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ORIGINAL ARTICLE

# High serum Aspartate transaminase levels on day 3 postliver transplantation correlates with graft and patient survival and would be a valid surrogate for outcome in liver transplantation clinical trials

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## Summary

Aspartate transaminase, a liver specific enzyme released into serum following acute liver injury, is used in experimental organ preservation studies as a measure of liver IR injury. Whether post-operative serum transaminases are a good indicator of IR injury and subsequent graft and patient survival in human liver transplantation remains controversial. A single centre prospectively collected liver transplant database was analysed for the period 1988–2012. All patients were followed up for 5 years or until graft failure. Transaminase levels on the 1st, 3rd and 7th post-operative days were correlated with the patient demographics, operative outcomes, post-operative complications and both graft and patient survival via a binary logistic regression analysis. Graft and patient survival at 3 months was 80.3% and 87.5%. AST levels on the 3rd ($P = 0.005$) and 7th ($P = 0.001$) post-operative days correlated with early graft loss. Patients were grouped by their AST level (day 3): <107iU, 107–1213iU, 1213–2744iU and >2744iU. The incidence of graft loss at 3 months was 10%, 12%. 27% and 59% and 1-year patient mortality was 12%, 14%, 27% and 62%. Day 3 AST levels correlate with patient and graft outcome postliver transplantation and would be a suitable surrogate endpoint for clinical trials in liver transplantation.

## Introduction

The recent widening of the listing criteria for liver transplantations has led to increased demand for organs and the implantation of more grafts from extended criteria donors. The use of grafts from donors following cardiac death (DCD) in the UK has increased from 6.9% in 2005 [1] to 19.1% of grafts implanted in 2013 [2]. Grafts from extended criteria donors are more prone to ischaemia reperfusion (IR) injury and a poorer outcome following transplantation [3]. Several donor and transplant variables have been reliably shown to reduce graft function and survival in multivariate analyses including a long cold ischaemic time, donor hospital stay and elderly donors [1,4–6]. The use of DCD grafts in UK centres is associated with a twofold increase in recipient mortality and graft loss up to 3 years post-transplantation [1].

As there is currently no accepted treatment for IR injury, strategies to ameliorate IR injury remain key research goals. Several strategies such as ischaemic preconditioning [7,8], pharmacological preconditioning [9] and adoptive transfer of cells [10] can ameliorate IR injury in small animal models. Clinical translation of these strategies has been poor, partly due to a lack of understanding of the mechanisms of these strategies but also due to significant difficulty in funding trials large enough to show benefits to

 

a single intervention in the complex circumstances of human liver transplantation. A key factor is that 90-day graft and patient survival are 89.3% and 90.8% [11] and hence powering trials with these as a primary endpoint is impossible due to trial size and cost. Surrogate endpoints (as in the use of creatinine clearance for kidney transplant outcomes) have not as yet been validated for liver transplant outcomes.

NIH defines a surrogate endpoint as a biomarker intended to substitute for a clinical endpoint that should predict clinical benefit or harm or lack of both [12]. Surrogate markers are biomarkers that reflect a pathological process at an earlier stage than death or significant illness. Surrogate markers are used routinely in cardiology trials [13] and trials in HIV [14,15].

Current endpoints in trials of liver transplantation include a reduction in total hospital stay, length of stay in intensive care, a reduction in post-operative comorbidities and a reduction in post-operative serum transaminases [16,17]. None of these have been properly validated with graft specific outcome.

Aspartate transaminase (AST) and alanine transaminase (ALT) are intracellular liver enzymes that are released into the circulation in high quantities following hepatocyte injury.

A reduction in serum AST and ALT levels at 48 h has been used as a primary endpoint for liver IR studies in small animal models [8,10,18].

Serum transaminases are monitored daily in patients following liver transplantation as a marker of IR injury. Serum transaminases are released from damaged hepatocytes; however, the use of peak transaminase levels to predict outcome following liver transplantation is controversial.

