CYP3A activity in severe liver cirrhosis correlates with Child–Pugh and model for end-stage liver disease (MELD) scores

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The clearance of hepatically eliminated drugs is reduced in patients with advanced liver disease and may correlate with the Child–Pugh (CP) score.
- Total midazolam clearance is substantially lower in advanced liver disease.

WHAT THIS STUDY ADDS

- The correlation between CP or model for end-stage liver disease (MELD) score and unbound midazolam clearance suggests that both scores can be used to predict the metabolic capacity of CYP3A.
- The correlation between CP and MELD scores suggests that, from a pharmacokinetic point of view, MELD score categories of <10, 10 to <15 and ≥15 roughly correspond to CP classes A, B and C.

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AIMS

Impaired liver function often necessitates drug dose adjustment to avoid excessive drug accumulation and adverse events, but a marker for the extent of the required adjustment is lacking. The aim of this study was to investigate whether Child–Pugh (CP) and model for end-stage liver disease (MELD) scores correlate with drug clearance.

METHODS

Midazolam was used as a CYP3A probe and its pharmacokinetics were analyzed in 24 patients with mild to severe liver cirrhosis ($n = 4, 10$ and 10 with CP class A, B and C, respectively) and six patients without liver disease.

RESULTS

Both scores correlated well with unbound midazolam clearance (CL_u), unbound midazolam fraction and half-life (all $P < 0.01$), whereas the unbound steady-state volume of distribution was not significantly changed. In patients with severe liver cirrhosis unbound midazolam clearance was only 14% of controls (CP C: $CL_u = 843 \pm 346 \text{ l h}^{-1}$, MELD ≥ 15 : $CL_u = 805 \pm 474 \text{ l h}^{-1}$, controls: $CL_u = 5815 \pm 2649 \text{ l h}^{-1}$, $P < 0.01$).

CONCLUSION

The correlation with unbound midazolam clearance suggests that either score predicts the metabolic capacity of CYP3A, the most relevant drug metabolizing enzyme subfamily in humans.

Introduction

Liver cirrhosis impairs most of the numerous specific liver functions including drug metabolism and may thus necessitate dose adjustments in order to avoid excessive drug accumulation and adverse events. Thus far, no simple method is available to quantify metabolic liver function reliably and predict pharmacokinetics in patients with liver cirrhosis that could be used as a guide for drug dose selection [1, 2].

The Child–Pugh (CP) score is most commonly used to classify liver impairment and to estimate its impact on pharmacokinetics and dose requirements for patients with liver cirrhosis [3–6]. Originally developed as an indicator of peri-operative mortality in patients with liver cirrhosis [7, 8] the CP score is likely not very sensitive to detect mild to moderate changes in liver function [1, 2]. A further drawback is that it is based on both laboratory and clinical findings, some of which may be prone to investigator bias and difficult to quantify accurately. Moreover, with the advent of new pharmacological or interventional options the clinical presentation of liver cirrhosis may have changed, thus further biasing reliable assessment.

The model for end-stage liver disease (MELD) score is used to predict survival of patients with liver cirrhosis and to determine the urgency of liver transplantation [9, 10]. This score is exclusively based on laboratory values thus avoiding distortion caused by ambiguous clinical assessment. However, it has not been evaluated whether the MELD score will predict individual elimination capacity in patients with liver cirrhosis and might thus help guiding drug dose adjustments.

Drug metabolism is largely based on cytochrome P450 (CYP) enzymes [11], with the CYP3A isoforms being most relevant. CYP3A activity is impaired in patients with liver cirrhosis, likely because of reduced liver cell mass, reduced liver blood flow and also impaired intestinal metabolism [12, 13]. Midazolam systemic clearance is thought to reflect overall CYP3A function in an individual because midazolam is metabolized largely by CYP3A enzymes (with 1'-hydroxymidazolam being a major metabolite) and is not a substrate of drug transporters, which might affect systemic clearance independently [14]. In patients with liver cirrhosis midazolam clearance was reduced to 47–64%, half-life was prolonged to 124–244% [15–18] and the unbound fraction was increased to 190–260% [16, 17] as compared with patients without liver disease [15, 16] or healthy volunteers [17, 18]. Unfortunately, these earlier studies did not analyze the correlation between CP score and the extent of pharmacokinetic changes, leaving unresolved whether the CP score will reflect the extent of CYP3A impairment and predict the required dose adjustment of drugs which are CYP3A substrates.

