





# The von Willebrand Factor Facilitates Model for End-Stage Liver Disease—Independent Risk Stratification on the Waiting List for Liver Transplantation

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**BACKGROUND AND AIMS:** The Model for End-Stage Liver Disease (MELD) is used for clinical decision-making and organ allocation for orthotopic liver transplantation (OLT) and was previously upgraded through inclusion of serum sodium (Na) concentrations (MELD-Na). However, MELD-Na may underestimate complications arising from portal hypertension or infection. The von Willebrand factor (vWF) antigen (vWF-Ag) correlates with portal pressure and seems capable of predicting complications in patients with cirrhosis. Accordingly, this study aimed to evaluate vWF-Ag as an adjunct surrogate marker for risk stratification on the waiting list for OLT.

**APPROACH AND RESULTS:** Hence, vWF-Ag at time of listing was assessed in patients listed for OLT. Clinical characteristics, MELD-Na, and mortality on the waiting list were recorded. Prediction of 3-month waiting-list survival was assessed by receiver operating characteristics and net reclassification improvement. Interestingly, patients dying within 3 months on the waiting list displayed elevated levels of vWF-Ag ( $P < 0.001$ ). MELD-Na and vWF-Ag were comparable and independent in their predictive potential for 3-month mortality on the waiting list (area under the curve [AUC], vWF-Ag = 0.739;

MELD-Na = 0.764). Importantly, a vWF-Ag cutoff at 413% identified patients at risk for death within 3 months of listing with a higher odds ratio (OR) than the previously published cutoff at a MELD-Na of 20 points (vWF-Ag, OR = 10.873, 95% confidence interval [CI], 3.160, 36.084; MELD-Na, OR = 7.594, 95% CI, 2.578, 22.372;  $P < 0.001$ , respectively). Ultimately, inclusion of vWF-Ag into the MELD-Na equation significantly improved prediction of 3-month waiting-list mortality (AUC, MELD-Na+vWF = 0.804).

**CONCLUSIONS:** A single measurement of vWF-Ag at listing for OLT predicts early mortality. Combining vWF-Ag levels with MELD-Na improves risk stratification and may help to prioritize organ allocation to decrease waiting-list mortality. (HEPATOLOGY 2020;72:584-594).

To this date, orthotopic liver transplantation (OLT) is the gold standard in treatment of patients suffering from end-stage liver disease.<sup>(1)</sup> Although OLT does improve the outcome of these patients in terms of overall survival and quality

*Abbreviations:* ALT, alanine aminotransferase; AP, alkaline phosphatase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; AUC, area under the curve; CI, confidence interval; GGT, gamma-glutamyltranspeptidase; HR, hazard ratio; MELD, Model for End-Stage Liver Disease; MELD-Na, MELD with Na concentration; MELD-Na+vWF, vWF-Ag incorporated into the MELD-Na system; MVA, multivariable analysis; NA, sodium; NRI, net reclassification improvement; OLT, orthotopic liver transplantation; OR, odds ratio; PT, prothrombin time; ROC, receiver operating characteristic; SBP, spontaneous bacterial peritonitis; UNOS, United Network for Organ Sharing; vWF, von Willebrand factor; vWF-Ag, vWF antigen.

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of life,<sup>(2)</sup> shortage of donor organs and the resulting high mortality on the waiting list for OLT remain a critical problem.<sup>(3-5)</sup> In fact, the mortality rate in Eurotransplant and the United Network for Organ Sharing is approximately 25% and might even be subject to underreporting,<sup>(6-8)</sup> which clearly illustrates the need for improved risk stratification in this patient cohort.

The Model for End-Stage Liver Disease (MELD) was introduced to standardize assessment of waiting-list mortality nearly 2 decades ago and has become a central part of liver allocation throughout the United States and Europe.<sup>(7,9)</sup> Subsequently, the original MELD was adapted through inclusion of serum sodium (NA) concentrations (MELD-Na), which led to improved prediction of early mortality on the waiting list for OLT.<sup>(10,11)</sup> Still, MELD-Na is prone to well-known limitations that comprise the lack of assessment of subclinical infection while on the waiting list and an underestimation of complications resulting from portal hypertension.<sup>(9,12-15)</sup> Accordingly, further optimization of the organ allocation process seems inevitable to reduce mortality before OLT.

