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ORIGINAL ARTICLE

Prospective Assessment of Liver Function by an Enzymatic Liver Function Test to Estimate Short-Term Survival in Patients with Liver Cirrhosis

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Abstract

Background MELD attempts to objectively predict the risk of mortality of patients with liver cirrhosis and is commonly used to prioritize organ allocation. Despite the usefulness of the MELD, updated metrics could further improve the accuracy of estimates of survival.

Aims To assess and compare the prognostic ability of an enzymatic ^{13}C-based liver function test (LiMAx) and distinct markers of liver function to predict 3-month mortality of patients with chronic liver failure.

Methods We prospectively investigated liver function of 268 chronic liver failure patients without hepatocellular carcinoma. Primary study endpoint was liver-related death within 3 months of follow-up. Prognostic values were calculated using Cox proportional hazards and logistic regression analysis.

Results The Cox proportional hazard model indicated that LiMAx ($p < 0.001$) and serum creatinine values ($p < 0.001$) were the significant parameters independently associated with the risk of liver failure-related death. Logistic regression analysis revealed LiMAx and serum creatinine to be independent predictors of mortality. Areas under the receiver-operating characteristic curves for MELD (0.86 [0.80–0.92]) and for a combined score of LiMAx and serum creatinine (0.83 [0.76–0.90]) were comparable.

Conclusions Apart from serum creatinine levels, enzymatic liver function measured by LiMAx was found to be an independent predictor of short-term mortality risk in patients with liver cirrhosis. A risk score combining both determinants allows reliable prediction of short-term prognosis considering actual organ function.

Trial Registration Number (German Clinical Trials Register) # DRKS00000614.

Keywords End-stage liver disease · Liver function test · LiMAx · MELD · Risk assessment · Survival

Abbreviations

AUROC	Area under the receiver-operating characteristics
CI	Confidence interval
CPS	Child–Pugh score
ESLD	End-stage liver disease
GI	Gastrointestinal

Tomasz Dziodzio and Martin Stockmann have equally contributed to this work.

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HCV	Hepatitis C virus
HR	Hazard ratio
ICC	Intraclass correlation coefficient
INR	International normalized ratio
IQR	Interquartile range
LiMAx	Maximum liver function capacity
LTx	Liver transplantation
MELD	Model for end-stage liver disease
MELDNa	Sodium MELD
NAFLD	Nonalcoholic fatty liver disease
RC	Regression coefficient
ROC	Receiver-operating characteristic
SBP	Spontaneous bacterial peritonitis
SE	Sensitivity
SP	Specificity
SD	Standard deviation
UKELD	United Kingdom Model for End-Stage Liver Disease

Introduction

In several clinical situations, mathematical scores are used to assess disease severity and determine prognosis. Somehow uniquely, liver transplant societies all over the world have advocated a mathematical model to facilitate the most equitable and objective allocation of donor organs to liver transplant candidates—the model for end-stage liver disease (MELD) [1, 2]. Recently, alternative mathematical indices have been suggested that refine the MELD by incorporating additional prognostic parameters [3], by adjusting the formula [4], or by replacing the MELD policies [5]. Although it has contributed to a significant reduction in waitlist mortality since its implementation in 2002 [6], certain cohorts of patients may be disadvantaged in the MELD-based liver allocation era [7]. Bedside tests of hepatic synthetic function (bilirubin, albumin, and INR), high serum creatinine [8], and hyponatremia [9] represent good predictors of outcome in cirrhotic patients. However, the components of MELD might only represent surrogate markers for actual liver function and updated models are awaited [10]. In particular, the composition of coagulatory factors shows wide variability in patients with liver cirrhosis and INR measurements appear to be a less reliable tool to assess bleeding risk [11, 12]. Further, recently concepts of cirrhotic coagulation shifted to a thrombosis-prone construct with the need of anticoagulant treatments [13, 14]. Thus, an appropriate question to be raised is whether the consideration of approaches to quantify residual hepatic metabolic capacity to assess "true liver function" provides additional information on short-term prognosis of patients with end-stage liver disease (ESLD).

The initial work has shown LiMAx as a new ^{13}C-based breath test for the determination of maximum liver function capacity, useful for the assessment of individual risk prior to hepatic surgery [15, 16]. Its predictive value has been reported in patients with acute liver failure [17] and patients with bacterial sepsis [18]. We have previously demonstrated LiMAx as a noninvasive and simple tool for the assessment of enzymatic liver function in a pilot study of cirrhotic patients and healthy subjects [19].