The aim of the present study was to analyze the relationship between CYP3A activity and severity of liver cirrhosis as estimated by CP or MELD scores.

Methods

Study design

Patients with liver cirrhosis and patients without liver disease (control group) were enrolled in a prospective, open label, single centre, single dose pharmacokinetic study. They were either scheduled for endoscopy (and therefore sedated with midazolam according to routine clinical care) or were given a small midazolam dose under controlled conditions in our Clinical Research Centre.

Inclusion criteria were an age of at least 18 years and planned sedation with midazolam in patients undergoing endoscopy. Exclusion criteria were any administration of midazolam in the previous 3 days, any intake of a substance known to inhibit CYP3A activity within 10 times the drug's half-life (including planned sedation with propofol in patients undergoing endoscopy), any intake of a substance known to induce CYP3A activity within the last 4 weeks, intake of compounds interfering with blood coagulation or affecting international normalized ratio (INR) measurements, an estimated creatinine clearance $<30 \text{ ml min}^{-1}$, liver transplant, and known pregnancy or lactation. Patients included as controls had no history of liver disease and normal laboratory findings. No patient received anticoagulants affecting the INR.

In patients undergoing endoscopy, midazolam (Dormicum®, Roche Pharma, Grenzach-Wyhlen, Germany) was administered intravenously at the beginning of the procedure and according to clinical needs (usually 2–5 mg) and the actual dose was documented. In patients studied in the Clinical Research Centre a small, non-sedating midazolam dose was administered intravenously (1–2 mg). Venous blood samples were drawn into 2.7 ml lithium-heparin tubes before and 3, 15, 30 min and 1, 2, 3, 4, 5, and 6 h after midazolam administration using a vein of the arm contralateral to the midazolam administration site. Plasma was separated after centrifugation and kept frozen at -20°C until analysis.

All participants provided written informed consent. The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg and the Federal Institute for Drugs and Medical Devices (BfArM, Bonn, Germany; EudraCT No: 2009–011993-14). The study was planned and conducted according to the principles of the Declaration of Helsinki, the rules of good clinical practice and the specific legal requirements in Germany.

Clinical and laboratory data

Serum albumin, total bilirubin, creatinine and INR were quantified on the study day. In patients with liver cirrhosis ultrasonography of the abdomen was performed to quantify ascites and hepatic encephalopathy was assessed by an experienced gastroenterologist. The CP score was calculated following standard rules [5] using the INR instead of prothrombin time (INR $<1.7 = 1$ point, INR $1.7\text{--}2.3 = 2$ points and INR $>2.3 = 3$ points). A specific rule for bilirubin

in patients with primary biliary cirrhosis was not applied and is also not proposed in the current guidelines [5,6]. The MELD score was calculated as $9.57 \times \log_e(\text{creatinine mg dl}^{-1}) + 3.78 \times \log_e(\text{total bilirubin mg dl}^{-1}) + 11.20 \times \log_e(\text{INR}) + 6.43$ and rounded to the nearest whole number [9]. Laboratory values <1.0 were set to 1.0 for the purpose of MELD score calculation [10]. Creatinine clearance was estimated using the Cockcroft & Gault equation ($e\text{CL}_{\text{Cr,CG}}$) [19]. Glomerular filtration rate was estimated using the abbreviated (four variable) modification of diet in renal disease study equation ($e\text{GFR}_{\text{MDRD}}$) [20] and converted to ml min^{-1} using body surface area as estimated by Mosteller's equation [21].

Analytical assays

Midazolam, 1'-hydroxymidazolam, and isotopically labelled internal standards (D_5 -midazolam and $^{13}\text{C}_3$ -1'-hydroxymidazolam) were purchased from Toronto Research Chemicals (TRC, Toronto, Canada). All other reagents and solvents (water, methanol, acetonitrile, ammonia solution (25%) and formic acid (100%)) used for chromatography, mass spectrometry, and sample preparation were of highest analytical quality (UHPLC or LC/MS/MS grade from Promochem and Biosolve) and were purchased from LGC (Wesel, Germany).

