrolled during the outpatient follow-up. The patients had histologically proven liver cirrhosis or the liver cirrhosis was diagnosed based on clear clinical, morphological and biochemical parameters. The presence of portal hypertension was defined by the presence of enlarged spleen and/or varices. Exclusion criteria were : aged under 18 years, pregnancy, hepatocellular carcinoma or other extrahepatic neoplasm, significant organ insufficiency (cardiac, respiratory, renal), diabetes, portal vein thrombosis or other previous thrombotic event, active infection, active alcohol consumption (consumption of up until one month or less), blood transfusion, antiplatelet or anticoagulant medication.

Data collection

When enrolled in the study, all patients underwent a complete blood count and biochemical analysis of blood sample, basic coagulation tests (prothrombin time, activated partial thromboplastin time, thrombin time), D-dimer test, measurement of vWF-Ag concentration, urine sample analysis, leukocyte count in ascites (when present), abdominal ultrasound, gas analysis from capillary blood sample, measurement of the vital parameters and daily urine output (in hospitalized patients). We also calculated the Child-Turcotte-Pugh (CTP) and MELD score, we registered the complications of liver cirrhosis and portal hypertension and the presence of acute decompensation (AD) (17). Patients were prospectively followed for a period of 90 days and the three-month mortality was the primary end-point.

vWF-Ag assay

By using a vacutainer system (VACUETTE Greiner Bio-one, 9NC coagulation sodium citrate 3.2%) a blood sample in a citrate-containing tube was drawn from a cubital/antecubital vein. After centrifugation at 1000 x g cycle for 10 minutes a platelet rich plasma sample was aliquoted. The analysis was performed shortly afterwards, within the next three hours. vWF-Ag level was measured by immunoturbidimetric method using SIEMENS vWR Ag Test Kit (suspension of small polystyrene particles coated with rabbit anti-human vWF-Ag antibodies) on automatic coagulometer (BCS XP System-Siemens Healthineers Global device). The normal range for vWF-Ag was between 50%-150% and the values above 150% were obtained after plasma samples were diluted and the measured values were calculated according to the dilution factor.

Ethical consideration

All patients signed an informed consent for participation in the study. The research and the study protocol were in line with the ethical principles of the Helsinki declaration and the study protocol was approved by the Ethics commission for human research of the Medical Faculty at Ss Cyril and Methodius University in Skopje, Macedonia.

Statistical analysis

The statistical analysis was made by using Statistica for Windows 7.0 and SPSS 17.0 (Windows, Chicago, IL, USA). Descriptive statistics are provided as mean±SD, median and IQR. Correlation between vWF-Ag and the scoring systems were analyzed by Spearman's correlation. Differences between the numeric parameters of the censored and uncensored group of patients were analyzed by Mann-Whitney's U test (Mann-Whitney-U test was corrected for multiple comparisons). Univariate regression analysis was performed to identify a relation between vWF-Ag, CTP and MELD score and the three-month mortality. Receiver operating characteristic (ROC) analysis was performed to compare the diagnostic performance of vWF-Ag and the MELD score for the prediction of three-month mortality. P values <0.05 were considered significant.

Result

Patient characteristics

A total of 70 patients with liver cirrhosis and portal hypertension were included in the study. Of these, 55 (78.6%) were men and 15 (21.4%) women (gender ratio 3.7:1). The average age was 57.2±10.4 years [95% CI (54.8–59.6)], ranging from 31 to 84 years. According to the CTP classification, there were 18 patients (25.71%) in class A, 24 patients (34.29%) in class B and 28 patients (40%) in class C [CTP mean 8.9±2.9, median 9 IQR (6-11) and MELD mean 19.6±9.9, median 18 IQR (11-25)]. Regarding the etiology of cirrhosis, 36 patients (51.43%) were diagnosed with alcoholic liver disease, 11 patients (15.71%) with chronic hepatitis B, 6 patients (8.57%) with chronic hepatitis C, 5 patients (7.14%) with autoimmune hepatitis, 1 patient (1.43%) with primary biliary cholangitis and in 11 patients (15.71%) the cirrhosis was cryptogenic. According to the Baveno classification, 11 patients were clinically compensated (4 in stage 1 and 7 in stage 2) and 59 patients were classified as clinically decompensated (43 in stage 3 and 16 in stage 4) and AD was diagnosed in 32 patients (45.71%).

