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ORIGINAL ARTICLE

Prognostic value of enzymatic liver function for the estimation of short-term survival of liver transplant candidates: a prospective study with the LiMAx test

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Conflicts of interest
M. Stockmann is the inventor of the LiMAx test and has capital interest in Humedics, the company marketing this test. M. Jara discloses receiving research grants in order of the d-LIVER European Commission Framework Program. Remaining 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.

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Summary

LiMAx has been recently proposed as a new quantitative liver function test. Thus, we aimed to evaluate the diagnostic ability of LiMAx to assess short-term survival in liver transplant candidates and compare its performance to the model for end-stage liver disease (MELD) and indocyanine green plasma disappearance rate (ICG-PDR). Liver function of 167 chronic liver failure patients without hepatocellular carcinoma was prospectively investigated when they were evaluated for liver transplantation. Primary study endpoints were liver-related death within 6 months of follow-up. Within 6 months of follow-up, 18 patients died and 36 underwent liver transplantation. Median LiMAx results on evaluation day were significantly lower in patients who died (99 μg/kg/h vs. 55 μg/kg/h; $P = 0.024$), while median ICG-PDR results did not differ within both groups (4.4%/min vs. 3.5%/min; $P = 0.159$). LiMAx showed a higher negative predictive value (NPV: 0.93) as compared with ICG-PDR (NPV: 0.90) and the MELD (NPV: 0.91) in predicting risk of death within 6 months. In conclusion, LiMAx provides good prognostic information of liver transplant candidates. In particular, patients who are not at risk of death can be identified reliably by measuring actual enzymatic liver function capacity.

Introduction

Prognosis of chronic liver disease represents the basis for any decision-making process. At present in Germany and many other countries, there is a discrepancy between available donor organs and patients on the waiting list for liver transplantation. The model for end-stage liver disease (MELD) has been identified as a precise tool to predict short-term survival of cirrhotic patients and is currently used in many countries for priority ranking to objectively allocate donor organs [1–3]. However, even in the MELD era, some groups of patients might still be underserved, and thus, the optimal system for prognostic discrimination of liver transplant candidates remains a keenly debated

topic. Alternative parameters besides MELD components have been suggested being of individual prognostic value for assessment of pretransplant survival and with respect to the actual sickest first allocation policy (to offer donor livers to recipients in greatest need with substantial risk of dying); also, the consideration of distinct variables is suggested to not only focus on waiting list mortality but also post-transplant outcome [4–6].

LiMAx was recently proposed as a quantitative liver function test allowing the measurement of the enzymatic liver function capacity [7]. LiMAx reflects actual enzymatic liver function capacity based on hepatocellular-specific metabolism of intravenously administered ^{13}C-labeled methacetin – a substrate of the hepatic cytochrome P450 1A2 enzyme. ^{13}C-methacetin is selectively demethylated into acetaminophen and $^{13}CO_2$, which results in increasing $^{13}CO_2$ concentration in the exhaled air. The ratio of $^{13}CO_2/^{12}CO_2$ is measured over the test period. The more ^{13}C-methacetin is metabolized, the more $^{13}CO_2$ is exhaled resulting in higher LiMAx.

Previous studies reported the clinical utility and prognostic potential of LiMAx in liver transplant recipients, and recently, we demonstrated its prognostic value also in acute liver failure [8–10]. To date, LiMAx has not been evaluated as a prognostic tool in liver transplant candidates. Therefore, we investigated the prognostic ability of LiMAx and compared it with ICG-PDR and the MELD in a large cohort of liver transplant candidates without hepatocellular carcinoma.

Patients and methods

The study population comprised adult patients in stable end-stage liver disease evaluated for their first liver transplantation at the Department of Transplantation Surgery, Charité-Universitätsmedizin Berlin, Campus Virchow Klinikum between July 2009 and April 2013. Exclusion criteria were previous liver transplantation, acute on chronic liver failure, patients under liver support therapy, and patients evaluated for liver transplantation due to hepatocellular carcinoma. Patients fulfilling these criteria were enrolled after obtaining their written informed consent. The institutional ethics committee approved the study protocol, and the study was performed in accordance with ethical standards of the 1964 Declaration of Helsinki. One hundred and sixty-seven consecutive patients fulfilling the inclusion criteria were enrolled, and their data was retrieved for statistical analysis.

