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Low C-reactive Protein and Urea Distinguish Primary Nonfunction From Early Allograft Dysfunction Within 48 Hours of Liver Transplantation

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Background. Primary nonfunction (PNF) is a life-threatening complication of liver transplantation (LT), but in the early postoperative period, it can be difficult to differentiate from early allograft dysfunction (EAD). The aim of this study was to determine if serum biomarkers can distinguish PNF from EAD in the initial 48 h following LT. **Materials and Methods.** A retrospective study of adult patients that underwent LT between January 2010 and April 2020 was performed. Clinical parameters, absolute values and trends of C-reactive protein (CRP), blood urea, creatinine, liver function tests, platelets, and international normalized ratio in the initial 48 h after LT were compared between the EAD and PNF groups. **Results.** There were 1937 eligible LTs, with PNF and EAD occurring in 38 (2%) and 503 (26%) patients, respectively. A low serum CRP and urea were associated with PNF. CRP was able to differentiate between the PNF and EAD on postoperative day (POD)1 (20 versus 43 mg/L; $P < 0.001$) and POD2 (24 versus 77; $P < 0.001$). The area under the receiver operating characteristic curve (AUROC) of POD2 CRP was 0.770 (95% confidence interval [CI] 0.645-0.895). The urea value on POD2 (5.05 versus 9.0 mmol/L; $P = 0.002$) and trend of POD2:1 ratio (0.71 versus 1.32 mmol/L; $P < 0.001$) were significantly different between the groups. The AUROC of the change in urea from POD1 to 2 was 0.765 (95% CI 0.645-0.885). Aspartate transaminase was significantly different between the groups, with an AUROC of 0.884 (95% CI 0.753-1.00) on POD2. **Discussion.** The biochemical profile immediately following LT can distinguish PNF from EAD; CRP, urea, and aspartate transaminase are more effective than ALT and bilirubin in distinguishing PNF from EAD in the initial postoperative 48 h. Clinicians should consider the values of these markers when making treatment decisions.

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Graft dysfunction is common in the early postoperative period after liver transplantation (LT), as an unavoidable consequence of the preservation-reperfusion injury process.[1,2] The clinical manifestations of this dysfunction are variable depending on the extent of graft impairment and subsequent physiological insult, ranging from minimal systemic instability to life-threatening multiorgan failure.[3] LT recipients are classified into 3 main risk groups, those with immediate graft function (IGF), early allograft dysfunction (EAD), or primary nonfunction (PNF).[4] EAD has been reported to occur in 23%–30% of LT recipients and may require additional organ support until graft function and consequently the recipient's physiology improve.[4-6] A period of EAD, as defined with numerous different criteria, has been associated with poor graft and overall patient survival.[5,7] The commonly accepted definition of PNF is graft failure resulting in patient death or the need for emergency retransplantation within 7 to 14 d,[7] in the absence of another identifiable cause. Even with rescue retransplantation for PNF, the early postoperative mortality is >50%.[8-10] Due to rapid physiologic deterioration,

early identification of PNF is key to facilitate early listing for retransplantation.[1,7-10] Conversely, excluding PNF in favor of EAD may also prevent unnecessary retransplantation when graft recovery is possible. However, distinguishing between PNF and EAD remains a significant clinical challenge.

In an effort to ensure early access to a suitable graft and to prevent death from PNF, transplant authorities have implemented various biochemical criteria that allow urgent relisting within 7 d of transplant.[11,12] The current National Health Service Blood and Transplant[11] and Organ Procurement and Transplantation Network[12] guidelines recommend the use of alanine transaminase (ALT), international normalized ratio (INR), lactatemia, and metabolic acidosis as criteria for PNF and are mandatory to relist for this indication. However, these markers can all be influenced by factors other than primary hepatocellular dysfunction. In addition, previously validated predictive models for early allograft failure cannot be applied within the initial 48 h and therefore are of little value to clinicians trying to make time-critical decisions in the setting of severe early graft dysfunction.[7,13,14]

Further biomarkers that can more reliably indicate graft function in the early postoperative period would be useful. C-reactive protein (CRP) is exclusively produced by the hepatocyte as part of the acute phase response in response to interleukin-6 (IL-6)[15] (Figure 1) and a failure to increase CRP after surgery has been shown to correlate with posthepatectomy liver failure[17] as well as severe EAD after liver transplantation previously.[18] The production of urea is also dependent on hepatocyte function.[19] These markers, including adjusting for renal dysfunction by determining the creatinine:urea ratio, have not been evaluated as markers of PNF. Therefore, the aim of this study was to use a cohort of LT patients to determine the accuracy of CRP and blood urea for differentiating PNF from EAD in the early postoperative period.

MATERIALS AND METHODS

Study Population

The study received institutional approval (CARMS-16399). All adult (≥18 y) recipients of liver transplants (LTs) between January 2010 and December 2020 at the Queen Elizabeth Hospital Birmingham, United Kingdom, were considered for inclusion in this retrospective, observational cohort study. Exclusion criteria included intraoperative death, graft loss, or death in the initial 14 d posttransplant for an established diagnosis other than PNF. Patients were grouped according to whether they demonstrated IGF, EAD, or PNF.

Study Definitions

EAD was defined using the criteria reported by Olthoff et al[5]: AST or ALT >2000 IU/L within the first 7 d, or bilirubin ≥177 μmol/L (10mg/dL) on day 7, or INR ≥1.6. Graft dysfunction resulting in death or retransplant within 14 d, in the absence of a vascular complication and not explained by any other cause, was considered PNF. At our institution, the decision to perform an early retransplant (and therefore be considered PNF) is based on multidisciplinary input from medical, surgical, and critical care specialists taking into account the overall clinical condition of the patient. The remainder of patients that did not meet the predefined criteria for PNF or EAD were considered to have IGF.

