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The association of pretransplant ferritin level with waiting list and post-transplant survival. Does ferritin actually predict outcome?

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Abstract

Recent data suggest an association of serum ferritin (SF) with waiting list (WL) and postliver transplant (LT) outcomes. To assess the predictive capacity of SF on pre- and post-LT outcomes, and to identify whether recipient or donor liver siderosis is associated with post-LT survival; a retrospective analysis of 1079 patients assessed for first LT, 2000–2007 was performed. Iron deposition in the liver tissue was assessed using a semi-quantitative grading system. Median age was 54 (18–82) years and 67% were male. Seventeen per cent had hepatocellular carcinoma (HCC). Median Model for End-stage Liver Disease MELD score was 14 (6–40), ferritin was 174 μg/l (4–4597) with 36.5% had a SF ≥ μg/l. Age (OR = 1.028) and MELD score (OR = 1.158) were independently associated with WL mortality ($P < 0.001$), whilst SF was not (P = NS). Age (OR = 1.018), HCC (OR = 1.542) and cold ischemia time (CIT) ≥ 10 h (OR = 1.418) were independently associated with post-LT survival ($P < 0.05$). Explant siderosis grade <2 was seen in 376 (71.7%) patients. Patients with explant siderosis grade ≥2 had inferior 12-month post-LT survival ($P = 0.030$). Presence of graft siderosis (15.8% of patients) was not associated with survival. In conclusion, we found a limited role for SF as a prognostic indicator for pre- or post-transplant survival.

Introduction

The introduction of Model for End-stage Liver Disease (MELD) by the United Network of Organ Sharing (UNOS) in the United States of America (USA) in 2002 offered an objective, reproducible and potentially fairer system of organ allocation for patients on the transplant waiting list (WL) to overcome increasing WL mortality [1]. Over time, additional models have been proposed to improve the clinical prediction of patients who are at risk of death on the transplant WL. In this context, serum sodium (Na) integration with MELD was assessed in a number of studies such as the MELD-Na in the USA and the United Kingdom End-Stage Liver Disease (UKELD) score in the United Kingdom (UK) [2–5]. In 2010, Walker *et al.* described pretransplant serum ferritin (SF) as a predictor of WL mortality [6]. The addition of SF to MELD resulted in an increase in the area under the receiver operator curve (ROC) by 7.6% and 7.5% for prediction of 6- and 12-month WL mortality, respectively [6]. This however, was not statistically significant.

It was suggested that an optimum prognostic model for organ allocation is a model that should take into consideration post-transplant survival in addition to its ability to predict WL mortality [7]. The ability of MELD to predict postliver transplant (LT) survival was investigated in various studies and these have produced mixed results [8,9]. Therefore, organ allocation based on MELD score only, although it predicts WL mortality accurately; it may overlook post-transplant survival benefit and also may be at risk of promoting futile transplants. Weismuller *et al.* recently investigated pretransplant ferritin as a predictor of post-transplant survival [10]. Elevated SF in a small group of

patients with low transferrin saturation (n = 33) was independently, negatively associated with post-transplant survival. SF is a simple test, easily measured, reproducible, cheap and it forms part of the routine investigations of patients assessed for liver transplantation to assess iron homeostasis. Therefore, it is important to determine whether SF can predict pre- and post-LT outcome.

The aims of this study were fourfold. Firstly, to assess the predictive capacity of SF on 1-year survival of patients with ESLD (patients with decompensated cirrhosis not responding to medical therapy and cirrhotics with hepatocellular carcinoma) assessed for LT. Secondly, to assess pretransplant ferritin on WL mortality. Thirdly, to assess whether ferritin levels are associated with post-LT patient and graft survival. Finally, to identify whether explant (recipient liver) siderosis or donor liver siderosis (liver biopsy at time 0) is associated with post-LT patient or graft outcome.

Patients and methods

A retrospective analysis of all patients assessed at our centre for LT between 01 January 2000 and 31 December 2007 was undertaken. A total of 1482 patients were assessed for LT over an 8-year period at our centre. Patients assessed for acute liver failure (n = 175), hereditary amyloid polyneuropathy (n = 43), patients assessed for re-transplantation (n = 150), patients with haemochromatosis (n = 11) and 24 patients with incomplete information were excluded. Data were analyzed on 1079 patients who were assessed for first LT.

Data

Demographical, clinical and laboratory data were extracted through a review of clinical notes and electronic patient records. All patients assessed for LT at our centre had their clinical history, physical examination findings, laboratory variables, histology reports and outcome of transplant assessment entered at the time of LT assessment into a prospectively collated electronic database. This database was interrogated in addition to the clinical notes. SF was measured routinely for all patients at the time of assessment for LT. SF measurement was repeated on a small subset of patients according to clinical indications. We used SF level measured at the time of assessment for LT only.