The von Willebrand factor (vWF) antigen (vWF-Ag) represents a key component of primary hemostasis that is stored and released by thrombocytes and endothelial cells.<sup>(16,17)</sup> Interestingly, previous studies have shown that vWF-Ag can predict long-term outcomes of patients with chronic liver disease.<sup>(18,19)</sup> Indeed, vWF-Ag is a surrogate marker for endothelial dysfunction, a key pathophysiologic driver for complications associated with portal hypertension and/or infections.<sup>(20)</sup> Although the first studies showed a strong correlation of vWF-Ag with portal hypertension,<sup>(21)</sup> recent data underline a more universal

role of vWF-Ag in predicting outcomes within this patient cohort.<sup>(22)</sup> In particular, vWF-Ag was shown to be even more accurate in predicting variceal bleeding than hepatic venous pressure gradient (HVPG). Further, an association of vWF-Ag with markers of bacterial translocation and, even more interestingly, with spontaneous bacterial peritonitis (SBP) was found and shown to be independent of portal pressure.<sup>(22)</sup> As both variceal bleeding and SBP are predominant causes of mortality in patients listed for transplantation,<sup>(23,24)</sup> and because of the multifactorial involvement of vWF-Ag in liver pathophysiology, we aimed to evaluate whether vWF-Ag could improve the apparent shortcomings of the MELD-Na score in risk assessment of patients listed for transplantation.

Accordingly, we aimed to explore differences in waiting-list survival regarding levels of vWF-Ag and to assess whether addition of vWF-Ag improves mortality risk prediction on the waiting list on top of MELD-Na.

## Patients and Methods

### STUDY POPULATION

All consecutive patients listed for OLT at our center between 2003 and 2016 were included in this study. Routine blood samples at the time of listing included baseline coagulation parameters (prothrombin time [PT], activated partial thromboplastin time [aPTT]), liver function tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [AP], gamma-glutamyltranspeptidase [GGT], and bilirubin), and vWF-Ag levels. Patients undergoing transplantation for acute hepatic failure or undergoing

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retransplantation were excluded from this study. As the primary goal of this study was to evaluate a potential benefit of vWF-Ag in comparison with MELD-Na alone, standard exception points on the waiting list (e.g., patients with hepatocellular carcinoma) were not taken into consideration, and patients were analyzed according to their calculated MELD-Na score. Thereby, we aimed to reflect the individual patient risk for early waiting-list mortality based on the MELD-Na system without any inherent bias. The study protocol was approved by the institutional review board (Ethical Committee of the Medical University of Vienna, Vienna, Austria, EK-1196/2018). All clinical research has been performed in adherence to the Declarations of Helsinki and Istanbul.

## CALCULATION OF MELD-Na AND EVALUATION OF vWF-Ag

MELD was calculated based on the formula given by Malinchoc et al., which includes serum bilirubin, creatinine, and international normalized ratio.<sup>(25)</sup> In addition, MELD-Na was calculated based on the standard formula under inclusion of serum Na.<sup>(10)</sup> After comparison with vWF-Ag, MELD-Na was used for incorporation of vWF-Ag as reported in the following.

To render vWF-Ag a standardized and broadly applicable factor, it was evaluated within our clinical routine laboratory. Citrated blood was taken at listing, and concentration of vWF-Ag was assessed in the subsequently prepared plasma.

## STATISTICAL ANALYSES

Statistical analyses were performed using SPSS software (version 24; SPSS, Inc., Chicago, IL) as well as by using the R statistical software environment (R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org>, version 3.5.3, packages *survival*, *mgcv*, *cmprsk*) and were based on nonparametric tests for either paired or independent samples (Mann-Whitney U test, Wilcoxon test, chi-squared test). Baseline (i.e., at time of listing) vWF-Ag and MELD-Na were obtained and compared between patients who underwent transplantation and patients who died on the waiting list before they reached transplantation. Furthermore, receiver operating characteristic (ROC) analysis was applied to assess the discriminatory potential of the

mentioned variables for 3-month mortality. In addition, this statistical approach was used to identify the optimal cut-off level with the greatest accuracy of distinguishing high-risk and low-risk groups through the use of the Youden J statistic. To determine whether the vWF-Ag as a continuous parameter had a non-linear effect on the risk of 3-month mortality, generalized additive models with smoothing splines were used. Multivariable analysis (MVA) was used to investigate independence of predictive markers for 3-month waiting-list survival. Initially, all baseline characteristics were evaluated in a univariate logistic regression model. Subsequently, all markers shown to be significant on univariate analysis were included in the final model, which was computed using stepwise backward elimination. Parameters shown to be significant on MVA were considered independent predictors. To further provide a useful tool for clinical applicability of the presented results, incorporation of vWF-Ag into the MELD-Na scoring system was sought. Of note, the aim was to maximize the predictive potential for identification of patients who would die within 3 months while awaiting OLT. Thus, Cox regression analysis was computed to evaluate a potential additional value of vWF-Ag for MELD-Na based on predicted 3-month survival. Finally, net reclassification improvement (NRI) analysis was performed to validate the results regarding the predictive potential and to explore a possible improvement of MELD-Na after addition of vWF-Ag. *P* values <0.05 were considered statistically significant.