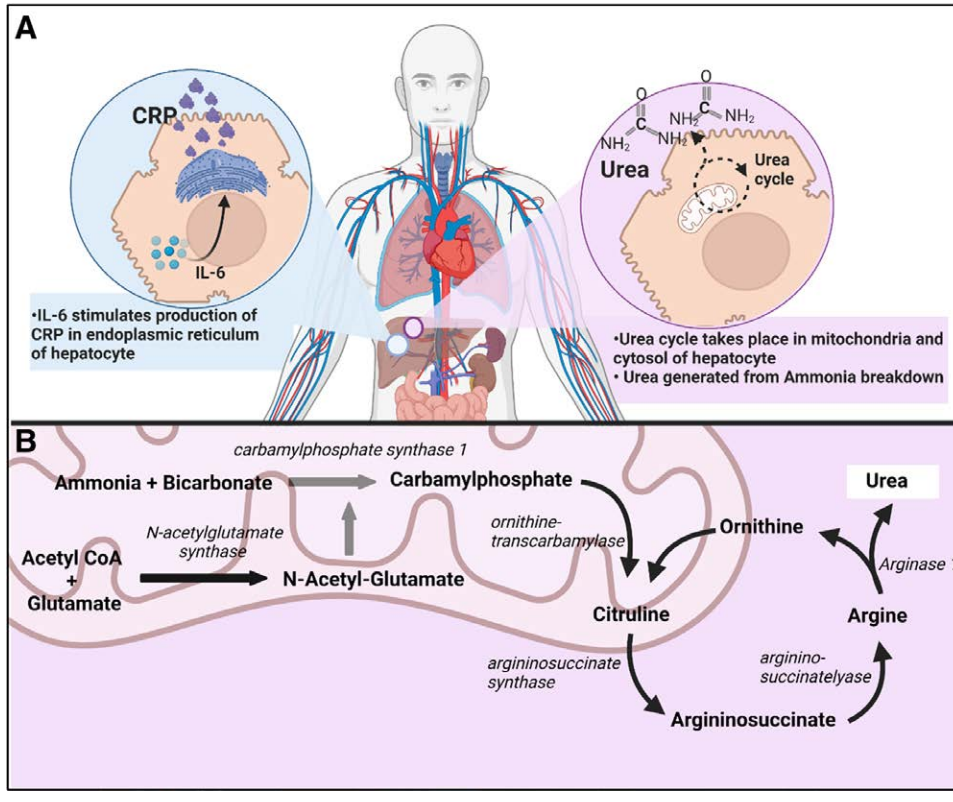


FIGURE 1. Schematic representation of CRP and urea production pathways in the hepatocyte. A, Diagram demonstrating the process of CRP and urea production within the hepatocyte. B, Urea cycle showing metabolites and enzymes (italics) involved in sequential steps. Diagram based on that provided by Bigot et al.[16] CRP, C-reactive protein; IL-6, interleukin-6.

Data Collection

Clinical variables collated included donor and recipient demographics, indication for transplant, recipient United Kingdom Model for End-Stage Liver Disease (UKELD) and Model for End-Stage Liver Disease (MELD), urgency of transplant at listing for each transplant. Absolute values of biochemical test results from postoperative day (POD) 1 and 2 were collected. These included urea, creatinine, platelets, fibrinogen, INR, bilirubin, AST, ALT, and CRP.

Data and Statistical Analysis

Absolute values of biochemical test results from POD1 and 2 were compared between PNF and EAD. The trends of these biochemical variables under study, as evidenced by the ratio of the POD 2:1 result, were also compared. In the situation of multiple results from within 24 h, the POD1 result was considered the first result of the calendar day following the completion of the operation.

The 3 groups (PNF, EAD, and IGF) were first compared with a univariate analysis, utilizing the Kruskall–Wallis test used to analyze trends in continuous variables and Chi-squared test for categorical variables. The PNF and EAD groups were then compared using an independent samples t-tests, and categorical variables were compared using the Fisher exact test and Chi-squared tests. Two sided tests of significance were utilized, and a P value ≤0.05 was considered statistically significant. With the number of events and significant variables, a multivariate analysis was not performed as it would have resulted in overfitting of the model due to a low events per variable ratio.[20]

Biochemical factors found to be significantly different between the PNF and EAD groups on univariate analysis were further investigated using receiver operating characteristic (ROC) curves. The area under the ROC curve (AUROC) value was reported, and the Youden index was used to identify the optimum cutoff values and the sensitivity and specificity of this value. The biomarkers with an AUROC >0.750 for their absolute values were then subject to a binary logistic regression model which displays, in graphical form, how the rate of PNF varies with the level of the marker. The lines on these graphs were produced with the entire cohort of the study and the quartiles displayed. This allows assessment visualization of the goodness of fit. Statistical analyses were performed using SPSS (Version 25.0. IBM Corp, Armonk, NY).

RESULTS

During the study period, 2102 LTs were performed at our institution. Of these, 503 (23.9%) and 38 (1.9%) patients developed EAD and PNF, respectively, according to definitions described in the methods (Figure 2). There were 165 patients excluded from the study because they experienced graft failure due to an alternative cause, such as hepatic artery thrombosis (Figure 2). Demographic and graft details for patients with PNF, EAD, and IGF are presented and compared in Table 1. The 3 groups differed significantly in age and donor type, with donors after cardiac death (DCD) graft significantly more prevalent among the PNF cohort (45%) (Table 1). As expected, there were multiple differences of

