Dig Dis Sci
DOI 10.1007/s10620-014-3250-z

ORIGINAL ARTICLE

Enzymatic Liver Function Capacity Correlates with Disease Severity of Patients with Liver Cirrhosis: A Study with the LiMAx Test

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Received: 7 April 2014 / Accepted: 6 June 2014

Abstract
Background Assessment and quantification of actual liver function is crucial in patients with chronic liver disease to monitor disease progression and predict individual prognosis. Mathematical models, such as model for end-stage liver disease, are used for risk stratification of patients with chronic liver disease but do not include parameters that reflect the actual functional state of the liver.
Aim We aimed to evaluate the potential of a ^{13}C-based liver function test as a stratification tool by comparison with other liver function tests and clinical parameters in a large sample of healthy controls and cirrhotic patients.
Methods We applied maximum liver function capacity (LiMAx) to evaluate actual liver function in 347 patients with cirrhosis and in 86 controls.
Results LiMAx showed strong negative correlation with Child-Pugh Score ($r = -0.707$; $p < 0.001$), MELD ($r = -0.686$; $p < 0.001$) and liver function tests. LiMAx was lower in patients with liver cirrhosis compared to healthy controls [99 (57–160) µg/kg/h vs. 412 (365–479) µg/kg/h, $p < 0.001$] and differed among Child-Pugh classes [a: 181 (144–227) µg/kg/h, b: 96 (62–132) µg/kg/h and c: 52 (37–81) µg/kg/h; $p < 0.001$]. When stratified patients according to disease severity, LiMAx results were not different between cirrhotic patients and cirrhotic patients with transjugular intrahepatic portosystemic shunt.
Conclusions LiMAx appears to provide reliable information on remnant enzymatic liver function in chronic liver disease and allows graduation of disease severity.

Keywords LiMAx · Cirrhosis · Liver function · Liver function tests · Disease severity · Surrogate marker

Introduction

Early diagnosis of impaired liver function and determination of disease severity in patients with chronic liver disease

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Published online: 04 July 2014

(CLD) remains an important issue to optimize clinical management and therapeutic treatment (e.g., partial hepatectomy or organ transplantation) [1]. Several liver indices and prognostic scores utilizing standard blood tests have been developed to overcome this problem [2–5]. Although these composite scores are widely used, they are relatively insensitive at detecting slight alterations of liver function due to the large functional reserve of the liver. Thus, actual parenchymal function might not be reflected sufficiently. Functional hepatic reserve might contribute to a more exact stratification of these patients and has led clinicians to advocate measuring liver function directly using tests of liver metabolism. Over the last decades, several studies have evaluated different quantitative liver function tests (QLFTs) for determination of liver function and stratification of patients with chronic liver disease [6–15].

LiMAx was recently proposed as a ^{13}C-liver function breath test for the perioperative assessment of actual liver function in liver surgical patients and has been shown to predict postoperative outcome [16]. LiMAx appeared to accurately reflect residual enzymatic function, which was suggested as a surrogate marker for remnant vital parenchymal tissue in a clinical model [17]. This is likely since the target enzymes of the cytochrome P 450 (CYP) 1A2 system are exclusively expressed in hepatocytes, distributed throughout the entire liver acinus and their activity is assumed being proportional to parenchymal volume [18]. The procedure is based on bodyweight-adjusted intravenous ^{13}C-labeled methacetin injection and its subsequent metabolization by CYP 1A2 enzyme into paracetamol and $^{13}CO_2$ [19]. The higher the degree of liver injury, the less ^{13}C-methacetin will be metabolized and the less $^{13}CO_2$ will be produced leading to decreased LiMAx values. LiMAx has been reported not to be influenced by age, gender or obesity [16, 20]. The test can be easily performed either bedside in hospitalized patients but also in an outpatient setting. To date, LiMAx has not been evaluated in patients with cirrhosis.

In the present study, the aim was to evaluate LiMAx in cirrhotic patients of various etiologies and to explore the associations of enzymatic liver function capacity and accepted surrogate markers of liver function.

