Transferrin as a Predictor of Survival in Cirrhosis

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Patients with cirrhosis frequently present with high serum ferritin and low transferrin concentrations, reflecting impaired liver function and inflammation. Recent studies have shown that transferrin and its saturation with iron are Model for End-Stage Liver Disease–independent predictors of mortality in patients with acute-on-chronic liver failure or decompensated cirrhosis. The aim of this study was to evaluate the prognostic utility of serum iron parameters in relation to markers of liver function and immune activation. Clinical, demographic, and biochemical data were retrospectively analyzed from a cohort of 1255 consecutive patients with cirrhosis (age ≥ 18 years) who presented from August 1, 2004 until December 31, 2014 at the University Hospital of Innsbruck. Patients with malignancies at diagnosis including hepatocellular carcinoma were excluded. Survival analysis was carried out by Cox regression by using baseline laboratory parameters, and findings were validated in an independent patient cohort. During a median follow-up of 2.4 years, 193 deaths occurred and 254 patients underwent liver transplantation. In patients with transferrin < 180 mg/dL, 3-month, 1-year, and 5-year transplant-free survival estimates were significantly lower (91.7%, 79.0%, and 30.5%) when compared with the group of patients with transferrin ≥ 180 mg/dL (98.9%, 95.5%, and 68.0%, $P < 0.001$). Transferrin predicted transplant-free survival independently of Model for End-Stage Liver Disease–sodium (MELD-Na) and C-reactive protein (CRP) in multivariate regression analysis including all patients. When patients with alcoholic or nonalcoholic fatty liver disease were excluded, transferrin was in addition an albumin-independent predictor of transplant-free survival. In conclusion, the association of transferrin with transplant-free survival is independent of MELD-Na score and CRP. In patients without fatty liver disease, transferrin also predicts survival independently of albumin.

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The Model for End-Stage Liver Disease (MELD) has evolved as a reference staging system for cirrhosis and is used for prognosis, evaluation for liver transplantation, and donor liver allocation.[1-3] Incorporating sodium, age, or albumin into modified MELD scores improves accuracy in predicting survival in patients with cirrhosis.[4-8] In search for additional prognostic parameters, high ferritin at the time of listing for liver transplantation was identified as an independent predictor of wait-list mortality,[9] but the prognostic utility of ferritin could not be reproduced in a larger cohort.[10] When transferrin saturation was taken into consideration, ferritin could predict mortality on the waiting list and after orthotopic liver transplantation.[11]

Iron overload was therefore proposed as a risk factor for mortality in patients with liver disease. Accordingly, transferrin and transferrin saturation have also been identified as predictors of survival in patients with decompensated cirrhosis and acute-on-chronic liver failure (ACLF).[12,13] Transferrin is lower in patients with cirrhosis and impaired synthetic function.[14] High ferritin and low transferrin can indicate inflammation,[15,16] which is also a risk factor for disease progression and mortality in cirrhosis. In addition, alcohol and metabolic factors can directly affect iron metabolism.[17-19] Hence,

Abbreviations: α1-ATD, alpha 1-antitrypsin deficiency; ACLF, acute-on-chronic liver failure; ALD, alcoholic liver disease; AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; HBV, hepatitis B virus; HCV, hepatitis C virus; HH, hereditary hemochromatosis; HR, hazard ratio; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; MELD-Na, Model for End-Stage Liver Disease–sodium; NAFLD, nonalcoholic fatty liver disease; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; ROC, receiver operating characteristic; UNOS, United Network for Organ Sharing.

it remains unclear if altered iron metabolism is cause or consequence of advanced liver disease in different etiologies.

The effect of inflammation and hepatic function on serum iron parameters was assessed by correlating serum ferritin, transferrin, and transferrin saturation with C-reactive protein (CRP) and albumin. The prognostic utility of all these parameters was determined by survival analysis in a large cohort of unselected patients with cirrhosis.

Patients and Methods

PATIENT DEMOGRAPHICS AND LABORATORY DATA

Derivation Cohort

From a cohort of consecutive 1437 patients (age $\geq$ 18 years) with liver cirrhosis who presented from August 1, 2004 to December 31, 2014 at the University Hospital of Innsbruck, 182 patients with hepatocellular carcinoma or other malignancy at diagnosis were excluded, resulting in a final study cohort of 1255 patients (Fig. 1). Diagnosis of cirrhosis was made according to clinical, biochemical, and imaging criteria.[20] We received ethical approval by the ethics committee of the Medical University of Innsbruck to conduct this study.