vWF-Ag in patients with liver cirrhosis

We established vWF-Ag mean 341.9±155.8%, median 312% IQR (214-410) in examined patients. The analysis confirmed a significant linear moderate positive correlation between vWF-Ag and CTP score (Spearman Rank Order Correlation : R=0.3356 ; p<0.05) (Figure 1A) and a significant linear moderate positive correlation between vWF-Ag and MELD score (Spearman Rank Order Correlation : R=0.3713 ; p<0.05) (Figure 1B). vWF-Ag level was higher in patients with advanced stage of cirrhosis, in patients with pronounced manifestations of portal hypertension, in patients with severe hepatic encephalopathy, in patients with significantly impaired liver synthetic function and in patients with AD (Table 1).

vWF-Ag as a predictor of three-month mortality

Of the 70 enrolled patients, at the end of the study we registered 14 (20%) noncensored (deceased) and 56 (80%) censored (alive) patients. The median of vWF-Ag, CTP and MELD score was significantly higher (p=0.0067 vs. p=0.00001 vs. p=0.00009) in noncensored compared to censored patients (Table 2). According to univariate logistic regression analysis, both, vWF-Ag and MELD score were significant predictors of three-month mortality. With each unit increase of vWF-Ag, the probability for three-month mortality was significantly increasing by 1.005 [p=0.006, 95% CI=1.002-1.009] times. With each unit increase of MELD, the probability for three-month mortality was significantly increasing by 1.198 [p=0.0001, 95% CI=1.088-1.318] times (Table 3). According to ROC analysis and AUC values, both, vWF-Ag and MELD score had a diagnostic performance for three-month mortality (Table 4), but the difference between AUC values of vWF-Ag and MELD score was not statistically significant [vWF-Ag-AUC=0.735 (0.573-0.898) CI 95%, p=0.007 and MELD-AUC=0.885 (0.807-0.964) CI 95%, p=0.000], (Z=-1.473 p=0.1407) (Figure 2).