Thus, it seems reasonable to evaluate LiMAx in patients with chronic liver disease to investigate its diagnostic value for the estimation of short-term prognosis in patients with ESLD.

Patients and Methods

Patients and Study Design

Between July 2009 and May 2013, we performed a prospective cohort study to evaluate and compare the prognostic ability of LiMAx and established scores for the prediction of short-term survival in consecutive patients with clinically suspected cirrhosis [the presence of at least two recognized complications such as endoscopy proven varices, history of encephalopathy, ascites of liver origin, imaging suggestive of cirrhosis (irregular outline of the liver, increased spleen size > 12 cm)], additional biochemical evidence of cirrhosis [such as platelet count < 120/nL in the absence of hematologic disorder, INR greater 1.5, and albumin levels lower 30 g/L], or histologically proven cirrhosis. Exclusion criteria were the previous liver surgery or liver transplantation, acute on chronic liver failure, patients undergoing liver support therapy, known or suspected hepatocellular carcinoma, current parenteral nutrition therapy, and history of illicit drug use or the presence of significant comorbidities potentially influencing short-term survival.

Only patients in a stable state were recruited from the outpatient and inpatient departments of the Charité - Universitätsmedizin Berlin. Inpatients were included after successful treatment of the underlying reason for hospital admission before discharge. Ethical approval was granted by the ethics committee of the Charité - Universitätsmedizin Berlin. The study was performed in accordance with ethical standards of the 1964 Declaration of Helsinki. Patients fulfilling inclusion criteria provided informed consent prior to study enrollment. Figure 1 shows the flowchart for study inclusion. Patients were followed from the date of study enrollment until death or liver transplantation or up to the time of analysis on January 12, 2014. Patients who underwent a liver transplantation within the 3 months of follow-up were censored at that time. One patient was lost to follow-up and was censored the date last known to be alive. The median

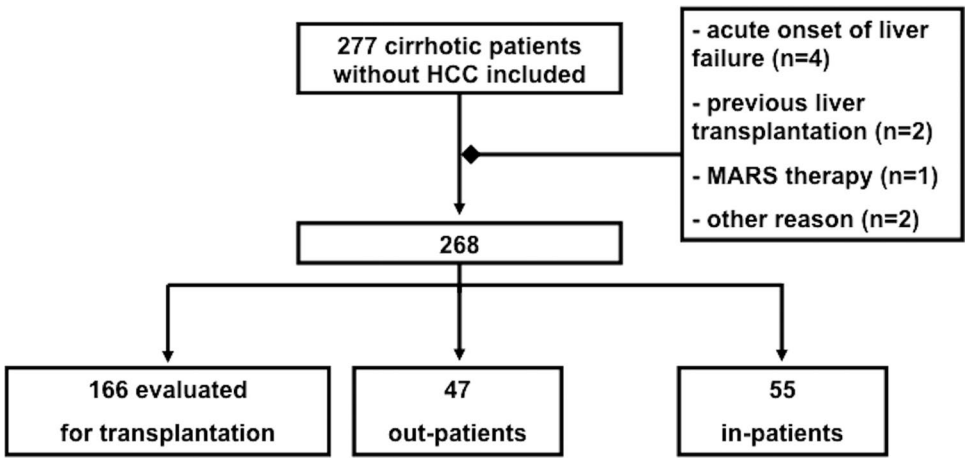

Fig. 1 Flow diagram of identified study population

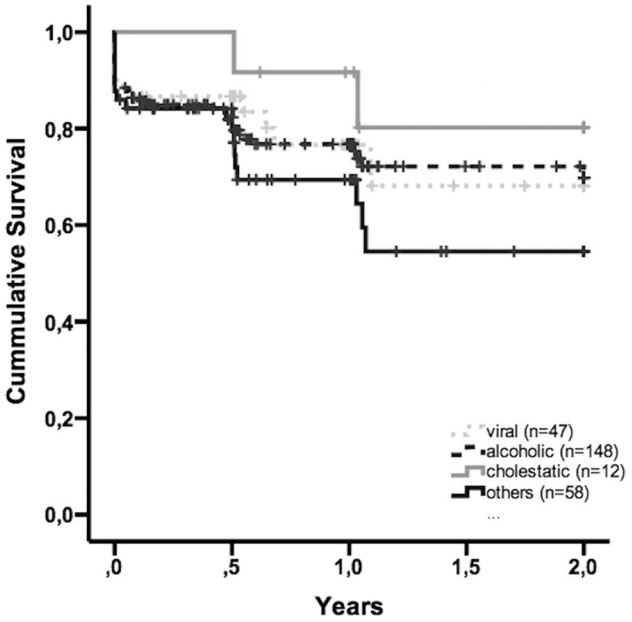

Fig. 2 Kaplan–Meier estimates for cirrhotic patients with alcoholic liver disease, viral hepatitis, and liver disease of "other" underlying reasons. The survival of all patient groups was similar (Breslow test over all 4 survival curves $p = 0.411$)