Iron deposition in the liver tissue (explant liver and liver graft baseline biopsy) was assessed routinely by one of three histopathologists using a semiquantitative grading system on a scale of 0–4 [11]. Child–Turcotte–Pugh (CTP) score was calculated according to Pugh modification [12]. MELD score was calculated according to UNOS adjustments [1]. UKELD score was calculated according to Barber *et al.* [5]. Donor and graft variables were recorded and donor risk index (DRI) was calculated according to Feng *et al.* [13]. Patient survival was documented according to the recorded survival status in our hospital information system and further validated using the National Health Service (NHS) electronic portal. This is a UK National database, up-dated according the generation of death certificate in the UK. This large anonymized data set was given ethical approval for interrogation, analysis and publication by the Southeast London Research Ethics Committee 3 (previously known as King's College Hospital Research Ethics Committee).

Outcome and definitions

For the total cohort of patients with end-stage liver disease, 1-year survival was defined as the time from LT assessment to death or transplantation and if alive censored at 1-year. WL outcome was defined for this study by death on the WL, delisting because of significant deterioration or hepatocellular carcinoma (HCC) progression beyond Milan criteria whilst awaiting LT [14]. Post-transplant survival was defined as time from transplantation to death; and if alive, censored on 20 December 2010. Graft survival was defined as time from transplantation to retransplantation or death; and if alive, censored on 20 December 2010. Patients lost to follow-up were censored as alive on the date of last clinic or hospital review.

Statistical Analysis

Continuous variables were expressed using median (range) and analyzed using nonparametric methods for non-normally distributed data (Mann–Whitney U-test and Kruskal–Wallis Test as appropriate). Categorical variables were reported as numbers (percentages) and analyzed using Chi-square test or Fisher's exact test as appropriate. We used transplant-free survival (time from listing to death, delisting or to transplant) to eliminate the artificial impact of transplantation on patients survival on the WL. Univariate and multivariate analyses were performed using Cox proportional hazard analysis to determine factors associated with assessment, listing and transplant outcomes. Factors associated with outcome on univariate analysis (P-value < 0.05) were entered into multivariate analysis. Kaplan–Meier analysis was performed to determine survival according to ferritin level. Data were analyzed according to SF as continuous and as categorical variable. Patients were classified into 2 groups of SF levels (μg/l) of ≤300 or >300 according to our laboratory normal reference values (20–300). Statistical analyses were performed with SPSS software (SPSS® 17.0 for Windows, SPSS, Chicago, IL, USA).

Results

Descriptives

One thousand and seventy-nine patients were assessed for first LT over an 8-year period, of whom 814 (75.4%) were listed for transplant. Of those not listed (n = 265), 92 (34.7%) did not meet minimal listing criteria, 18 (6.8%) were suitable for alternative therapy and 67 (25.3%) were deemed as having excessive comorbidities. Forty six (17.4%) were too sick for transplant or had HCC outside Milan criteria and 24 (9.1%) patients had evidence of ongoing alcohol or other substance misuse. Eighteen of 265 (6.8%) were not listed for other reasons. Table 1 summarizes baseline characteristics of the cohort. Median follow-up was 3.64 years (0.00–10.96). SF concentration was available on 1025/1079 patients (95%). Therefore, SF was available on 767/814 (94%) of listed patients and 553/589 (94%) of transplanted patients.

Table 1. Baseline characteristics of 1079 patients with end-stage liver disease.

Variable	n = 1079
Demographic	
Age (years)	54 (18–82)
Gender (male, %)	725 (67.2)
Blood group	
O (%)	460 (42.8)
A (%)	438 (40.7)
B (%)	136 (12.7)
AB (%)	41 (3.8)
HCC (%)	185 (17.1)
Aetiology	
ALD (%)	344 (31.9)
HCV (%)	230 (21.3)
HBV (%)	71 (6.6)
Cholestatic and autoimmune (%)	227 (21.0)
Cryptogenic (%)	112 (10.4)
Other (%)	95 (8.8)
Biochemical	
Na (mmol/l)	135 (116–151)
Bilirubin (μmol/l)	46 (3–1170)
Creatinine (μmol/l)	90 (39–603)
Albumin (g/l)	30 (9–49)
SF (μg/l)	174 (4–4597)
SF > 300 μg/l (%)	374 (36.5)
INR	1.25 (0.80–5.00)
Clinical	
Ascites (%)	666 (63.0)
Mild/Moderate (%)	423 (63.5)
Severe (%)	243 (36.5)
Encephalopathy (%)	347 (32.8)
Low grade (%)	288 (83.0)
High grade (%)	59 (17.0)
CTP score	9 (5–15)
CTP class A (%)	162 (15.4)
CTP class B (%)	438 (41.6)
CTP class C (%)	453 (43.0)
MELD score	14 (6–40)
UKELD score	55 (47–77)

HCC, hepatocellular carcinoma; ALD, alcohol-related liver disease; SF, serum ferritin; CTP, Child–Turcotte–Pugh; MELD, model for end-stage liver disease, UKELD, United Kingdom End-Stage Liver Disease.