Table 1. — **Patients characteristics and vWF-Ag values***

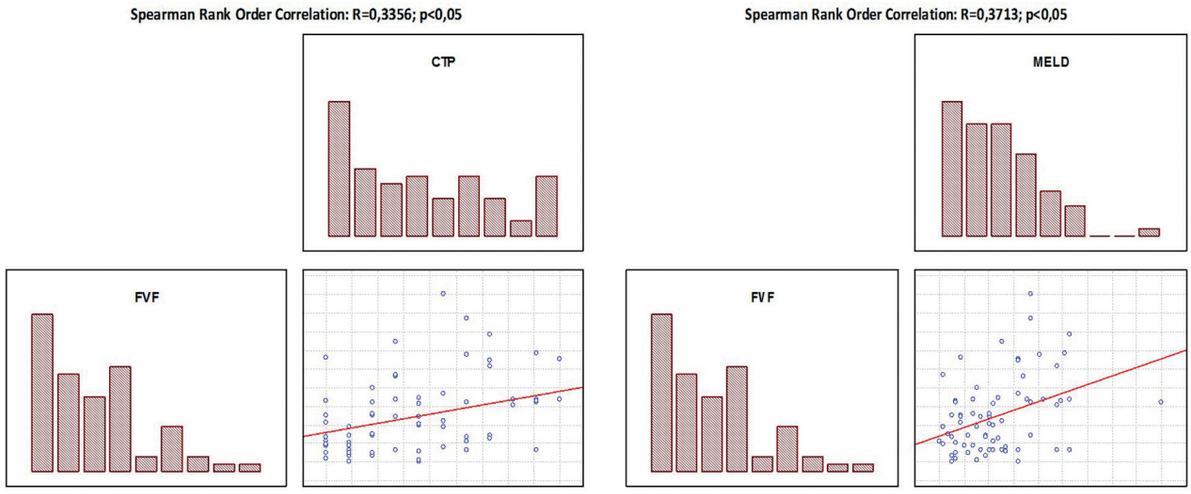
	vWF-Ag (%)	N
Male	323.88 ±145.27	55
Female	407.80 ±179.65	15
Age:≤40	290.20±39.88	2
Age: 41-50	356.77±199.90	17
Age: 51-60	344.74±129.65	22
Age: 61-70	364.94±174.84	21
Age: 71-80	285.86±73.11	7
Age: ≥81	414.00	1
CTP A	256.89 ±106.91	18
CTP B	333.82±124.43	24
CTP C	411.53±182.17	28
MELD≤9	273.89±106.63	9
MELD 10-19	301.02±115.88	33
MELD≥20	421.49±182.94	28
No encephalopathy	339.44±159.01	50
Mild encephalopathy	346.52±171.29	12
Severe encephalopathy	389.40±134.04	8
No ascites	306.04±134.06	17
Mild/moderate ascites	345.33±163.02	36
Severe/refractory ascites	382.06±157.72	17
Bilirubin < 34 micromol/l	315.45±129.05	31
Bilirubin 34-50 micromol/l	260.84±112.01	7
Bilirubin > 50 micromol/l	388.00±176.67	32
Albumin > 35 g/L	260.39±99.73	17
Albumin 28-35 g/L	312.26±147.73	29
Albumin < 35 g/L	430.04±159.73	24
INR <1.7	295.08±118.35	39
INR 1.7-2.3	361.20±170.58	19
INR >2.3	485.82±165.50	12
Creatinin <110 micromol/l	344.30±159.39	54
Creatinin 110-170 micromol/l	300.90±134.89	9
Creatinin 171-300 micromol/l	364.43±214.83	4
Creatinin >300 micromol/l	446.70±103.06	4
Na >135 micromol/l	320.22±140.54	46
Na 131-135 micromol/l	413.92±161.71	17
Na 126-130 micromol/l	492.83±256.85	3
Na 120-125 micromol/l	183.33±28.87	3
Na < 120 micromol/l	405.00	1
Compensated	301.45±128.53	11
Decompensated	353.98±161.18	59
Without AD	294.90±119.02	38
With AD	406.07±175.85	32

* The vWF-Ag values are expressed as mean±SD. vWF-Ag, von-Willebrand factor. CTP, Child-Turcotte-Pugh. MELD, model for end-stage liver disease. INR, international normalized ratio. AD, acute decompensation. The vWF-Ag levels were higher in patients with advanced stage of cirrhosis according to the CTP and MELD score, in patients with pronounced manifestations of portal hypertension, in patients with significantly impaired liver synthetic function, in decompensated and in patients with AD.

Discussion

The results in our study have confirmed that in patients with liver cirrhosis vWF-Ag was elevated, the values were higher in decompensated and in patients in advanced stage of disease and vWF-Ag significantly moderately correlated with CTP and MELD score. Our results have confirmed that in patients with liver cirrhosis, both vWF-Ag and MELD score were significantly associated with three-month mortality, with no significant difference in the diagnostic performance between the two parameters.

Liver cirrhosis is accompanied by many disorders in the process of hemostasis and fibrinolysis (18). The prolonged prothrombin time and the accompanying thrombocytopenia for a long period of time defined liver cirrhosis as a condition that is closely related to hemorrhagic tendency (19,20). However, at present there is strong evidence suggesting that prothrombin time and international normalized ratio (INR) are not reliable indicators of increased bleeding risk (21) and that cirrhosis, especially cirrhosis in advanced stage is in fact more substantively associated with hypercoagulable state (19,22,23). Actually, it is considered that in patients with liver cirrhosis there is a kind of unstable balance



A **B**

Figure 1. — A : nonparametric correlation between CTP score and vWF-Ag ; B : nonparametric correlation between MELD score and vWF-Ag. Significant linear moderate positive correlation between vWF-Ag and CTP score and between vWF-Ag and MELD score. CTP, Child-Turcotte-Pugh. vWF-Ag, von-Willebrand factor. MELD, model for end-stage liver disease.