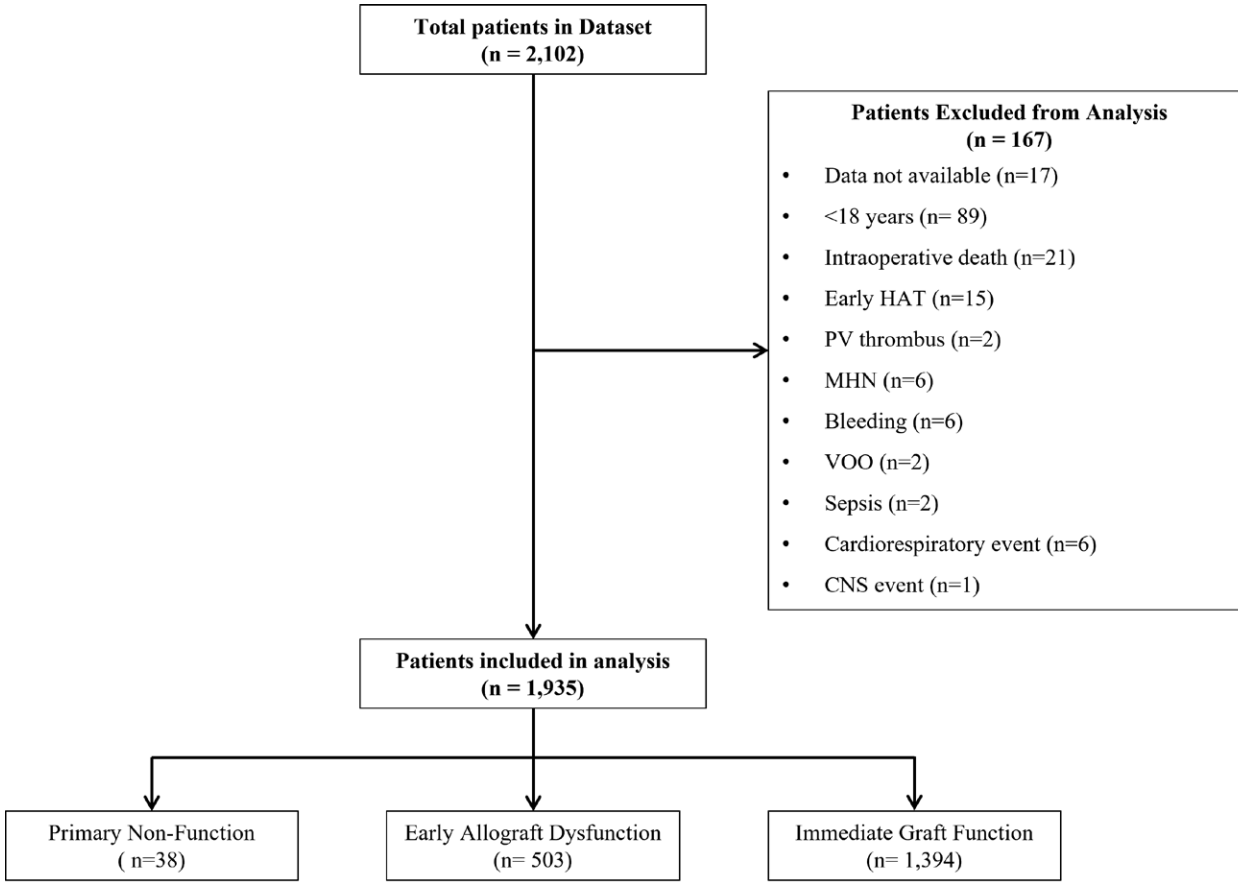


FIGURE 2. Patients included and excluded in the study. CNS, central nervous system; HAT, hepatic artery thrombosis; MHN, massive hemorrhagic necrosis ; PV, portal vein; VOO, venous outlet obstruction.

TABLE 1.

Comparison of demographic and graft details for patients with PNF, EAD, and IGF

		PNF (n = 38)	EAD[a] (n = 503)	IGF (n = 1394)	*P*
Demographics					
	Recipient age	55 (42–60)	52 (40–60)	54 (44–61)	**0.002**
	Male	23 (61)	305 (61)	843 (61)	0.999
Transplant details					
Transplant indication					0.848
	ALD	13 (34)	110 (22)	351 (25)	
	Viral hepatitis	8 (21)	73 (15)	261 (14)	
	PBC	1 (3)	55 (11)	151 (11)	
	PSC	5 (13)	72 (14)	179 (13)	
	AIH	1 (3)	19 (4)	44 (3)	
	NASH	2 (5)	44 (9)	138 (10)	
	HCC	1 (3)	9 (2)	39 (3)	
	Drug induced	1 (3)	16 (3)	45 (3)	
	Seronegative hepatitis	3 (8)	29 (6)	49 (4)	
	Cryptogenic cirrhosis	0 (0)	13 (3)	38 (3)	
	Hemochromatosis	1 (3)	3 (1)	15 (1)	
	Wilsons	0 (0)	2 (1)	9 (1)	
	Biliary atresia	0 (0)	4 (1)	7 (1)	
	A1AD	0 (0)	4 (1)	12 (1)	
	Noncirrhotic portal hypertension	0 (0)	8 (2)	20 (1)	
	Polycystic liver	1 (3)	13 (3)	40 (3)	
	Other genetic	0 (0)	2 (1)	12 (1)	
	Other biliary	1 (3)	3 (1)	12 (1)	
	Other tumor	0 (0)	3 (1)	4 (1)	
	Budd Chiari	0 (0)	5 (1)	4 (1)	
	Liver failure unknown cause	0 (0)	6 (1)	16 (1)	
	Other	0 (0)	10 (2)	23 (2)	
Donor type					**<0.001**
	DBD	20 (53)	354 (70)	1089 (79)	
	DCD	17 (45)	144 (29)	282 (20)	
	Living	0 (0)	3 (1)	8 (1)	
	Domino	1 (3)	2 (1)	9 (1)	
Graft number					0.1
	First	34 (90)	441 (88)	1278 (92)	
	Second	3 (8)	51 (10)	95 (7)	
	Third	1 (3)	11 (2)	14 (1)	
	Fourth	0 (0)	0 (0)	1 (1)	
Graft type					0.301
	Whole	37 (97)	472 (94)	1282 (92)	
	Split	1 (3)	31 (6)	106 (8)	
Urgency					0.372
	Super-urgent	6 (16)	53 (11)	128 (9)	
	Routine	32 (85)	444 (90)	1246 (91)	
UKELD at listing		52 (49–56)	54 (49–58)	54 (50–58)	0.164
MELD at listing		13 (9–18)	14 (10–18)	15 (11–19)	0.346

Bold values indicate significant at the $P < 0.05$ level.
[a]EAD defined as per Olthoff et al.[5]
A1AD, alpha-1 antitrypsin deficiency; AIH, autoimmune hepatitis; ALD, alcoholic liver disease; DBD, donor after brain death; DCD, donor after cardiac death; EAD, early allograft dysfunction; HCC, hepatocellular carcinoma; IGF, immediate graft function; MELD, Model for End-stage Liver Disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; PNF, primary nonfunction; PSC, primary sclerosing cholangitis; UKELD, United Kingdom Model for End-Stage Liver Disease.

TABLE 2.

Comparison of biochemical markers of patients with PNF, EAD, and IGF

	PNF (n = 38)	EAD[a] (n = 503)	IGF (n = 1394)	*P*
CRP (mg/L)				
POD 1	20 (10–40)	43 (21–71)	53 (28–80)	**<0.001**
POD 2	24 (7–72)	77 (53–112)	77 (53–103)	**<0.001**
POD 2:1	1.44 (0.94–1.85)	1.75 (1.18–2.84)	1.44 (1.02–2.74)	**<0.001**
Urea (mmol/L)				
POD 1	6.75 (5.35–8.28)	6.7 (5.0–9.2)	6.8 (5.1–9.1)	0.753
POD 2	5.05 (2.55–10.60)	9.0 (6.3–12.7)	9.4 (6.5–12.6)	**<0.001**
POD 2:1	0.71 (0.49–1.14)	1.32 (1.04–1.62)	1.33 (1.09–1.64)	**0.004**
Creatinine (μmol/L)				
POD 1	122 (103–143)	95 (73–138)	85 (64–113)	**<0.001**
POD 2	104 (91–156)	113 (76–175)	96 (65–141)	**<0.001**
POD 2:1	1.01 (0.84–1.24)	1.12 (0.89–1.39)	1.08 (0.89–1.37)	0.141
Bilirubin (μmol/L)				
POD 1	80 (64–112)	84 (47–128)	64 (38–99)	**<0.001**
POD 2	110 (73–153)	83 (48–137)	46 (25–80)	**<0.001**
POD 2:1	1.22 (0.89–1.51)	0.99 (0.76–1.25)	0.75 (0.56–1.00)	**<0.001**
ALT (IU/L)				
POD 1	1569 (991–2631)	1497 (712–2400)	633 (372–1002)	**<0.001**
POD 2	1676 (958–3049)	1230 (652–1955)	501 (294–789)	**<0.001**
POD 2:1	0.84 (0.72–1.38)	0.78 (0.64–1.05)	0.77 (0.65–0.91)	**0.035**
AST (IU/L)				
POD 1	5186 (3379–8089)	2510 (1433–3884)	677 (410–1108)	**<0.001**
POD 2	4692 (2479–6655)	1087 (645–1915)	378 (220–632)	**<0.001**
POD 2:1	0.79 (0.55–1.10)	0.41 (0.29–0.61)	0.52 (0.39–0.72)	**<0.001**
INR				
POD 1	2.2 (1.8–2.68)	1.7 (1.5–2.1)	1.6 (1.4–1.8)	**<0.001**
POD 2	2.3 (1.9–4.3)	1.5 (1.3–2.0)	1.3 (1.2–1.6)	**<0.001**
POD 2:1	1.08 (0.88–1.53)	0.9 (0.79–1.0)	0.85 (0.76–0.93)	**<0.001**
Fibrinogen (g/L)[b]				
POD 1	1.01 (0.65–1.28)	1.50 (1.00–1.80)	1.6 (1.3–2.0)	**<0.001**
POD 2	0.75 (0.63–1.15)	1.75 (1.23–2.40)	2.2 (1.3–2.6)	**<0.001**
Platelets ($\times 10^9$)				
POD 1	83 (69–119)	87 (59–122)	84 (60–119)	0.857
POD 2	47 (27–68)	59 (42–90)	60 (42–86)	0.062
POD 2:1	0.62 (0.47–0.68)	0.73 (0.58–0.92)	0.75 (0.59–0.92)	**0.016**