## Results

### PATIENT DEMOGRAPHICS

A total of 1,096 patients were listed for OLT at our center between 2003 and 2016 and were included in our prospective database. Of these 1,096 patients, 1,058 were listed for their first transplantation. Further, 19 patients were excluded from the analysis, as they were listed for fulminant liver failure. Ultimately, data on vWF-Ag were available in 269 patients listed for OLT who were included in the final analysis. Supporting Fig. S1 illustrates the process of patient exclusion. Of note, vWF-Ag evaluation occurred haphazardly and without clinical consequence in these patients. Notably, levels of

vWF-Ag were not available for patients with clinical deterioration and subsequent exclusion. Hence, the statistical analysis relied on comparing patients who did reach the endpoint of transplantation within 3 months and patients who survived on the list until this time point with patients who died within 3 months on the waiting list.

Supporting Table S1 shows the comparison between patients included in the analysis and patients excluded because of missing vWF-Ag levels. Patients' ages at listing differed significantly between the two cohorts, explained by a significantly higher frequency of patients younger than 15 years in the population of patients without available data on vWF-Ag. However, all remaining parameters showed an equal distribution. Characteristics of patients included in the final analysis are displayed in Table 1. In total, 31 patients (11.5%) died within the first 3 months after listing.

### PATIENTS DYING WITHIN 3 MONTHS ON THE WAITING LIST HAVE SIGNIFICANTLY ELEVATED LEVELS OF vWF-Ag AND MELD-Na AT TIME OF LISTING

Initially, evaluation of differences regarding MELD-Na and vWF-Ag between patients with an on-list survival of more or less than 3 months was sought. Indeed, patients dying within 3 months on the waiting list displayed elevated levels of MELD-Na (median MELD-Na, no mortality = 19, median MELD-Na, mortality = 24,  $P < 0.001$ ). Similar results were observed for vWF-Ag (median vWF-Ag, no mortality = 405%, median vWF-Ag, mortality = 420%,  $P < 0.001$ ). Strikingly, vWF-Ag and MELD-Na seemed to be comparable in their predictive potential for 3-month survival on the waiting list (area under the curve [AUC], vWF-Ag = 0.739, AUC, MELD-Na = 0.764).

TABLE 1. Patient Demographics

Parameters	Entire Cohort (n = 269)	M↓ W↓* (n = 94)	M↑ W↓* (n = 36)	M↓ W↑* (n = 49)	M↑ W↑* (n = 90)
Sex, n (%)					
Male	203 (75.5)	71 (75.5)	29 (80.6)	38 (77.6)	65 (72.2)
Female	66 (24.5)	23 (24.5)	7 (19.4)	11 (22.4)	25 (27.8)
Age at listing (years), median (range)	56 (15-73)	56 (22-72)	53 (19-68)	59 (40-72)	56 (15-73)
Parameters at listing, median (range)					
vWF-Ag (%)	419 (76-1,270)	297 (76-410)	342 (224-411)	420 (415-830)	420 (417-1,270)
MELD-Na	19 (7-40)	14 (7-19)	23 (20-40)	17 (10-19)	24 (20-38)
PT (%)	51 (14-142)	64 (37-136)	45 (14-142)	58 (40-112)	36 (15-76)
aPTT (seconds)	41.6 (31.0-67.0)	40.6 (31.2-51.2)	44.2 (31.5-61.8)	40.6 (34.2-49.5)	45.0 (31.0-67.0)
AST (U/L)	62 (17-2,187)	63 (17-2,187)	59 (23-270)	56 (24-202)	65 (22-453)
ALT (U/L)	40 (10-1,663)	44 (10-1,663)	33 (12-161)	35 (15-126)	38 (10-256)
Bilirubin (mg/dL)	2.95 (0.42-52.02)	1.69 (0.42-6.71)	5.80 (0.65-52.02)	2.32 (0.68-10.33)	5.69 (0.87-48.81)
AP (U/L)	128 (45-2,552)	111 (45-927)	129 (62-2,552)	134 (47-377)	150 (51-1,000)
GGT (U/L)	88 (12-2,733)	98 (12-695)	95 (15-2,733)	102 (14-481)	72 (17-689)
Creatinine (mg/dL)	0.90 (0.48-3.56)	0.84 (0.54-1.95)	1.07 (0.53-2.74)	0.89 (0.58-2.04)	1.02 (0.48-3.56)
Na (mmol/L)	136 (117-145)	138 (130-144)	135 (123-144)	138 (129-145)	132 (117-143)
Indication for LTx, n (%)					
Alcohol-associated cirrhosis	89 (33.1)	14 (14.9)	14 (38.9)	19 (38.8)	42 (46.7)
Tumor	62 (23.0)	46 (48.9)	4 (11.1)	8 (16.3)	4 (4.4)
Viral hepatitis	42 (15.6)	14 (14.9)	4 (11.1)	10 (20.4)	14 (15.6)
Biliary disorders	23 (8.6)	11 (11.7)	7 (19.4)	2 (4.1)	3 (3.3)
AI hepatitis	14 (5.2)	2 (2.1)	2 (5.6)	1 (2.0)	9 (10.0)
Cryptogenic cirrhosis	13 (4.8)	2 (2.1)	2 (5.6)	2 (4.1)	7 (7.8)
Other indications	26 (9.7)	5 (5.3)	3 (8.3)	47 (14.3)	11 (12.2)