The unbound plasma fraction of midazolam and its main metabolite 1'-hydroxymidazolam was isolated by plasma ultrafiltration. For this purpose plasma (1.5 ml) was ultrafiltered (Amicon Ultra, Ultracel-30 K; Millipore Corp. Carrigtwohill, Ireland) in a centrifuge for 40 min at 30°C and 4000 g. The resulting protein-free ultrafiltrate was used for the quantification of unbound midazolam and 1'-hydroxymidazolam in plasma. Within the validation procedures adsorption of midazolam and 1'-hydroxymidazolam at the ultrafiltration device and membrane was tested using blank plasma ultrafiltrate which was spiked with both analytes at three different concentrations and ultrafiltered again. The mean recovery varied between 85.8% and 95.9%, which corresponds to an adsorption of 5–15% to the ultrafiltration devices. For the quantification of the unbound fraction (f_u) three plasma samples (1, 2 and 3 h) were pooled to achieve the volume of plasma necessary for the ultrafiltration.

Total and unbound plasma concentrations of midazolam and 1'-hydroxymidazolam were determined by solid phase extraction and ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC/MS/MS) using a Waters Acquity UPLC and Waters Xevo TQ-S mass spectrometer (Waters Corp. Milford, MA, USA). These measurements were conducted at the Analytical Chemistry Laboratory of the Department of Clinical Pharmacology and Pharmacoepidemiology, which previously developed and validated an assay for the femtomolar quantification of midazolam and 1'-hydroxymidazolam in human plasma [22]. Plasma and plasma ultrafiltrate (0.25 ml) were extracted using 96 well

based solid phase extraction (Waters $\mu\text{Elution MCX}$). Extraction recoveries ranged between 75 and 92% for both analytes. Extracts were chromatographed within 2 min on a Waters BEH C18 $1.7\ \mu\text{m}$ UPLC column with a fast gradient consisting of formic acid, ammonia and acetonitrile. Midazolam and 1'-hydroxymidazolam were quantified using deuterium- and ^{13}C -labelled internal standards and positive electrospray tandem mass spectrometry in the multiple reaction monitoring mode with lower limits of quantification of $50\ \text{fg ml}^{-1}$ ($154\ \text{fmol l}^{-1}$) and $250\ \text{fg ml}^{-1}$ ($733\ \text{fmol l}^{-1}$). The accuracies in plasma and urine were always within $100 \pm 15\%$. The calibrated concentration ranges were linear for midazolam (0.05 – $250\ \text{pg ml}^{-1}$) and 1'-hydroxymidazolam (0.25 – $125\ \text{pg ml}^{-1}$) with correlation coefficients >0.9900 . Within-batch and batch-to-batch precision (coefficient of variation (CV)) in the calibrated ranges for both analytes were $<14\%$ and $<12\%$. No ion suppression was detectable and plasma matrix effects were minimized to $<15\%$ for midazolam and $<25\%$ for 1'-hydroxymidazolam.

Midazolam pharmacokinetics

Pharmacokinetic parameters were calculated using standard non-compartmental equations. The area under the curve (AUC) and the area under the first moment curve (AUMC) were calculated using the linear-trapezoidal rule with extrapolation to infinity. The slope (λ_z) was estimated by linear regression of the logarithmically transformed values from the terminal concentration decline. Systemic drug clearance was estimated using the individual midazolam dose (D) as $\text{CL} = D/\text{AUC}$. Half-life was calculated as $t_{1/2} = \log_e(2)/\lambda_z$. The apparent terminal volume of distribution was calculated as $V_d = \text{CL}/\lambda_z$ and the steady-state volume of distribution as $V_{ss} = \text{CL} \times \text{AUMC}/\text{AUC}$. The maximum concentration C_{max} was obtained directly from the data and divided by the administered dose. Unbound clearance (CL_u), which is considered to reflect metabolic capacity most closely [23], unbound steady-state volume of distribution ($V_{ss,u}$), which is considered to reflect changes in distribution space independently from changes in drug elimination [24] and unbound midazolam fraction were considered as primary parameters.