Bold values indicate significant at the $P < 0.05$ level.
[a]EAD defined as per Olthoff et al.[5]
[b]Fibrinogen values were not available for the whole cohort.
Comparison of routinely measured biochemical investigations for patients that experienced PNF, EAD, and IGF. Values given as median (IQR). Mann–Whitney test used to derive *P* values.
ALT, alanine aminotransferase; AST, aspartate transaminase; CRP, C-reactive protein; EAD, early allograft dysfunction; IGF, immediate graft function; INR, international normalized ratio; PNF, primary nonfunction; POD, postoperative day.

statistical significance between the biochemical markers in the 3 groups over the first 2 PODs (Table 2). In the PNF group, 24 of 38 (63%) patients required retransplantation or died before the end of POD3.

On direct comparison of demographic and graft details for the PNF and EAD groups, the only significant difference in demographics and transplant details was the donor type, with a significantly greater proportion of DCD donors in the PNF group (Table 3). Biochemical markers were then analyzed to investigate their ability to distinguish between PNF and EAD in the early postoperative period (Tables 4 and 5). In terms of

TABLE 3.

Comparison of demographic and graft details for patients with PNF and EAD

		PNF (n = 38)	EAD[a] (n = 503)	P
Demographics				
Recipient age		55 (42–60)	52 (40–60)	0.575
Male		23 (61)	305 (61)	0.999
Transplant details				
Transplant indication				0.818
	ALD	13 (34)	110 (22)	
	Viral hepatitis	8 (21)	73 (15)	
	PBC	1 (3)	55 (11)	
	PSC	5 (13)	72 (14)	
	AIH	1 (3)	19 (4)	
	NASH	2 (5)	44 (9)	
	HCC	1 (3)	9 (2)	
	Drug induced	1 (3)	16 (3)	
	Seronegative hepatitis	3 (8)	29 (6)	
	Cryptogenic cirrhosis	0 (0)	13 (3)	
	Hemochromatosis	1 (3)	3 (1)	
	Wilsons	0 (0)	2 (1)	
	Biliary atresia	0 (0)	4 (1)	
	A1AD	0 (0)	4 (1)	
	Noncirrhotic portal hypertension	0 (0)	8 (2)	
	Polycystic liver	1 (3)	13 (3)	
	Other genetic	0 (0)	2 (1)	
	Other biliary	1 (3)	3 (1)	
	Other tumor	0 (0)	3 (1)	
	Budd Chiari	0 (0)	5 (1)	
	Liver failure unknown cause	0 (0)	6 (1)	
	Other	0 (0)	10 (2)	
Donor type				**0.044**
	DBD	20 (53)	354 (70)	
	DCD	17 (45)	144 (29)	
	Living	0 (0)	3 (1)	
	Domino	1 (3)	2 (1)	
Graft number				0.895
	First	34 (90)	441 (88)	
	Second	3 (8)	51 (10)	
	Third	1 (3)	11 (2)	
	Fourth	0 (0)	0 (0)	
Graft type				
	Whole	37 (97)	472 (94)	0.717
	Split	1 (3)	31 (6)	
Urgency				0.334
	Super-urgent	6 (16)	53 (11)	
	Urgent	32 (85)	444 (90)	
UKELD at Listing		52 (49–56)	54 (49–58)	0.355
MELD at Listing		13 (9–18)	14 (10–18)	0.546

Bold values indicate significant at the $P < 0.05$ level.
[a]EAD defined as per Olthoff et al.[5]
A1AD, alpha-1 antitrypsin deficiency; AIH, autoimmune hepatitis; ALD, alcoholic liver disease; DBD, donor after brain death; DCD, donor after cardiac death; EAD, early allograft dysfunction; HCC, hepatocellular carcinoma; IGF, immediate graft function; MELD, Model for End-stage Liver Disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; PNF, primary nonfunction; PSC, primary sclerosing cholangitis; UKELD, United Kingdom Model for End-Stage Liver Disease.

TABLE 4.

Comparison of biochemical markers for patients with PNF and EAD

		PNF (n = 38)	EAD[a] (n = 503)	P
Biochemistry				
CRP (mg/L)				
	POD 1	20 (10–40)	43 (21–71)	**<0.001**
	POD 2	24 (7–72)	77 (53–112)	**<0.001**
	Day 2:1	1.44 (0.94–1.85)	1.75 (1.18–2.84)	**0.013**
Urea (mmol/L)				
	POD 1	6.75 (5.35–8.28)	6.7 (5.0–9.2)	0.459
	POD 2	5.05 (2.55–10.60)	9.0 (6.3–12.7)	**0.002**
	POD 2:1	0.71 (0.49–1.14)	1.32 (1.04–1.62)	**<0.001**
Creatinine (μmol/L)				
	POD 1	122 (103–143)	95 (73–138)	**0.008**
	POD 2	104 (91–156)	113 (76–175)	0.886
	POD 2:1	1.01 (0.84–1.24)	1.12 (0.89–1.39)	0.101
Creatinine:urea ratio				
	Day 1	15.0 (12.9–20.1)	14.4 (11.7–19.1)	0.261
	Day 2	22.9 (11.3–40.7)	12.5 (9.6–17.7)	**<0.001**
	Day 2:1	1.38 (0.98–1.89)	0.87 (0.72–1.09)	**<0.001**
Bilirubin (μmol/L)				
	POD 1	80 (64–112)	84 (47–128)	0.867
	POD 2	110 (73–153)	83 (48–137)	**0.049**
	POD 2:1	1.22 (0.89–1.51)	0.99 (0.76–1.25)	0.051
ALT (IU/L)				
	POD 1	1569 (991–2631)	1497 (712–2400)	0.147
	POD 2	1676 (958–3049)	1230 (652–1955)	**0.033**
	POD 2:1	0.84 (0.72–1.38)	0.78 (0.64–1.05)	0.15
AST (IU/L)				
	POD 1	5186 (3379–8089)	2510 (1433–3884)	**<0.001**
	POD 2	4692 (2479–6655)	1087 (645–1915)	**<0.001**
	POD 2:1	0.79 (0.55–1.10)	0.41 (0.29–0.61)	**0.001**
INR				
	POD 1	2.2 (1.8–2.68)	1.7 (1.5–2.1)	**<0.001**
	POD 2	2.3 (1.9–4.3)	1.5 (1.3–2.0)	**<0.001**
	POD 2:1	1.08 (0.88–1.53)	0.9 (0.79–1.0)	**0.002**
Fibrinogen (g/L)[b]				
	POD 1	1.01 (0.65–1.28)	1.50 (1.00–1.80)	**0.002**
	POD 2	0.75 (0.63–1.15)	1.75 (1.23–2.40)	**0.002**
Platelets ($\times 10^9$)				
	POD 1	83 (69–119)	87 (59–122)	0.791
	POD 2	47 (27–68)	59 (42–90)	**0.022**
	POD 2:1	0.62 (0.47–0.68)	0.73 (0.58–0.92)	**0.018**