Baseline characteristics according to serum ferritin level

There were 374 patients with SF > 300 μg/l (high SF group) and 651 patients with SF ≤ 300 μg/l (low SF group). As shown in Table 2, patients with high SF were older, with higher representation of male gender, higher proportion of patients with alcohol-related liver disease (ALD), but lower proportion of patients with cholestatic and autoimmune liver disease. All liver prognostic scores including MELD and UKELD were significantly higher in the high SF group, other than for serum sodium (Na) level which was significantly lower.

Three hundred and sixteen patients died within 1 year of assessment. The main cause of death was known in 224 (71%). The main cause of death was sepsis with multi-organ failure (MOF) in 116 (51.8%), malignancy (progression of HCC or development of cholangiocarcinoma in patients with primary sclerosing cholangitis) in 46 (20.5%), bleeding with MOF in 35 (15.6%). Cardiac-related death occurred in 13 (5.8%), intracranial pathology in 8 (3.6%) and death from miscellaneous causes in 6 (2.7%). Median SF for those who died of sepsis was higher 235 (10–4083) compared to those died from other causes 173 (7–2628), but this was not statistically significant (P = NS).

Table 2. Demographic, clinical and outcome measures according to SF of 1025 patients with ESLD.

Variables	SF ≤ 300 μg/l (n = 651)	SF > 300 μg/l (n = 374)	P-Value
Demographic			
Age (years)	53 (17–82)	55 (19–72)	0.070
Gender (male, %)	400 (61.4)	293 (78.3)	<0.001
Aetiology			
ALD (%)	183 (28.1)	149 (39.8)	<0.001
Viral (%)	173 (26.6)	112 (29.9)	0.246
Cholestatic and autoimmune (%)	164 (25.2)	51 (13.6)	<0.001
HCC (%)	112 (17.2)	59 (18.8)	0.555
Clinical			
Na (mmol/l)	136 (118–151)	133 (116–147)	<0.001
MELD score	13 (6–39)	16 (6–40)	<0.001
UKELD score	54 (43–77)	58 (43–77)	<0.001
CTP score	9 (5–14)	10 (5–15)	<0.001
CTP Class C (%)	217 (33.9)	218 (59.7)	<0.001

HCC, hepatocellular carcinoma; ALD, alcohol-related liver disease; SF, serum ferritin; CTP, Child–Turcotte–Pugh; MELD, model for end-stage liver disease, UKELD, United Kingdom End-Stage Liver Disease.

Table 3. Univariate and multivariate Cox proportional hazard analysis of factors associated with 1-year mortality in patients with end-stage liver disease assessed for liver transplant.

	Univariate			Multivariate	
Category	Factors	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Demographic	Age (year)	1.019 (1.007–1.030)	0.001	1.026 (1.013–1.039)	<0.001
Haematologic	Hb (g/dl)	0.817 (0.763–0.874)	<0.001	0.878 (0.815–0.947)	0.001
	Platelet ($\times 10^9$/l)	0.998 (0.996–1.000)	0.027	1.000 (0.998–1.001)	0.690
	Ferritin (μg/l)	1.001 (1.000–1.001)	<0.001	1.000 (1.000–1.000)	0.187
	Ferritin >300 μg/l	2.223 (1.733–2.852)	<0.001	0.970 (0.711–1.322)	0.846
Liver Prognostic Indicators	Na (mmol/l)	0.923 (0.901–0.945)	<0.001	0.966 (0.940–0.992)	0.011
	MELD score	1.135 (1.114–1.155)	<0.001	1.126 (1.101–1.152)	<0.001
	UKELD score	1.139 (1.115–1.164)	<0.001	1.128 (1.100–1.158)	<0.001
	CTP score	1.398 (1.315–1.486)	<0.001	1.323 (1.228–1.426)	<0.001
	CTP class C	3.462 (2.674–4.482)	<0.001	2.993 (2.235–4.006)	<0.001

HCC, hepatocellular carcinoma; ALD, alcohol-related liver disease; CTP, Child–Turcotte–Pugh; MELD, model for end-stage liver disease, UKELD, United Kingdom End-Stage Liver Disease.