Clinical, demographic, and biochemical data were collected by retrospective review of patient charts. MELD score was calculated according to the United Network for Organ Sharing (UNOS) model, in which a MELD score over 11 requires serum sodium correction (Model for End-Stage Liver Disease–sodium [MELD-Na]).[3,6,7]

The majority of patients (79%; 989 patients) were outpatients. The remaining 266 patients were hospitalized; 65 (5%) of whom were treated in an intermediate care unit. The start date of this cohort corresponds to the first assessment of serum iron parameters, and study end points were death, liver transplantation, or last follow-up. Accordingly, prognosis was calculated using 3-month, 1-year, and 5-year transplant-free survival, in which patients who underwent liver transplantation or were lost to follow-up were right-censored.

Validation Cohort

In order to validate the results of this investigation, a cohort of 596 unselected patients with cirrhosis was included in the 2 study centers: Innsbruck (302 patients) and Vienna (294 patients) from January 1, 2004 to March 31, 2016. Inclusion criteria were identical to those applied to the derivation cohort.

STATISTICAL ANALYSIS

SPSS Statistics, version 22.0.0.1 (IBM Corp., Armonk, NY) and MedCalc, version 17.7.2 (MedCalc Software, Ostend, Belgium) were used for statistical analysis, and a significance value of 0.05 was considered in all statistic tests. Kolmogorov-Smirnov test was used to test normality of distribution. Not normally distributed variables were reanalyzed after logarithmic transformation. Continuous variables were reported by using median and interquartile range and analyzed by using Student t test or the Mann-Whitney U test, as appropriate. Categorical variables were expressed in frequencies (with percentages in parentheses) and tested for significance using the χ^2 test or the Fisher's exact test. Survival models were built based on univariate and multivariate Cox regression analysis. For survival analysis, 3-month, 1-year, and 5-year transplant-free survival rates were analyzed. Receiver operating characteristic (ROC) calculations were performed, and the Youden index was applied to calculate the optimal transferrin cutoff point. Kaplan-Meier analysis was then carried out to determine survival according to transferrin levels and groups were compared using the log-rank test. Correlation analysis for transferrin, CRP, and albumin were carried out using the Spearman's rank correlation coefficient because all 3 variables did not show a normal distribution.

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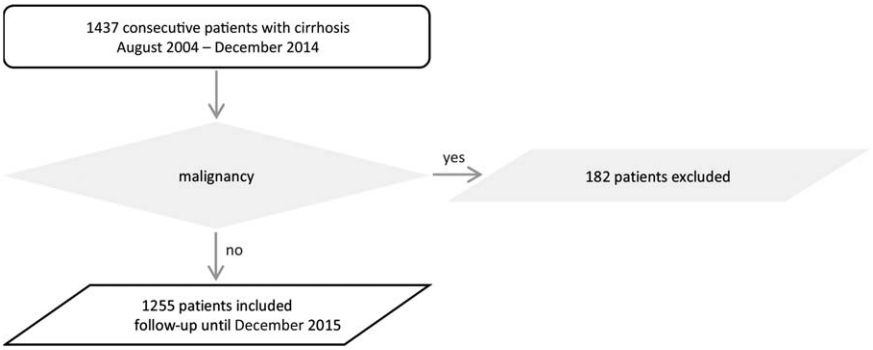

FIG. 1. Study flowchart.

Results

To determine whether transferrin is a predictor of survival in unselected patients with cirrhosis, clinical and biochemical parameters were retrospectively assessed in a cohort of 1255 cirrhosis patients. During a median follow-up of 2.4 years (95% confidence interval [CI], 0.1-9.0), 193 (15%) deaths occurred, and 254 (20%) patients underwent liver transplantation. For validation, an independent cohort of patients was recruited at the liver centers in Innsbruck and Vienna. Baseline patient characteristics of the derivation and validation cohort are shown in Table 1.