\*M = MELD-Na (cutoff at 20 points), W = vWF-Ag (cutoff at 413%). Abbreviations: AI, autoimmune; LTx, liver transplant.



### MELD-Na AND vWF-Ag ARE INDEPENDENT PREDICTORS FOR 3-MONTH MORTALITY ON THE WAITING LIST

Comparative analysis of vWF-Ag and serum Na revealed that there was only a little redundancy for both parameters (Supporting Fig. S2). Interestingly, the majority of patients (i.e., 77.32%) showed high levels of serum Na accompanied by low levels of vWF-Ag, whereas only 3.72% of patients displayed severe hyponatremia (<130 mmol/L) while having concomitantly increased vWF-Ag levels. Intriguingly, the incidence of 3-month mortality showed a significantly different distribution in the specific cohorts according to vWF-Ag and Na (*P* = 0.019, Supporting Fig. S2). Of note, besides the cohort defined by high vWF-Ag and low levels of serum Na, a specifically high frequency of early waiting-list mortality was observed in patients with Na levels above 130 mmol/L and vWF-Ag levels above 500% (18.2%).

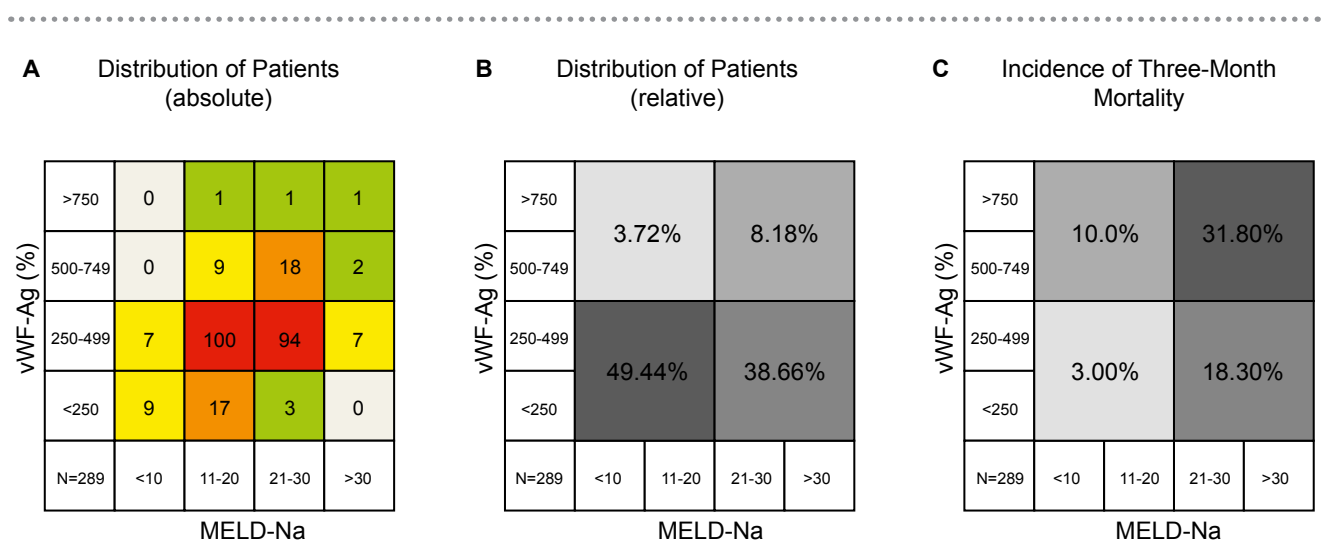
In contrast, comparative analysis for vWF-Ag and MELD-Na showed a more linear relationship. Most patients were found to have both low levels of vWF-Ag and low MELD-Na scores (Fig. 1A,B). Further, a clear difference between high and low MELD-Na scores was observed in regard to 3-month mortality (Fig. 1D). Nevertheless, the additional stratification of

patients with specifically high vWF-Ag levels at listing (i.e., >500%) revealed a pronounced incidence of 3-month mortality in this cohort, which was independent of MELD-Na points.