Table 2. — **Analysis of vWF-Ag, CTP and MELD score in terms of three-month mortality**

Parameters	Mean	number (N)	Standard deviation	Percentiles			p
				25 th	50th	75th	
vWF-Ag							
censored	314.01	56	138.37	207.00	281.50	395.5	Mann Whitney U test: Z=-2.709 p=0.0067*
noncensored	453.31	14	176.29	385.00	409.50	598.5	
CTP							
Censored	8.14	56	2.58	6.00	8.00	10.00	Mann Whitney U test: Z=-4.331 p=0.00001*
noncensored	12.21	14	2.12	11.00	12.50	14.00	
MELD							
Censored	16.86	56	7.71	10.50	15.50	21.00	Mann Whitney U test: Z=-4.434 p=0.00009*
noncensored	30.78	14	10.13	25.00	29.00	35.00	

vWF-Ag, CTP and MELD score were significantly higher in the noncensored compared to the censored group of patients. CTP, Child-Turcotte-Pugh. vWF-Ag, von-Willebrand factor. MELD, model for end-stage liver disease.

Table 3. — **Univariate logistic regression analysis of the predictive value of vWF-Ag and MELD score regarding the three-month mortality**

Variable	Beta (B)	Standard error	Wald	Degrees of freedom	Significance	Expected (B)	95% Confidence Interval for Expected (B)	
							Lower	Upper
vWF-Ag	.005	.002	7.455	1	.006	1.005	1.002	1.009
MELD	.180	.049	13.683	1	.000	1.198	1.088	1.318

vWF-Ag and MELD score were significantly associated with three-month mortality. With each unit increase of vWF-Ag, the probability for three-month mortality was significantly increasing by 1.005 times and with each unit increase of MELD the probability for three-month mortality was significantly increasing by 1.198 times. vWF-Ag, von-Willebrand factor. MELD, model for end-stage liver disease.

Table 4. — **ROC analysis of AUC values for the diagnostic performance of vWF-Ag and MELD score regarding the three-month mortality**

Area Under the Curve					
variable	Area	Standard Error[a]	Asymptotic Significance	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
vWF-Ag	.735	.083	.007	.573	.898
MELD	.885	.040	.000	.807	.964

vWF-Ag and MELD score had a diagnostic performance for three-month mortality, but the difference between the AUC values was not statistically significant (Z=-1.473 p=0.1407). vWF-Ag, von-Willebrand factor. MELD, model for end-stage liver disease.

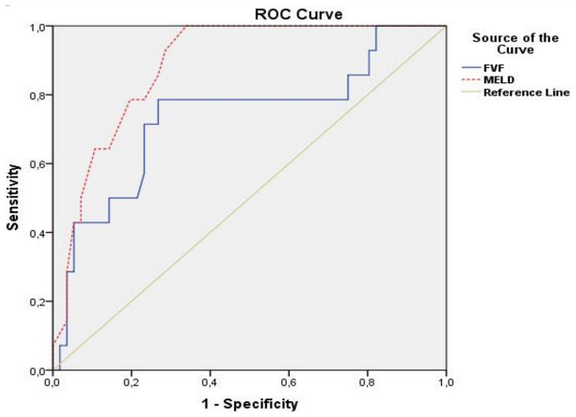


Figure 2. — ROC analysis of the diagnostic performance of vWF-Ag and MELD score regarding the three-month mortality. The AUC for vWF-Ag was 0.735 (0.573-0.898) CI 95%, p=0.007 and the AUC for MELD was 0.885 (0.807-0.964) CI 95%, p=0.000]. The difference between the AUC values was not statistically significant (Z=-1.473 p=0.1407). vWF-Ag, von-Willebrand factor. MELD, model for end-stage liver disease. ROC, receiver operating characteristic. AUC, area under the curve. CI, confidence interval.

