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CLINICAL STUDIES

Cystatin C is a strong predictor of survival in patients with cirrhosis: is a cystatin C-based MELD better?

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Keywords
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Abbrevations
GFR glomerular filtration rate; INR international normalized ratio; MELD model of end stage liver disease; UNOS United Network for Organ Sharing..

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Abstract

Background/Aims: The model of end stage liver disease (MELD) includes serum creatinine, which is a poor surrogate marker of renal function in patients with cirrhosis. Especially in women and patients with advanced disease creatinine underestimates true renal function. Our objective was to assess whether or not the substitution of creatinine by cystatin C improves the prognostic performance of the model. *Methods:* The association between MELD parameters and cystatin C with survival was investigated using a Cox proportional hazards model. A cystatin C-based MELD score was calculated from the results and compared with creatinine-based MELD in terms of discrimination and calibration. *Results:* Four hundred and twenty-nine patients were included in the study; 19% died and 12% underwent liver transplantation during a median follow-up of 602 days. In multivariate Cox regression, cystatin C was an independent predictor of 90-day mortality with a hazard ratio of 8.0 (95% CI: 2.2–29.6). The median cystatin C-based MELD was 15, the median creatinine-based MELD was 12. Calibration and discrimination for 3 month and 1 year mortality was similar between the scores (AUC > 0.85 for both scores). Gender differences in cystatin C-based MELD were less pronounced than those in the creatinine-based model, because creatinine but not cystatin C was affected by gender. *Conclusion:* Substitution of creatinine by cystatin C does not improve the predictive power of MELD.

To assess the short-term mortality in patients undergoing transjugular intrahepatic portosystemic shunt procedure Malinchoc and co-workers developed a prognostic model that included international normalized ratio (INR), total bilirubin, plasma creatinine and the cause of cirrhosis (1). A modified version of this original score has been validated as a predictor of survival in cirrhotic patients independent of TIPS and is now known as model for end stage liver disease (MELD) (2). The following modifications from the original Malinchoc score were made: (i) a lower bound of 1.0 for all variables, (ii) an upper bound of 4.0 for creatinine, (iii) a value of 4.0 for creatinine if the patient has been dialysed twice within the last 7 days, (iv) the cause of cirrhosis is no longer considered and (v) an upper bound of 40 for the resulting score. This United Network for Organ Sharing (UNOS) modified MELD score has replaced the Child-Turcotte-Pugh score for organ allocation for liver transplantation within the UNOS and within Eurotransplant (3).

Model for end stage liver disease score limitations are its poor accuracy in patients with less severe stages of liver disease and the fact that age, plasma sodium concentration and the degree of portal hypertension (HVPG) as important predictors of mortality in cirrhosis are not incorporated into the model (4). Several studies have shown that combined models which also include age, serum sodium or hepatic venous pressure gradient (HVPG) have an improved prognostic value compared to MELD alone (5–8).

The association between impaired renal function and poor prognosis in patients with cirrhosis is reflected by the incorporation of creatinine in the MELD score. However, serum creatinine does not reliably reflect renal function in patients with cirrhosis (9, 10). This is particularly true for female patients and Child Pugh class C patients with low muscle mass where plasma creatinine concentrations overestimate renal function (9). Although glomerular filtration rate (GFR) estimated by the modification of diet in renal disease (MDRD) equa-

tion is a more accurate parameter of renal function, its incorporation into the MELD score does not improve the prognostic power of the model (11).

A more accurate surrogate marker of renal function in patients with end stage liver disease is the protease inhibitor cystatin C (12). In contrast to creatinine its serum concentration is independent of muscle mass or gender and can be reliably determined in the patients with hyperbilirubinaemia (13, 14). Cystatin C also has a higher sensitivity for the detection of impaired renal function compared to creatinine and is an independent predictor of hepatorenal syndrome and death in patients with cirrhosis (9, 15).

The aim of our study was to assess if a modified MELD score that incorporates cystatin C has a better performance for the prediction of 90 days and 1 year mortality compared to the creatinine-based MELD score.

Patient selection and statistical methods

Cystatin C was prospectively determined in all adult patients with cirrhosis referred to the Department of Gastroenterology and Hepatology at the University Hospital of Innsbruck, Austria, between August 2007 and September 2009. In addition, cystatin C was determined retrospectively in serum samples stored at −80°C from a cohort of consecutive patients who were treated at our unit between November 2005 and January 2007.