duration of follow-up was 234 [interquartile range (IQR): 84–390] days. Hence, survival data were available for 265 patients. We compiled a Kaplan–Meier survival estimate for patients with alcoholic liver disease, viral hepatitis, or "other" types of liver disease (Fig. 2). To evaluate the repeatability of LiMAx in cirrhotic livers, we performed the test in 13 patients on two consecutive days.

Procedures and Definitions

At the time of inclusion, a diagnostic workup was carried out in all eligible patients including physical examination, laboratory tests, and quantitative liver function testing. Test procedures were performed after a minimum of 12 h of fasting preferably in the morning in order to avoid influence of smoking or drug intake. Hepatic encephalopathy was graded using the West Haven criteria [20] and ascites according to published guidelines [21]. The MELD score was calculated using the standard formula considering UNOS modifications. The Child–Pugh score (CPS), MELDNa, UKELD were computed according to published formulas [3, 22, 23].

LiMAx Test Protocol

LiMAx (maximum liver function capacity) reflects the actual enzymatic capacity of the liver. The test is performed at the patient's bedside. The procedure is based on hepatocellular-specific metabolism of intravenously administered ^{13}C-labeled methacetin—a selective substrate of the hepatic cytochrome P450 1A2 enzyme. ^{13}C-methacetin is rapidly demethylated by the liver specific enzyme cytochrome P450 1A2 into acetaminophen and $^{13}CO_2$, which is subsequently exhaled [24]. As a consequence, metabolism of ^{13}C-methacetin results in increased $^{13}CO_2$ concentration in exhaled air [15]. The ratio of $^{13}CO_2/^{12}CO_2$ concentration is constantly measured in the exhaled air to analyze the maximal difference with respect to baseline values. As the $^{13}CO_2/^{12}CO_2$ ratio is determined, the test is not influenced by pulmonary disease or other factors influencing absolute CO_2 exhalation. Results are given in [µg/kg/h] and available directly after test termination. The one-sided reference range for LiMAx values was found to be greater 315 µg/kg/h [25].

Statistical Methods

Continuous descriptive data are given as median and IQR. Categorical data are summarized as number and frequencies. Comparison between two independent groups was performed with the Mann–Whitney *U* test and with the

Kruskal–Wallis test between more than two groups. Spearman's rank correlation test was used to determine correlation between variables. Repeatability within subjects was calculated as the agreement between LiMAx tests one and two and was estimated in a reliability analysis by the intraclass correlation coefficient (ICC), in a two-way mixed effects model where patient effects are random and measure effects are fixed.

Survival Analysis

In order to identify prognostic values for patient survival, we performed statistical concepts based on the initial publication of Malinchoc et al. [26] who introduced the MELD score in year 2000. We included single components considered for calculation of MELD, MELDNa, and Child–Pugh Score to compare the predictive values of individual clinical and laboratory parameters for patient survival. Moreover, we focused solely on 3-month survival according to the initial paper.

Univariate Cox proportional hazard analysis was performed with baseline clinical and laboratory parameters obtained at the time of study enrollment in a per-protocol manner. To identify independent prognostic effects of variables, we applied a multivariate Cox proportional hazard regression analysis including the same variables.

We used the *backward* stepwise variable selection method. Moreover, we assessed the interaction between candidate variables and 3-month survival using a multivariate logistic regression model. Herein we excluded patients who were lost to follow-up or underwent liver transplantation. With respect to these results, we developed a new score based on actual enzymatic liver and renal function.

Once the predictive model had been finalized, we compared the performance of the new model to the MELD and CPS. Receiver-operating characteristic (ROC) curves were assessed to estimate the ability of LiMAx, MELD, and CPS to discriminate between patients who died and survived. Optimal cutoff values for each tool were chosen for maximal Youden's index [sensitivity (Se) + (specificity (Sp) − 1]. Diagnostic performance of each test was expressed as area under the receiver-operating characteristics (AUROC), and differences between AUROCs of several ROC curves were calculated using the DeLong test [27].

