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Neutrophil-to-lymphocyte ratio predicts mortality in patients listed for liver transplantation

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Joanna A Leithead; designed research study, performed research study, collected data, analysed data, wrote paper.

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Bridget K Gunson; collected data.

James W Ferguson; wrote paper.

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Key words: Liver transplantation, neutrophil-to-lymphocyte ratio, mortality, inflammation

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Abbreviations: CRP, c-reactive protein; c-statistics, concordance statistics; eGFR, estimated glomerular filtration rate; INR, international normalised ratio; IQR, inter-quartile range; LBP, lipopolysaccharide binding protein; MELD, Model for End Stage Liver Disease; NLR, neutrophil-to-lymphocyte ratio; ROC, receiver-operating characteristic; SD, standard deviation; SIRS, systemic inflammatory response syndrome; TIPSS, transjugular intra-hepatic porto-systemic shunt; UKELD, UK Score for Patients with End-Stage Liver Disease.

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ABSTRACT

Background and aims: In the absence of overt infection, the systemic inflammatory response is increasingly recognised as a pathogenetic factor in the circulatory dysfunction of advanced cirrhosis. Our aim was to determine whether the neutrophil-to-lymphocyte ratio, a marker of systemic inflammation, is predictive of mortality in patients with end stage cirrhosis listed for liver transplantation.

Methods: A single centre study of 570 patients listed for first elective single organ liver transplantation January 2007-June 2011.

Results: The median listing neutrophil-to-lymphocyte ratio was 2.9 (IQR 1.9-4.7). Neutrophil-to-lymphocyte ratio demonstrated a positive correlation with listing serum bilirubin ($p<0.001$), negative correlation with serum sodium ($p<0.001$), and positive correlation with the MELD score ($p<0.001$). Neutrophil-to-lymphocyte ratio increased with increasing severity of ascites ($p<0.001$). A higher neutrophil count ($p<0.001$) and lower lymphocyte count ($p=0.001$) were predictors of wait-list death. In a multivariate competing risk Cox model, neutrophil-to-lymphocyte ratio remained independently associated with mortality (HR 1.10; 95% CI 1.05-1.15, $p<0.001$). The proportion of patients with a neutrophil-to-lymphocyte ratio <2, 2-4.9 and ≥5 who had died by 3-months of listing was 3%, 13.8% and 37.3%, respectively ($p<0.001$). After adjusting for MELD, increasing increments of neutrophil-to-lymphocyte ratio were predictive of death by 3-months ($p=0.043$).

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Conclusions: The blood neutrophil-to-lymphocyte ratio, a simple and readily available marker of systemic inflammation, is an independent predictor of mortality in patients with liver failure listed for liver transplantation.

KEY POINTS BOX

- The ability of the blood neutrophil-to-lymphocyte ratio, a marker of systemic inflammation, to predict prognosis in stable patients with end-stage cirrhosis listed for transplantation has not been examined previously.
- We observed that the neutrophil-to-lymphocyte ratio increased with increasing severity of liver failure, correlating positively with jaundice, ascites and MELD.
- In a multivariate competing risk Cox model, neutrophil-to-lymphocyte ratio was an independent predictor of wait-list death.
- Our findings suggest a new prognostic marker to aid wait-list prioritisation and organ allocation, and add weight to the hypothesis that low-grade endotoxaemia and a systemic inflammatory response play a pathogenetic role in this setting.

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In the absence of overt infection, the systemic inflammatory response is increasingly recognised as a pathogenetic factor in the circulatory dysfunction of advanced cirrhosis. Patients with Child-Pugh Class C cirrhosis and ascites demonstrate an increased frequency of bacterial translocation, circulating bacterial DNA and raised plasma levels of lipopolysaccharide binding protein (LBP) [1,2,3,4,5]. Bacterial translocation is associated with nitric oxide overproduction in the mesenteric vasculature of cirrhotic rats, which appears to aggravate the arterial vasodilatation [6]. In humans with end stage liver disease there is increased mesenteric lymph node tumour necrosis factor–alpha (TNF-α) expression, and increased LBP levels are accompanied by greater circulating levels of inflammatory mediators and a more pronounced circulatory dysfunction [5,7]. The exaggerated immuno-haemodynamic derangement is reversed by the administration of norfloxacin [5,8]. Moreover, long-term prophylactic antibiotics reduce the incidence of hepatorenal syndrome and improve survival, independent of the prevention of infection [9]. It is therefore postulated that bacterial translocation and the secondary systemic response exacerbate the pre-existing portal hypertensive syndrome, with implications for hepatic function.