Univariate Cox regression analysis showed that ferritin, transferrin, and transferrin saturation, but not

TABLE 1. Clinical Characteristics and Outcome of Patients Stratified According to MELD Score

	Derivation Cohort (n = 1255)	Validation Cohort (n = 596)	*P* Value
Age, years	57 (49-64)	55 (48-63)	0.002
Sex, female/male	404/851 (32.2/67.8)	166/430 (27.9/72.1)	0.06
Underlying liver disease			<0.001
ALD	222 (17.7)	21 (3.5)	
NAFLD	563 (44.9)	121 (20.3)	
HBV/HCV	254 (20.2)	140 (23.5)	
Cryptogenic	80 (6.4)	5 (0.8)	
Biliary (PSC/PBC)	52 (4.1)	45 (7.6)	
Autoimmune	26 (2.1)	66 (11.1)	
Metabolic (HH/α1-ATD/Wilson's)	23 (1.8)	94 (15.8)	
Other	35 (2.8)	104 (17.4)	
White cell count, G/L*	6.0 (4.4-7.8)	—	—
Hemoglobin, g/L*	123 (107-140)	—	—
Platelet count, G/L*	116 (75.8-168)	—	—
CRP, mg/dL	0.60 (0.21-1.42)	0.35 (0.11-1.18)	<0.001
Albumin, mg/dL	3530 (2998-4080)	3630 (3093-4082)	0.07
Creatinine, mg/dL	0.84 (0.68-1.06)	0.84 (0.72-0.10)	0.94
MELD-Na score	13 (9-18)	11 (8-17)	<0.001
Bilirubin, mg/dL	1.61 (0.90-3.09)	1.24 (0.78-2.59)	<0.001
INR	1.3 (1.1-1.5)	1.3 (1.1-1.44)	0.001
Sodium, mmol/L	138 (135-140)	138 (135-140)	0.53
Serum iron, μmol/L	17.6 (10.3-25.9)	19.8 (11.4-27.8)	0.005
Serum ferritin, μg/L	204 (70-510)	215 (68-596)	0.18
Transferrin, mg/dL	232 (170-285)	248 (180-295)	0.005
Transferrin saturation, %	32 (17-54)	33 (20-57)	0.20
Transplant-free survival rates, % (n)			
3-month	97.0 (1085/1118)	95.9 (534/557)	0.25
1-year	91.6 (779/850)	86.2 (413/479)	0.002
5-year	58.8 (227/386)	49.5 (147/297)	0.002

NOTE: Data are given as n (%) or median (interquartile range).
*n = 1254.

TABLE 2. Univariate and Multivariate Cox Regression: Serum Iron Parameters

	Derivation Cohort (n = 1255)				Validation Cohort (n = 596)			
	Univariate Cox Regression HR (95% CI)	*P* Value	Multivariate Cox Regression HR (95% CI)	*P* Value	Univariate Cox Regression HR (95% CI)	*P* Value	Multivariate Cox Regression HR (95% CI)	*P* Value
Age, years	1.023 (1.010-1.036)	<0.001	1.026 (1.013-1.040)	<0.001	1.047 (1.033-1.061)	<0.001	1.047 (1.033-1.062)	<0.001
Serum iron, μmol/L	0.995 (0.982-1.008)	0.47	—	—	0.991 (0.978-1.003)	0.14	—	—
Serum ferritin, g/L	1.038 (1.021-1.054)	<0.001	1.008 (0.983-1.035)	0.54	1.010 (0.999-1.021)	0.07	1.000 (0.983-1.016)	0.95
Transferrin, g/L	0.496 (0.411-0.598)	<0.001	0.563 (0.419-0.755)	<0.001	0.498 (0.413-0.600)	<0.001	0.522 (0.406-0.672)	<0.001
Transferrin saturation, %	1.011 (1.006-1.017)	<0.001	1.005 (0.994-1.016)	0.37	1.006 (1.002-1.010)	0.002	1.002 (0.996-1.008)	0.53

serum iron, were significantly associated with transplant-free survival. Of all serum iron parameters, transferrin is the only independent predictor of survival in the derivation and validation cohort (Table 2).

ROC curve analysis showed that the best cutoff for transferrin to predict 3-month transplant-free survival is 180 mg/dL (Fig. 2). The 3-month, 1-year, and 5-year transplant-free survival estimates were significantly lower in the group of patients with transferrin < 180 mg/dL (91.7%, 79.0%, and 30.5%) when compared with the group of patients with transferrin ≥ 180 mg/dL (98.9%, 95.5%, and 68.0%; Table 4; Fig. 3A). Stratification based on transferrin also showed that patients with transferrin < 180 mg/dL were more likely to be male and have cirrhosis due to alcoholic liver disease (ALD) or nonalcoholic fatty liver disease (NAFLD) as an underlying etiology. Patients with low transferrin also had more advanced liver disease (higher MELD-Na score), higher white cell count, and higher levels of CRP (Table 4).