As a consequence, the independence of vWF-Ag in relation to MELD-Na was assessed in the MVA, including PT and bilirubin, as well as the presence of autoimmune hepatitis, tumor, or cryptogenic cirrhosis. Ultimately, MELD-Na, vWF-Ag, and cryptogenic cirrhosis remained significant and hence independent, as visualized in Table 2.

### A CUTOFF AT 413% vWF-Ag IS VITAL FOR IDENTIFICATION OF PATIENTS DYING EARLY ON THE WAITING LIST

The cutoff, maximizing the Youden index with a sensitivity of 90.3% and a specificity of 53.4%, was found to be 413% of vWF-Ag at listing. Of note, the optimal cutoff for MELD-Na in this cohort was 20 points (sensitivity = 87.1%, specificity = 64.7%). Subsequently, the cohort was subdivided into two risk groups: vWF-Ag<sup>HIGH</sup> (>413%) and vWF-Ag<sup>LOW</sup> (≤413%). Indeed, patients within the high-risk group were found to have a significantly higher incidence of mortality within 3 months on the waiting list (28 of 139 [20.1%] in vWF-Ag<sup>HIGH</sup> vs. 3 of 130 [2.3%] in



**FIG. 1.** Association of vWF-Ag with Na and MELD-Na in patients awaiting OLT. To evaluate a potential redundancy of vWF-Ag and MELD-Na, correlative analysis was performed. Absolute distribution of patients in regard to their (A) vWF-Ag levels and MELD-Na is visualized. Similarly, the (B) relative distribution is shown. Ultimately, the (C) incidence of 3-month waiting-list mortality is shown in accordance to the respective parameters.

TABLE 2. MVA for 3-Month Survival on Waiting List

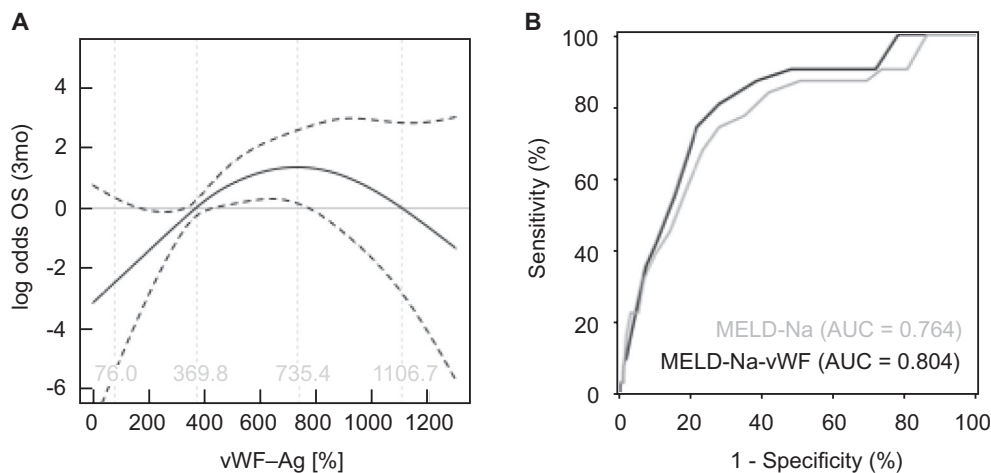
Parameter	Univariate Analysis			MVA		
	OR	95% CI	PValue	OR	95% CI	PValue
vWF-Ag (%)	1.005	1.002, 1.007	0.001	1.003	1.001, 1.006	0.036
MELD-Na	1.180	1.098, 1.268	<0.001	1.154	1.068, 1.246	<0.001
CLIF-C AD	1.082	0.974, 1.203	0.141	—	—	—
Sex	0.770	0.335, 1.767	0.537	—	—	—
Age at listing	1.021	0.981, 1.062	0.310	—	—	—
Circulating parameters at listing						
PT (%)	0.970	0.945, 0.995	0.021	1.015	0.987, 1.044	0.284
aPTT (seconds)	1.046	0.984, 1.113	0.149	—	—	—
AST (U/L)	0.999	0.994, 1.004	0.683	—	—	—
ALT (U/L)	0.997	0.988, 1.007	0.586	—	—	—
Bilirubin (mg/dL)	1.058	1.011, 1.108	0.016	0.974	0.910, 1.043	0.450
AP (U/L)	1.000	0.998, 1.002	0.832	—	—	—
GGT (U/L)	0.997	0.992, 1.003	0.331	—	—	—
Indication for LTx						
Alcohol-associated cirrhosis	1.790	0.838, 3.821	0.132	—	—	—
Tumor	0.097	0.013, 0.724	0.023	0.000	NA	0.998
Viral hepatitis (B+C)	0.547	0.158, 1.888	0.340	—	—	—
Biliary disorders	0.327	0.043, 2.517	0.283	—	—	—
Autoimmune hepatitis	4.893	1.525, 15.703	0.008	1.527	0.295, 7.900	0.613
Cryptogenic cirrhosis	3.770	1.087, 13.072	0.036	3.851	1.031, 14.384	0.045
Other indications	1.002	0.282, 3.552	0.998	—	—	—