Bold values indicate significant at the $P < 0.05$ level.
[a]EAD defined as per Olthoff et al.[5]
[b]Fibrinogen values were not available for the whole cohort.
Comparison of routinely measured biochemical investigations for patients that experienced PNF and EAD. Values given as median (IQR). Mann–Whitney test used to derive *P* values.
ALT, alanine aminotransferase; AST, aspartate transaminase; CRP, C-reactive protein; EAD, early allograft dysfunction; IGF, immediate graft function; INR, international normalized ratio; PNF, primary nonfunction; POD, postoperative day.

the more traditionally used biomarkers, ALT was only able to differentiate between the 2 groups on POD2 where it was significantly higher in the PNF group (1676 versus 1230 IU/L; $P = 0.033$) (Table 4). INR was significantly higher in PNF versus EAD on POD1 (2.2 versus 1.7; $P < 0.001$) and 2 (2.3 versus 1.5; $P < 0.001$), as well as demonstrating a worsening trend (POD1:POD2; 1.08 versus 0.90; $P = 0.002$) (Table 4). AST was also significantly different between the 2 groups, with an area under the receiver operating characteristic curve (AUROC) of 0.884 (95% confidence interval [CI] 0.753-1.00) on POD2 (Table 5).

CRP was significantly lower in the PNF compared with the EAD group on POD 1 (20 versus 43 mg/L; $P < 0.001$) and 2 (24 versus 77 mg/L; $P < 0.001$), as well as showing a significance difference in the trend from POD1 to POD2

TABLE 5.

Biochemical characteristics/serum markers and their ability to distinguish PNF from EAD after liver transplantation

	AUROC (95% CI)	Cutoff value	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Biochemistry						
CRP (mg/L)						
Day 1	0.696 (0.578-0.815)	26.5	62.50	68.60	13.1	96.1
Day 2	0.770 (0.645-0.895)	58.5	72	69.50	14.9	97.0
Day 2:1	0.650 (0.552-0.748)	1.52	58.30	56.90	9.1	94.7
Urea (mmol/L)						
Day 1	NS	NS	NS	NS		
Day 2	0.670 (0.536-0.803)	8.15	60.00	58.20	9.8	95.1
Day 2:1	0.765 (0.645-0.885)	1.05	75	74.40	18.0	97.7
Creatinine (μmol/L)						
Day 1	0.626 (0.555-0.697)	115.5	62.50	66.50	14.2	96
Day 2	NS	NS	NS	NS		
Day 2:1	NS	NS	NS	NS		
Creatinine:urea ratio						
Day 1		NS	NS	NS		
Day 2	0.707 (0.576-0.838)	17.03	66.7	72.4	15.2	96.5
Day 2:1	0.786 (0.686-0.886)	1.05	66.7	71.7	14.8	96.5
Bilirubin (μmol/L)						
Day 1	NS	NS	NS	NS		
Day 2	0.617 (0.518-0.716)	1.09	60.00	57.80	9.8	95.1
Day 2:1	NS	NS	NS	NS		
ALT (IU/L)						
Day 1	NS	NS	NS	NS		
Day 2	0.641 (0.507-0.775)	1531	65	61.50	11.4	96
Day 2:1	NS	NS	NS	NS		
AST (IU/L)						
Day 1	0.767 (0.628-0.905)	3319	78.30	68.80	15.0	97.2
Day 2	0.884 (0.753-1.00)	2409	81.30	81.40	24.8	98.3
Day 2:1	0.760 (0.623-0.897)	0.54	71.40	71.00	15.6	97.0
INR						
Day 1	0.719 (0.610-0.828)	1.85	68.40	60.80	11.6	96.2
Day 2	0.764 (0.649-0.879)	1.85	73.90	69.80	15.5	97.2
Day 2:1	0.691 (0.571-0.811)	0.98	60.90	64.40	11.3	95.6
Platelets ($\times 10^9$)						
Day 1	NS	NS	NS	NS		
Day 2	0.636 (0.518-0.754)	48.5	60	65.70	11.7	95.7
Day 2:1	0.640 (0.516-0.764)	0.64	60	64.20	11.3	95.6

Youden index used to identify optimum cutoff values. Not significant at the $P < 0.05$ level.
ALT, alanine aminotransferase; AST, aspartate transaminase; AUC, area under the receiver operating characteristic curve; CRP, C-reactive protein; EAD, early allograft dysfunction; IGF, immediate graft function; INR, international normalized ratio; NPV, negative predictive value; PNF, primary nonfunction; POD, postoperative day; PPV, positive predictive value.

(1.44 versus 1.75 mg/L; $P = 0.013$) (Table 4). The AUROC of the POD 2 CRP has a higher sensitivity value of 0.770 (95% CI 0.645-0.895) (Table 5; Figure 2A). Although urea was not able to significantly differentiate between the 2 groups on POD 1, the urea was significantly lower in the PNF group on POD2 (5.05 versus 9.0 mmol/L; $P = 0.002$) and had a worse trend from POD1 to POD2 ratio (0.71 versus 1.32; $P < 0.001$). The AUROC of the POD2:1 change in urea was 0.765 (95% CI 0.645-0.885) (Table 5; Figure 2A). To summarize these findings, there was a failure of urea and CRP to increment in the serum (and therefore a failure of production) in the PNF group compared with the EAD group.

The ability of other commonly measured biochemical markers to distinguish PNF from EAD are shown in Tables 4 and 5, as well as visualized in Figure 3A and B. The threshold that provided the optimal sensitivity and specificity of each biomarker, along with its positive and negative predictive value are demonstrated in Table 5. The relationship between biochemical marker levels and the incidence of PNF are shown in Figure 4 for the absolute values of tests that had an AUROC of >0.75.