between prothrombotic and antithrombotic processes that appear to be going on simultaneously (21,24). Also, it is considered that the routine coagulation tests are not capable to reflect this imbalance properly (25) and to indicate which process predominates in a patient at a certain point. The coexisting prothrombotic state favors microthrombosis and thrombotic vascular obliteration in the hepatic circulation which emphasizes the portal hypertension and worsens the liver function (26). This means that vWF-Ag is in a way involved in the pathogenesis of portal hypertension as well as in the liver disease progression.

Not only quantitative, but in patients with liver cirrhosis there is also a qualitative imbalance in the presence of different vWF-Ag multimer subtypes. Also, the data in the literature regarding the functional properties of vWF-Ag and the distribution of the different multimer subtypes are conflicting. Usually, most of the circulating vWF-Ag is in a form of small multimers that are continuously released from the endothelial cell and megakaryocytes, while most functional ultra-large molecular weight multimers (ULMWM) are collected in the cytoplasmic granules and their active release is stimulated by some physiological agonists. The local damage of the endothelial cells could result in disruption of the cytoplasmic granules and release of vWF-Ag multimers. Also, in certain proportion of patients with AD the inflammatory cytokines within the systemic inflammation inhibit the ADAMTS13 activity which could lead to the presence of ULMWM in these patients (24,27). However, Lisman et al. did not report presence of ULMWM and in individual patients, despite reduction, strongly elevated ADAMTS13 activity was also registered. In terms of hemostasis ULMWM are the most active vWF-Ag forms and are considered prothrombotic (24,28) which explains the increased prothrombotic tendency in patients with AD compared to patients with stabile cirrhosis (24). This fact also imposes the question about the role of anticoagulant therapy in terms of improving clinical outcomes in patients with AD. All the above suggests that elevated vWF-Ag level is a factor of liver disease progression and significant ED estimated through elevated vWF-Ag level has a strong negative prognostic influence by itself. This is the reason why vWF-Ag has a strong prognostic value and in patients with liver cirrhosis it can be used as a valuable prognostic indicator (13).

ED and elevated vWF-Ag level in cirrhosis are generally related to endotoxemia due to bacterial translocation (15,18), especially in advanced disease stage. In a large group of 249 patients with advanced liver cirrhosis Li et al. analyzed the relation between the haemostatic abnormalities, fibrotic markers and the level of endotoxemia. The abnormalities in the haemostatic parameters (rise of prothrombin time, INR, activated partial thromboplastin time, thrombin time, fibrin(ogen) degradation product, D-Dimer and decrease of antithrombin-III and fibrinogen) were significantly higher in advanced stage of disease according to the CTP score and they significantly gradually increased from patients without to patients with ascites and then to patients with spontaneous bacterial peritonitis. This indicates that endotoxemia is closely related to the procoagulant activity and that both are involved in the development of ascites and liver fibrosis (29). The higher vWF-Ag level in the hepatic veins than the level in the peripheral circulation suggests that the liver endothelium is probably the main source of vWF-Ag in these patients (12,30). Still, vWF-Ag is also elevated in patients without clinically significant portal hypertension suggesting that there are probably other factors that stimulate vWF-Ag release or in some other way lead to elevated vWF-Ag. Among mechanisms that contribute to vWF-Ag elevation in cirrhosis are the reduced vWF-Ag clearance due to reduced ADAMTS13 production in the liver stellate cells (30-32), the increased vWF-Ag liver production in systemic inflammation (23), the increased vWF-Ag release from the increased endothelial surface due to angiogenesis and collateralization (15,18) and the effect of vasoconstriction mediators that elevate as compensation to the hyperdynamic circulation.