Markers of systemic inflammation have been linked with prognosis in patients in this setting. Hospitalised cirrhotics with the systemic inflammatory response syndrome (SIRS) have more severe hepatic encephalopathy, and are more likely to develop hepatorenal syndrome and have non reversible renal dysfunction [10,11,12]. Moreover, the presence of SIRS is associated with greater in hospital mortality [10,11,12]. However, the individual parameters of SIRS may be influenced by the clinical features of cirrhosis, and the presence or absence of SIRS does not always correlate with the systemic inflammatory response in these patients [13,14]. An alternative marker of systemic inflammation, c-reactive protein (CRP) has been shown to be a superior prognostic marker if persistently elevated [14]. Yet, CRP is

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synthesised by the liver and is only marginally increased even in patients with active infection [14]. Furthermore, CRP is influenced by other factors such as body mass index, weight loss, smoking, active alcohol consumption and diabetes [15].

The studies examining SIRS and CRP as prognostic markers in cirrhosis included patients with decompensated disease and a high prevalence of overt infection or acute alcoholic hepatitis [10,11,12,13]. Only a single study has evaluated the usefulness of a marker of subclinical inflammation in predicting prognosis in stable patients [16]. Biyik et al found that in a population of cirrhotics with less advanced disease (mean MELD score 10 and child pugh score 7) a greater neutrophil-to-lymphocyte ratio (NLR) was associated with an increased risk of long-term death [16]. Neutrophilia occurs in chronic inflammation and lymphopenia accompanies malnutrition; increasing NLR has been shown to predict outcome in various other disease processes including cardiac disease, malignancy and renal failure [17,18,19,20]. To our knowledge the utility of the NLR as a predictor of death in stable patients with end stage cirrhosis has not been examined previously. Our aim was therefore to determine whether the NLR, a simple marker of chronic systemic inflammation, is predictive of mortality in patients with end stage cirrhosis listed for liver transplantation.

Methods

This was a single centre study of 570 consecutive adults listed for first elective single-organ liver transplantation for chronic liver disease between January 2007 and June 2011. Patients transplanted for acute liver failure were not included, and no patient had end stage renal disease requiring renal replacement therapy.

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Institutional approval was obtained (Clinical Audit department, Queen Elizabeth Hospital; reference CAB-05300-13). Written patient consent was not required.

Patients were identified from a prospectively collected database. The following variables at time of listing for liver transplantation were recorded: age, gender, weight, height, aetiology of liver disease, presence of diabetes mellitus, previous variceal haemorrhage, refractory ascites and transjugular intra-hepatic porto-systemic shunt (TIPSS); prescription of nonselective betablockers, prophylactic antibiotics (the indication for antibiotics was usually the secondary prophylaxis of spontaneous bacterial peritonitis, or end-stage liver disease, and was clinician dependent) and immunosuppressive medications (the indication for immunosuppressive medications was autoimmune disease, predominantly autoimmune liver disease and inflammatory bowel disease); laboratory data (serum bilirubin, albumin, international normalised ratio (INR), neutrophil count, lymphocyte count, creatinine, serum sodium).

Refractory ascites and type 2 hepatorenal syndrome were defined according the International Ascites Club criteria [21,22]. The MELD (Model for End Stage Liver Disease) score was determined [23]. The UK Score for Patients with End-Stage Liver Disease (UKELD), a recently devised scoring system that incorporates serum sodium in addition to the MELD variables used routinely in the UK to prioritise graft allocation, was also calculated [24]. Glomerular filtration rate (eGFR) was estimated using the Modification of Diet in Renal Disease (MDRD) Study 4-variable equation (eGFR = 186 x creatinine(mg/dl)$^{-1.154}$ x age(years)$^{-0.203}$ x 1.212(if black) x 0.742(if female) [25].