The importance of these early biomarker trends is shown in Figure 5, which visualizes the timing of graft failure due to PNF in our cohort. This clearly shows that more than half of the grafts that demonstrate PNF do not survive long enough to have existing scoring systems, such as the Model for Early Allograft Failure, Liver Graft Assessment Following Transplantation (L-GrAFT$_7$), and the Early Allograft Failure Simplified Estimation (EASE), applied in a manner that is of clinical benefit.

DISCUSSION

This was a retrospective study with the primary aim of exploring whether routinely collected biochemical markers that are dependent upon hepatocyte function (namely, CRP and urea) can distinguish PNF from EAD within the first 48 h after transplantation. The key findings were that this hypothesis was supported, and furthermore, appears more useful that existing criteria. A number of serum biomarkers including low CRP, low urea, fibrinogen, AST, and INR differ significantly between those with PNF, and those experiencing EAD, although the latter biochemical tests are frequently reported and implicated in categorizing the graft function. The high negative predictive value of POD2 AST, CRP, and Urea 2:1 at the provided thresholds allows PNF to be excluded with a high degree of accuracy (97%–100%). Several markers within existing criteria are influenced by factors other than graft function and are less predictive in the early postoperative phase. The early identification of adverse clinical sequalae of a liver graft following LT is crucial for optimal management. However, a single and ideal test that accurately distinguishes between a graft destined for early failure and one that will recover following a period of organ support does not currently exist. This is highly relevant as the proportion of marginal donors and grafts being transplanted is increasing.

Previous authors have developed multivariable prognostic formulas to estimate survival, complications, and the likelihood of PNF using large transplant databases.[7,13,14] However, these formulas require variables obtained from POD 3 (Model for Early Allograft Failure), POD 7 (L-Gr-AFT), and POD 10 (EASE). This prevents the early application of these complex models in clinical situations where patient deterioration is seen within 24–48 h of graft reperfusion. As demonstrated in Figure 5, more than half of the grafts that demonstrate PNF do not survive long enough to have these scores applied in a manner that is of clinical benefit. More sophisticated investigations like functional graft assessment via serum clearance of indocyanine green and LiMAx have been investigated but have failed to provide a meaningful clinical benefit.[21-23] We hypothesize that incorporating CRP and blood urea in the decision-making algorithm may enhance early prediction of PNF.

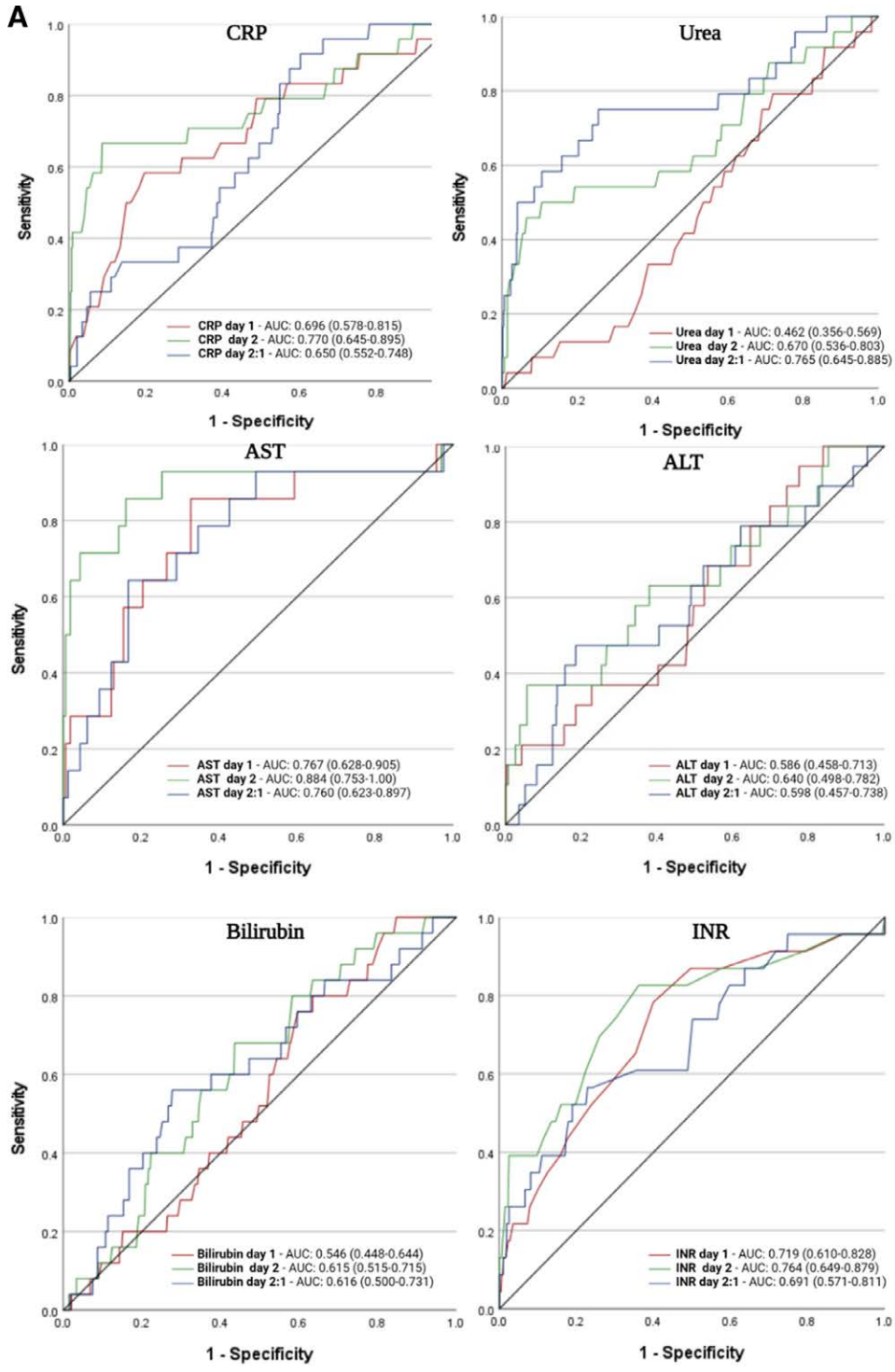


FIGURE 3. ROC curves demonstrating the ability of different biomarkers to predict PNF, grouped according to biomarker. A, ROC curves for predicting PNF from commonly measured biochemical markers. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; INR, international normalized ratio; PNF, primary nonfunction; ROC, receiver operating characteristic.

The liver has a key role in the production of numerous serum proteins commonly measured in the postoperative period, such as CRP and fibrinogen.[24] CRP is produced in hepatocytes under stimulation of IL-6 and therefore from a mechanistic perspective could serve as a marker of graft function. Following liver resection, low CRP levels on POD1 are an independent predictor of posthepatectomy liver failure, which indicates its value as a marker of synthetic function.[17] The findings of this study confirm that in grafts that ultimately fail to function, the absolute value and trajectory of CRP produced is significantly lower on both POD1 and 2. Measurement of both IL-6 and CRP may be of benefit in future studies, as a low CRP in the setting of a raised IL-6 may provide further evidence to support this theory.