Statistics

The relationship between pharmacokinetic parameters, CP and MELD scores was summarized by Spearman's rank correlation coefficient ρ with corresponding 95% confidence intervals (CI) estimated based on Fisher's z transformation. The primary end point of the study was midazolam clearance. A linear regression was also fitted to infer MELD score ranges based on CP classes using data from the cirrhosis patients. Cohen's κ with linear weighting was calculated as a measure of concordance between MELD score categories and CP classes. Differences in pharmacokinetic parameters between subgroups were compared using one way ANOVA and Tukey's *post hoc* test. Statistical analyses were

Table 1

Demographics

	Liver cirrhosis				
	All	Child–Pugh grade			Controls
		A	B	C	
Patients (female / male)	4 / 20	1 / 3	3 / 7	0 / 10	3 / 3
Age (years)	55 ± 8 (40–70)	56 ± 4 (52–61)	54 ± 9 (40–70)	55 ± 9 (42–66)	57 ± 23 (19–83)
Weight (kg)	81 ± 17 (50–120)	91 ± 30 (50–120)	73 ± 13 (55–98)	85 ± 13 (70–108)	61 ± 15 (44–78)
BSA (m²)	1.97 ± 0.25 (1.45–2.45)	2.09 ± 0.44 (1.45–2.45)	1.86 ± 0.20 (1.57–2.22)	2.04 ± 0.16 (1.79–2.31)	1.68 ± 0.22 (1.42–1.91)
Type of cirrhosis (n)*					
Alcoholic	11	1	6	4	NA
Autoimmune hepatitis	1	0	0	1	NA
Chronic hepatitis B	3	0	1	2	NA
Chronic hepatitis C	7	2	3	2	NA
Hemochromatosis	1	0	0	1	NA
Primary biliary cirrhosis	2	0	1	1	NA
Cryptogenic	1	1	0	0	NA
Creatinine (mg dl ^{−1})	1.07 ± 0.34 (0.61–2.03)	0.91 ± 0.19 (0.71–1.12)	1.08 ± 0.35 (0.61–1.74)	1.14 ± 0.38 (0.72–2.03)	0.87 ± 0.29 (0.51–1.24)
Bilirubin (mg dl ^{−1})	2.88 ± 2.39 (0.70–9.40)	1.30 ± 0.81 (0.70–2.50)	1.74 ± 1.09 (0.70–3.8)	4.65 ± 2.67 (1.40–9.40)	0.62 ± 0.29 (0.30–1.10)
INR	1.30 ± 0.26 (0.94–2.08)	1.09 ± 0.05 (1.04–1.16)	1.19 ± 0.15 (0.94–1.45)	1.51 ± 0.26 (1.06–2.08)	1.04 ± 0.04 (0.99–1.11)
eCL _{crea,CG} (ml min ^{−1})	97 ± 41 (36–175)	115 ± 37 (66–145)	87 ± 39 (45–154)	100 ± 46 (36–175)	81 ± 38 (41–142)
eGFR _{MDRD} (ml min ^{−1})	89 ± 35 (34–146)	100 ± 27 (70–133)	84 ± 40 (40–143)	89 ± 36 (34–146)	88 ± 43 (52–168)
MELD score	13 (7–24)	8 (7–11)	12 (7–16)	17.5 (11–24)	7.5 (6–10)
MELD <10 (n)	6	3	3	0	5
MELD 10–<15 (n)	9	1	5	3	1†
MELD ≥15 (n)	9	0	2	7	0

*The number of diagnoses may be higher than patient numbers in some cases because in some patients more than one cause for cirrhosis was present (e.g. hepatitis B and alcohol).

†This patient with lower gastrointestinal bleeding had a calculated MELD score of 10. Values are reported as mean ± SD (minimum – maximum), absolute number (n), or median (minimum – maximum). BSA, body surface area (as calculated by Mosteller's equation); eCL_{crea,CG}, estimated creatinine clearance; eGFR_{MDRD}, estimated glomerular filtration rate; INR, international normalized ratio; MELD, model of end-stage liver disease; NA, not applicable.

conducted using SAS v9.2 and GraphPad Prism v5.02 (GraphPad Software, San Diego, CA, USA). A *P* value <0.05 was considered statistically significant.

Results

Overall 24 patients with liver cirrhosis and six patients without liver disease were included into the study (Table 1). Patients with liver cirrhosis had CP scores from 5 to 13 and MELD scores from 7 to 24.

The correlation between CP and MELD scores was $\rho = 0.88$ (95% CI 0.75, 0.94, $P < 0.01$). A linear relationship was observed between the two scores ($\text{MELD} = 1.825 \times \text{CP} - 2.308$, $r^2 = 0.68$, $P < 0.01$). When CP classes were extrapolated to MELD scores on the basis of this equation, CP A corresponded to a MELD score <10, CP B to MELD 10–<15 and CP C to MELD ≥15 (Figure 1). Concordance between categories was moderate as indicated by $\kappa = 0.54$.