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Statistical analyses

Normally distributed continuous variables and non-parametric continuous variables were compared using the Student's t-test and Mann-Whitney test, respectively. Chi-squared analysis or Fisher's exact test were used for comparison of categorical data. Correlations were determined by Spearman's rank test.

Survival modelling was performed using Cox proportional hazards analysis. The outcome of interest was wait-list death. In the first instance traditional Cox modelling was performed with patients censored at the time of liver transplantation, removal from the list because of clinical improvement or refusal, or last follow-up if still active. Thereafter, competing risk Cox regression analysis was used, according to the method of Fine and Gray [26]. In patients listed for liver transplantation the outcome transplantation is a competing risk that may impact on the probability of death, and vice versa. Competing risk analysis provides event specific (death or transplant) hazard ratios that are adjusted for inter-dependence. Competing risk analysis is considered the most appropriate statistical method for assessing the relationship between covariates and death in patients on a transplant waiting list, whilst traditional Cox regression analysis estimates the probability of death in the absence of transplantation [27]. In the competing risk analysis patients were censored at the time of removal from the list because of clinical improvement or refusal, or last follow-up if still active. Clinically relevant variables were included simultaneously in the multivariate models. Hyponatraemia was not included because of colinearity. In view of small patient numbers, all patients with follow-up beyond 1-year were censored at this time point.

Logistic regression analysis was used to examine the MELD adjusted association between NLR and 3-month wait-list mortality. Receiver-operating characteristic (ROC) curves were

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then generated to assess the accuracy of variables in predicting death by 3-months. To examine the impact of the addition of NLR to the MELD and UKELD scores competing risk Cox regression of variables transformed into their natural logarithms provided regression coefficients. Concordance statistics (c-statistics) were compared using the method described by Hanley and McNeil [28]. All patients censored prior to the specified time point were excluded from these analyses.

Data was analysed using the SPSS 21 package (SPSS Inc, Chicago, IL, USA) except the competing risk analyses, which were performed using Stata 13.1 (Stat Corp, College Station, Texas). All values are expressed as mean and standard deviation (SD), median and inter-quartile range (IQR) and number and percent (%) as appropriate. $P<0.05$ was considered statistically significant at all times.

Results

Characteristics of the patients at time of listing for liver transplantation are outlined in Table 1. The mean age was 54.1 (SD 9.9) years and the male to female ratio was 1.8:1. The mean listing MELD score was 15 (SD 5).

The median listing neutrophil count was 3.2 (IQR 2.2-4.5) $x10^9$/l. Ninety-six patients (17.0%) had a neutrophil count below the reference range, and 27 patients (4.8%) had a neutrophil count above. The median listing lymphocyte count was 1.1 (0.7-1.5) $x10^9$/l. Two hundred and twenty-five patients (39.8%) and 1 patient (0.2%) had a lymphocyte count below and above the normal range, respectively. The median NLR was 2.9 (IQR 1.9-4.7).

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Parameters associated with NLR

There was no relationship between listing NLR and age (r=0.030, p=0.473) or gender (male, 2.8 (1.8-4.7); female, 3.0 (2.1-4.7), median (IQR), p=0.137). Patients with cholestatic disease had a higher NLR than other aetiologies of liver disease (alcoholic liver disease, 3.1 (2.0-4.7); hepatitis C, 2.2 (1.5-3.5); cholestatic, 3.7 (2.5-5.7); NAFLD, 2.7 (2.0-4.5) other, 2.8 (1.8-4.5); median (IQR), p<0.001). The NLR was lower in patients with hepatocellular carcinoma (hepatocellular carcinoma, 2.0 (1.4-2.8); no hepatocellular carcinoma, 3.3 (2.3-5.2); median (IQR), p<0.001).

NLR demonstrated a positive correlation with listing serum bilirubin (r=0.277, p<0.001) and listing INR (r=0.156, p<0.001), and a negative correlation with listing serum albumin (r=-0.090, p=0.033). NLR was positively related to serum creatinine (r=0.104, p=0.013), and negatively related to eGFR (r=-0.137, p=0.001) and serum sodium (r=-0.453, p<0.001). NLR correlated with the MELD (r=0.297, p<0.001) and UKELD scores (r=0.460, p<0.001). The median listing NLR increased with increasing severity of ascites (no ascites, 2.2 (1.5-3.2); controlled ascites, 3.1 (2.2-4.7); refractory ascites, 4.6 (3.0-6.4); median (IQR), p<0.001, Figure 1), and was higher in patients fulfilling the diagnostic criteria for type 2 hepatorenal syndrome (hepatorenal syndrome, 4.8 (3.7-8.4); no hepatorenal syndrome 2.9 (1.9-4.6), p=0.008).