Serum ferritin in association with 1-year mortality in patients with ESLD

As summarized in Table 3, SF as a continuous variable and SF > 300 μg/l were associated with 1-year mortality in patients with ESLD on univariate analysis. However, SF neither as a continuous nor as a categorical variable was associated with 1-year mortality in patients with ESLD on multivariate analysis. Age, haemoglobin level, CTP, MELD and UKELD scores were independently associated with 1-year mortality in this group.

Serum ferritin in association with waiting list mortality

Eight hundred and fourteen patients were listed for LT of whom 589 (72.4%) were transplanted. One hundred and sixty-one patients (19.8%) died awaiting a graft and 64 (7.9%) were delisted. Of those delisted ($n = 64$), 26 (40.6%) patients were delisted because of significant clinical improvement and were included in the favourable outcome group. Thirty-eight (59.4%) patients were delisted because of significant deterioration, HCC progression beyond Milan criteria or recidivism to alcohol or other substances of misuse whilst on the WL. As shown in Table 4, patients with high SF (>300 μgm/l) were significantly older, more likely to be male and more likely to have alcohol-related cirrhosis. Patients with low SF were more likely to have cholestatic and autoimmune liver disease. The high SF group had increased median scores of liver prognostic models (CTP, MELD, UKELD) and lower Na level. This group also had increased WL mortality. This was also true for time points 3-, 6- and 12-month following listing.

Patients with high SF who reached transplantation had significantly reduced waiting time for a liver graft. This is likely to reflect their underlying liver disease severity. Table 5 demonstrates the factors associated with WL mortality on Cox proportional hazard analysis. Age, ferritin level (μg/l), SF > 300 μg/l, sodium level, MELD score, UKELD score and CTP score were associated with WL mortality on univariate analysis. Age, serum sodium, MELD score and SF level or SF > 300 μg/l were examined in a multivariate analysis. To avoid cross interaction of

Table 4. Demographic, clinical and outcome measures according to SF of 767 patients listed for liver transplantation with available SF measurements.

Variables	SF ≤ 300 μg/l ($n = 474$)	SF > 300 μg/l ($n = 293$)	*P*-Value
Demographic			
Age (years)	53 (17–72)	55 (19–72)	0.010
Gender (male, %)	281 (59.3)	237 (80.9)	<0.001
Aetiology			
ALD (%)	125 (26.4)	11 (37.9)	0.001
Viral (%)	122 (25.7)	92 (31.4)	0.089
Cholestatic and autoimmune (%)	139 (29.3)	43 (14.7)	<0.001
HCC (%)	81 (17.1)	52 (17.7)	0.815
Clinical			
Na (mmol/l)	136 (118–145)	133 (116–147)	<0.001
MELD score	13 (6–39)	16 (6–40)	<0.001
UKELD score	55 (44–77)	58 (43–75)	<0.001
CTP score	9 (5–14)	10 (5–14)	<0.001
CTP Class C (%)	171 (36.6)	176 (61.3)	<0.001
WL Mortality			
Time on WL (days)	110 (1–786)	82 (2–673)	0.001
Died/delisted (%)	106 (22.4)	84 (28.7)	0.049
3 Months (%)	46 (9.7)	54 (18.4)	<0.001
6 Months (%)	73 (15.4)	82 (28.0)	<0.001
12 Months (%)	102 (21.5)	98 (33.4)	<0.001

HCC, hepatocellular carcinoma; ALD, alcohol-related liver disease; SF, serum ferritin; CTP, Child–Turcotte–Pugh; MELD, model for end-stage liver disease, UKELD, United Kingdom End-Stage Liver Disease; WL, waiting list.

Table 5. Factors associated with waiting list mortality on univariate and multivariate analysis using Cox proportional hazard analysis.

	Univariate		Multivariate	
Factors	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age (years)	1.025 (1.011–1.038)	<0.001	1.028 (1.014–1.043)	<0.001
Ferritin (μg/l)	1.000 (1.000–1.001)	0.001	1.000 (1.000–1.000)	0.278
Ferritin >300 μg/l	1.654 (1.240–2.208)	0.001	0.862 (0.630–1.179)	0.353
Na (mmol/l)	0.926 (0.901–0.951)	<0.001	0.973 (0.945–1.002)	0.070
MELD score	1.141 (1.114–1.169)	<0.001	1.158 (1.125–1.191)	<0.001
UKELD score	1.129 (1.102–1.158)	<0.001	1.135 (1.105–1.167)	<0.001
CTP score	1.441 (1.338–1.551)	<0.001	1.380 (1.261–1.511)	<0.001

CTP, Child–Turcotte–Pugh; MELD, model for end-stage liver disease, UKELD, United Kingdom End-Stage Liver Disease.

individual components of liver prognostic models, CTP score was tested after removal of MELD and UKELD scores. UKELD was tested after removal of MELD score, CTP score and Na level on multivariate analysis. Age, MELD score, UKELD score and CTP score were independently associated with WL outcome. Neither SF as a continuous variable nor SF > 300 μg/l were independently associated with WL mortality.