3-month survival rates were estimated using Kaplan–Meier characteristics. Differences between survival curves were determined using the Breslow–Wilcoxon test. Statistical analysis was performed with R 3.0.2 open-source software package and with IBM SPSS Statistics 22 software package (Armonk, NY, USA).

Results

Study Population

Table 1 summarizes clinical baseline data of the 268 patients studied. Twenty-seven patients (10.1%) died, and twenty-seven patients (10.1%) received a liver transplant within 3 months of enrollment. Two deaths were not liver related (both after surgical interventions) and were excluded from survival analysis.

LiMAx as a Marker of Hepatic Dysfunction and Disease Severity

Strong correlations were found between surrogate markers of liver function and LiMAx (INR: $r_s = -0.673$; $p < 0.001$; serum albumin: $r_s = 0.459$; $p < 0.001$ and serum bilirubin: $r_s = -0.596$; $p < 0.001$). LiMAx showed significant strong negative correlations with MELD and CPS ($r_s = -0.603$; $p < 0.001$ and $r_s = -0.632$; $p < 0.001$, respectively). Figure 3 shows the variation in LiMAx values across CP classes.

In a subset of 13 cirrhotic patients, we analyzed the repeatability of LiMAx on two consecutive days. One patient was classified as CP class A (7.7%), six as CP class B (46.6%), and six as CP class C (46.6%). Median MELD was 19 (IQR: 13–24) points. Median LiMAx values measured on day one [79 (IQR: 53–114) µg/kg/h] did not differ significantly from LiMAx values measured on day two [82 (IQR: 63–117) µg/kg/h; $p = 0.843$] indicating a mean difference of $-0.3 \pm 22\%$ standard deviation (SD) (2 ± 18 µg/kg/h) between both days (Suppl. Table 1; Suppl. Figure 1). The corresponding intraclass correlation coefficient was excellent with 0.98 (95% confidence interval 0.94–0.99).

Factors Predictive of Short-Term Survival

Single components of the MELD, CPS, and enzymatic liver function capacity as measured by LiMAx were tested univariately using a Cox proportional hazard model to estimate their single predictive potential with respect to 3-month mortality. By applying a multivariate Cox proportional hazard model including the same variables at the time of study enrollment, only serum creatinine and LiMAx were independent predictors (hazard ratios (HR): creatinine: 2.11; 95% CI 1.44–3.08; $p < 0.001$ and LiMAx: 0.98; 95% CI 0.97–0.99; $p < 0.001$) (Table 2).

Logistic regression analysis revealed these two parameters as being significantly associated with 3-month mortality. Regression coefficients (RC) were as follows: LiMAx

Table 1 Epidemiological, clinical, and biochemical characteristics of 268 cirrhotic patients with a follow-up period of 3 months

Variables	Patient Cohort
Age (years)	55 (49–60)
Gender *n* (%)	
Female	103 (38.4)
Male	165 (61.6)
BMI (kg/m^2)	26.1 (23.0–29.4)
Etiology *n* (%)	
Alcoholic	151 (56.3)
Autoimmune	14 (5.2)
Cholestatic	12 (4.5)
Cryptogenic	20 (7.5)
NAFLD	14 (5.2)
Viral	47 (17.6)
Others	10 (3.7)
Serum albumin (g/L)	31.6 (27.3–35.6)
Serum bilirubin (g/dL)	2.9 (1.5–4.8)
INR	1.46 (1.27–1.73)
Platelet count (nL)	90 (66–133)
Serum creatinine (mg/dL)	0.88 (0.70–1.15)
Serum sodium (mmol/L)	137 (133–139)
Ascites grade *n* (%)	
None/mild	74 (27.6)
Moderate	131 (48.9)
Severe	63 (23.5)
SBP episodes	
No	222 (82.8)
Once	39 (14.6)
Multiple	7 (2.6)
HE grade *n* (%)	
Grade 0	205 (76.5)
Grade I	51 (19.0)
Grade II	11 (4.1)
Grade III	1 (0.4)
Esophageal varices *n* (%)	
Yes	213 (79.5)
No	55 (20.5)
Previous GI hemorrhage *n* (%)	95 (35.4)
Child–Pugh classes *n* (%)	
A	43 (16.0)
B	118 (44.0)
C	107 (39.9)
MELD	16 (13–20)
MELDNa	19 (14–23)
UKELD	52 (49–55)
LiMAx (µg/kg/h)	93 (52–147)
Underwent LTx *n* (%)	27 (10.1)
Died *n* (%)	27 (10.1)