Patients and Methods

Study Design and Population

Adult patients with diagnosed cirrhosis—either histologically or clinically—in a stable state were recruited from in- and outpatient departments of the Charité—Universitätsmedizin Berlin between July 2008 and August 2013. Exclusion criteria were acute on chronic liver failure, acute manifestation of complications of liver disease and patients undergoing extracorporeal liver support therapy. Among the 347 included patients, 212 were evaluated for liver transplantation, 77 were outpatients and 58 inpatients. Inpatients were included after successful treatment of the underlying reason for hospital admission prior discharge. Out of the 347 cirrhotic patients, 78 (22.5 %) had a transjugular intrahepatic portosystemic shunt (TIPS). To account for any hemodynamic differences within these patients, we analyzed this group separately. In addition, healthy volunteers with no history or laboratory evidence (serum albumin, serum bilirubin, alkaline phosphatase (ALP) prothrombin time (PT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT) within the reference range) of liver disease were used. Subjects gave written informed consent. The study protocol was approved by the ethics committee Charité Universitätsmedizin Berlin and was performed in accordance with ethical standards of the 1964 Declaration of Helsinki.

Methods

Liver tests were performed at the time of inclusion after fasting for a minimum of 3 h. Blood samples were drawn prior to test initiation from a peripheral vein. Child-Pugh Score (CPS), the UNOS-modified model for end-stage liver disease (MELD) and MELDNa were computed as previously described [2, 4, 21]. Hepatic encephalopathy was assessed using West Haven criteria and ascites according to Moore et al. [22, 23].

Maximum liver function capacity (LiMAx) reflects actual enzymatic liver function capacity. The procedure is based on bodyweight-adjusted (2 mg/kg BW) intravenous ^{13}C-labeled methacetin bolus injection and subsequent injection of 20 ml 0.9 % sodium chloride as previously described [16]. Exhaled breath is collected by a distinct two-way face mask and analyzed by means of a special device (Humedics, Berlin, Germany) using a refined online protocol. Herein, we are able to achieve a continuous real-time sampling rate and optimal analysis of delta-over-baseline (DOB) curves of $^{13}CO_2/^{12}CO_2$ ratio. ^{13}C-methacetin is a substrate of the hepatic CYP 1A2 enzyme. ^{13}C-methacetin is exclusively metabolized by the liver into paracetamol and $^{13}CO_2$ [19]. Prior to the substrate injection, the baseline ratio of $^{13}CO_2/^{12}CO_2$ concentration is recorded in the native expired air to calculate an individual baseline. Using the individual actual ratio of $^{13}CO_2/^{12}CO_2$ concentration test, results are not influenced by obstructive pulmonary disease or ventilation and thus can be applied in every clinical situation. Maximum delta-over-baseline (DOB_{max}) of the $^{13}CO_2/^{12}CO_2$ ratio was determined by analyzing the continuous DOB curve over a maximum of 60 min. According to the following formula, LiMAx value is calculated [16]:

$$\text{LiMAx} = \frac{\text{DOB}_{\max} * \text{standard}^{13}\text{CO}_2/^{12}\text{CO}_2\text{ratio} * \text{CO}_2\text{production} * \text{molar mass } (^{13}\text{C} - \text{methacetin})}{\text{Body weight}}$$

Results are given in μg/kg/h and considered as normal when greater 315 μg/kg/h. To date, ^{13}C-methacetin for intravenous application is not commercially available but may be produced by hospital pharmacies.

Statistical Methods

All data were analyzed using statistical software IBM SPSS Statistics 21. We used the Shapiro–Wilk test to determine the distribution of our data. Continuous variables were expressed as median and interquartile range (IQR; 25th–75th percentile) and categorical variables as numbers and percentage. For comparison between two groups of normally distributed data, unpaired student's *t* test was used. Where data were not normally distributed, we applied the Mann–Whitney *U* test and the Kruskal–Wallis test to determine differences between two groups and more than two groups, respectively. Correlations were calculated using Spearman's correlation coefficient. A *p* value of less than 0.05 was considered significant.