Serum ferritin in association with post-transplant outcome

The 1-, 3-, 5-year patient survival post-LT was 88%, 82% and 77%, respectively. Thirty-eight patients (6.5%) required re-transplantation. The 1-, 3-, 5-year graft survival was 85%, 78% and 73%, respectively. As shown in Table 6, the high SF group were older, had larger proportion of patients with ALD and viral aetiology; had increased liver prognostic scores (CTP, MELD, UKELD) and had lower Na levels. Although 6- and 12-month mortality after transplant was significantly higher in the high SF group; long-term patient and graft survival were not significantly different. Figure 1 demonstrates that the high SF group had inferior survival to those in the low SF group, but this was not statistically significant ($P = 0.099$). Similar findings were observed for graft survival (data not shown).

We also examined whether any pretransplant recipient related, donor related or graft related variables were associated with post-LT patient and graft survival. Recipient variables tested included recipient age, gender, aetiology of liver disease, liver prognostic scoring models and ferritin level as continuous and as a categorical variable. Donor and graft factors tested included DRI and individual variables included in the calculation of DRI. We also tested for blood group mismatch, gender mismatch and body mass index (both as a continuous variable and as categorical variable with cut-off value of 30 kg/m^2 (definition of obesity according to WHO classification) [15]. On univariate analysis, recipient age, the presence of HCC and CIT ≥ 10 h were associated with reduced post-LT patient survival. Cut-off value for CIT was chosen according to coordinates of ROC curve characteristics associated with the best Youden index [16]. SF as a continuous variable was not statistically associated with decreased post-LT survival ($P = 0.054$). SF > 300 μg/l, DRI (as a continuous variable) and DRI ≥ 1.7 [17], MELD score, UKELD score and CTP score were not associated with patient survival. On multivariate analysis, we tested recipient age, HCC, CIT ≥ 10 h, and SF as a continuous variable. Age, HCC and CIT ≥ 10 h were independently associated with post-LT patient survival on multivariate analysis (Table 7). With regard to graft

Table 6. Demographic, clinical and outcome measures according to SF of 553 transplanted patients with available SF measurements.

Variables	SF ≤ 300 μg/l (*n* = 352)	SF > 300 μg/l (*n* = 201)	*P*-Value
Demographic			
Age (years)	53 (18–70)	54 (19–72)	0.041
Gender (male)	206 (58.5)	163 (81.1)	0.410
Aetiology			
ALD (%)	83 (23.6)	73 (36.3)	0.001
Viral (%)	57 (25.6)	70 (34.8)	0.021
Cholestatic and autoimmune (%)	113 (32.1)	30 (14.9)	<0.001
HCC (%)	57 (16.2)	39 (19.4)	0.338
Clinical			
Na (mmol/l)	136 (122–145)	134 (116–145)	<0.001
MELD score	13 (6–32)	15 (6–40)	<0.001
UKELD score	54 (45–70)	56 (43–73)	<0.001
CTP score	8 (5–14)	10 (5–14)	<0.001
CTP Class C (%)	101 (29.1)	103 (52.8)	<0.001
Post-Transplant Outcomes			
3 Months mortality (%)	19 (5.4)	15 (7.5)	0.331
6 Months mortality (%)	26 (7.4)	27 (13.4)	0.020
12 Months mortality (%)	37 (10.5)	40 (19.9)	0.002
Overall Patient survival (%)	263 (74.7)	138 (68.7)	0.125
Overall Graft survival (%)	249 (70.7)	132 (65.7)	0.216

HCC, hepatocellular carcinoma; ALD, alcohol-related liver disease; SF, serum ferritin; CTP, Child–Turcotte–Pugh; MELD, model for end-stage liver disease, UKELD, United Kingdom End-Stage Liver Disease.