Continuous variables are displayed as median and interquartile range and categorical variables as number and percentage

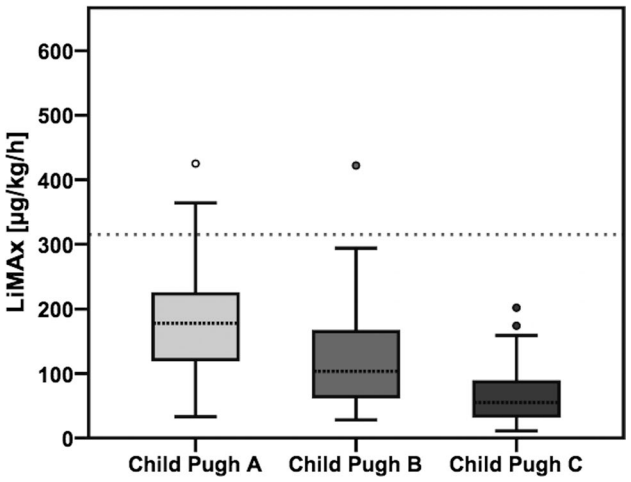

Fig. 3 Box and whiskers plot of LiMAx values across Child–Pugh classes. Median LiMAx differed significantly between Child–Pugh classes [A vs. B (178 (IQR: 121–225) µg/kg/h vs. 104 (IQR: 64–166) µg/kg/h; $p < 0.001$ and B vs. C (55 (IQR: 34–88) µg/kg/h; $p < 0.001$]. The horizontal dashed line represents the LiMAx cutoff for normal (> 315 µg/kg/h)

Table 2 Uni- and multivariate Cox regression analysis (backward stepwise selection) of parameters associated with liver-related 3-month mortality ($n = 265$)

Parameter	*p* univariate	*p* multivariate	HR	95% CI
Nonmild ascites	0.045	Excluded		
Moderate ascites	0.014	Excluded		
Severe ascites	0.246	Excluded		
No HE	< 0.001	Excluded		
HE grade 1	0.105	Excluded		
HE grade 2	< 0.001	Excluded		
Serum albumin	0.001	Excluded		
INR	0.006	Excluded		
Serum bilirubin	< 0.001	Excluded		
Serum sodium	0.026	Excluded		
Serum creatinine	0.014	< 0.001	2.11	1.44–3.08
LiMAx	0.002	< 0.001	0.98	0.97–0.99

Multivariate model contained the following parameters: INR, serum creatinine, total serum bilirubin, serum sodium, serum albumin (all continuous), grade of ascites, and HE (categorical) and LiMAx (continuous)

RC: − 1.344; RC standard error: 0.335; $p < 0.001$, and serum creatinine RC: − 1.751; RC standard error: 0.504; $p < 0.001$.

Prediction of Survival Probability Considering Enzymatic Liver Function and Renal Function

Based on the results of the Cox proportional hazard analysis, we developed a model considering LiMAx and serum

creatinine for the prediction of survival probability of individual patients. Therefore, we generated the following equation using the coefficients of respective parameters:

$$\text{CreLiMAx} = -0.532 * \log(\text{LiMAx}) + 1.373 * \log(\text{serum creatinine})$$

To obtain the likelihood of survival for each patient, we used the estimated baseline hazard values. Individual risk score (R) is computed on the basis of the survival function [S_0(t)] and calculated S(t) according to the following equitation $S(t) = S_0(t)^{\exp(R-Rm)}$. R_m represents the mean risk score of the present study cohort with a value of − 2.472 (Suppl. Table 2).

For example, a patient with LiMAx = 55 and creatinine = 2.2, respectively, log(LiMAx) = 4 and a log (creatinine) = 0.79 results in a risk score of $R = -0.532 \times 4 + 1.373 \times 0.79 = -1$. That leads to a probability of survival of $S(t) = S_0(t)^{\exp(R-Rm)} = 0.919^{\exp(-1+2.472)} = 0.7 \hat{=} 70\%$.