Our results are comparable with the results of most studies in this area. The vWF-Ag values are significantly higher in patients with cirrhosis compared to those in healthy controls (12,14,15,18,33), there is a significant correlation between vWF-Ag and MELD score (12,18), and significant association between vWF-Ag and mortality (12,13,19). La Mura et al. analyzed a group of 42 patients with compensated cirrhosis and clinically significant portal hypertension. They confirmed that vWF-Ag correlated with hepatic venous pressure gradient, CTP and MELD score and that vWF-Ag was a significant predictor of cirrhosis-associated clinical

events and mortality independent of MELD score. They also defined a cut-off value of 216 U/dl that discriminated compensated from decompensated patients with different survival. Ferlitsch et al. in their study comprising 286 patients with cirrhosis proved that vWF-Ag is a predictor of portal hypertension, decompensation and mortality. They showed that cut-off value of 241% defined the presence of clinically significant portal hypertension and cut-off value of 315% differentiated compensated from decompensated patients with a significantly different survival. The ROC analysis of the AUC values in both previous studies did not establish a significant difference in the diagnostic efficiency of vWF-Ag and MELD score in the mortality prediction {0.739 for MELD vs. 0.740 for vWF-Ag (12) and 0.65 for MELD vs. 0.71 for vWF-Ag (13). However, the patient population in both previous studies mainly included compensated patients with liver cirrhosis in early stage according to the CTP score. Unlike these, the population in our study was mainly consisted of decompensated patients in advanced stage (59 decompensated patients, 28 patients in CTP class C, MELD mean 19.6±9.9). Since the prognostic value of vWF-Ag is most relevant in advanced disease with significant portal hypertension we assumed that our population would be more informative for conducting a research that would analyze the predictive value of vWF-Ag in short-term mortality prediction. Since MELD score is the most relevant three-month mortality predictor in cirrhotic patients, we decided to compare the predictive value of vWF-Ag to the predictive value of MELD score in terms of three-month mortality, hoping that our research could contribute in the general knowledge regarding this matter. There are several studies that analyzed the predictive value of vWF-Ag in patients with liver cirrhosis, but as far as we are aware, in this type of population vWF-Ag was not specifically related to three-month mortality previously.

Our results once again have confirmed the prognostic value of vWF-Ag in patients with liver cirrhosis, which also emphasizes the crucial role of ED in the pathogenesis of portal hypertension as the main determinant of the liver disease progression (6,34,35). Still, our group consisted of a rather small number of patients in a different stage of liver cirrhosis and portal hypertension, hence we did not analyze and compare the prediction role of vWF-Ag in different stage of liver cirrhosis and also we did not analyze the dependence between different predictive indicators. Most studies that performed multivariable regression analysis have demonstrated that vWF-Ag is a predictor of mortality independent of MELD score which means that among patients with similar degree of liver damage and portal hypertension, those with higher vWF-Ag level would have worse outcome (12,13). Therefore, markedly elevated vWF-Ag in patients with early stage of disease could suggest increased prothrombotic potential and consequently more intense disease progression which could also have certain prognostic implication.

In conclusion, vWF-Ag is a single, simple-to-obtain, noninvasive, widely available and relatively cheap biomarker characterized with low interlaboratory variability (36) that in patients with liver cirrhosis can be used as a relevant predictor of three-month mortality with diagnostic efficiency similar to MELD score. As to the MELD score's limitations, prothrombin time is clearly insufficient in expressing hemostasis abnormalities in cirrhotic patients and creatinin can be elevated as a result of primary renal condition. The rise of vWF-Ag indicates development of a prothrombotic state that is related to most complications of advanced liver disease and it seems that it adds prognostic information in a more straightforward and essential way. Implementation of vWF-Ag into the currently used prognostic scores could improve the mortality prediction in these patients. Still, additional research is needed in order to determine in which circumstances, conditions or cirrhosis-associated complications vWF-Ag would have a greater prognostic value.

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Contribution

ECR : study concept and design, analysis and interpretation of data, responsible for the integrity of the work as a whole ; MGD : critical revision of the manuscript for important intellectual content ; VCI : acquisition of data and supervision ; JM : study concept and design, drafting of the manuscript.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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