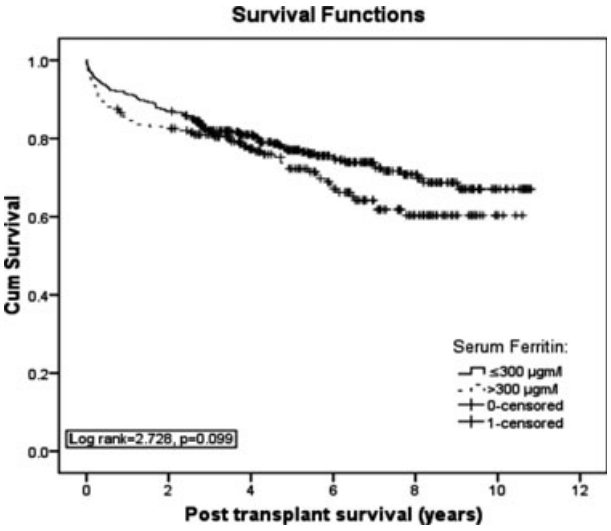


Figure 1 Survival analysis demonstrating post liver transplant LT patient survival according to pre-LT ferritin level on Kaplan–Meier survival analysis (Log rank $\chi^2 = 2.728$, $P = 0.099$).

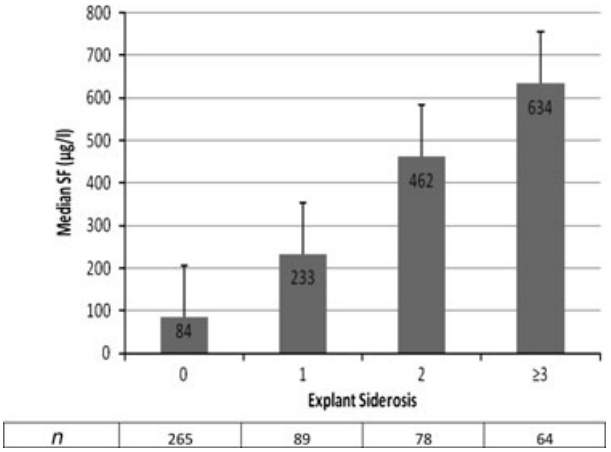


Figure 2 Median serum ferritin SF (µg/l) according to explant siderosis. Kruskal–Wallis = 158.398, $P < 0.001$.

survival, the presence of HCC (OR = 1.634, 95% CI = 1.168–2.287, $P = 0.004$) and CIT ≥ 10 h (OR = 1.627, 95% CI = 1.189–2.226, $P = 0.002$) were associated with increased graft loss on Cox proportional hazard analysis.

Histological data

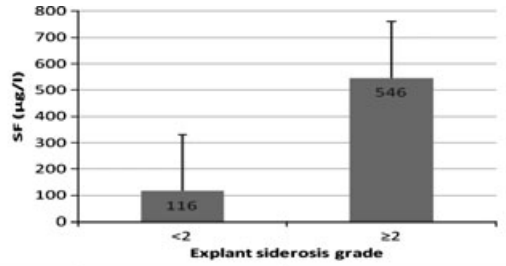
Data on explant siderosis was available on 524/589 (89%) of patients. Explant siderosis grade 0 was seen in 282 (53.8%), grade 1 in 94 (17.9%), grade 2 in 81 (15.5%), grade 3 in 59 (11.3%) and grade 4 in only 8 patients (1.5%). Therefore, we classified our cohort according to explant siderosis grades 0, 1, 2 and ≥3. Figure 2 shows a progressive increase in median SF according to explant siderosis grade ($P < 0.001$). Patients with explant siderosis grade ≥2 had significantly inferior 12-month post-LT survival on Kaplan–Meier survival analysis (Log rank $\chi^2 = 4.717$, $P = 0.030$). Figure 3, demonstrates that patients with explant siderosis grade ≥2, also had significantly increased median SF levels, MELD score, CTP score, UKELD score and significantly lower median Na levels. Explant siderosis grade ≥2, was not associated with long-term patient or graft survival.

A reperfusion liver biopsy was available on 501/589 (85%) of patients. Graft siderosis was evaluated in these as described above. There were 422 (84.2%) grafts with grade 0 siderosis, 68 (13.6%) with grade 1 siderosis, 9 (1.8%) with grade 2 siderosis and 2 (0.4%) with grade 3 siderosis. No grafts had grade 4 siderosis detectable. Therefore, we divided grafts according to siderosis grade into 2 groups: those with no siderosis (grade 0, $n = 422$) and those with siderosis (grade ≥1, $n = 79$). There was no association between graft siderosis ≥1 with 1-year or long-term patient or graft survival on Cox proportional hazard analysis (P = NS).

Table 7. Factors associated with postliver transplant mortality on Cox proportional hazard analysis.