Inclusion criteria were age of 18 years and above and the diagnosis of cirrhosis, which was based on imaging studies (CT scan and/or ultrasound) showing morphological signs compatible with end stage liver disease, oesophageal/cardiac varices or portal hypertensive gastropathy in upper GI endoscopy and/or biochemical signs of cirrhosis. Exclusion criteria were missing laboratory parameters required for calculation of MELD score, prior liver or kidney transplantation, renal replacement therapy prior to entry into the study, malignancies (including hepatocellular carcinoma) and loss to follow-up within 90 days. The start date of survival analysis was the date of the first cystatin C determination. Patients lost to follow-up after 90 days were censored with the last day they were known to be alive and patients who underwent liver transplantation were censored with the date of transplantation. Patients were evaluated for liver transplantation if the MELD was ≥ 12 and within our centre graft allocation was based on MELD. The study was approved by the local ethics committee.

Calculation of the three different models for end stage liver disease models

Association between MELD parameters and cystatin C with survival was determined by Cox regression analysis. To study the predictive power of different scores with and without cystatin C, three models were calculated. The 'original MELD' was calculated according to the formula published by Malinchoc *et al.*, where MELD = 0.957 * ln(creatinine) + 0.378 * ln(bilirubin) + 1.120 * ln(INR) + 0.643 (1). The resulting score was multiplied by 10 and the cause of cirrhosis was not considered. The second model is referred to as 'UNOS MELD' and is based on the same formula with the following constraints: all parameters <1.0 are bound to 1.0, the maximum value for creatinine is 4.0 and all scores exceeding 40.0 are bound to 40. In the cystatin C-based model ('MELD-Cys') creatinine and its regression coefficient were replaced by cystatin C and the regression coefficient of cystatin C derived from our multivariate Cox regression: MELD-Cys = 10 * [2.357 * ln(cystatin C) + 0.378 * ln(bilirubin) + 1.120 * ln(INR) + 0.643]. This model is subject to the same constraints as the UNOS MELD model.

Laboratory methods

Creatinine was determined with an enzymatic method (Creatinin plus; Roche Diagnostics, Basel, Switzerland). This method has a day to day imprecision at our centre of 2.41% at a concentration of 0.95 mg/L, and 1.85% at a concentration of 3.56 mg/L. The employed method is validated to provide reliable results in icteric samples up to a total bilirubin concentration as high as 25 mg/dL (428 μMol/L). To eliminate the interference of bilirubin on creatinine determination, samples with a total bilirubin concentration exceeding 25 mg/dL were diluted according to a routine algorithm, but dilution was only necessary in 10 samples. For total bilirubin (Bilirubin DPD; Roche Diagnostics) the respective coefficients of variation (CV) are 3.31% at 1.10 mg/dL and 2.2% at 4.12 mg/dL respectively. The imprecision of INR (Thromborel S; Siemens, Marburg, Germany; CV 4.69% at an INR of 2.2) and cystatin C (Tinaquant Cystatin C; Roche Diagnostics, CV's 2.52% at 1.14 mg/L and 2.48% at 4.48 mg/L) is within the same range as that in the other methods.

Statistical methods

Results are shown as absolute numbers, means (with SD), or medians [with range or inter-quartile range (IQR)]. Analysis of variables with a non-normal distribution was carried out by Mann–Whitney tests and means of normally distributed variables were compared with *t*-tests. A two sided *P*-value of <0.05 was considered statistically significant. Patient survival was calculated using the Kaplan–Meier method and differences between subgroups compared by the log-rank test. Multiple Cox proportional hazards regression was used to estimate the coefficient of Cystatin C for the MELD-Cys model. Each model's predictive performance was assessed through calibration and discrimination evaluation. For calibration analysis the study cohort was divided into quintiles according to the MELD score and

then compared via the outcome rates. Receiver operating characteristic (ROC) analyses were applied to summarize discrimination with the area-under-the-curve (AUC) statistic and 95% confidence interval (CI). The statistical analysis of the data was performed using SPSS 17.0.

Results

Demographic and clinical features of 429 patients included in the study are summarized in Table 1. The mean age of the study cohort was 57 years (range 22–93 years) and the median follow-up was 1.6 years (IQR 0.6–3.5). The most common underlying diseases were alcoholic or non-alcoholic fatty liver cirrhosis followed by viral hepatitis and cryptogenic cirrhosis. During follow-up, 50 patients (12%) underwent liver transplantation and 83 patients (19%) died. The main causes of death were multi organ failure with or without sepsis (59%), variceal or non-variceal bleeding (19%) and hepatic decompensation (17%). Mean transplant free survival was 1470 days with a 3-month, 1-year, and 3-year transplant free survival rate of 92, 84, and 77% respectively. No significant difference in survival between men and women was found in the log-rank test.