Results

Patient Characteristics

A total of three hundred forty-seven consecutive patients [236 males, 111 females; median age 56, 50–61 years] and eighty-six healthy subjects [48 males, 38 females; median age 28, 24–32 years] were included and analyzed. Among

Table 1 Characteristics of study subjects

	Controls ($n = 86$)	Cirrhotic patients ($n = 269$)	Cirrhotic patients with TIPS ($n = 78$)	*p* value[a]
Age (years)	28 (24–32)	56 (49–61)	56 (51–61)	0.692
Gender (M/F)	48 (55.8 %)/38 (44.2 %)	148 (68.4 %)/85 (31.6 %)	52 (66.7 %)/26 (33.3 %)	0.784
BMI (kg/m^2)	23.0 (20.9–25.1)	26.8 (23.6–29.7)	25.9 (23.1–29.7)	0.338
Etiology				
Alcohol	–	131 (36.9 %)	51 (65.4 %)	
Viral hepatitis	–	66 (18.6 %)	12 (15.4 %)	
Cholestatic (PBC/PSC)	–	11 (3.1 %)	1 (1.3 %)	0.058
Others	–	61 (24.2 %)	14 (17.9 %)	
AST (U/L)	24 (20–27)	60 (44–94)	50 (37–68)	0.002
ALT (U/L)	19 (15–25)	37 (25–63)	29 (21–43)	0.003
GGT (U/L)	15 (11–21)	89 (43–168)	106 (49–231)	0.107
Serum bilirubin (mg/dL)	0.6 (0.4–0.8)	2.8 (1.4–4.4)	2.2 (1.1–3.8)	0.027
ALP (U/L)	48 (41–59)	123 (92–177)	136 (92–180)	0.369
Serum albumin (g/dL)	4.4 (4.2–4.6)	3.2 (2.8–3.6)	3.1 (2.8–3.5)	0.252
PT (%)	94 (89–100)	59 (49–72)	62 (51–74)	0.347
Serum sodium (mmol/L)	140 (139–141)	137 (133–139)	136 (132–138)	0.098
Creatinine (mg/dL)	0.84 (0.72–0.96)	0.86 (0.69–1.06)	0.91 (0.78–1.29)	0.007
Platelet count ($\times 10^9$/L)	226 (188–253)	89 (64–1,317)	108 (77–162)	0.009
LiMAx (μg/kg/h)	412 (365–479)	95 (53–153)	112 (66–173	0.121
MELD (pts)		15 (11–19)	15 (12–19)	0.988
CPS		8 (7–10)	9 (7–10)	0.730

Data are medians and interquartile range (IQR; 25th percentile–75th percentile) or number and percentages

TIPS transjugular intrahepatic protosystemic shunt, *BMI* body mass index, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma glutamyltransferase, *ALP* alkaline phosphatase, *PT* prothrombin time, *MELD* model for end-stage liver disease, *CPS* Child-Pugh Score

[a] Comparison of cirrhotic patients versus cirrhotic patients with TIPS

Table 2 Results of bivariate correlation analysis of LiMAx to liver tests

Variables	*n*	r_s value	*p* value[a]
Serum albumin	336	0.761	<0.001
ALT	347	−0.311	<0.001
AST	348	−0.573	<0.001
Serum bilirubin	348	−0.778	<0.001
PT	330	0.814	<0.001
Platelet count	335	0.494	<0.001
Creatinine	346	−0.127	0.018
MELD score	269[b]	−0.686	<0.001
CPS	269[b]	−0.707	<0.001

[a] Spearman's correlation coefficient

[b] Only cirrhotic patients analyzed

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *PT* prothrombin time, *MELD* model for end-stage liver disease, *CPS* Child-Pugh Score

the cirrhotic patients, 78 (22.5 %) were TIPS patients. Alcoholic liver disease (ALD) was the predominant etiology, followed by viral hepatitis and non-alcoholic fatty liver disease. Table 1 shows demographical and clinical characteristics of study subjects for each group (controls/cirrhotic patients/cirrhotic patients with TIPS).