The use of antibiotic prophylaxis (antibiotics, 3.4 (2.5-5.3); no antibiotics, 2.7 (1.8-4.6); median (IQR), p<0.001) and immunosuppressive medications (immunosuppression, 4.8 (2.4-7.0), no immunosuppression, 2.8 (1.9-4.5); median (IQR), p<0.001) were associated with a greater NLR. There was no difference in the median listing NLR of patients prescribed

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NSBB (2.2 (IQR 2.0-4.5)) compared to patients not prescribed NSBB (2.9 (IQR 1.9-4.7), p=0.893).

NLR and wait-list mortality

Eighty-seven patients (15.3%) died and 410 patients (71.9%) were transplanted during a median followup time of 77 (IQR 27-199) days. The estimated 3-month, 6-month and 12-month survival was 89.0%, 79.8% and 65.0%, respectively. The documented causes of death were liver failure (52 patients), multi-organ failure cause unspecified (10 patients), sepsis (9 patients), cardiac (4 patients), hepatocellular carcinoma (3 patients) and other (9 patients).

Variables associated with wait-list death on univariate analysis are presented in Table 2. A higher neutrophil count ($p<0.001$) and lower lymphocyte count ($p=0.001$) were predictors of mortality within 1 year of listing. The greater the NLR, the greater the risk of death ($p<0.001$).

In a multivariate competing risk Cox model, NLR was independently associated with wait-list mortality (HR 1.10; 95% CI 1.05-1.15, $p<0.001$, Table 3 and Figure 2). Other variables associated with death were MELD score ($p=0.007$) and refractory ascites ($p=0.010$). The higher the NLR, the lower the likelihood of transplantation ($p=0.023$). Substitution of MELD with UKELD in the multivariate model did not affect the statistical relationship between NLR and mortality (HR 1.08; 95% CI 1.03-1.13, $p=0.001$, model not shown). NLR remained independently associated with wait-list death in the following subgroups of patients: absence of hepatocellular carcinoma, presence of hepatocellular carcinoma, refractory ascites, no refractory ascites, listing MELD ≥18, listing MELD <18 (Table 4).

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NLR and 3-month mortality

263 patients (46.1%) and 44 patients (7.7%) were transplanted or had died by 3-months after listing. The listing neutrophil count was higher in patients who died (dead, 4.6 (2.6-7.0); alive, 3.1 (2.1-4.0); median (IQR), p<0.001), and the listing lymphocyte count was lower (dead, 1.0 (0.6-1.3); alive, 1.0 (0.7-1.5); median (IQR), p=0.018). The median listing NLR of the non surviving patients was 4.7 (IQR 2.8-8.8) and for the surviving patients was 2.8 (IQR 2.0-4.5, p<0.001, Figure 3).

The proportion of patients with a listing NLR <2, 2-4.9, and ≥5 who had died within 3-months was 3%, 13.8% and 37.3%, respectively (p<0.001). After adjusting for MELD, increasing increments of NLR were associated with greater 3-month mortality (NLR <2, OR 1.00; NLR 2-4.9, OR 3.17 (95% CI 0.70-14.37); NLR ≥5, OR 6.02 (95% CI 1.28-28.41), p=0.043).

The c-statistic for the listing NLR for predicting death by 3-months was 0.709 (95% CI 0.624-0.794). The addition of NLR to MELD increased the c-statistic from 0.768 (95% CI 0.689-0.847) to 0.792 (95% CI 0.716-0.868, p=0.579). The addition of NLR to UKELD did not affect the c-statistic for predicting 3-month death (UKELD alone, 0.821 (95% CI 0.749-0.892); UKELD and NLR, 0.820 (95% CI 0.750-0.890).