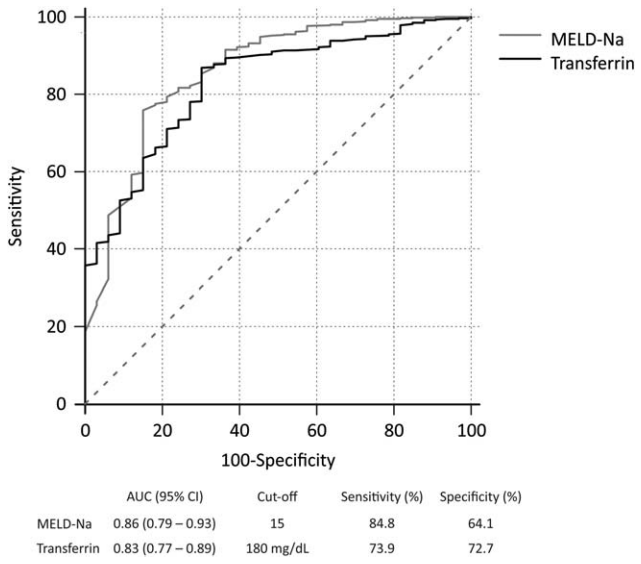

FIG. 2. ROC curves: diagnostic accuracy of MELD-Na and transferrin to predict 3-month transplant-free mortality. AUC with 95% CIs are indicated. For means of comparison, transferrin is indicated in opposite test direction compared with MELD-Na.

To test if etiology had an impact on the predictive power of transferrin, we next grouped patients according to underlying disease. This analysis showed that transplant-free survival was not significantly different in the ALD- or NAFLD-associated cirrhosis group when compared with all other etiologies (log-rank $P = 0.10$). Transferrin independently predicted transplant-free survival in the group of patients with cirrhosis caused by diseases other than ALD or NAFLD (hazard ratio [HR], 0.988; 95% CI, 0.985-0.992; $P < 0.001$). Individual subgroup analysis for patients with ALD or NAFLD showed that transferrin was significantly associated with transplant-free survival (ALD—HR, 0.535; 95% CI, 0.318-0.900; $P = 0.02$; NAFLD—HR, 0.572; 95% CI, 0.443-0.739; $P < 0.001$), but this association was lost for both subgroups when MELD or MELD-Na was added to a multivariate model (data not shown).

To further study the impact of cirrhosis stage on the utility of transferrin for prognosis, we analyzed patient subgroups with MELD-Na ≥ 15 and patients with MELD-Na < 15, which is the accepted cutoff for transplant evaluation.[21] As shown in Fig. 3B, transplant-free survival was significantly higher in patients with transferrin ≥ 180 mg/dL in the cohort with a MELD-Na score < 15 ($P < 0.001$). In the group of patients with MELD-Na score ≥ 15, this association

TABLE 3. Univariate and Multivariate Cox Regression: Iron and Inflammation

	Derivation Cohort (n = 1255)				Validation Cohort (n = 596)			
	Univariate Cox Regression HR (95% CI)	*P* Value	Multivariate Cox Regression HR (95% CI)	*P* Value	Univariate Cox Regression HR (95% CI)	*P* Value	Multivariate Cox Regression HR (95% CI)	*P* Value
Age, years	1.023 (1.010-1.036)	<0.001	1.031 (1.018-1.044)	<0.001	1.047 (1.033-1.061)	<0.001	1.044 (1.030-1.059)	<0.001
MELD-Na score	1.122 (1.097-1.148)	<0.001	1.104 (1.075-1.134)	<0.001	1.084 (1.065-1.104)	<0.001	1.058 (1.034-1.083)	<0.001
CRP, mg/dL	1.127 (1.078-1.179)	<0.001	1.036 (0.975-1.102)	0.26	1.088 (1.048-1.129)	<0.001	1.044 (0.993-1.097)	0.09
Transferrin, g/L	0.496 (0.411-0.598)	<0.001	0.735 (0.592-0.913)	0.005	0.498 (0.413-0.600)	<0.001	0.654 (0.524-0.816)	<0.001

was lost, showing that transferrin is a better predictor of survival at earlier liver disease stages (Fig. 3C).