Overall, the midazolam concentration decline was slower in patients with higher CP classes and MELD score categories (Figure 2), which was reflected by an increase in half-life (Table 2). CP C patients had longer half-lives than CP B patients, but the latter had higher dose-normalized midazolam concentrations, suggesting differences in the

Figure 1

Correlation between Child–Pugh and model of end-stage liver disease (MELD) scores in patients with liver cirrhosis (small circles and squares; squares indicate two patients with transjugular intrahepatic portosystemic shunts, filled symbols indicate two patients with primary biliary cirrhosis, $r^2 = 0.68$, $P < 0.01$). Control patients without liver disease are indicated by large grey circles

Figure 2

Dose-normalized midazolam (black continuous lines) and 1'-hydroxymidazolam (blue broken lines) plasma concentration–time profiles in patients with liver cirrhosis grouped according to Child–Pugh class and MELD score categories (thin lines) and patients without liver disease (Control, thick lines). Error bars indicate SEMs

Table 2

Pharmacokinetic parameters of midazolam in patients with and without liver disease

	Liver cirrhosis						Controls
	Child–Pugh grade			MELD score			
	A	B	C	<10	10–<15	≥15	
Patients (n)	4	10	10	6	9	9	6
Unbound midazolam							
CL _u (l h ^{−1})	6 133 ± 3 397 ^{ab}	1 315 ± 995 ^{ac}	843 ± 346 ^{bd}	3 844 ± 3 961	1 755 ± 1 458 ^e	805 ± 474 ^f	5 815 ± 2 649 ^{cdef}
V _{ss,u} (l)	21 701 ± 8 653 ^g	9 948 ± 4 273 ^g	13 322 ± 5 023	13 017 ± 9 963	14 409 ± 5 568	12 414 ± 5 566	14 416 ± 5 294
f _u (%)	0.45 ± 0.13 ^h	0.95 ± 0.45	1.12 ± 0.47 ^{hi}	0.73 ± 0.38	0.82 ± 0.29	1.20 ± 0.59 ^j	0.45 ± 0.10 ^{ij}
t _{1/2} (h)	2.9 ± 0.9	10.6 ± 10.3	12.9 ± 6.5 ^k	3.9 ± 3.0 ^l	10.6 ± 9.3	14.2 ± 8.0 ^{lm}	2.3 ± 1.0 ^{km}
C _{max,u} (ng ml ^{−1} mg ^{−1})	0.10 ± 0.06	0.39 ± 0.33	0.16 ± 0.10	0.32 ± 0.29	0.19 ± 0.09	0.26 ± 0.34	0.15 ± 0.09
V _{d,u} (l)	24 202 ± 9 146 ^{no}	10 640 ± 4 604 ⁿ	13 518 ± 4 968 ^o	14 616 ± 11 207	15 321 ± 6 077	12 533 ± 5 416	17 299 ± 7 169
Total midazolam							
CL (l h ^{−1})	25.5 ± 8.2 ^{pq}	9.8 ± 6.6 ^{pr}	8.4 ± 2.1 ^{qs}	17.9 ± 10.4	12.1 ± 8.5 ^t	7.5 ± 2.2 ^u	24.8 ± 8.1 ^{rstu}
V _{ss} (l)	95 ± 42	85 ± 37 ^v	146 ± 61 ^{wv}	72 ± 33	111 ± 39 ^x	141 ± 67 ^{xy}	62 ± 16 ^{wy}
C _{max} (ng ml ^{−1} mg ^{−1})	25.0 ± 19.4	39.1 ± 18.9	19.0 ± 21.3	39.8 ± 24.5	25.3 ± 12.5	23.9 ± 25.5	33.1 ± 19.6
V _d (l)	107 ± 43	92 ± 40	148 ± 59 ^z	80 ± 35	118 ± 41	142 ± 66 ¹	75 ± 22 ²¹
1′-hydroxymidazolam							
f _u (%)	1.11 ± 0.33	2.81 ± 1.95	3.24 ± 1.48	2.01 ± 1.34	2.16 ± 1.04	3.71 ± 2.10 ²	1.36 ± 0.41 ²

Values are reported as mean ± SD. ^{a–z} Small letters indicate a statistically significant difference in Tukey's *post hoc* test between results with the respective letters. CL, systemic drug clearance; C_{max}, maximum concentration; f_u, unbound fraction expressed as percent; t_{1/2}, half-life; V_d, apparent volume of distribution; V_{ss}, steady-state volume of distribution.