Median serum creatinine was 0.88 mg/dL in the study cohort and subgroup analysis showed that creatinine but not cystatin C was significantly lower in women compared to that in men (median creatinine: 0.78 mg/dL vs. 0.93 mg/dL; $P < 0.001$; median cystatin C: 1.22 mg/L in women and 1.24 mg/L in men, $P = 0.27$). Creatinine and cystatin C were well correlated in the study cohort ($r = 0.776$; $P < 0.001$) suggesting a linear relationship between these parameters.

To identify independent predictors of survival, Cox regression was carried out, where creatinine, cystatin C, bilirubin and INR were included in the model. Univariate regression analysis showed that increasing serum bilirubin, creatinine, INR and cystatin C significantly predicted death (data not shown). In multivariate analysis, bilirubin and cystatin C but not creatinine were independent predictors of death (Table 2). Of all variables included in the model, cystatin C was the strongest predictor with a hazard ratio of 8.0 (95% CI: 2.2–29.6).

Based on the finding that cystatin C was a better predictor of survival than creatinine, a MELD score that included cystatin C instead of creatinine was calculated for each patient. When the original MELD, the UNOS MELD and the cystatin C based MELD scores were compared by paired analysis, MELD-Cys was significantly higher than that in the other models (median original MELD: 10, median UNOS MELD: 12, and median MELD-Cys: 15; range −6–51 for original MELD and range 6–40 for UNOS MELD and MELD-Cys; $P < 0.001$).

The discriminative power of the scores in predicting 90-day mortality was compared by ROC curve analysis (Fig. 1). The 90-day mortality AUCs for the original

Table 1. Demographic, clinical and biochemical features of the study cohort

	Study cohort (n = 429)
Demographic	
Mean age (year) (SD)	57.2 (12.0)
Sex (f/m)	136/293
Cause of cirrhosis (%)	
ALD/NASH	58.7
Viral hepatitis	25.6
Cryptogenic	5.8
Other	9.8
Biochemical (median, IQR)	
Serum bilirubin (mg/dL)	1.6 (0.9–3.3)
Serum creatinine (mg/dL)	0.9 (0.7–1.2)
Cystatin C (g/L)	1.2 (1.0–1.7)
INR	1.2 (1.1–1.5)
Median MELD (IQR)	12 (9–17)
Median follow-up (year) (IQR)	1.3 (0.6–3.5)
Deaths/liver transplantation (n)	83/50
75‰ transplant free survival time (year)	4.2

ALD, alcoholic liver disease; INR, international normalized ratio; IQR, inter-quartile range; NASH, non-alcoholic steatohepatitis; SD, Standard deviation.

Table 2. Results from the multivariate Cox regression

	HR	HR 95% CI	P
Creatinine	1.29	0.52–3.19	0.59
Bilirubin	2.19	1.52–3.13	<0.005
INR	3.83	0.98–14.98	0.05
Cystatin C	8.00	2.16–29.64	<0.005

CI, confidence interval; HR, Hazard ratio; INR, international normalized ratio.

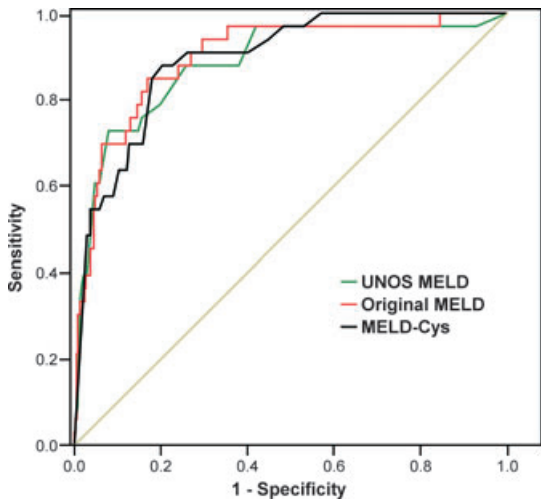

Fig. 1. Discrimination. ROC curves of the original MELD, UNOS MELD and MELD-Cys score for the prediction of death within 91 days. The differences between the three models were not statistically significant and AUC was >0.85 for each model.