Abbreviations: CLIF-C AD, chronic liver failure acute decompensation score; LTx, liver transplant; NA, not available.

vWF-Ag<sup>LOW</sup>,  $P < 0.001$ ; Supporting Fig. S3A), which was comparable with the high-risk group according to MELD-Na (27 of 139 [19.4%] in MELD-Na<sup>HIGH</sup> vs. 4 of 130 [3.1%] in MELD-Na<sup>LOW</sup>,  $P < 0.001$ ; Supporting Fig. S3A). Strikingly, the cutoff at 413% of vWF-Ag at listing was found to have a higher odds ratio (OR) for mortality within 3 months on the list when compared with a MELD-Na cutoff at 20 points (vWF-Ag, OR = 10.679, 95% confidence interval [CI] = 3.160, 36.084,  $P < 0.001$ ; MELD-Na, OR = 7.594, 95% CI = 2.578, 22.372,  $P < 0.001$ ). The subsequently computed subgroup analysis for 3-month mortality in patients with low MELD-Na ( $\leq 20$ ) and patients with high MELD-Na ( $> 20$ ) showed an independent prediction of vWF-Ag (MELD-Na  $\leq 20$ , 4 of 44 [9.1%] in vWF-Ag<sup>HIGH</sup> vs. 0 of 86 [0.0%] in vWF-Ag<sup>LOW</sup>,  $P = 0.005$ ; MELD-Na  $> 20$ , 24 of 95 [25.3%] in vWF-Ag<sup>HIGH</sup> vs. 3 of 44 [6.8%] in vWF-Ag<sup>LOW</sup>,  $P = 0.005$ ; Supporting Fig. S3B), whereas no patient in the low-risk group according to vWF-Ag and having less than 20 MELD-Na points died within 3 months on the waiting list (Supporting Fig. S3B).

## VWF-Ag INCREASES PREDICTIVE POTENTIAL FOR 3-MONTH SURVIVAL OF MELD-Na ALONE IN PATIENTS AWAITING OLT

To evaluate a continuous nonlinear relationship between vWF-Ag and risk for 3-month mortality, a generalized additive model with smoothing splines was applied and visualized in Fig. 2A. An increased risk (OR  $\geq 1$ ) was observed for values of vWF-Ag between 369.8% and 1,106.7%. Further, a linear increase in risk for 3-month mortality was observed for levels of vWF-Ag under 369.4%. As a consequence, extreme values for vWF-Ag were defined at the upper limit of normal (185%) and at 1,100% as the upper limit. These limits included 95.9% of all values for vWF-Ag in this cohort. In case of patients with higher or lower levels for vWF-Ag, the value was reduced to the defined limits, respectively. Next, a multivariable Cox regression analysis including MELD-Na and vWF-Ag at listing was fitted. Indeed, both factors were significant for survival on the



**FIG. 2.** vWF-Ag allows additional risk stratification on the waiting list for OLT. (A) Continuous risk for 3-month mortality (OR) was calculated for vWF-Ag and is visualized. VWF-Ag was incorporated as described in the main text. (B) Indeed, incorporation of vWF-Ag was able to increase the AUC of MELD-Na alone for prediction of 3-month mortality on the waiting list. (\* $P < 0.05$ , \*\* $P < 0.005$ ).

waiting list on univariate analysis (MELD-Na, hazard ratio [HR] = 1.154, 95% CI = 1.103, 1.208,  $P < 0.001$ ; vWF-Ag, HR = 1.004, 95% CI = 1.002, 1.006,  $P < 0.001$ ) and MVA (MELD-Na, HR = 1.135, 95% CI = 1.082, 1.192,  $P < 0.001$ ; vWF-Ag, HR = 1.002, 95% CI = 1.000, 1.004,  $P = 0.031$ ). To incorporate vWF-Ag into the MELD-Na system, the following equation including the regression coefficients for MELD-Na and vWF was fitted:

$$\text{MELD-Na-vWF} = ([1.127 \times \text{MELD-Na}] + [0.002 \times \text{vWF-Ag}]) \times 5.5$$