LiMAx and Surrogate Markers of Liver Function

LiMAx values differed significantly between healthy controls [412 (365–479) µg/kg/h] and cirrhotic patients [99 (57–160) µg/kg/h] ($p < 0.001$). LiMAx showed very good correlation with established biological scores and surrogate markers of hepatic function. Strong negative correlations were found between LiMAx and predictive models (MELD: $r_s = -0.686$; $p < 0.001$, CPS: $r_s = -0.707$; $p < 0.001$, respectively), whereas a strong positive correlation was found between LiMAx and prothrombin time and LiMAx and albumin ($r_s = 0.814$; $p < 0.001$ and $r_s = 0.761$; $p < 0.001$, respectively). LiMAx showed moderate correlations with platelet count ($r_s = 0.494$; $p < 0.001$) (Table 2). Analysis of enzymatic liver function among Child-Pugh (CP) classes revealed significantly different LiMAx results: CP class A patients scored lower [181 (144–227) µg/kg/h] compared to healthy controls ($p < 0.001$) and higher compared to CP class B patients [96 (62–132) µg/kg/h] ($p < 0.001$). Moreover, LiMAx results were significantly lower in CP class C [52 (37–81) µg/kg/h] compared to CP class B ($p < 0.001$). Figure 1 illustrates LiMAx results across Child-Pugh and MELD scores.

LiMAx and Etiology of Liver Disease

In order to assess the influence of etiology of liver disease on LiMAx, we compared the results of 131 alcoholic, 66 viral, 11 cholestatic and 61 liver disease patients of other reasons (Table 3). Interestingly, scores showed different results across etiologies. MELD was different among ALD and viral hepatitis ($p = 0.003$) and ALD and cholestatic liver disease ($p = 0.015$) but not among viral hepatitis and cholestatic liver disease ($p = 0.199$). In contrast, LiMAx was significantly higher in patients with cholestatic liver disease [202 (108–331) µg/kg/h] when compared to other groups [ALD 90 (49–129) µg/kg/h, $p = 0.001$ and viral hepatitis 99 (60–168) µg/kg/h, $p = 0.004$, respectively]. To determine the effects related to the etiology of liver