Next, the effect of inflammation on transferrin concentration and its association with mortality were assessed using CRP as a surrogate parameter. Figure 4 shows a nonlinear but significantly negative correlation between transferrin and CRP, which confirms that transferrin is a negative acute phase reactant. As shown in Table 3, multivariate Cox regression analysis including all patients showed a stronger prediction of mortality for transferrin than for CRP. These results were confirmed in the validation cohort.

To assess the interactions between hepatic synthetic function and transferrin, albumin was next included in the analysis. Pearson correlation showed a stronger and linear association between transferrin and albumin (Fig. 4B). Multivariate Cox regression analysis including albumin revealed different results for the derivation and validation cohort. In search for factors accounting for these differences, patients were grouped by disease stage (MELD-Na cutoff 15) or by etiology. As shown in Table 5 and in accordance with previous studies,[22] MELD-Na is a poor predictor of survival in patients with early cirrhosis (MELD-Na < 15). In this patient group, albumin remained the only independent predictor of survival in the derivation and validation cohort. When patients with ALD or NAFLD were excluded, on the basis that alcohol and metabolic syndrome directly affect serum iron parameters, transferrin was a predictor of transplant-free survival independently of MELD-Na, CRP, and albumin in both cohorts (Table 6).

Discussion

Beyond the immediate implications for predicting outcomes, studying prognostic factors in patients with

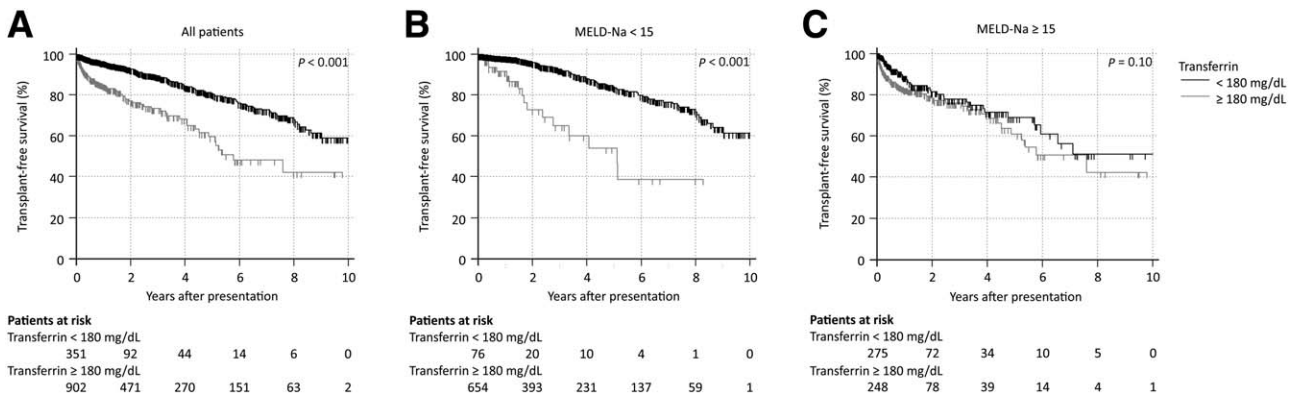

FIG. 3. Kaplan-Meier analysis: transplant-free survival according to a transferrin cutoff of 180 mg/dL in (A) all patients, (B) patients with a MELD-Na score < 15, and (C) a MELD-Na score ≥ 15.

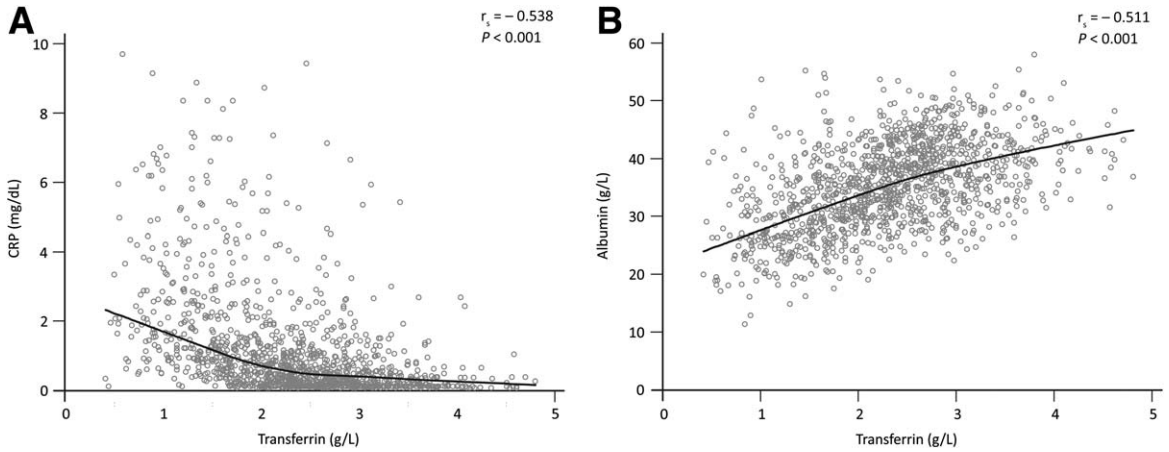

FIG. 4. Correlation analysis: transferrin versus (A) CRP and (B) transferrin versus albumin.