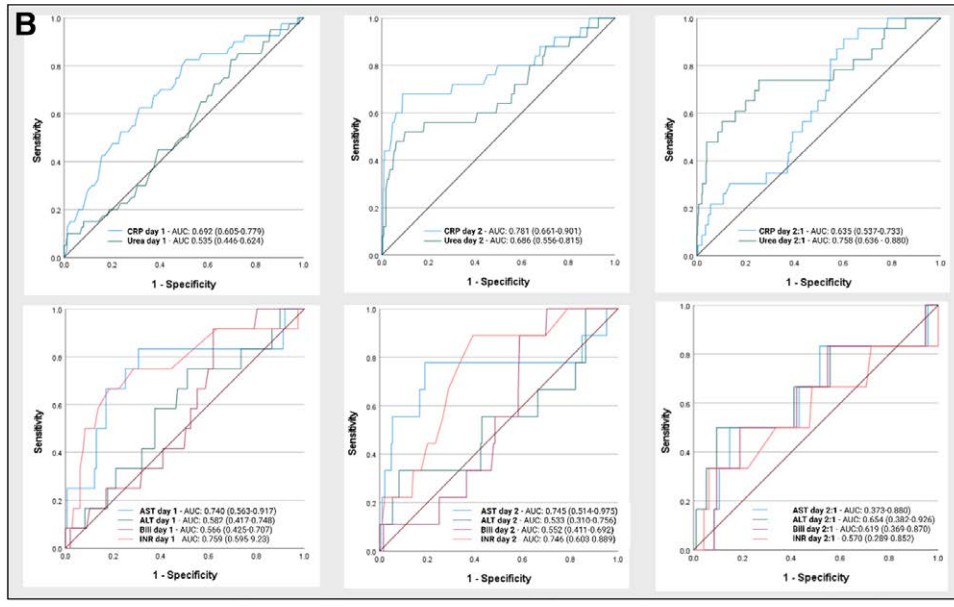
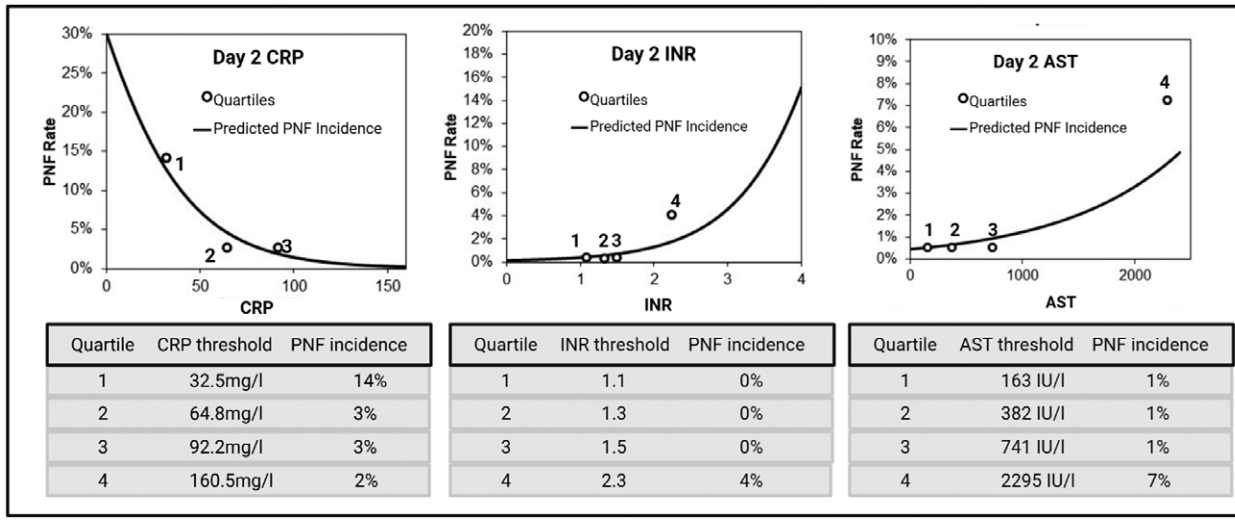


FIGURE 3. Continued. B, ROC curves separated by postoperative day for the commonly measured biochemical markers.



Quartile	CRP threshold	PNF incidence
1	32.5mg/l	14%
2	64.8mg/l	3%
3	92.2mg/l	3%
4	160.5mg/l	2%

Quartile	INR threshold	PNF incidence
1	1.1	0%
2	1.3	0%
3	1.5	0%
4	2.3	4%

Quartile	AST threshold	PNF incidence
1	163 IU/l	1%
2	382 IU/l	1%
3	741 IU/l	1%
4	2295 IU/l	7%

FIGURE 4. Plots demonstrating the relationship between biomarker levels and PNF rate. Binary logistic regression model graphs for the markers (absolute values only) that had an area under the receiver operating characteristic curve of ≥0.75. The solid line on the graph demonstrates the PNF incidence (y-axis) that can be expected at the given value demonstrated on the x-axis. AST, aspartate transaminase; CRP, C-reactive protein; INR, international normalized ratio; PNF, primary nonfunction.

Urea is produced through the multiple enzymatic reactions that comprise the urea cycle, which occurs exclusively within periportal hepatocytes.[25] This waste product primarily undergoes renal excretion and therefore is mostly used a marker of renal function, rather than hepatic synthetic function. The findings of this study indicate that urea is a useful serum marker for differentiating grafts with PNF from those with EAD. The liver is essential for the deamination of ammonia, and consequentially, generating urea.[19] The hepatocyte has the ability to substantially increase urea production per gram of liver parenchyma remaining following hepatic resection, therefore demonstrating that some physiological reserve exists. The finding that serum urea levels fail to increase in the setting of PNF may suggest that this compensation mechanism is exhausted. However, it must be acknowledged that the use of intra- and postoperative hemofiltration may affect urea levels. A weakness of the present study is that we were unable to control for this intervention retrospectively.