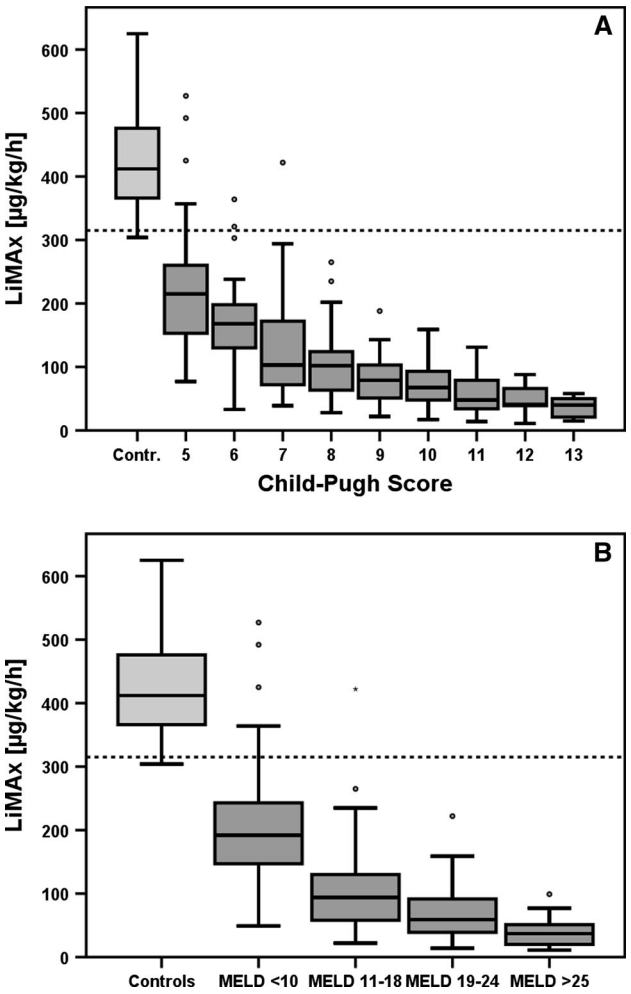

Fig. 1 Variation of LiMAx (*Box and whiskers plot*) in healthy subjects ($n = 68$) and in cirrhotic patients ($n = 269$) across Child-Pugh Scores (**a**) and MELD classes (**b**). **a** Differences between Child-Pugh Scores were for controls vs. CPS 5, $p < 0.001$; CPS 5 versus CPS 6, $p = 0.009$; CPS 6 versus CPS 7 $p = 0.010$; CPS 7 versus CPS 8, $p = 0.244$; CPS 8 versus CPS 9, $p = 0.044$; CPS 9 versus CPS 10, $p = 0.343$; CPS 10 versus CPS 11, $p = 0.054$; CPS 11 versus CPS 12, $p = 0.489$; CPS 12 versus CPS 13, $p = 0.595$, respectively. **b** Differences between MELD levels were for controls versus MELD < 10, $p < 0.001$; MELD < 10 versus MELD 19–24, $p < 0.001$; MELD 11–18 versus MELD 19–24, $p < 0.001$ and MELD 19–24 versus MELD 25, $p = 0.016$, respectively

Table 3 Main clinical and biochemical data of 269 chronic liver failure patients

	Alcoholic ($n = 131$)	Viral ($n = 66$)	Cholestatic ($n = 11$)	Others ($n = 61$)	p value
Age (years)	57 (50–61)	56 (52–60)	52 (45–58)	56 (47–66)	0.585
Gender (M/F)	91 (69.5 %)/40 (30.5 %)	49 (74.2 %)/17 (25.8 %)	8 (73.7 %)/3 (27.3 %)	36 (59.0 %)/25 (41.0 %)	<0.297
Ascites grade					
None	37 (28.2 %)	28 (42.4 %)	6 (54.5 %)	28 (45.9 %)	0.1094
Mild/moderate	69 (52.6 %)	32 (48.4 %)	3 (27.3.4 %)	24 (39.3 %)	
Sever	25 (19.1 %)	6 (9.1 %)	2 (18.2 %)	9 (14.8 %)	
Hepatic encephalopathy					
Stage 0	100 (76.3 %)	54 (81.8 %)	11 (100 %)	52 (85.2 %)	0.168
Stage I	24 (18.3 %)	9 (13.6 %)	0 (0 %)	7 (11.5 %)	
Stage II	7 (5.3 %)	2 (3.0 %)	0 (0 %)	2 (3.3 %)	
Stage III	0 (0 %)	1 (1.5 %)	0 (0 %)	0 (0 %)	
Stage IV	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	
Esophageal varices	106 (80.9 %)	49 (74.2 %)	5 (45.5 %)	40 (65.6 %)	0.063
AST [U/L]	52 (38–70)	87 (61–123)	80 (44–123)	58 (46–93)	<0.001
ALT [U/L]	29 (20–41)	57 (41–117)	73 (27–116)	38 (27–66)	<0.001
Serum albumin [g/dL]	3.2 (2.9–3.6)	3.1 (2.7–3.5)	3.9 (3.3–4.2)	3.2 (2.6–3.8)	0.035
Serum bilirubin [mg/dL]	3.0 (1.6–5.2)	2.2 (1.2–3.4)	1.5 (0.9–6.9)	3.5 (1.6–5.3)	0.017
ALP [U/L]	124 (90–172)	115 (88–156)	225 (158–352)	117 (96–212)	0.008
PT [%]	57 (47–69)	60 (52–77)	74 (63–106)	62 (48–75)	0.007
Platelet count [$\times 10^9$/L]	97 (71–135)	81 (53–121)	103 (82–264)	79 (57–119)	0.032
LiMAx [µg/kg/h]	90 (49–129)	99 (60–168)	202 (108–331)	97 (52–152)	0.004
MELD [pts]	16 (12–20)	14 (9–17)	10 (6–16)	16 (12–20)	0.002
CPS [pts]	8 (7–10)	8 (6–10)	7 (5–8)	9 (67–11)	0.063

Data are medians and interquartile range (IQR; 25th percentile–75th percentile) or number and percentages

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *PT* prothrombin time, *MELD* model for end-stage liver disease, *CPS* Child-Pugh Score

disease, we stratified patients according to disease severity. Herein, cholestatic patients showed a tendency to score higher in LiMAx compared to other disease groups (Fig. 2).