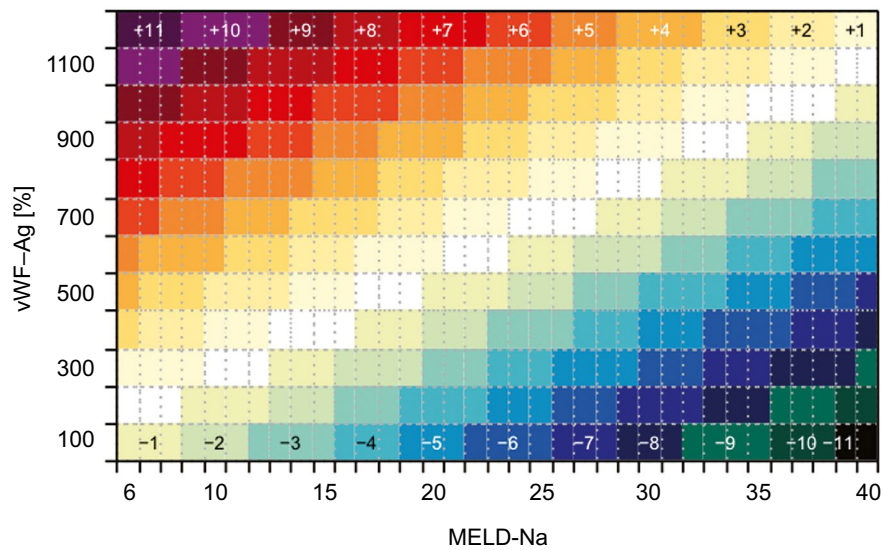
Of note, the multiplier 5.5 was introduced to render the scale comparable to the original MELD-Na score, while automatically leading to a range from 6 to 40 points. Further, values for MELD-Na-vWF were rounded to the next integer. Thereby, a bidirectional reclassification was achieved allowing patients to both gain or lose MELD points in accordance to vWF-Ag. Indeed, the introduction of vWF-Ag leads to a potential change of 11 points in both directions, as visualized in Fig. 3. Further, Table 3 was computed to visualize the reclassification pattern in the studied cohort.

ROC analysis was performed and revealed an improved discriminatory potential of MELD-Na-vWF for 3-month survival on the waiting list (AUC, MELD-Na = 0.764; AUC, MELD-Na-vWF = 0.804,  $P < 0.001$  respectively; Fig. 2B). Based on this ROC analysis, the optimal cutoff for

MELD-Na-vWF was identified at 20 points with a sensitivity of 74.2% and a specificity of 78.2%. This cutoff was found to specifically identify patients who died within 3 months on the waiting list (6 of 177 [3.4%] in MELD-Na-vWF<sup>LOW</sup> vs. 25 of 92 [27.2%] in MELD-Na-vWF<sup>HIGH</sup>,  $P < 0.001$ ; Supporting Fig. S3C). Of note, the use of MELD-Na-vWF lead to a higher incidence of early waiting-list mortality in the high-risk group, when compared with the high-risk group assessed by MELD-Na alone using the Youden index for MELD-Na at 20 points (4 of 130 [3.1%] in MELDNa<sup>LOW</sup> vs. 27 of 139 [19.4%] in MELDNa<sup>HIGH</sup>,  $P < 0.001$ ), whereas the incidences of death within 3 months after listing in the low-risk groups were comparable. This improvement was further characterized by NRI (Supporting Fig. S3D). In total, 47 patients were reclassified correctly when using MELD-Na-vWF, whereas only 4 patients were reclassified inappropriately. This is further illustrated by a total NRI of 0.125, meaning that the introduction of MELD-Na-vWF leads to an improvement in accurate prediction of 12.5% in this cohort.

## Discussion

Although MELD was initially established for prediction of survival in patients receiving transjugular intrahepatic portosystemic shunts,<sup>(26,27)</sup> it has become



**FIG. 3.** Computation of MELD-Na-vWF on the Basis of the MELD-Na after incorporation of vWF-Ag. This figure illustrates the individual change of MELD-Na when including vWF-Ag as an additional variable.

**TABLE 3. Visualization of Reclassification**

		MELD-Na-vWF				Total
		<10	10-19	20-29	30-40	
MELD-Na	<10	13	3	—	—	16
	10-19	3	120	4	—	127
	20-29	—	38	78	—	116
	30-40	—	—	6	4	4
	Total	16	161	88	4	269

a central part of liver allocation in many countries throughout the world.<sup>(28,29)</sup> In 2008, Kim et al. documented that the incorporation of Na in the MELD scoring system was able to improve prediction of 3-month waiting-list survival. Still, recent data show major shortcomings of MELD-Na, mainly associated with an overestimation of creatinine within its calculation, leading to underestimation of certain subpopulations awaiting OLT.<sup>(30-32)</sup> Further, MELD was shown to vary in its prediction of survival according to the underlying cause of cirrhosis, giving patients with viral hepatitis a special disadvantage on the waiting list.<sup>(33)</sup> Hence, adaptation of the MELD-Na score is a central task for further improvement of allocation policy and for reducing waiting-list mortality. In particular, the identification of high-risk patients with a low MELD score at listing and further stratification

of patients with a very high MELD score pose difficult challenges.<sup>(34-37)</sup>