Methods

Patients underwent quantitative liver function tests (QLFT) and blood tests after study enrollment. Blood samples were drawn prior to test initiation from a peripheral vein, and MELD score was calculated according to UNOS modifications:

$$11.2 \times \ln(\text{INR}) + 9.57 \times \ln[\text{creatinine (mg/dl)}] + 3.78 \times \ln[\text{totalbilirubin (mg/dl)}] + 6.43$$

Survival and death were assessed 6 months after study enrollment by contacting patients, carers, or general practitioners.

Liver function tests

LiMAx (maximum liver function capacity) reflects the actual enzymatic liver function capacity. The LiMAx procedure is based on bodyweight adjusted intravenous ^{13}C-labeled methacetin bolus injection as previously described [7]. Exhaled breath was continuously analyzed using a special device [Fast liver investigation package (FLIP)®; Humedics GmbH, Berlin, Germany]. Prior to substrate injection, the baseline ratio of $^{13}CO_2/^{12}CO_2$ concentration was recorded in the native exhaled air. Using the mean, individual baseline was set for the delta-over-base calculation $^{13}CO_2/^{12}CO_2$ values. After intravenous administration of 2 mg/kg bodyweight ^{13}C-methacetin followed by 20 ml 0.9% sodium chloride, dynamic of $^{13}CO_2$ production was measured online over a period of 60 min maximum, and the LiMAx value was calculated following the previously described formula [7]. Results are given in μg/kg/h.

Indocyanine green plasma disappearance rate (ICG-PDR) evaluates hepatic clearance and is suggested to provide additional information on liver function [11,12]. After intravenous application, ICG is bound to albumin and eliminated by hepatic parenchymal cells via an energy dependent transport mechanism for conjugated and unconjugated bilirubin. ICG-PDR was noninvasively measured over a period of 15 min by a commercially available pulse dye densitometer (Dye Densitogram Analyzer DDG2001, Nihon Kohden, Japan) using a finger sensor [13]. Each individual received an intravenous bolus injection of 0.5 mg/kg bodyweight ICG solution (ICG-Pulsion, Pulsion Medical Systems, Feldkirchen, Germany) dissolved in aqueous solvent immediately flushed with 10 ml normal saline. The ICG-PDR was determined. Results are given as %/min, and normal ICG-PDR values are considered to be over 18%/min [14].

Statistical analysis

Data were analyzed using statistical software IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as median and interquartile range (IQR;

25th–75th percentile) and categorical variables as frequencies and percentage. We applied the chi-squared test for categorical variables and the Mann–Whitney *U*-test for comparison of quantitative variables. Correlations were calculated using Spearman's correlation coefficient. To evaluate the optimal cut-off of selected parameters to discriminate between survivors and nonsurvivors, we used receiver operating characteristic (ROC) curves. Six-month survival rates were estimated using Kaplan–Meier methods with patients undergoing liver transplantation censored at the time. Differences between survival curves were determined using the Breslow–Wilcoxon test. A *P* value of less than 0.05 was considered statistically significant.

Results

Clinical and epidemiological characteristics of 167 liver transplant candidates without HCC are shown in Table 1. Predominant cause of liver disease was alcoholic liver disease (57%). Within 6 months of follow-up, 36 patients underwent liver transplantation and 18 died. Interval between study enrollment and death was 46 (22–114) days and 60 (43–120) days between study enrollment and liver transplantation. Time between listing and death was 23 (10–117) days, between listing and liver transplantation 42 (22–104) days, and between listing and follow-up visit 175 (160–188) days, respectively. Among them, 17 deaths were related to liver disease, while one was not liver-related (sepsis after partial nephrectomy due to new diagnosed renal clear cell carcinoma). Thus, this patient was excluded for subsequent survival analysis.

Table 1. Patient characteristics.