Discussion

In this large single centre study we have examined for the first time the ability of the blood NLR, a simple marker of systemic inflammation, to predict prognosis in stable patients with end stage cirrhosis listed for liver transplantation. We have made two important observations. Firstly, the NLR increases with increasing severity of liver failure. We observed that the NLR

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correlated positively with jaundice, ascites and MELD, and the higher the NLR the greater was the risk of death. This supports the hypothesis that subclinical inflammation is a pathogenetic factor in advanced liver disease. Secondly, the NLR is a useful prognostic indicator in patients listed for liver transplantation. In a multivariate competing risk Cox model, listing NLR predicted wait-list mortality independent of the MELD score.

In contrast to previous studies of markers of systemic inflammation in advanced cirrhosis, our findings were made in the absence of acute decompensation precipitated by for example infection or acute alcoholic hepatitis [10,11,12,14]. In stable cirrhotic patients the prevalence of circulating bacterial DNA has been reported to be approximately 40% [29,30]. Low dose endotoxaemia without clinical signs or symptoms is associated with a systemic inflammatory response, and induces a rise in blood neutrophil count and fall in total lymphocyte count [31]. Therefore, in cirrhosis the increased NLR may be an indicator of subclinical endotoxaemia. Lymphocytopenia is also a well recognised sequelae of malnutrition. The high prevalence of lymphocytopenia in our patients, and the association of a lower lymphocyte count with mortality, may have at least in part been a consequence of poor nutritional status [20,32].

A greater listing NLR was associated with more severe liver failure, as demonstrated by a higher serum bilirubin level, INR and MELD score. Moreover, an increasing NLR was related to the severity of ascites, hyponatraemia and renal dysfunction. Our study does not clarify whether the systemic inflammatory response contributes to the hepatic and circulatory dysfunction of cirrhosis, or is a secondary phenomenon and a surrogate marker of advanced disease. However, the previous observation that norfloxacin administration in ascitic patients increased vascular resistance, in the context of normalisation of plasma LBP and reduced cytokine levels, is consistent with an active role [5,8]. Further support for this theory is

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provided by Fernandez et al's randomised controlled trial in which long-term prophylactic antibiotics reduced the incidence of hepatorenal syndrome and improved survival, independent of the prevention of infection [9].

Blood NLR has been linked with outcome in other aspects of liver disease. In patients with hepatocellular carcinoma, the NLR correlates with tumour stage, and a greater NLR has been shown to be related to non response to treatment, tumour recurrence, all-cause mortality and post liver transplantation death [33,34,35,36,37]. Similarly, in patients receiving chemotherapy or undergoing resection of colorectal liver metastasis, a higher NLR was associated with an increased mortality risk [38,39]. Only one paper has examined the role of NLR for staging severity of liver disease: in patients undergoing liver biopsy for a clinical suspicion of non-alcoholic fatty liver disease the NLR was predictive of the presence of steatohepatitis and fibrosis [40].

The majority of deaths in this series were as a result of liver failure or sepsis. We hypothesise that the increased NLR in our patients was a sign of subclinical endotoxaemia, and that the relationship between increasing NLR and death reflected the role that the systemic inflammatory response plays in the hepato-circulatory dysfunction of advanced liver disease. It should be mentioned that blood NLR has also been shown to be a marker of worse outcome in other disease processes where chronic inflammation has pathogenetic significance. In particular, the NLR has recently emerged as a risk stratification tool in cardiovascular disease. An elevated NLR is associated with arterial stiffness and the coronary artery calcification score, and is a poor prognostic indicator in acute coronary syndrome and stable coronary artery disease [17]. It is postulated that neutrophils have a causal link in such

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conditions. Following on from this, in patients scheduled for angiography the blood neutrophil count outperformed CRP as a predictor of cardiovascular mortality [41].

The main limitation of this study is the NLR was calculated from a single blood sample taken at the time of listing for transplantation. It is possible that low grade infection could have been undiagnosed, and impacted on the association between NLR and death. However, our unit has a high suspicion for infection in cirrhotic patients, and infection is a contraindication to activation on the transplant waiting list. Moreover, the incidence of death increased linearly over a prolonged follow-up time period.

The clinical implications of our findings are two-fold. Firstly, NLR is predictive of liver transplant wait-list mortality, independent of current scoring systems. In an era of organ shortage and growing wait-list death tools to optimise wait-list prioritisation and organ allocation are necessary. Our findings support the use of the NLR as a prognostic marker in this setting. Secondly, interventions that modify the systemic inflammatory response are likely to be beneficial. Prophylactic antibiotics in high risk individuals may reduce the incidence of circulatory complications and prolong survival to transplantation [9].