TABLE 4. Clinical Characteristics and Outcome of Patients Stratified According to Transferrin Concentration

	Derivation Cohort			Validation Cohort		
	Transferrin <180 mg/dL (n = 352)	Transferrin ≥180 mg/dL (n = 903)	*P* Value	Transferrin <180 mg/dL (n = 147)	Transferrin ≥180 mg/dL (n = 449)	*P* Value
Age, years	56 (48-63)	57 (49-64)	0.16	56 (49-64)	54 (47-62)	0.08
Sex, female/male	96/256 (27.3/72.7)	308/595 (34.1/65.9)	0.02	36/111 (24.5-75.5)	130/319 (29.0-71.0)	0.30
Underlying liver disease			<0.001			<0.001
ALD	89 (25.3)	133 (14.7)		5 (3.4)	16 (3.6)	
NAFLD	177 (50.3)	386 (42.7)		31 (21.1)	90 (20.0)	
HBV/HCV	40 (11.4)	214 (23.7)		14 (9.5)	126 (28.1)	
Cryptogenic	20 (5.7)	60 (6.6)		2 (1.4)	3 (0.7)	
Biliary (PSC/PBC)	3 (0.9)	49 (5.4)		14 (9.5)	31 (6.9)	
Autoimmune	4 (1.1)	22 (2.4)		11 (7.5)	55 (12.2)	
Metabolic (HH/α1-ATD/Wilson)	10 (2.8)	13 (1.4)		37 (25.2)	57 (12.7)	
Other	9 (2.6)	26 (2.9)		33 (22.4)	71 (15.8)	
White cell count, g/L	7.0 (5.1-9.1)*	5.6 (4.3-7.2)	<0.001	—	—	—
Hemoglobin, g/L	111 (98-124)*	128 (113-144)	<0.001	—	—	—
Platelet count, g/L	105 (69-150)*	118 (77-171)	0.01	—	—	—
CRP, mg/dL	1.54 (0.86-3.28)	0.38 (0.14-0.93)	<0.001	1.28 (0.49-2.74)	0.23 (0.10-0.64)	<0.001
Albumin, mg/dL	2950 (2520-3470)	3740 (3250-4210)	<0.001	3070 (2700-3440)	3780 (3320-4195)	<0.001
Creatinine, mg/dL	0.88 (0.66-1.26)	0.83 (0.68-1.00)	0.001	0.86 (0.72-1.12)	0.83 (0.71-0.96)	0.04
MELD-Na score	19 (15-25)	12 (9-15)	<0.001	19 (15-24)	10 (8-14)	<0.001
Bilirubin, mg/dL	3.35 (1.72-6.27)	1.27 (0.79-2.21)	<0.001	3.38 (1.69-6.82)	1.04 (0.74-1.79)	<0.001
INR	1.5 (1.3-1.7)	1.2 (1.1-1.4)	<0.001	1.5 (1.3-1.8)	1.2 (1.1-1.3)	<0.001
Sodium, mmol/L	135 (131-138)	139 (136-141)	<0.001	135 (132-138)	138 (136-140)	<0.001
Serum iron, μmol/L	17.1 (10.7-24.6)	17.7 (10.3-26.3)	0.21	19.8 (11.6-26.9)	19.8 (11.3-28.1)	0.69
Serum ferritin, μg/L	540 (245-1031)	141 (48-310)	<0.001	660 (286-1344)	153 (50-420)	<0.001
Transferrin, mg/dL	136.5 (106-160)	261 (226-303)	<0.001	136 (103-160)	272 (234-315)	<0.001
Transferrin saturation, %	58 (32-84)	27 (15-42)	<0.001	65 (36-88)	28 (17-44)	<0.001
Transplant-free survival rate, % (n)						
3-month	91.7 (265/289)	98.9 (820/829)	<0.001	83.5 (106/127)	99.5 (428/430)	<0.001
1-year	79.0 (158/200)	95.5 (621/650)	<0.001	62.6 (62/99)	92.4 (351/380)	<0.001
5-year	30.5 (29/95)	68.0 (198/291)	<0.001	20.5 (15/73)	58.9 (132/224)	<0.001

NOTE: Data are given as n (%) or median (interquartile range).
*n = 351.