MELD, UNOS MELD and MELD-Cys were 0.90, 0.88 and 0.89 respectively (Table 3). These differences did not reach statistical significance. Optimal cut-off values for all three MELD sores were derived from ROC curve analysis and sensitivity and specificity for predicting death within 90 days are shown in Table 3. Similar results without significant differences among the three scores were found for prediction of death within 1 year (AUC >0.85 for all three scores; data not shown). Also when a competing risk model (event defined as death or liver transplantation within 3 months) was applied, no significant difference was found in the discriminative ability of the three models (AUC >0.85 for all three scores; data not shown).

Calibration of all three models for 3-month mortality was poor for scores within lower three quintiles but seemed to be fairly good in the fourth and fifth quintile of each score (Fig. 2a). As shown in Fig. 2b, the calibration of the scores for 1 year mortality was better but still remained imprecise within the lower two quintiles.

A comparison between UNOS MELD and MELD-Cys in individual patients showed that MELD-Cys is higher as illustrated by the upward shift of the scatter plot shown in Fig. 3. UNOS MELD ≥ 12 is a widely accepted cut-off for enrolling patients on the liver transplant waiting list. Transplant free survival in this subgroup was comparable to survival of patients with MELD-Cys ≥ 17. A total of 35 patients with a UNOS MELD <12 had a MELD-Cys of ≥ 17 (Group A) and 32 patients with a MELD ≥ 12 had a MELD-Cys of <17 (Group B). Survival analysis showed overall survival was better in Group A than in Group B but this difference did not reach statistical significance.

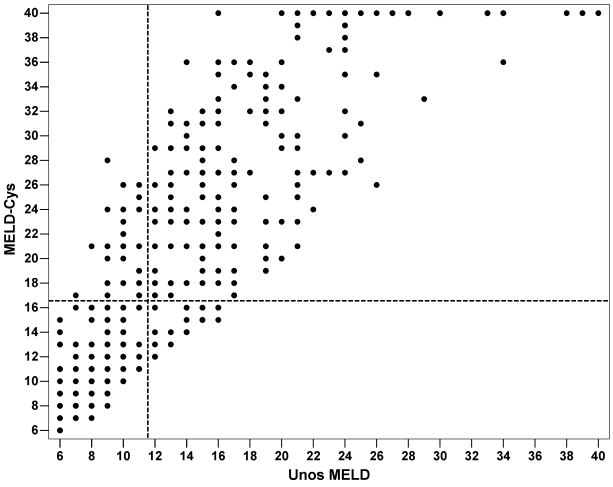

Fig. 3. Scatter plot of UNOS MELD vs. MELD-Cys. Thresholds for comparable mortality of both scores are marked by horizontal and vertical dashed lines (UNOS MELD ≥ 12 and MELD-Cys ≥ 17). Resulting quadrants show theoretical shifts in patients fulfilling criteria for enrolment on the liver transplant waiting list depending on the applied model. One dot can indicate more than one patient.

Discussion

The present study shows that cystatin C is a better predictor of survival than creatinine, but including this parameter in a modified MELD score does not increase

Table 3. Discriminative performance and thresholds for 90-day mortality of the three models

	Cut-off value	AUC (95% CI)	Sensitivity (%)	Specificity (%)
Original MELD	≥ 16	0.90 (0.84–0.96)	85	83
UNOS MELD	≥ 21	0.88 (0.82–0.95)	73	92
MELD-Cys	≥ 26	0.89 (0.84–0.94)	88	80

AUC, area-under-the-curve; MELD, model of end stage liver disease; UNOS, United Network for Organ Sharing.

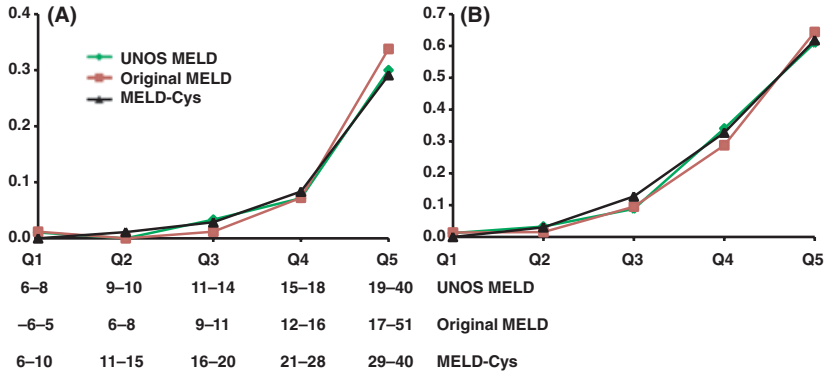

Fig. 2. Calibration analysis. The probability of death within 3 months (a) and within 1 year (b) for each quintile of the original MELD, UNOS MELD and MELD-Cys score. Corresponding scores for each quintile are shown below panel A. All three models are poorly calibrated in patients in the lower two quintiles, whereas calibration is good for patients with higher scores. No significant differences in calibration of the three models were observed.