	Liver transplant candidates, $n = 167$
Age (years)	55 (48–59)
Gender (m/f)	100 (59.9%)/ 67 (40.1%)
BMI (kg/m^2)	26.57 (23.15–29.59)
Etiology	
Alcohol	96 (58%)
Viral hepatitis	27 (16.2%)
NAFLD	11 (6.6%)
AIH	9 (5.4%)
Cholestatic (PBC/PSC)	7 (4.2%)
Cryptogenic	12 (7.2%)
Others	5 (3%)
MELD	16 (13–20)
Child-Pugh classes (A/B/C)	24 (14.4%)/ 79 (47.3%)/ 64 (38.3%)
LiMAx (μg/kg/h)	90 (51–135)
ICG-PDR (%/min)	4.2 (3.0–5.9)
Underwent liver transplantation	36 (21.6%)
Liver related deaths	17 (10.2%)

BMI, body mass index; NAFLD, non alcoholic fatty liver disease; AHI, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; MELD, model for end-stage liver disease; LiMAx, maximum liver function capacity; ICG-PDR, indocyanine green plasma disappearance rate.
Data are medians and interquartile range (IQR; 25th percentile–75th percentile) and frequencies and percentage.

LiMAx and ICG-PDR showed significant negative correlation with MELD score ($r_s = -0.55$; $P < 0.001$ and $r_s = -0.51$; $P < 0.001$, respectively) and Child-Pugh score (CPS) ($r_s = -0.60$; $P < 0.001$ and $r_s = -0.44$; $P < 0.001$, respectively). Table 2 summarizes clinical, biochemical, and QLFT results of patients after a follow-up period of 6 months. Median MELD and CPS were significantly

Table 2. Clinical and biochemical characteristics of patients according to 6-month survival – patients receiving a liver transplantation within 6 months of follow up were excluded for this analysis.

	Survivors ($n = 113$)	Liver-related deaths ($n = 17$)	*P* value
Age (years)	54 (47–58)	57 (48–60)	0.415
BMI (kg/m^2)	25.83 (22.77–29.21)	24.69 (23.50–30.65)	0.959
Gender (m/f)	63/50	10/7	0.812
Etiology (ALD/viral/ others)	61/ 22/ 30	11/ 1/ 5	0.388
Serum bilirubin (mg/ dl)	2.6 (1.4–3.8)	4.1 (3.2–7.9)	**0.001**
Serum albumin (g/l)	32.0 (28.0–36.0)	29.0 (25.3–33.5)	0.061
INR	1.4 (1.3–1.7)	1.7 (1.4–1.9)	0.063
Serum creatinine (mg/dl)	0.85 (0.66–1.07)	0.97 (0.74–1.35)	0.099
Ascites grade*			
Mild	69	8	0.373
Moderate	23	6	
Severe	21	3	
HE grade†			
Grade 0	94	12	0.212
Grade I-II	19	5	
Jaundice (yes/no)	74/39	15/2	0.060
Varices (yes/no)	89/24	15/2	0.363
Child-Pugh score	8 (7–10)	10 (8–11)	**0.005**
MELD score	15 (12–18)	19 (16–23)	**0.001**
LiMAx (μg/kg/h)	99 (63–150)	50 (40–114)	**0.024**
ICG-PDR (%/min)	4.4 (3.2–6.2)	3.5 (2.2–5.6)	0.159

BMI, body mass index; INR, international normalized ratio; HE, hepatic encephalopathy; MELD, model for end-stage liver disease; LiMAx, maximum liver function capacity; ICG-PDR, indocyanine green plasma disappearance rate.
Data are medians and interquartile range (IQR; 25th percentile–75th percentile) or frequencies and percentage.
Bold *p*-values indicate significance $p < 0.05$.
*****Ascites was classified as mild: no ascites or only detectable by ultrasound, moderate: causing distention of the abdomen and shifting dullness, severe: marked abdominal distension with or without transmitted fluid wave [29].
†According to West Heaven criteria [30].