Influence of Frequent Complications of Chronic Liver Disease

Out of 269 cirrhotic patients, 52 were diagnosed with hepatic encephalopathy. Median LiMAx was significantly lower in patients with manifest hepatic encephalopathy (HE) when compared to non-HE patients [59 (42–94) µg/kg/h vs. 102 (61–168) µg/kg/h; $p < 0.001$]. Similarly patients with ascites ($n = 170$) scored lower in LiMAx results than patients with no ascites [76 (48–120) µg/kg/h vs. 131 (77–192) µg/kg/h; $p < 0.001$]. When we grouped patients in CP classes, there was no significant difference between patients with and without manifest ascites in patients with advanced liver disease (CP B vs. CP C patients) ($p = 0.076$).

When we compared liver function of the 269 cirrhotic patients and 78 cirrhotic patients with TIPS, neither surrogate markers of synthetic function (serum albumin and PT) nor LiMAx values differed significantly, and both groups were comparable in terms of severity of liver disease (Table 1). When we grouped patients according to CPS to compare LiMAx between cirrhotics and cirrhotics with TIPS, analysis revealed no influence of TIPS on test results (Fig. 3).

Discussion

The current study focuses assessment of hepatic function in cirrhosis using the LiMAx test. LiMAx appears to be a useful flow-independent tool to grade liver dysfunction.

LiMAx has been previously evaluated in patients receiving oncological liver surgery and transplantation [16, 17, 20, 24, 25]. The test was shown to be a reliable tool for the evaluation of pre- and postoperative individual remnant

Significance levels between Child-Pugh classes across etiologies were as follows:

	Child-Pugh A	Child-Pugh B	Child-Pugh C
alcoholic vs. viral	p=0.784	p=0.297	p=0.480
alcoholic vs. cholestatic	p=0.119	p=**0.009**	p=0.390
alcoholic vs. others	p=0.748	p=0.873	p=0.857
viral vs. cholestatic	p=0.154	p=**0.041**	p=0.249
viral vs. others	p=0.514	p=0.489	p=0.594
cholestatic vs. others	p=**0.039**	p=**0.020**	p=0.433

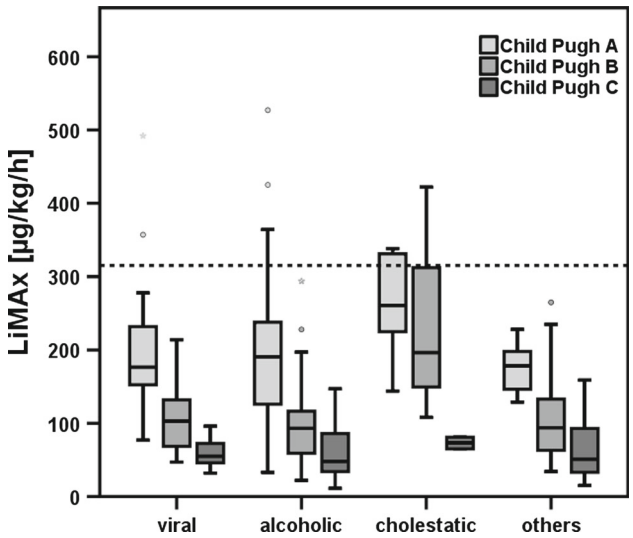

Fig. 2 LiMAx (*Box and whiskers plot*) by disease etiology and Child-Pugh classes in cirrhotic patients ($n = 269$)

liver function and prediction of postoperative outcome. Recent work by the authors could demonstrate its potential to predict outcome in acute liver failure [26]. Thus, we applied the test to determine liver function in chronic liver disease. LiMAx might be a useful tool to assess actual hepatic dysfunction in cirrhosis, which might provide additional information on disease severity and in turn allow better stratification of these patients.