Figure 3

Midazolam plasma clearance in patients with liver cirrhosis (small circles and squares; squares indicate two patients with transjugular intrahepatic portosystemic shunts, filled symbols indicate two patients with primary biliary cirrhosis) and control patients without liver disease (large grey circles). All correlations were highly significant ($P < 0.01$, Table 3)

apparent volume of distribution. In contrast, patients in the highest MELD score category also had the highest midazolam concentrations (Figure 2). Similar relationships were observed for 1'-hydroxymidazolam concentrations (Figure 2).

CL_u of midazolam decreased with increasing CP and MELD scores (Figure 3, $P < 0.01$) whereas f_u and $t_{1/2}$ increased (Figure 4, $P < 0.01$). The unbound volume parameters ($V_{ss,u}$, $V_{d,u}$) tended to be lower in patients with higher scores, but clear statistical associations were not found in correlation analyses. $C_{max,u}$ was not associated with both scores. Total midazolam clearance showed similar relationships (Figure 3, $P < 0.01$). The volume parameters (V_{ss} , V_d) significantly increased with higher scores ($P < 0.01$ for both CP and MELD scores), which could be explained by the increasing f_u . C_{max} tended to be lower in patients with higher scores, but clear statistical associations were not found. 1'-hydroxymidazolam f_u increased in parallel with midazolam f_u (Table 2) and was significantly associated with CP and MELD scores in correlation analysis ($P < 0.01$).

In four patients with advanced stages of liver cirrhosis (one CP B, MELD 13 and three CP C, MELD 19, 23 and 24) no concentration decline was observed between 2 and 6 h. Thus, individual elimination rate constants could not be calculated for these patients and their profiles were conservatively extrapolated using an elimination half-life of 12.7 h as derived from the average concentration course in our CP C patients. Statistical analyses after exclusion of these four patients led to similar results.

The estimated correlations between scores and midazolam clearances are shown in Table 3. The strongest

association was found between the CP score and unbound clearance ($\rho = -0.78$) using all patients ('primary analysis'). Exclusion of four patients with advanced stages of liver cirrhosis ('secondary analysis') or exclusion of control patients did not modify this result. Differences between the scores were minor and confidence intervals were similar (Table 3).

Analysis of pharmacokinetic parameters normalized to body weight led to similar results. Generally, clearance parameters showed slightly stronger statistical associations (e.g. $\rho = -0.81$, 95% CI $-0.91, -0.64$, and $\rho = -0.76$, 95% CI $-0.88, -0.55$, for the correlation between CL_u and CP and MELD scores, Supporting information Figure S1) than without normalization. The normalized $V_{ss,u}$ showed a slightly stronger trend to lower values in patients with higher scores that was significantly correlated with the CP score ($P = 0.02$) but not with the MELD score ($P = 0.058$, supporting information Figure S1). However, after excluding control patients, no correlation was observed between normalized $V_{ss,u}$ and the two scores.

No serious adverse events occurred. Prolonged sedation was observed in two patients (one control and one patient with liver cirrhosis, CP A, MELD 7). Both had received rather high midazolam doses according to clinical needs.

Discussion

A large body of evidence suggests that the elimination of drugs with significant hepatic metabolism will deteriorate

Figure 4
Unbound midazolam fraction and plasma half-life in patients with liver cirrhosis (small circles and squares; squares indicate two patients with transjugular intrahepatic portosystemic shunts, filled symbols indicate two patients with primary biliary cirrhosis) and control patients without liver disease (large grey circles). Unbound fraction: Spearman $\rho = 0.75$ (95% CI 0.52, 0.88) for Child–Pugh score and 0.61 (95% CI 0.31, 0.80) for MELD score. Half-life: Spearman $\rho = 0.75$ (95% CI 0.52, 0.88) for Child–Pugh score and 0.76 (95% CI 0.50, 0.88) for MELD score (all $P < 0.01$)