Further, we validated the described Cox proportional hazard model by using the method of cross-validation with a number of ten repetitions. To evaluate the result, we used Somers D_{xy} as described by Harrell [28]. The average D_{xy} for the original, the training, and the test data set was very similar to − 0.4432, − 0.4443, and − 0.4479. That led to a small optimism of 0.0036. Therefore, the corrected original D_{xy} was − 0.4468. On the basis of these results, we assume no critical overfit with an expected prediction regarding the below described predictive values.

Comparison of the Prognostic Value of CreLiMAx Risk Score to Other Prognostic Scores

We analyzed the discriminative ability of LiMAx, CPS, MELD, MELDNa, UKELD, and the herein suggested risk score to prioritize patients validly according to their risk of death by means of ROC analysis. With respect to 3-month survival, the discriminative ability of LiMAx alone to identify patients at risk of death revealed good results (AUROC: 0.75; 95% CI 0.65–0.85) although combined prognostic models showed better accuracy. Combination of LiMAx and MELD did not yield significant additional prognostic value (AUROC: 0.86; 95% CI 0.80–0.92).

The proposed CreLiMAx risk score performed similar to MELD with an AUROC of 0.83 (95% CI 0.76–0.90). Comparison of AUROCs revealed consistent diagnostic accuracy of the CreLiMAx risk score when compared with the MELD ($p = 0.455$). Respective values of diagnostic accuracy are demonstrated in Table 3.

Discussion

Although MELD forms the current basis of an organ allocation system based on medical urgency, there remains room for improvement [10]. Several studies have shown acceptable predictive ability of quantitative liver function tests in patients with cirrhosis, but did not exceed those of established biological indices [29–31]. ^{13}C-liver function breath tests have been shown to be particularly useful, but there is still an unmet need for a widely accepted method [32]. We have extensively evaluated the LiMAx test methodology [16, 17, 19, 25] and its prognostic value in different clinical situations [17, 18]. However, its application and prognostic ability need to be evaluated in cirrhotic patients before enzymatic liver function can be advocated for the estimation of prognosis in ESLD.

The results of the present study indicate that, apart from serum creatinine, enzymatic liver function represents the major independent factor influencing short-term prognosis of patients with liver cirrhosis. To our knowledge, this is the first study that analyzes the prognostic value of an enzymatic liver function test in a formal statistical model containing single components of the MELD and CPS. Such an analysis appears mandatory in order to support the merging potential of determinants of metabolic liver function for the assessment of survival in ESLD. Moreover, in-between day repeatability of LiMAx appeared to be excellent in this study indicating robustness and reliability of test results in such patients.

LiMAx correlated well with accepted surrogate markers of liver failure and prognostic scores. We showed that LiMAx appears to be a valid tool for the assessment of the actual liver function, which mirrors the CP grading system excellently, with sicker patients scoring significantly

Table 3 Diagnostic accuracy for prediction of 3-month survival for prognostic tools

	AUROC	Sensitivity (%)	Specificity (%)	PPV	NPV	Cutoff
CPS	0.82	84	70	25	97	10
MELD	0.86	100	55	21	100	16
MELDNa	0.87	96	65	29	97	21
UKELD	0.83	92	67	26	98	53
LiMAx (µg/kg/h)	0.75	64	81	29	95	53
CreLiMAx	0.83	88	70	26	98	0.1

lower than patients with less severe liver disease. Further, LiMAx appears to reliably measure enzymatic liver function in patients with liver cirrhosis. These results suggest that LiMAx is a robust and reliable marker of the enzymatic liver function in cirrhotic patients. The previous studies reported the clinical application of the test to determine functional hepatic reserve in patients undergoing liver surgery [15, 16], liver transplantation [33, 34], and bariatric surgery [35]. More importantly, recent work also showed that LiMAx accurately reflects the residual liver function in patients with cirrhosis and reliably distinguishes between early Child–Pugh classes and noncirrhotic patients [19]. Its extensive first-pass bioavailability, instant enzymatic metabolization into acetaminophen and $^{13}CO_2$ [36], and lack of toxicity [15, 37] support the use of methacetin metabolism to provide information on true liver function. Compared to the methacetin breath test (MBT) with oral substrate administration, we refined the methodology by the introduction of intravenous substrate administration to overcome the limitation of impaired intestinal transport [38] and to assess exclusively the metabolism of hepatic enzymatic activity in real time. Additionally, LiMAx is a noninvasive test, which can be performed easily, repeatedly, and at low cost in an ambulant setting.