In this study, we were able to evaluate the potential of vWF-Ag to substratify patients, irrespective of their MELD-Na score. Indeed, patients with vWF-Ag above 413% at the time of listing were found to have a diminished survival on the waiting list independent of their MELD-Na score at listing. Of note, these results held through after exclusion of patients suffering from tumors, who were previously shown to have an advantage in terms of waiting-list survival (Supporting Fig. S4). As a key protein for hemostasis and coagulation, vWF-Ag is secreted by endothelial cells and platelets as a response to alterations and augmentation of shear stress.<sup>(17,38-40)</sup> Hence, vWF-Ag could be established as a noninvasive marker for portal hypertension and as a predictor of long-term outcomes in patients suffering from this clinically important condition.<sup>(18,19,21,41,42)</sup> Although the predictive potential of vWF-Ag in these patients was thought to be due to its accurate ability to stratify the individual severity of portal hypertension, a recent analysis found an independent predictive potential of vWF-Ag.<sup>(22)</sup> Interestingly, vWF-Ag was found to predict bleeding from esophageal varices and SBP better than HVPG as a measure of portal venous pressure/hypertension. A potential explanation of this finding was that vWF-Ag might serve as a pleiotropic



marker including endothelial cell dysfunction and bacterial translocation, as shown through correlation with lipopolysaccharide-binding protein in circulation. Thus, vWF-Ag can be seen as a multifactorial marker in patients with end-stage liver disease, which unifies portal hypertension and associated comorbidities within one single parameter.

The presented data suggest that the incorporation of vWF-Ag in the MELD-Na score provides a relevant improvement in terms of risk stratification for patients awaiting OLT. In this context, it was of interest to note that very high vWF-Ag levels were predominantly found in patients with normal or even high Na levels, suggesting that vWF-Ag adds additional insight to the MELD score as compared with Na. However, when both criteria were met (low Na and high vWF-Ag), 3-month mortality was excessively high at 40%, again suggesting that the dual addition of Na and vWF-Ag to MELD might be particularly specific as different aspects of advanced liver disease are considered. In addition, an independent predictive potential of vWF-Ag assessed through MVA was observed in this study. Hence, a model based on Cox regression was fitted and the newly developed MELD-Na-vWF model resulted. Indeed, this incorporation of vWF-Ag into the MELD-Na system was found to increase the potential of predicting 3-month mortality on the waiting list to a clinically relevant extent, as seen in ROC and NRI analyses. Interestingly, after incorporation of vWF-Ag and reclassification of patients into the newly developed MELD-Na-vWF system, an overestimation of MELD-Na was observed. Although vWF-Ag is a sensitive marker for portal hypertension and complications associated with cirrhosis, low levels of vWF-Ag might identify patients who are not prone to disease progression and potentially life-threatening complications. Indeed, 21 patients of 31 patients dying within 3 months after listing (68%) died because of complications that were reported to be predicted by high levels of vWF-Ag. Further, vWF-Ag was recently found to play a direct role in the progression of fibrosis and cirrhosis.<sup>(43-45)</sup> In particular, experimental data suggest not only a deleterious effect of vWF-Ag in acute liver injury but also a beneficial effect of vWF-Ag deficiency in a rodent model of liver fibrosis.<sup>(43,45)</sup> Thus, a reduction of risk for early waiting-list mortality in patients with low levels of vWF-Ag as found in this study might be explained both by reduced risk for severe complications and preservation from rapid progression of chronic liver

diseases. Nevertheless, the findings regarding the cutoff for MELD-Na-vWF at 20 points should be considered exploratory. Although these data clearly support the importance of vWF-Ag assessment for patients on the waiting list for OLT, the sample size as well as the study type are not suitable for definition of a precise cutoff. In addition, it has to be acknowledged that this study relies on a single-center data bank without external or internal validation. Yet, MELD-Na-vWF clearly shows an improvement when compared with MELD-Na alone, mostly through identification of patients with the highest risk for death while on the waiting list, which obtains a potential for renovation of the currently applied allocation system.

In conclusion, we found that vWF-Ag, a cheap and easily accessible plasma protein, is able to independently predict 3-month mortality in patients listed for OLT. Importantly, incorporation of vWF-Ag in the MELD-Na score was vital to significantly improve risk assessment for on-list mortality. Ultimately, the data provided within this analysis suggest that the adoption of vWF-Ag for the process of organ allocation could eventually result in a higher number of patients reaching the endpoint of transplantation, hence improving survival.

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## Supporting Information

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