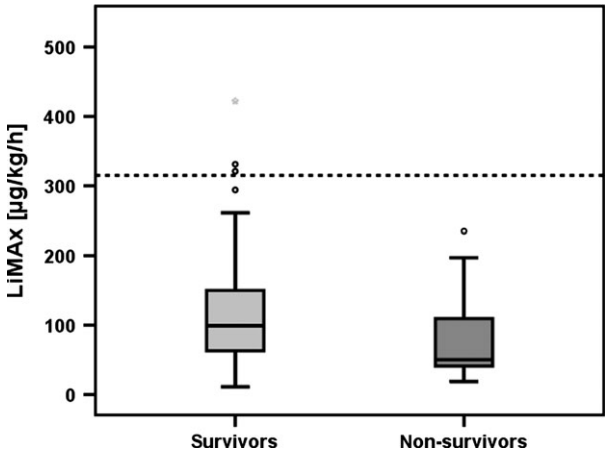

Figure 1 Outcome of patients according to LiMAx values measured on evaluation day. Bold lines indicate medians, boxes the range from lower to upper quartile, whiskers represent 1.5 interquartile range and circles outliers. Bold dotted horizontal line indicates the cut-off for normal (>315 µg/kg/h). Differences between survivors and nonsurvivors were 99 (63–150) µg/kg/h and 50 (40–114) µg/kg/h; $P = 0.024$.

Table 3. Cut-off value of quantitative liver tests and MELD for probability of death within 6 months.

Variable	Cut-off	Positive predictive value	Negative predictive value	Negative diagnostic likelihood ratio
LiMAx	55 µg/kg/h	0.29	0.93	0.07
MELD	20	0.35	0.91	0.09

LiMAx, maximum liver function capacity; MELD, model for end-stage liver disease.

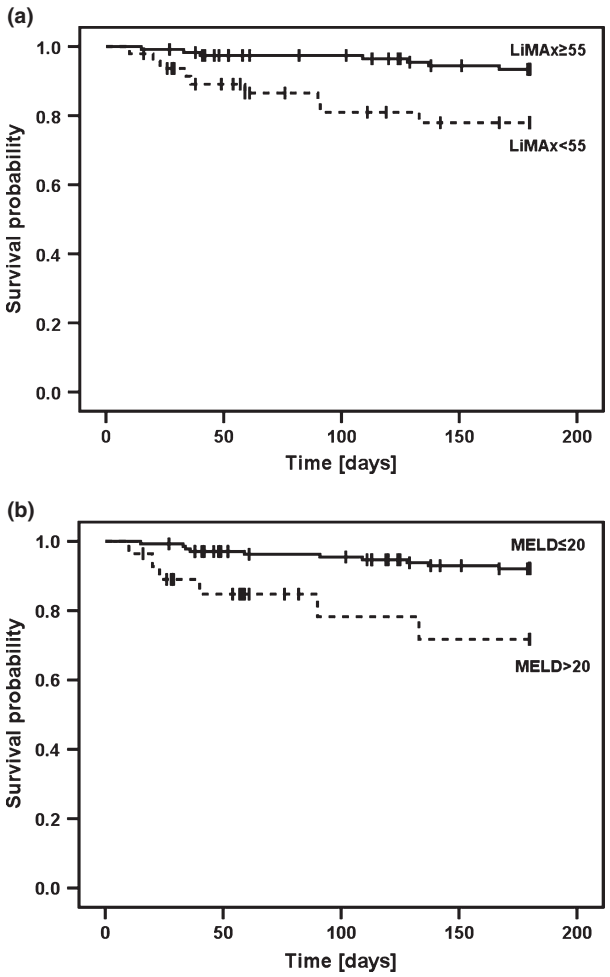

Figure 2 Kaplan–Meier estimates according to the optimal cut-off for (a) LiMAx (b) model for end-stage liver disease (MELD). Differences between survival curves were determined using the Breslow–Wilcoxon test and were for LiMAx estimates $P = 0.003$ and for MELD estimates $P = 0.002$.

different between patients who survived and those who died, while among single parameters solely serum bilirubin and LiMAx values differed significantly between both groups. Median LiMAx values in survivors were 99 (63–150) µg/kg/h, while median values in nonsurvivors were 50 (40–114) µg/kg/h ($P = 0.024$) (Fig. 1).