TABLE 5. Univariate and Multivariate Cox Regression: Iron, Inflammation, and Hepatic Function at Different Cirrhosis Stages

	Derivation Cohort				Validation Cohort			
	Univariate Cox Regression HR (95% CI)	*P* Value	Multivariate Cox Regression HR (95% CI)	*P* Value	Univariate Cox Regression HR (95% CI)	*P* Value	Multivariate Cox Regression HR (95% CI)	*P* Value
Model A: all patients		n = 1255				n = 596		
Age, years	1.023 (1.010-1.036)	<0.001	1.035 (1.021-1.049)	<0.001	1.047 (1.033-1.061)	<0.001	1.042 (1.028-1.056)	<0.001
MELD-Na score	1.122 (1.097-1.148)	<0.001	1.096 (1.066-1.127)	<0.001	1.084 (1.065-1.104)	<0.001	1.047 (1.021-1.074)	<0.001
CRP, mg/dL	1.127 (1.078-1.179)	<0.001	1.020 (0.958-1.087)	0.53	1.088 (1.048-1.129)	<0.001	1.022 (0.971-1.076)	0.41
Albumin, g/L	0.921 (0.903-0.940)	<0.001	0.949 (0.926-0.973)	<0.001	0.914 (0.894-0.934)	<0.001	0.955 (0.930-0.981)	0.001
Transferrin, g/L	0.496 (0.411-0.598)	<0.001	0.917 (0.725-1.160)	0.47	0.498 (0.413-0.600)	<0.001	0.750 (0.593-0.948)	0.02
Model B: MELD-Na < 15		n = 731				n = 404		
Age, years	1.024 (1.006-1.042)	0.009	1.022 (1.004-1.040)	0.02	1.044 (1.027-1.062)	<0.001	1.041 (1.024-1.058)	<0.001
MELD-Na score	1.144 (1.057-1.239)	0.001	1.075 (0.986-1.171)	0.10	1.153 (1.068-1.244)	<0.001	1.071 (0.986-1.163)	0.10
CRP, mg/dL	1.164 (1.065-1.271)	0.001	1.060 (0.946-1.188)	0.32	1.105 (1.035-1.180)	0.003	1.071 (0.990-1.159)	0.09
Albumin, g/L	0.925 (0.899-0.953)	<0.001	0.952 (0.919-0.985)	0.005	0.900 (0.872-0.929)	<0.001	0.913 (0.881-0.946)	<0.001
Transferrin, g/L	0.473 (0.343-0.654)	<0.001	0.689 (0.485-0.980)	0.04	0.613 (0.456-0.823)	0.001	0.842 (0.614-1.155)	0.29
Model C: MELD-Na ≥ 15		n = 524				n = 192		
Age, years	1.031 (1.012-1.051)	0.001	1.044 (1.023-1.065)	<0.001	1.043 (1.018-1.068)	0.001	1.045 (1.020-1.070)	<0.001
MELD-Na score	1.155 (1.106-1.207)	<0.001	1.155 (1.104-1.209)	<0.001	1.053 (1.013-1.095)	0.009	1.049 (1.006-1.094)	0.02
CRP, mg/dL	1.055 (0.991-1.124)	0.10	—	—	1.035 (0.976-1.098)	0.25	—	—
Albumin, g/L	0.944 (0.915-0.974)	<0.001	0.944 (0.911-0.978)	0.001	0.985 (0.948-1.023)	0.43	—	—
Transferrin, g/L	0.701 (0.530-0.927)	0.01	1.032 (0.745-1.431)	0.85	0.649 (0.475-0.886)	0.007	0.663 (0.472-0.932)	0.02

cirrhosis helps improve our understanding of disease pathogenesis and could ultimately lead to the identification of new therapeutic targets. Recent studies have shown that serum iron parameters are independent predictors of survival in patients awaiting liver transplantation, ACLF, and critically ill patients.[11-13,23]