LiMAx examination revealed clear differences in patients stratified according to the CP criteria: Patients with minor hepatic alterations scored significantly lower compared to healthy controls and sicker patients showed more severe impairment of enzymatic liver function. Although LiMAx results excellently mirror the CP grading system, the question arises whether the variation of LiMAx across CPS reflects a certain degree of individual enzymatic liver function or might carry additional information on the real functional state of the liver. When we grouped patients according to different etiologies of liver disease, cholestatic patients had higher LiMAx compared to other subgroups, but lower than healthy subjects. These findings partly reflect the results of other studies which showed similar liver function to healthy controls within this subgroup [7, 27]. It seems likely that patients with PBC and PSC show only slight alteration of liver function since both diseases are initially characterized by immune-mediated destruction of the intrahepatic bile ducts. In consequence, progressive inflammation resulting in portal hypertension leads to cirrhosis in the advanced disease state [28, 29]. Thus, it can be hypothesized that present results might reflect this pathophysiological model. CP A and B patients with cholestatic liver disease showed superior LiMAx compared to CP A and B patients of other etiologies, mirroring the predominantly cholestatic-related effects rather than parenchymal damage (and thus metabolic dysfunction) at this stage, whereas cholestatic CP C patients score as low as CP C patients of other diseases indicating parenchymal injury and the presence of cirrhosis. However, this finding should be interpreted with caution since the number of patients with cholestatic liver disease was small.

An interesting finding is that LiMAx shows greater variation in patients with minor hepatic dysfunction (CPS

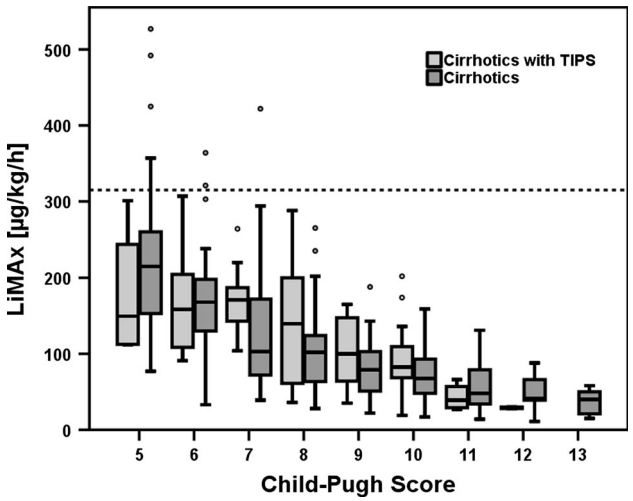

Fig. 3 Variation of LiMAx (*Box and whiskers plot*) in cirrhotic patients ($n = 269$) and TIPS patients ($n = 78$) across Child-Pugh Scores. Differences between cirrhotic patients and cirrhotic patients with TIPS were for CPS 5, $p = 0.276$; CPS 6, $p = 0.908$; CPS 7 $p = 0.019$; CPS 8, $p = 0.213$; CPS 9, $p = 0.127$; CPS 10, $p = 0.127$; CPS 11, $p = 0.342$; CPS 12, $p = 0.327$, respectively

5–8) compared to patients with greater impairment of liver function (CPS 9–13). CP classification remains a clinical score with subjective elements dependent on clinical signs. Hypothetically, patients with decreased LiMAx might suffer from highly impaired liver function regardless of their CPS. Within this context, LiMAx might improve the assessment of disease severity in selected patients by providing precise information on remnant enzymatic liver function capacity. In particular, patients with low CPS but severely decreased LiMAx might have a poor prognosis similar to patients with higher CP scores, in spite of not developing clinical complications. Future studies assessing the outcome of those patients are needed to provide evidence for the potential of LiMAx as a predictor of morbidity and mortality.