Increased risk of death associated with impaired enzymatic liver function capacity and renal dysfunction is indicated in both multivariate Cox proportional hazard analysis and multivariate logistic regression analysis. Since LiMAx reflects the actual and individual enzymatic liver function, it should be considered as a complement to establish surrogate markers of degree of liver failure (INR, serum albumin, and serum bilirubin levels) in order to achieve a more accurate and refined representation of hepatic function [39]. In turn, enzymatic capacity of the liver appears to be of greater importance compared to clinical parameters such as decompensation with ascites or hepatic encephalopathy [8, 40].

By contrast, renal dysfunction could be repeatedly confirmed as an important, but also indirect marker of advanced liver cirrhosis, which strongly influences individual prognosis [41]. In turn, the creation of a predictive model combining both parameters appears noteworthy, as established scores do not include any parameters reflecting "true" quantitative liver function.

When we calculated a risk score including LiMAx and serum creatinine, predictive accuracy of established models was comparable. Although MELDNa has not been widely adopted as an organ allocation system, it had the highest prognostic value in this cohort study. These results are in line with the findings of numerous high-quality studies, in which decreased serum sodium concentration was found to be associated with significant morbidity and mortality of patients on the liver transplant waiting list [42–45]. A recent multicenter study analyzing the application of a MELDNa exception for organ allocation in this subpopulation concluded that serum sodium concentration seems to allow a high rate of transplant in patients normally underserved in the MELD era without reducing efficacy of transplantation [46].

Possible limitations of the present study need to be mentioned. First, the potential influence of porto-systemic shunts and in turn modulated hepatic blood flow is frequent causes of concerns when applying functional tests in cirrhotic patients. We have previously demonstrated that the presence of transjugular intrahepatic porto-systemic shunts does not affect LiMAx test results in cirrhotic patients [19]. Moreover, in-between day repeatability appeared to be excellent in this study, indicating that hemodynamic variables seem not to influence test methodology. Second, although we have tried to enroll patients with a wide spectrum of chronic liver disease, the generalizability of current findings is limited due to the single-center design of the present study. Third, we did not validate the proposed score in a separate cohort. Although an external validation seems favorable, we have performed an extensive internal validation using a state-of-the-art statistical approach. Statistically speaking, this approach is of equal value as an external validation.

In the light of the raised concerns and ongoing debates about the utility of MELD [47], the reported findings represent an important step toward a possible endorsement and refinement of MELD through integration of a parameter representing enzymatic liver function. Portal vein thrombosis has been shown to be associated with significantly higher posttransplant mortality after liver transplantation [48]. Current expert opinion suggests anticoagulant treatment for the management of portal vein thrombosis in cirrhotic patients on the transplant waiting list, which in turn might affect the applicability of established prognostic models in those patients [13, 14]. Hence, in such patients the actual enzymatic liver function and CreLiMAx risk score is appealing as an INR independent adjunct to the MELD to gauge the individual risk of mortality. Unfortunately, a subgroup analysis of diagnostic accuracy of both scores could not be performed due to the small number of patients receiving anticoagulatory treatment.

In this context, we want to highlight recently published results from a pilot study reporting the methacetin breath test as a suitable tool to predict individual cirrhotic complications, especially in patients with low MELD classes [49]. These findings depict the ancillary value of objective tests of liver function to the MELD policy in order to optimize management of cirrhotic patients and maintain the highest possible priority ranking on the liver transplant waiting list. Considering the limited availability of LiMAx, we see the strength of such tests in the early discrimination of patients at increased risk of developing complications that are not adequately captured by the MELD.

In conclusion, the present cohort study revealed enzymatic liver function measured by means of LiMAx and serum creatinine as major independent factors influencing the prognosis of patients with liver cirrhosis. The proposed new risk score showed very good diagnostic accuracy. Taking everything into consideration, the current model proposes to be a valuable new score in general and might complement current risk stratification models based on the MELD.

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Compliance with ethical standards

Conflict of interest Martin Stockmann is the inventor of the LiMAx test and has capital interest in Humedics, the company marketing the LiMAx test. Maximilian Jara and James Orr disclose having received research Grants in order of the d-LIVER European Commission's Seventh Framework Programme/European Research Council, Grant Agreement Number 287596—www.d-liver.eu. Martin Stockmann was also steering committee member for the d-LIVER project. Remaining authors who have taken part in this study declared no conflict of interest with respect to this manuscript.

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