Table 3
Correlation between unbound and total midazolam clearance and CP and MELD scores

	n	Primary analysis		n	Secondary analysis	
		CP score	MELD score		CP score	MELD score
Unbound clearance (CL _u)						
All patients	30	−0.78 [−0.89, −0.58]***	−0.72 [−0.85, −0.48]***	26	−0.78 [−0.90, −0.56]***	−0.68 [−0.84, −0.39]***
Liver cirrhosis	24	−0.63 [−0.83, −0.31]***	−0.55 [−0.78, −0.19]**	20	−0.63 [−0.84, −0.26]**	−0.49 [−0.77, 0.06]*
Total clearance (CL)						
All patients	30	−0.65 [−0.82, −0.38]***	−0.67 [−0.83, −0.41]***	26	−0.71 [−0.86, −0.45]***	−0.72 [−0.86, −0.46]***
Liver cirrhosis	24	−0.43 [−0.71, −0.04]*	−0.51 [−0.76, −0.13]**	20	−0.55 [−0.80, −0.15]**	−0.63 [−0.84, −0.26]**

Values are reported as Spearman’s rank correlation coefficient with 95% confidence interval. *, ** and *** indicate $P < 0.05$, $P < 0.01$ and $P < 0.001$. In the primary analysis the four patients were included who had advanced metabolic impairment and in whom no decline in midazolam concentrations was observed between 2 and 6 h after administration. In these patients midazolam clearance was estimated based on an extrapolation using the average elimination rate of CP C patients.

as liver cirrhosis progresses. To quantify liver dysfunction the CP classification is usually applied and it is also used in the summaries of product characteristics as approved by the legal authorities to restrict drug use (contraindications) or to establish dose adjustment rules. A correlation of CP scores with metabolic liver function has been shown, e.g. for the metabolism of caffeine, a substrate of CYP1A2 [25–27]. Similarly, correlations between CP score and CYP3A substrates such as lidocaine [26] and amprenavir [28] have been reported. However, CYP3A assessment is often flawed. Indeed erythromycin kinetics do not correlate well with midazolam kinetics probably because erythromycin is also a substrate of P-glycoprotein [29, 30] and organic

anion transporting polypeptides (OATP) [30] as opposed to midazolam, which is a CYP3A substrate but not a substrate of common drug transporters [14]. Lidocaine is a substrate of CYP3A and CYP1A2 [31], limiting accurate quantification of the activity of a specific CYP.

We aimed to define CYP3A activity under well controlled conditions [32] using the i.v. route of administration and unbound concentrations and to correlate it with CP and MELD scores. This appeared necessary, because these classifications were established for another purpose and have never been thoroughly validated in this regard. Moreover, since the 1960s, treatment of cirrhotic patients has changed considerably and likely also the prevalence

and extent of clinical symptoms like ascites and encephalopathy, indicating that in recent years the clinical significance of specific score values might have changed. Hence, if CP scores are to be used to guide dosing, the relationship between CP scores and metabolic clearance of drugs should be well established and it should be known whether CP values can be used to predict drug clearance and whether they will ultimately suggest dose adjustment.

With this study we aimed to assess the relationship between CP scores in well characterized patients with cirrhosis and midazolam clearance as a well established marker for CYP3A activity [33], which represents the most relevant drug metabolizing enzymes in humans [11]. Concurrently we aimed to describe the relationship between MELD and CP score in a current patient population to test whether MELD scores could be used for dose adjustment. This would have the advantage of being based exclusively on laboratory values and not requiring the judgment of clinical parameters that are sometimes difficult to assess. A further advantage of MELD scores would be that laboratory values are easily accessible and would thus enable computerized assessment as a basis for electronic clinical decision support. To our best knowledge no study has previously assessed unbound midazolam clearance in liver cirrhosis. The present findings point to similar correlations between unbound clearance and the two scores.

Our analysis revealed a highly significant correlation between individual CP scores and unbound i.v. midazolam clearance. In CP B and C the unbound clearance was reduced to 23% and 14% as compared with normal controls. CP A patients had similar clearances to normal controls. The reduction of unbound and total clearance was more pronounced than earlier reported where total clearance was reduced to 47–64% [15–18], likely indicating more advanced liver impairment in our patients.

The correlation between MELD and CP scores was good ($r^2 = 0.68$), suggesting that either score can be used as a marker for metabolic CYP3A capacity. Based on this observation we derived for each CP class a corresponding MELD score category and compared the midazolam clearances of these groups. In MELD < 10 patients the unbound clearance was reduced to 66%, in MELD 10–<15 to 30% and in MELD ≥ 15 to 14%. Hence, in contrast to CP A patients midazolam clearance appears to be already impaired in MELD < 10 patients. However, this difference was statistically not significant and the observed decrease was small, and would alter exposure of pure CYP3A compounds only modestly. Thus, clinically relevant changes are not expected in MELD < 10 patients for most drugs, but cannot be excluded for narrow therapeutic index drugs. If our patients were classified into three categories (mild, moderate, severe) according to the CP score or the MELD score (using our derived categories) 62.5% would be in the same category.