Hemodynamic alterations and altered body fluid distribution are frequent in cirrhotic patients and are deemed being major limitations of such functional test. However, our results demonstrate that TIPS surgery and the presence of ascites do not seem to influence LiMAx. As a result of the refined LiMAx test methodology, the maximum of the metabolic product of ^{13}C-methacetin–$^{13}CO_2$ is accurately determined at a defined time point up to 60 min after injection. By considering this threshold, the results are closely connected to the actual enzymatic capacity of the liver despite of the presence of portosystemic shunts and related hemodynamic effects.

This study is certainly not the first evaluating a test to assess liver function in chronic liver disease. Over the last two decades, other liver function tests have been used and need to be discussed in this context. Aminopyrine breath test (ABT) was shown to be a useful tool to differentiate between different stages of liver function impairment in patients with chronic liver disease and cirrhosis [7, 10, 15, 30]. However, in cholestatic liver disease, ABT has limited potential to identify disease severity [27]. Studies identified the ABT as a prognostic tool in cirrhosis but conflicting results also emerged showing inferiority to CP classification in the prediction of survival [31–33]. Although this test is one of the best studied QLFT, it has not entered the mainstay of clinical practice. Galactose elimination capacity (GEC) is another well-studied test shown to identify patients with major hepatic impairment, but it failed to identify patients with moderately impaired liver function compared to healthy controls [7]. Although the GEC test procedure was modified over the years, the imprecision of test results still needs to be considered [34]. Indocyanine green (ICG) test has been shown to be a reliable tool for stratification of liver function [35]. Although ICG is a popular tool to estimate hepatic function, one should be aware that it is plasma removal/clearance rate and not metabolic function which is measured. Beyond that, its excretion has been shown to be influenced by perfusion and increased cholestasis [36]. Although CP criteria and MELD do not provide satisfactory quantitative information on liver function, they are predominantly used for the assessment of disease severity in chronic liver failure patients.

Different dynamic functional tests have been shown to mirror hepatic function. Each method has its strengths and shortcomings, but up to now none of the previously mentioned tests has entered routine clinical practice. Certainly, there is a need for further and more exact stratification of chronic liver failure patients. Our data shows a certain variation of actual enzymatic liver function among patients with low CP scores. This suggests that LiMAx might provide additional information on hepatic enzymatic dysfunction in the early stage of liver disease and may allow more precise stratification of these patients. In order to establish LiMAx as a robust and useful indicator of hepatic function in chronic liver failure, its predictive potential and, more importantly, its additional value in conjunction with established systems need to be demonstrated.

For the sake of completeness, some limitations of this study need to be discussed: Functional enzymatic pathways (e.g., cytochrome systems) have not been determined in this study but may be altered in chronic liver disease. Moreover, the influence of disease-related symptoms such as cholestasis/severe jaundice and the presence of portosystemic shunt on test results need to be investigated in more detail in future studies. Hence, more investigation is required to consistently correlate LiMAx results with hepatic function (e.g., test repeatability, correlation to liver

histology, comparison of test performance with other quantitative liver function tests) and survival in chronic liver failure before being able to advocate LiMAx as an additional useful tool for the evaluation of actual liver function.

The present study suggests the assessment of remnant parenchymal liver function in chronic liver disease by means of a ^{13}C-based test for the determination of the enzymatic liver function capacity—the LiMAx test. Consequently in addition to established markers of liver function, the further assessment of remnant enzymatic liver function might enable physicians to more accurately graduate hepatic dysfunction and disease severity in cirrhotic patients.

In summary, this study supports LiMAx being a robust method for the determination of enzymatic liver function in cirrhotic patients and enables grading of disease severity based on the assessment of enzymatic metabolic capacity of the liver.

Acknowledgments This study was funded in part by the European Union's 7th Framework Programme, d-LIVER Grant agreement no. 287596.

Conflict of interest Martin Stockmann is the inventor of the LiMAx test and has capital interest in Humedics, the company marketing the LiMAx test. In addition he is steering board member for the d-LIVER Project (funded by the European Commission Framework Program). Maximilian Jara and James Orr disclose receiving research Grants in order of the d-LIVER European Commission Framework Program (Grant agreement no. 287596). Remaining authors who have taken part in this study declared that they do not have anything to disclosure regarding funding or conflict of interest with respect to this manuscript.

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