A small number of analyses have correlated peak transaminase levels with early graft failure specifically primary graft nonfunction (PGNF) and late complications in DCD grafts. They have demonstrated that peak AST levels of >2000iU [19], 3500iU [20] or even 5000iU [21] can accurately predict PGNF. The development of nonanastomotic biliary strictures in DCD grafts is associated with higher peak ALT and AST levels [22]. United Network for Organ Sharing (UNOS) currently incorporate a peak AST value of >3000iU when describing PGNF and when deciding to relist a patient for transplantation. Although higher AST levels have been shown to correlate with IR injury on postreperfusion biopsies, there was significant overlap between grafts that functioned and those failed [23]. Although these studies demonstrate that peak AST levels correlate with PGNF and graft loss within 7 days, there are several limitations to current data. Peak AST levels can happen at different timepoints following transplantation and make it difficult to draw comparisons between patients. Secondly, these studies have identified the prognostic significance of only excessive post-operative AST levels that are not experienced in the majority of patients. Furthermore, the majority of these studies have correlated AST levels with the development of primary graft nonfunction within 7 days. Although a devastating complication following liver transplantation, PGNF accounts for a small proportion of graft loss and post-operative morbidity. The use of an endpoint in clinical trials must correlate with a more global prognosis to be of any significance and would ideally be measured at a specific time-point to allow reproducibility.

The aim of this study was to identify a reliable surrogate endpoint for clinical trials in liver transplantation.

## Methods

A prospectively collected transplant database was reviewed for the period 1988–2012 to allow an adequate sample size and adequate follow-up data. All donor types (DBD, DCD, split and domino grafts) were included in the analysis. Re-transplantations were also included.

Aspartate transaminase, ALT and bilirubin levels were measured in peripheral venous blood (Clinical Chemistry analyser, Modular analytics P module, Roche, Indianapolis) daily in the post-operative period.

### Total graft loss

Total graft loss was accepted as a graft lost for any reason following transplantation. Date of graft loss was accepted as date of death or date of re-transplantation. Grafts lost within the first 3 months were defined as early graft loss and those lost after 3 months were defined as late graft loss.

A further analysis was performed between the years of 1998 and 2012 – this included less grafts but was to ensure that transaminase values as a surrogate marker was valid in a modern cohort.

### Liver specific graft loss following transplantation

Liver specific graft loss was defined as a graft lost secondary to specific intrahepatic pathology and included grafts loss due to primary graft nonfunction, nonthrombotic infarction, acute rejection, ductopenic rejection and biliary complications (excluding anastomotic stricture or bile leak). Hepatic artery thrombosis and portal vein thrombosis were excluded as a cause of graft loss from the analysis as it would be difficult to rule out a technical failure as contributing to graft loss under these circumstances.

Date of graft loss was accepted as date of death or date of re-transplantation. Grafts lost within the first 3 months following liver transplantation was defined as early graft loss and those lost after 3 months were defined as late graft loss.

### Clinical outcomes

A ROC curve was performed to identify specificity and sensitivity of AST day 3 values at predicting liver specific graft loss. A likelihood ratio was calculated for specific AST values. Patients were grouped according to their AST values on the 3rd post-operative day and the percentage of total graft loss, liver specific graft loss at 3 months and percentage mortality at 30 days, 90 days and 1 year were measured along with mean number of days in ITU, mean number of days ventilated, percentage of patients requiring transient renal support (haemofiltration and haemodialysis) – patients with creatinine values of >150 μmol/l were removed from this analysis to avoid the bias of pre-existing renal impairment – and the percentage of patients that developed a bacteraemia or a site-specific infection (chest, wound and intra-abdominal). Infective complications were only accepted after a positive microbiology culture.

### Statistical analysis

Results were analysed on the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 21, IBM. Transaminase levels were correlated with both short-term (3 month) and long-term (5-year) outcome. Transaminase levels were adjusted for cold ischaemic time, secondary warm ischaemic time, donor and recipient age, recipient gender and pre-operative MELD score using a binary logistic regression analysis.

When statistical analyses were performed to analyse transaminase levels with long-term graft loss, grafts that had been lost in the first 3 months post-transplant were excluded from the analysis.

Patients with missing data were excluded from the analysis. A $P$ value of $<0.05$ was considered to be significant.

## Results

Data were analysed on 1272 patients (640 Male/628 Female/4 Unspecified) undergoing liver transplantation at the Royal Free Hospital, London between 1988 and 2012. Minimum follow-up was 3 months or until graft failure. A total of 181 patients were excluded due to missing data. The mean donor age was 42 (range 6–78). The majority of grafts were from donors after brain death (DBD) (97%). The mean cold ischaemic time was 604 min (range 42–1194). 99% of grafts were preserved in commercial University of Wisconsin preservation fluid (Organ Recovery Systems, Chicago, IL, USA). Further donor details are provided in Table 1. The mean recipient age at time of transplantation was 47 years (range 37–69) and the mean pre-operative MELD score was 18 (range 3–40). Further recipient characteristics are given in Table 2. The main indications for transplantation were hepatitis C virus (HCV)-related cirrhosis (22%) and alcoholic liver disease (ALD) (17%). Other indications are given in Table 3.