We calculated the best discriminative cut-off value for predicting the probability of death within 6 months after evaluation, which was 55 µg/kg/h for LiMAx, 3.9%/min for ICG-PDR, and 20 for the MELD. When these cut-off values were applied, LiMAx showed a positive predictive value (PPV) of 0.29 and a negative predictive value (NPV) of 0.93 (sensitivity: 0.56; specificity 0.81), ICG-PDR a PPV of 0.15 and a NPV 0.90 (sensitivity: 0.53; specificity 0.59) and MELD a PPV of 0.35 and a NPV of 0.91 (sensitivity: 0.38; specificity 0.90) (Table 3). Calculated negative diagnostic likelihood ratios (DLR) were good for LiMAx (DLR: 0.07) and MELD (DLR: 0.09) and with 0.10 moderate for ICG-PDR [15]. Kaplan–Meier survival estimates yielded significant differences of patient survival for MELD ($P < 0.001$) and LiMAx ($P = 0.003$), but not for ICG-PDR ($P = 0.291$) (Fig. 2). Further, Kaplan–Meier survival curves did not differ between patients with and without manifest ascites ($P = 0.662$), patients with and without manifest encephalopathy ($P = 0.128$) and patients with and without manifest esophageal varices ($P = 0.212$).

Discussion

The results emerging from the present study show that LiMAx appears to be a suitable predictor of 6-month survival of patients evaluated for liver transplantation with a good discriminative ability for the identification of transplant candidates who are not at risk of dying within this period.

LiMAx has been initially reported as a reliable tool for the evaluation of liver function and risk stratification of liver surgical patients in the perioperative work-up and has demonstrated its ability to predict postoperative outcome [7,16]. Recently, we could also show its prognostic value in patients with acute liver failure [10]. Hence, it seems reasonable to investigate the clinical utility of LiMAx in patients with chronic liver disease and to explore its discriminative ability in identifying patients at risk of death. Additionally, analysis of the prognostic potential of ICG-PDR, another well-studied QLFT, and the MELD allows a direct comparison of respective prognostic efficiencies.

Herein, we were able to demonstrate LiMAx as a suitable prognostic marker for 6-month survival in liver transplant candidates. Among the predictors analyzed, the MELD, CPS, serum bilirubin, and LiMAx appeared to be associated with the risk of death within 6 months of follow-up. When we compared the performance of diagnostic test using identified cut-offs, LiMAx showed the highest NPV value for the accurate identification of those patients who did not die within the 6 months of follow-up. This was superior as compared with the MELD and ICG-PDR. These findings suggest LiMAx as an appropriate tool to estimate mortality risk in patients evaluated for transplantation. This is of particular of interest because the reported optimal MELD cut-offs to identify patients with poor survival and best survival benefit after transplantation lie within the range of the identified cut-off in our cohort [17,18]. Hence, actual enzymatic liver function capacity measured by means of LiMAx appears to provide additional prognostic information on patients in clinically moderate stages of liver disease that already benefit from liver transplantation. In conjunction with the MELD and the results deriving from the evaluation work-up, actual enzymatic liver function capacity measured by means of LiMAx might also be considered as a variable before candidates are registered on the waiting list. By integrating LiMAx in the diagnostic work-up for liver transplant evaluation, decision-making would base on an additional objective method that reflects the actual parenchymal function of the liver. Thus, it may be possible to quantify liver function impairment and to predict not only to average likelihood of death but also the individual mortality risk of patients based on actual enzymatic liver function. As we could show that LiMAx validly identifies those patients with low mortality risk, it could be suggested to re-evaluate patients with moderate MELD and LiMAx values above the mentioned cut-off at a later point in time. Within this context, it seems obvious that tools based on biochemical components – such as the MELD or sodium MELD, which have been defined based on big data deriving from organ transplant networks – are practical and widely accepted as prognostic measures. However, renal dysfunction is known to influence individual prognosis in patients with end-stage liver disease, and serum creatinine is strongly powered in the MELD and sodium MELD formula. In contrast, LiMAx provides information on actual liver function alone and thus might enable physicians to assess the course of liver disease and prognosis relying exclusively on a parameter reflecting the actual functional state of the liver. Beyond that repeat re-evaluation of enzymatic liver function impairment using LiMAx is absolutely valid and easy to be performed even in an outpatient setting providing test results instantly after test termination, while MELD results derive usually with timely delay because blood samples need to be analyzed by clinical laboratories. Although acquisition costs of the special FLIP-device may hinder a rapid and widespread clinical usage in particular in times of economic restriction in the public health system, it should be acknowledged that if its demonstrable and quantifiable that LiMAx provides any benefit in terms of improved diagnostic ability and/or effects on an improved management of certain patients, it may be worth to be taken up in clinical practice. It should be born in mind that beside liver function prognosis is also related to the appearance of clinical complications that contribute to a worsening of the health status and prognosis, such as renal dysfunction, ascitic decompensation, and manifestation of esophageal varices [19–21]. Indeed, when we analyzing false negative test results of LiMAx, almost half of the patients died due to acute variceal bleeding, which represent a sudden event that is difficult to predict by the herein used measures. However, survival revealed being not different between patients presenting with and without ascitic formation or with and without overt hepatic encephalopathy at the time of study enrollment. Kaplan–Meier estimates differed significantly for the MELD and LiMAx, but not for ICG-PDR. Although previously published studies identified ICG half-life suitable for estimation of poor short and middle-term outcome, its diagnostic accuracy was reported diverse [22–24]. This might be partly explainable by the fact that ICG elimination appears to be considerably affected by hepatic perfusion because of its merely hepatic extraction without undergoing enzymatic metabolisation. This finding and the reported influence of cholestatis might hamper the value of ICG as an indicator of liver function and prognosis in end-stage liver disease [25–27].