the predictive performance of the model. This finding is surprising, because renal dysfunction is associated with poor outcome in patients with end stage liver disease and cystatin C is a better surrogate parameter of renal function than creatinine in these patients (9, 15). Despite this advantage, the widespread use of cystatin C as a routine parameter is mainly limited by its cost, which is more than 10-fold higher than enzymatic creatinine measurement (Reagent cost: 3.5€ for cystatin C per measurement compared to 0.25€ for creatinine).

A possible explanation for the similar predictive power of a Cystatin C-based MELD and the UNOS-modified MELD score is that the significant difference between creatinine and cystatin C is levelled by the lower bound of 1.0 for both parameters. However, even the original MELD score, where creatinine concentrations <1.0 are considered, does not have a better discrimination and calibration than those in the other models. Also an eGFR-based MELD score was reported to have no superior accuracy (11).

It remains an unresolved issue if including true renal function, determined by inulin or creatinine clearance, will improve the performance of the MELD score. All factors known to determine the prognosis of patients with cirrhosis can hardly be reflected by a prognostic model that only includes bilirubin, renal function and INR. Previous studies have shown that only more complex models which additionally include other parameters such as age, HVPG or serum sodium can result in a better prognostic tool (5–8).

Nevertheless, this study shows a good discriminative ability of all tested MELD scores for 3-month and 1-year overall and transplant free survival. Our study also confirms the good calibration of MELD in patients with advanced cirrhosis and shows similar results for MELD-Cys, but all MELD scores are poorly calibrated in patients with less advanced disease. This observation raises the question if the proposed cut-off of MELD >12 is a valid criterion for listing patients for transplantation. Our study also included patients with cirrhosis, who were not yet listed for liver transpln and suggests that for these patients additional factors need to be considered for prognostic considerations.

In the present cohort application of MELD-Cys ≥ 17 instead of UNOS MELD ≥ 12 as a criterion for enrolment on the liver transplant waitlist would result in a net increase of three patients on the list. Although this is a minor change in the total number of patients on the waitlist, using the MELD-Cys for listing 14% of patients with UNOS MELD ≥ 12 (Group B) would no longer fulfil the criteria for enrolment. At the same time 17% of patients with a UNOS MELD <12 (Group A) would now be evaluated for liver transplantation. Furthermore, MELD-Cys would not increase the number of women on the wait list with a proportion of 28% female patients regardless whether the UNOS MELD or the MELD-Cys cut-off was used. To assess if the observed trend of improved overall survival in group A is real, studies specifically addressing this question in larger cohorts are required.

Although mortality was not different between men and women in our cohort, the creatinine-based MELD scores, but not MELD-Cys, were significantly lower in women. This suggests that the predictive performance of UNOS MELD is gender dependent. An advantage of a cystatin C based MELD score may be that it is less affected by gender. Therefore, substituting creatinine by cystatin C may ameliorate the discrimination of women who are more likely to die on waiting list and less likely to receive a transplant since MELD-based organ allocation was introduced (16). However, our sample size did not allow separate calibration and discrimination for men and women and this question needs to be clarified in larger cohorts.

Another important factor influencing creatinine is malnutrition. BMI as a surrogate marker of nutritional status was available from 373 patients. In this subgroup, median creatinine and median cystatin C concentration were not different in patients with BMI <20 kg/m² ($n = 48$) as compared to those in patients with a BMI $\geq$ 20 kg/m² ($n = 325$). There was no correlation between BMI and different MELD sores, suggesting that Cys-MELD would not change decision-making in malnourished patients. A clear limitation of this retrospective analysis is that no formal assessment of muscle mass was available.

In conclusion, cystatin C is a better predictor of survival than creatinine but a cystatin C-based MELD score has an equal predictive performance compared to the creatinine-based model.

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