The reduction of total midazolam clearance in patients with severe liver cirrhosis (34% in CP C patients, 30% in

MELD ≥ 15 patients) was less pronounced as compared with unbound midazolam clearance, which can be explained by the higher unbound fraction in these patients. A similar observation has been reported after administration of erythromycin in patients with liver cirrhosis, where total erythromycin clearance was reduced to 71% and unbound erythromycin clearance to 37% [23].

The prolongation in midazolam half-life in patients with higher CP and MELD scores can be largely explained by reduced drug clearance. However, in patients with lower unbound midazolam clearance the unbound volume of distribution was also lower (supporting information Figure S2). This could explain the less pronounced change in half-life as would be expected based on the change in unbound midazolam clearance in patients with more severe liver disease.

We used the MELD score as adopted by the United Network for Organ Sharing (UNOS), i.e. we applied lower limits for the laboratory values. By using these limits negative MELD values are avoided. When calculating the MELD score without such limits we got essentially similar results, but statistical correlations were slightly weaker (e.g. $\rho = 0.82$ for the correlation between MELD and CP scores and $r^2 = 0.62$ for the linear regression of MELD and CP scores in patients with liver cirrhosis).

The unbound midazolam fraction in our patients (0.45% in controls, 1.1–1.2% in advanced liver cirrhosis) was lower than reported in previous studies (1.7–1.9% in controls, 3.2–4.9% in liver cirrhosis) [16, 17]. There appears to be no obvious explanation for this discrepancy, but we suspect that methodological aspects might play a role. We used ultrafiltration (significant adsorption to the membrane was excluded) and quantification by UPLC/MS/MS, whereas many previous publications used equilibrium dialysis after *ex vivo* addition of ^{14}C -midazolam and quantification of radioactivity [16, 17]. Osmotic water shift during equilibrium dialysis could lead to higher values if a correction is not made. To the best of our knowledge, only two studies used ultrafiltration and tried to quantify midazolam by HPLC, but unbound concentrations were below the detection limit [34, 35] and could be quantified only in spiked samples [35]. Finally, our values appear to be plausible because the relative change (control vs. advanced liver cirrhosis) and variability are similar to published values.

Our study has some limitations. First, we estimated systemic midazolam clearance after intravenous midazolam administration, which is assumed to reflect predominantly hepatic drug metabolism. Thus, our results may not be applicable to orally administered drugs with relevant intestinal first pass metabolism, where other factors might play a role. For example, patients with transjugular intrahepatic portosystemic shunts (TIPS) had unchanged systemic midazolam clearance but increased systemic availability after oral administration [18]. Second, we only measured unbound midazolam concentrations after pooling plasma

samples of a patient. This was considered appropriate because earlier studies in cirrhotic patients found linear protein binding in the observed midazolam concentration range [16]. Third, other factors potentially affecting CYP3A activity (e.g. gender, genetic polymorphisms) were not analyzed. However, the influence of single factors (other than drug–drug interactions) on CYP3A activity is considered to be minor. For example, weight-corrected total midazolam clearance was reported to be only 11% higher in women [36]. Patients taking known CYP3A inhibitors or inducers were excluded from our study.

In conclusion we found a good correlation between CP or MELD score and the unbound midazolam kinetics suggesting that it can be used to predict the metabolic capacity of CYP3A the most relevant drug metabolizing enzyme subfamily in humans. Whether it can ultimately be used to predict dose requirements of CYP3A substrates should now be assessed.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). AA was financed by Syrian Ministry of High Education. DC, AB, RE, JLB, JB, TMG, PS, and WEH declare: no support from any organization for the submitted work. No author had financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1

Unbound midazolam clearance and unbound steady-state volume of distribution normalized to body weight in patients with liver cirrhosis (small circles) and control patients without liver disease (large grey circles)

Figure S2

Steady-state and terminal volume of distribution in relation to drug clearance in patients with liver cirrhosis (small circles) and control patients without liver disease (large grey circles)