Although the MELD has been a major achievement allowing priority ranking and an objective allocation of donor livers to chronic liver failure patients, debates on the optimal scoring system have not yet been concluded. Certain cohorts of patients may be underserved in the MELD

based liver allocation era and may still die prematurely due to complications not reflected by its components [28]. Certainly, biochemical surrogate parameters of liver function are easy to obtain, but they might underlie to a certain degree influenceability, and thus, the consideration of actual enzymatic liver function might provide more liver specific diagnostic information for clinical decisions in patients with end-stage liver disease. Evaluation of remnant functional mass has already been investigated and Zipprich *et al.* suggested the incorporation of ICG clearance into the MELD to more accurately predict survival in patients with intermediate and advanced liver disease [22]. Similarly, MEGX test was found to be an independent predictor of poor prognosis in Child-Pugh B patients [1]. Although these findings suggest the utility of QLFT as prognostic tools in certain groups of end-stage liver disease, these particular tests are not routinely used for clinical decision finding.

An obvious limitation of this study is that results emerge from a single institution considering only elective evaluation candidates and not patients with acute onset of liver failure and in turn patients with high-urgency status. Therefore, baseline MELD of all studied patients appears relatively low compared with current trends toward an increasing MELD score of transplant candidates in Germany. Second, the relatively large percentage of patients undergoing liver transplantation within 6 months after evaluation might influence data on survival and therefore might limit the conclusion for subgroups. Beyond that, QLFTs were inclusion criteria and hence a sample bias cannot be excluded. Finally, we have included a subgroup of patients with cholestatic liver disease – although small in number ($n = 7$) – it has to be mentioned that the natural history of patients with biliary cirrhosis is usually different from that of patients with nonbiliary cirrhosis, and thus, study results might not be unrestricted transferable to all etiologies of liver disease.

In conclusion, the present study suggests LiMAx as a suitable tool for short-term survival of liver transplant candidates. In particular, actual enzymatic liver function capacity appears to be a strong indicator for the identification of those patients who are not at risk of death, which might provide additional prognostic value in conjunction with the MELD.

Authorship

MJ: wrote the paper, assisted in statistical analysis, and collected data. MM: performed statistical analysis and assisted in writing the manuscript. KL: collected data and critically reviewed the article. ES: contributed to the study design and research concept and critically reviewed the article. PN: designed the research and critically reviewed the article. MS: designed the research, wrote the study protocol, assisted in writing the manuscript, and critically reviewed the article.

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