The hepatocellular enzymes, ALT and AST, are utilized commonly in the assessment of graft function but are better considered to be markers of hepatocyte injury rather than synthetic function.[26] In this study, AST, rather than ALT, was shown to be significantly elevated in PNF patients compared to EAD patients. Similar to CRP, AST is not subject to other postoperative factors such as renal replacement therapy or the transfusion of blood products like urea and INR, respectively. However, although a useful marker of hepatocellular damage, AST values may be misleading in some cases, as they may be disproportionately elevated in certain grafts, for example, those in DCD donors, which may proceed to function well. Furthermore, AST is found in the mitochondria and cytoplasm of numerous other cell types (heart, skeletal muscle,

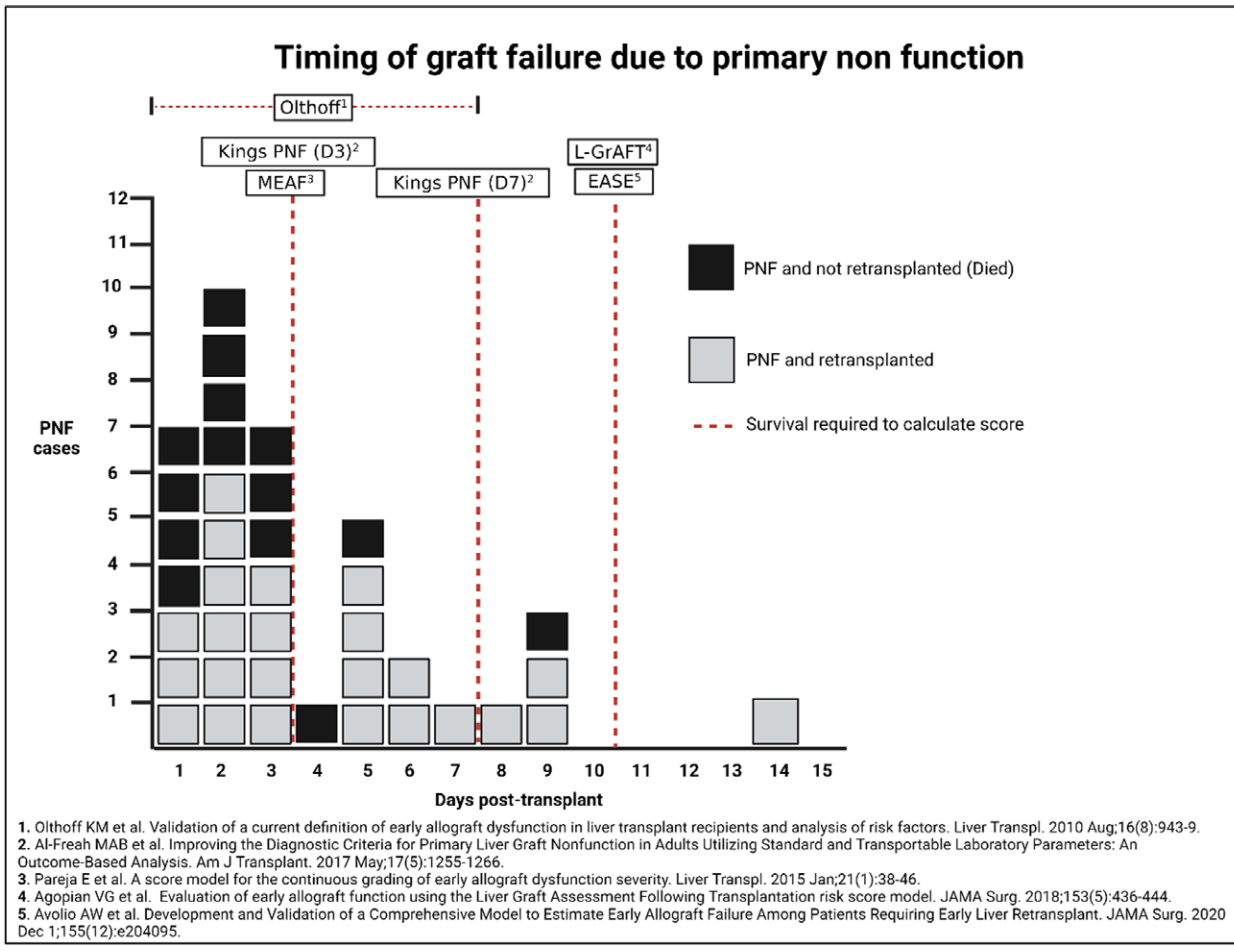


FIGURE 5. Timing of graft failure due to PNF and its relationship to existing scoring systems. Bar graph demonstrating the time point of either retransplant or death due to PNF following liver transplantation. The dashed lines demonstrate published early graft function prediction models. EASE, Early Allograft Failure Simplified Estimation; L-GrAFT, Liver Graft Assessment Following Transplantation; MEAF, Model for Early Allograft Failure; PNF, primary nonfunction.

kidney) throughout the body, and therefore, serum elevations are not always attributable to the liver.[26] Given that a multivariate analysis was not possible in this study, due to the low number of PNF patients, it was not possible to investigate whether the elevated AST values in this group may be related to the increased proportion of DCD grafts in the PNF group. Our proposal is that during the early postoperative period AST should be considered for quantifying the degree of hepatocellular damage, whereas CRP and blood urea levels should be considered for quantifying liver graft synthetic function. A combination of these, alongside the physiological status of the patient, may help differentiating between PNF and EAD and direct clinicians in appropriate decision-making such as early relisting for repeat transplantation.

The main limitations of this study are that it is retrospective in nature, from a single center, and there was a low incidence of PNF in the cohort. To further validate these findings, a prospective multicenter study is required. Furthermore, we have divided PNF and EAD into distinct categories based on the best definitions currently available for the purposes of analyzing the potential of different biomarkers. However, it is important to emphasize that PNF and EAD are not distinct and in fact represent points on a spectrum of graft dysfunction. This further emphasizes the need for clearer consensus definitions to all further, more robust, studies. In addition, there are further variables that warrant further study in the future. For example, we have not analyzed the impact of sarcopenia, given that urea is a product of muscle protein catabolism, or malnutrition. Even though lactate is incorporated in the definition of PNF, we opted not to analyze this variable at POD1 and 2, due to multiple variables that impact on serum lactate in this setting, for example, hemofiltration, fluid status, other organ ischemia, and its fluctuation in the early postoperative period. In this study, we also excluded other causes of early allograft failure such as early hepatic artery thrombosis and MHN, which will also affect the early postoperative trajectory and biomarker profile, to make the PNF group as homogenous as possible. The use of machine perfusion has increased substantially over the past few years in liver transplantation. We have not detailed the effect of this on PNF and EAD rates in this study, but previous reports from our institution have detailed this, showing no cases of PNF and a slightly increased rate of EAD, likely due to the use of more marginal grafts in higher-risk recipients.[27]

In conclusion, early postoperative CRP and urea may be more informative than more traditionally used biomarkers such as ALT and bilirubin for distinguishing between PNF and EAD in the initial 48 postoperative h and LT. AST may also be useful, but during the study period, it was not measured routinely, so was available for less than half of the patients.

Distinguishing PNF from severe EAD remains a clinical decision, which will always encompass several factors. The high negative predictive value of CRP and urea at the thresholds reported demonstrate that these markers can be utilized to assist clinical decision-making. These markers are inexpensive and routinely measured. A CRP < 20 mg/L on POD1 should be considered a strong predictor of subsequent PNF. This study should be repeated with prospective, multicenter datasets to further validate this conclusion.

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