	Univariate		Multivariate	
Factors	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age (years)	1.018 (1.004–1.033)	0.010	1.018 (1.003–1.034)	0.018
HCC	1.853 (1.310–2.622)	<0.001	1.542 (1.044–2.277)	0.030
Ferritin (µg/l)	1.000 (1.000–1.001)	0.054		
Ferritin >300 µg/l	1.311 (0.950–1.811)	0.100		
DRI	1.245 (0.689–2.251)	0.468		
DRI ≥ 1.7	1.258 (0.733–2.159)	0.405		
CIT ≥ 10 h	1.436 (1.036–1.991)	0.030	1.418 (1.022–1.967)	0.036

HCC, hepatocellular carcinoma; ALD, alcohol-related liver disease; SF, serum ferritin; CTP, Child–Turcotte–Pugh; MELD, model for end-stage liver disease, DRI, donor risk index; UKELD, United Kingdom End-Stage Liver Disease.



n	354	142	
Variable	Median		*P*-value
Na	136	135	0.012
CTP	9	10	<0.001
MELD	13	15	<0.001
UKELD	54	56	<0.001
SF	116	546	<0.001

Figure 3 Median serum ferritin and liver prognostic models according to explant siderosis.

Discussion

In this study of 1079 patients assessed for LT in a single centre, SF was associated with 1-year mortality in patients with ESLD and death on the WL but not independent of established liver prognostic models such as CTP score or MELD score. We identified that SF was not associated with post-transplant patient or graft survival. These findings are therefore contrary to recently published reports; but probably reflect the significantly smaller number of patients reported in these studies [6,10].

Serum ferritin is an intracellular protein that stores iron and plays a key role in iron homeostasis. Under steady-state conditions, SF correlates with body iron stores [18]. However, SF can be significantly increased in a number of non-liver-related and liver-related conditions. SF is increased significantly in hereditary iron overload conditions such as haemochromatosis in association with increased iron stores [19]. SF is also an acute phase reactant and can be elevated in infective and in inflammatory states such as rheumatoid arthritis and adult Still's disease [20]. SF may be elevated in patients with chronic kidney disease, diabetes and in the metabolic syndrome [21–23]. Elevated SF has also been reported in haematological malignancies such as acute leukaemia, myelodysplastic syndrome, haemophagocytic lymphohistiocytosis (HLH) and other cancers [24–26]. Furthermore, hyperferritinaemia was also seen in patients with hepatic inflammatory processes such as chronic hepatitis C virus infection, alcohol-related liver disease and non-alcoholic steatohepatitis [27–29] Therefore, it is unlikely that SF in isolation can provide the specificity required for a test to predict important outcome measures such as WL mortality or post-LT survival in patients with ESLD.

Our data suggest an association between SF and WL mortality. However, our high SF group also clearly had significantly elevated median CTP score, MELD score and UKELD score which indicate that the high SF group may have reduced WL survival because of the severity of their underlying liver disease. This was supported by the fact that after we controlled for liver prognostic scores, SF failed to predict WL mortality, whilst all other liver prognostic models remained significantly associated with WL mortality even after controlling for other factors such as age, ferritin and Na level. CTP score, MELD score and UKELD score were validated models for prediction of mortality risk in patients with ESLD and have been extensively used for prediction of mortality on the transplant WL [5,30–33]. Therefore, it is unsurprising that these models retained their capacity to predict mortality on the WL in our cohort.

Although Walker *et al.* tested and externally validated SF as a predictor of WL mortality; the study had a number of limitations worth exploring [6]. Firstly, SF was not tested as continuous variable to indicate the impact of each unit (or 10 units) of increase in ferritin level on WL mortality. Secondly, the test cohort had trichotomous cut-off values of <200, 200–400 and >400, whereas the validation cohort had only a dichotomous cut-off point of 500 µg/l. This may result in limited applicability of their findings on other cohorts of patients. Thirdly, despite these important short falls, the addition of SF to MELD did not result in significant improvement in MELD predictive capacity in relation to WL mortality [6]. Unsurprisingly, our analysis demonstrated that a higher proportion of patients in the high SF group had ALD (38% vs 26%, $P = 0.001$) or viral-related disease (31% vs 26%, $P = 0.089$) as an indication for transplantation which is consistent with published reports [27,28]. Our data also showed that recipient age was an independent factor in determining survival on the WL, consistent with published studies [2,34,35]. For every 1 year increase in recipient age, there was approximately 3% increased risk of death on the WL. Furthermore, patients listed for LT with age >50 years had twice the risk of death on the WL (data not shown, OR = 1.985, 95% CI = 1.441–2.735, $P < 0.001$).