TABLE 6. Univariate and Multivariate Cox Regression: Iron, Inflammation, and Hepatic Function in Patients Without Fatty Liver Disease (ALD or NAFLD)

	Derivation Cohort (n = 454)				Validation Cohort (n = 470)			
	Univariate Cox Regression HR (95% CI)	*P* Value	Multivariate Cox Regression HR (95% CI)	*P* Value	Univariate Cox Regression HR (95% CI)	*P* Value	Multivariate Cox Regression HR (95% CI)	*P* Value
Age, years	1.029 (1.010-1.048)	0.003	1.031 (1.012-1.051)	0.001	1.045 (1.029-1.062)	<0.001	1.040 (1.023-1.057)	0.000
MELD-Na score	1.142 (1.099-1.187)	<0.001	1.106 (1.057-1.159)	0.000	1.082 (1.061-1.104)	<0.001	1.037 (1.007-1.068)	0.014
CRP, mg/dL	1.175 (1.086-1.273)	<0.001	0.996 (0.889-1.116)	0.940	1.085 (1.040-1.131)	<0.001	1.022 (0.964-1.083)	0.469
Albumin, g/L	0.898 (0.868-0.930)	<0.001	0.928 (0.892-0.965)	0.000	0.911 (0.887-0.936)	<0.001	0.963 (0.932-0.995)	0.022
Transferrin, g/L	0.311 (0.211-0.458)	<0.001	0.613 (0.395-0.950)	0.029	0.406 (0.324-0.510)	<0.001	0.582 (0.432-0.784)	0.000

A main finding of this study is that of all serum iron parameters, transferrin is the best prognostic parameter and it is independent of MELD-Na. In contrast to the ACLF cohort, where the best cutoff for transferrin was 87 mg/dL, the best predictive power of transferrin in our cohort for a 3-month and 1-year survival was 180 mg/dL. This highlights both the severity of alterations in iron metabolism in ACLF, as well as the need to individualize cutoffs according to disease stage.

Reduced transferrin concentration in patients with cirrhosis can be attributed to impaired hepatic function, inflammation, alcohol consumption, or metabolic syndrome. In our study, transferrin correlates significantly with CRP but predicts survival independently of inflammation. In contrast, transferrin does not predict transplant-free survival independently of albumin. In patients with MELD-Na < 15, where this established score has poor discriminative accuracy,[22] albumin remained the best predictor of survival in both cohorts. When alcohol and metabolic effects were excluded from the model, by selecting only patients with viral, genetic, cholestatic, and autoimmune etiology, transferrin was confirmed as a predictor of transplant-free survival independently of MELD-Na, CRP, and albumin. The finding that patients with fatty liver disease have significantly lower transferrin concentration confirms that alcohol and metabolic factors directly suppress transferrin synthesis. This effect could explain why albumin is a stronger predictor of survival than transferrin when also patients with fatty liver disease are included in the survival analysis.

Association studies cannot discern if altered serum iron parameters are a mere consequence or a driver of disease progression in patients with cirrhosis. Regardless of the exact hierarchy of events, excess iron will impair different immune effector functions and can induce ferropotosis—a recently identified form of programmed cell death triggered by iron.[24,25] Toxic nontransferrin-bound iron species increase when plasma transferrin concentration decreases and are specifically taken up by hepatocytes.[26] Accordingly, hepatic iron overload has been histologically reported in 32.4% of patients with end-stage liver disease.[27] Impaired hepatocellular function has also been associated with decreased hepcidin production, which can lead to uncontrolled release of iron from cells. Reduced plasma iron binding capacity and increased iron release in cirrhosis could therefore promote hepatocellular iron toxicity in cirrhosis. The results from our study support the hypothesis that increasing iron binding capacity in plasma could improve survival in patients with cirrhosis. This can be achieved by administering apotransferrin[28] or hepcidin agonists, which are currently in clinical development.[29]

In conclusion, our study shows that transferrin predicts transplant-free survival independently of MELD-Na score in unselected patients with cirrhosis. Regardless of etiology and cirrhosis stage, transferrin ≥ 180 mg/dL is associated with significantly better prognosis. More complex survival modeling in this large cohort shows that the prognostic value of transferrin is independent of inflammation. Albumin appears to be a stronger predictor of survival than transferrin in patients with ALD and NAFLD. The albumin-independent association of transferrin with survival in patients without fatty liver disease confirms that alcohol and metabolic factors represent an additional burden on iron metabolism and influence how serum iron parameters predict survival in patients with cirrhosis.

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