In conclusion, in this large single centre study we have shown for the first time that a simple and readily available marker of systemic inflammation, the NLR, is an independent predictor of mortality in patients with liver failure listed for liver transplantation. Our findings suggest a new prognostic marker to aid wait-list prioritisation and organ allocation, and add weight to the hypothesis that low grade endotoxaemia and a systemic inflammatory response play a pathogenetic role in end stage cirrhosis.

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Table 1: Characteristics of all patients at time of listing for liver transplantation

	n:570
Age (years)	54.1(9.9)
Male gender	365(64.0)
Body mass index (kg/m^2)	27.7(10.1)
Diagnosis:	
Alcohol	145(25.4)
Hepatitis C	123(21.6)
Cholestatic	154(27.0)
NAFLD	54(9.5)
Other	94(16.5)
Hepatocellular carinoma	148(26.0)
Diabetes mellitus	133(23.5)
Blood parameters at listing:	
Bilirubin (μmol/l)	48(25-102)
Albumin (g/l)	33(7)
INR	1.3(1.2-1.6)
Creatinine (μmol/l)	87(25)
eGFR (ml/min/1.73m^2)	88(31)
Sodium (mmol/l)	138(134-141)
Neutrophil count (x10^9/l)	3.2(2.2-4.5)
Lymphocyte count (x10^9/l)	1.1(0.7-1.5)
NLR	2.7(1.9-4.7)
MELD score	15(5)
UKELD score	54(6)
Previous variceal haemorrhage	133(23.3)
Ascites	356(62.5)
Refractory ascites	129(22.6)
Hepatorenal syndrome (type 2)	9(1.6)
Nonselective beta-blockers	212(39.0)
Prophylactic antibiotics	123(21.6)
Immunosuppressive medications	43(7.5)
TIPSS	25(4.4)

Values expressed as mean (standard deviation), median (interquartile range) and number (percent) where appropriate

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Table 2: Univariate cox regression analyses of variables associated with death after listing for liver transplantation.

		Cox regression analysis Outcome death (censored at transplant)			Competing risk Cox regression analysis Outcome Death		
		HR	95% CI	P value	HR	95% CI	P value
Age ≥60 years		0.98	0.63-	0.943	1.21	0.78-	0.402
Female gender		0.65	1.53	0.066	0.75	1.87	0.217
Blood group	A	1.18	0.41-	0.490	1.22	0.47-	0.419
	B/AB	1.08	1.03	0.808	1.40	1.19	0.265
Hepatocellular		0.64	0.74-	0.151	0.43	0.76-	**0.006**
carcinoma		1.53	1.90	0.079	1.72	1.95	**0.026**
eGFR <60		3.31	0.60-	**<0.001**	2.78	0.77-	**<0.001**
ml/min/1.73m^2		2.59	1.95	**<0.001**	2.64	2.54	**<0.001**
Hyponatraemia		1.14	0.35-	**<0.001**	1.07	0.23-	**<0.001**
Refractory ascites		1.16	1.18	**<0.001**	1.10	0.78	**<0.001**
MELD score		1.22	0.95-	**<0.001**	1.16	1.07-	**<0.001**
UKELD score		0.58	2.47	**0.011**	0.52	2.76	**0.001**
Neutrophil count		1.14	2.17-	**<0.001**	1.14	1.83-	**<0.001**
(x10^9/l)			5.06			4.24	
Lymphocyte count			1.69-			1.72-	
(x10^9/l)			3.96			4.05	
NLR			1.09-			1.03-	
			1.19			1.10	
			1.12-			1.06-	
			1.21			1.14	
			1.13-			1.08-	
			1.31			1.25	
			0.39-			0.36-	
			0.88			0.76	
			1.09-			1.10-	
			1.19			1.18	

Reference group (relative risk 1.00): age <60 years, male gender, blood group O, no hepatocellular carcinoma, body mass index ≤30, eGFR ≥60 ml/min/1.73m^2, no hyponatraemia, no refractory ascites.