On assessment of post-transplant survival, SF as continuous variable and SF > 300 µg/l were not associated with long-term post-transplant survival in contrary to Weismuller *et al.* [10]. However, we note that in the latter study, the authors did not report on results of SF either as continuous variable or as a categorical variable in their Cox proportional hazard model analysis to determine factors associated with post-LT outcome. The authors purely reported on a sub-group of patients ($n = 33$) with SF ≥ 365 µg/l and transferrin saturation <55% who had significantly increased post-LT mortality. Transferrin saturation data were not available to us (because it is not part of our routine tests for patients assessed for LT) to be able to compare the results.

Furthermore, there is an important inconsistency in reporting SF data in previous studies compared to the current one. Obviously, cut-off points selected are different according to each range of normal values and possibly by other factors. In the study by Walker *et al.*, reference values used for the Australian cohort were 200 and 400 μg/l whilst cut-off point chosen for the US validation cohort was 500 μg/l [6]. In the Weismuller study, the cut-off point was chosen as 365 μg/l according to their laboratory reference range (Germany) [10]. The above cut-off points are different to our local laboratory reference values of 20–300 μg/l (UK). It is obvious from this description that reference values for SF are significantly different between countries and continents. Furthermore, it is not unusual to find different SF reference range between laboratories in the same country. This makes comparison between these studies, generalization and applicability of the published reports of SF as predictor of outcome in patients with ESLD limited. Therefore, it is unlikely that SF will have a role in the assessment of patients for LT or in the modification of current prognostic models such as MELD or UKELD to improve their prognostic capacity.

We found recipient age, HCC and CIT ≥ 10 h were independently associated with post-LT survival, consistent with previous reports [9,10,36,37]. Pre-LT MELD score and UKELD score were not associated with post-LT survival which was also consistent with published literature [5,7]. DRI, or individual components of DRI other than CIT were not associated with post-LT survival which is contrary to the published reports [13,38]. This is may be explained by the fact that the median DRI of our cohort (1.7) was significantly higher than median DRI reported for Feng *et al.* and Schaubel *et al.* data with median DRI reported by MELD group with a range of 1.2–1.5. Furthermore, donor-recipient matching may be influenced by local practices which may also explain the disparity in results.

We studied explants to determine whether pretransplant SF levels correlate with histological iron deposition. SF was associated with explant siderosis in our cohort. The higher the median SF, the higher the siderosis grade in recipient livers (Fig. 3); which is consistent with published literature [39,40]. By limiting post-transplant patient survival analysis to 1 year (data not shown), both SF > 300 μg/l (OR = 1.930, 95% CI = 1.208–3.045, $P = 0.006$) and explants siderosis grade ≥2 (OR = 1.737, 95% CI = 1.048–2.877, $P = 0.032$) were associated with 1-year patient survival. This is consistent with the work of Kowdley *et al.* who reported increased post-transplant mortality in non-haemochromatotic patients with increased hepatic iron deposition [41]. There was a suggestion that donor liver siderosis at reperfusion liver biopsy associated with post-LT hepatic fibrosis and other post-LT adverse events [42]. In this study, there was no association of donor hepatic siderosis with post-LT survival. This disparity may be explained in the fact that we investigated survival in isolation, rather than post-transplant adverse events such as acute rejection or early graft dysfunction.

There are number of strengths of this study. First, this is the largest study to-date to investigate the association of SF with pre- and post-LT outcomes. Second, despite its retrospective design, patient LT assessment data were entered prospectively at the time of LT assessment which limits any inconsistencies. Third, we have included multidimensional variables such as pre-LT variables, donor and graft quality data as well as histological variables to analyze post-LT outcome. Limitations of this study were that it is a single centre experience; therefore, applicability of the findings on other cohorts may be limited. The second limitation is unavailability of full iron profile tests on our patients such as serum iron and transferrin saturation which prevented inclusion of these variables into our analyses.

In conclusion, in this single centre study, we demonstrated an association between pre-LT ferritin level and WL mortality. However, this was dependent on other known variables that reflect severity of liver disease. Our analysis identified no association of pre-LT ferritin with long-term post-LT patient or graft survival. Therefore, we found limited role for ferritin as a prognostic indicator for assessment of pretransplant or post-transplant survival. However, giving the association of SF with outcome demonstrated in this study and by other groups, a large cohort, such as Eurotransplant, prospective study may provide a definitive evidence on the value of SF as an independent predictor of outcome in LT candidates.

Authorship

MABF: data collection, statistical analysis, writing of manuscript. SK: data collection. MRF: concept of study, review of statistical work and manuscript. AQ: review of histological data. AB: review of ferritin data and manuscript, NDH, JGO, KA and JAW: review of manuscript. MAH: concept and study design, final review of manuscript.

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