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Table 3: Multivariate cox regression analyses of variables associated with death after listing for liver transplantation.

		Cox regression analysis Outcome death (censored at transplant)		Competing risk Cox regression analysis Outcome Death		Outcome Transplant	
		HR(95% CI)	P value	HR(95% CI)	P value	HR(95% CI)	P value
Age ≥60 years		1.36(0.83-2.24)	0.223	1.47(0.88-2.43)	0.139	0.80(0.64-1.02)	0.069
Female gender			0.328		0.149		0.197
Blood group	A	0.78(0.48-1.28)	0.769	0.70(0.44-1.13)	0.812	1.15(0.93-1.43)	0.761
	B/AB		0.475		0.437		**0.003**
Hepatocellular carcinoma		0.93(0.57-1.52)	0.131	1.06(0.64-1.77)	0.439	0.97(0.78-1.20)	**<0.001**
			0.958		0.377		0.171
eGFR <60 ml/min/1.73m^2		0.80(0.43-1.49)	**0.001**	1.29(0.68-2.44)	**0.010**	0.64(0.47-0.86)	0.688
			<0.001		**0.007**		**0.005**
Refractory ascites		1.71(0.85-3.41)	**<0.001**	0.76(0.38-1.52)	**<0.001**	2.10(1.60-2.75)	**0.023**
MELD score		1.01(0.60-1.70)		1.26(0.76-2.10)		0.81(0.60-1.10)	
NLR		2.32(1.43-3.75)		1.89(1.16-3.08)		0.94(0.71-1.25)	
		1.15(1.10-1.21)		1.06(1.01-1.10)		1.03(1.01-1.06)	
		1.10(1.05-1.16)		1.10(1.05-1.15)		0.95(0.90-0.99)	

Reference group (relative risk 1.00): age <60 years, male gender, blood group O, eGFR ≥60 ml/min/1.73m^2, no refractory ascites.

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Table 4: Association between NLR and wait-list mortality on competing risk analysis in patient subgroups

Subgroups	No.	Median NLR (IQR)	Competing risk cox regression analysis	
			*Adjusted HR (95% CI)	P value
No hepatocellular	422	3.3(2.3-5.2)	1.11(1.07-1.16)	**<0.001**
carcinoma	148	2.0(1.4-2.8)	1.47(1.17-1.84)	**0.001**
Hepatocellular	441	2.6(1.8-4.0)	1.14(1.05-1.25)	**0.003**
carcinoma	129	4.6(3.0-6.4)	1.08(1.03-1.14)	**0.002**
No refractory ascites	371	2.7(1.8-4.2)	1.15(1.06-1.24)	**0.001**
Refractory ascites	151	3.6(2.5-5.8)	1.13(1.08-1.19)	**<0.001**
MELD <18				
MELD ≥18				

*Adjusted for age ≥60 years, gender, blood group and MELD score

Figure 1: Box plot of listing NLR in patients subdivided according to severity of ascites.

Figure 2: Cumulative incidence of death in patients with listed for liver transplantation subdivided based on the NLR; 0-1.9 solid black line, 2-4.9 dash line, ≥5 solid grey line (truncated at 365 days). Cumulative incidence calculated using competing risk analysis and adjusted for age, gender, blood group, hepatocellular carcinoma, eGFR <60 ml/min/1.73m^2, refractory ascites and MELD score.

Figure 3: Box-plot of NLR in surviving and non surviving patients by 3-months after listing for liver transplantation.

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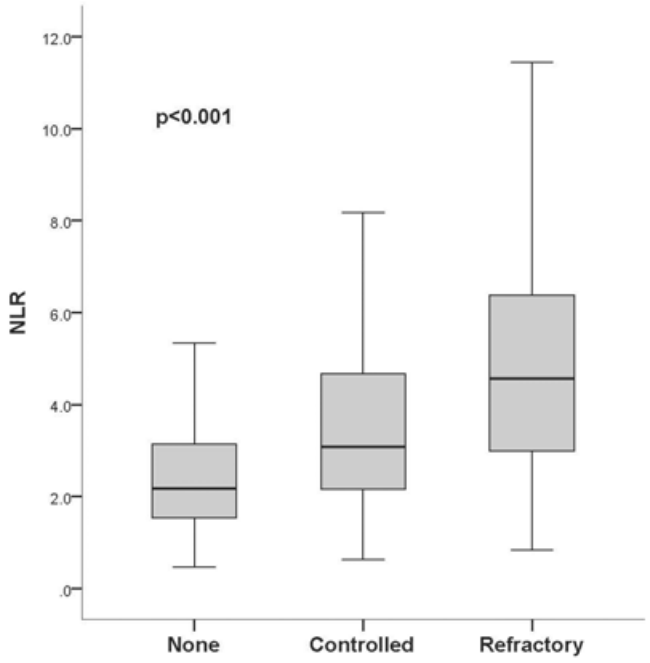

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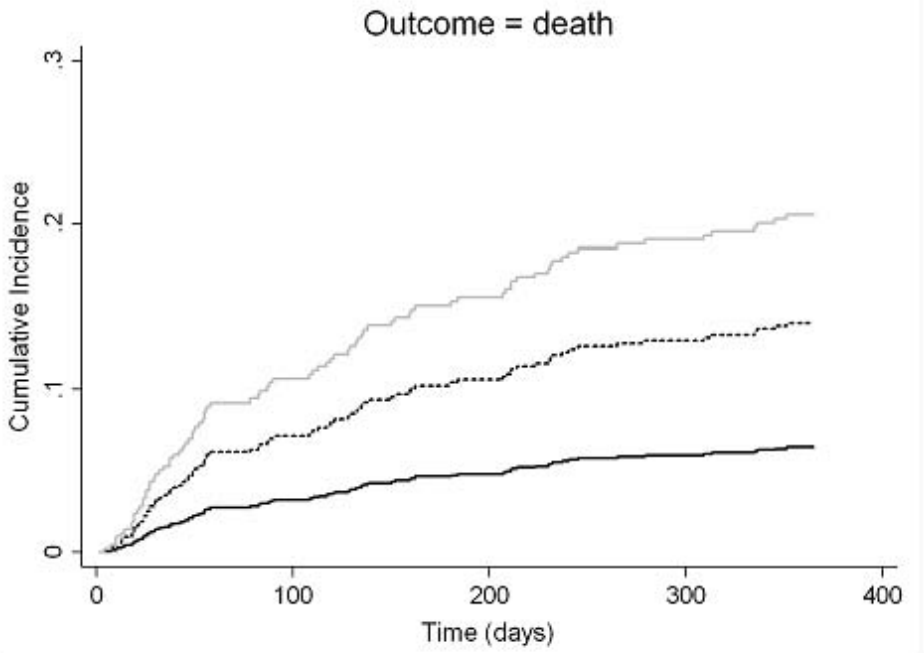

Number of ,				
NLR ≥5	119	50	26	15
NLR 2-4.9	299	134	76	46
NLR <2	147	63	38	22

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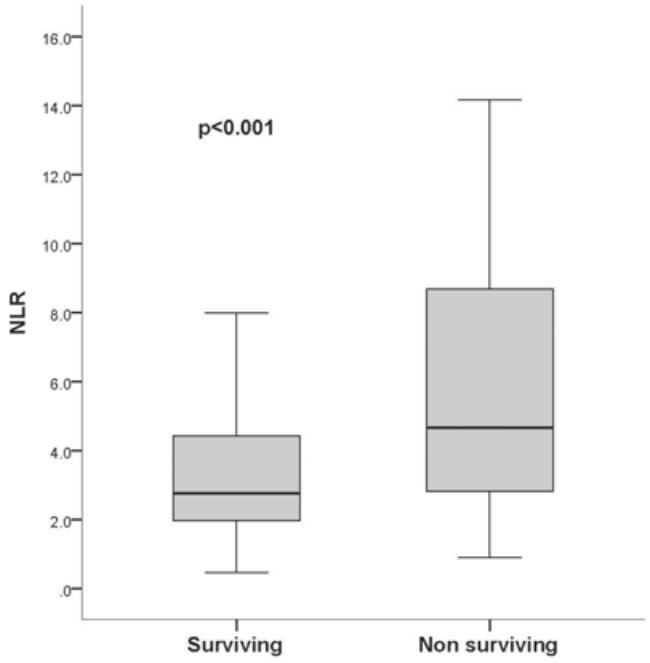

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