The median AST levels peaked on the first post-operative day (Table 4). A total of 206 grafts (18.9%) were lost in the first 3 months. A further 221 grafts (20.3%) were lost between 3 months and 5 years. The incidence of pathology encountered is in Table 5.

### Transaminase levels and early total graft failure (0–3 months)

For this analysis, 1062 grafts were analysed. AST levels on the 1st post-operative day were significantly higher in grafts that were lost within 3 months than those that were still functioning ($P = 0.013$). On the 3rd post-operative day, AST levels ($P < 0.001$) and ALT levels ($P < 0.001$) were significantly higher in grafts that were lost than those that were still functioning after 3 months.

A binary logistic regression model showed that AST levels on the 3rd post-operative day ($P = 0.002$) and that AST levels ($P < 0.001$) and ALT levels ($P = 0.006$) on the 7th post-operative day were strong predictors of graft loss. Transaminase levels on the 1st post-operative day were not indicative of early graft loss (AST $P = 0.645$, ALT

**Table 1.** Donor characteristics.

| | |
|---|---|
| Age | 43 (6–78) |
| Type of donor | |
| Deceased brain death | 1228 |
| Deceased cardiac death | 33 |
| Domino | 8 |
| Split | 3 |
| Number of grafts analysed at each time-point | |
| 0–3 months | 1062 |
| 3 months–5 years | 623 |
| Length of time in ITU | 3 (0–185) |
| Cold ischaemic time (minutes) | 604 (42–1194) |
| Implantation warm ischaemic time (minutes) | 43 (19–220) |

Values given are median and range.

**Table 2.** Recipient characteristics.

| | |
|---|---|
| Gender | |
| Male | 640 |
| Female | 628 |
| Unspecified | 4 |
| Age | 50 (2–69) |
| Length of time in ITU | 3 (0–148) |
| Pre-operative MELD score | 16 (3–40) |
| Pre-operative creatinine (μmol/l) | 85 (16–753) |
| Pre-operative haemoglobin (g/dl) | 11 (5.4–17.0) |

Values given are median and range.

$P = 0.363$). Although AST levels on the 3rd post-operative day correlated strongly with early graft failure, ALT levels did not ($P = 0.710$; Table 6).

The binary logistic regression analysis was rerun including only transplants performed between the years of 1998 and 2012. A total of 796 grafts were included in this analysis. AST levels on the 3rd post-operative day ($P = 0.003$) but not ALT levels on the 3rd post-operative day ($P = 0.548$) correlated with early graft loss in this recent but reduced size cohort.

### Transaminase levels and early liver specific graft failure (0 and 3 months)

For this analysis, 151 grafts that failed within 3 months for a nonliver specific reason were excluded. A total of 940 grafts were therefore included in this analysis. AST levels on the 1st post-operative day were significantly higher in grafts that failed within three months than grafts that survived ($P = 0.024$) three months or more. On the 3rd post-operative day, AST levels ($P < 0.001$) and ALT levels ($P = 0.025$) were significantly higher in grafts that failed than those that survived more than three months.

A binary logistic regression model showed that AST levels on the 3rd post-operative day ($P = 0.005$) and 7th post-operative day were strong predictors of early graft failure ($P = 0.001$). Transaminase levels on the 1st post-operative day were not indicative of early graft loss (AST $P = 0.092$, ALT $P = 0.962$). Although AST levels on the 3rd post-operative day correlated strongly with early graft failure, ALT levels did not ($P = 0.966$).

### Transaminase levels and late graft failure (3 months–5 years)

A total of 623 graft outcomes were analysed. There was no correlation between AST and ALT levels on day 3 and graft lost between 3 months and 5 years post-transplant (Table 6).

**Table 3.** Indication for transplantation.

| Indication for transplantation | Number | Percentage |
|---|---|---|
| Hepatitis C cirrhosis | 280 | 22.0 |
| Alcohol liver disease | 221 | 17.4 |
| Primary biliary cirrhosis | 149 | 11.7 |
| Hepatitis B cirrhosis | 114 | 9.0 |
| Primary sclerosing cholangitis | 109 | 8.6 |
| Acute liver failure (unknown cause) | 69 | 5.4 |
| Cryptogenic cirrhosis | 62 | 4.9 |
| Hepatic artery thrombosis | 39 | 3.1 |
| Auto-immune hepatitis | 34 | 2.7 |
| Metabolic diseases | 24 | 1.9 |
| Acute liver failure (paracetamol) | 23 | 1.8 |
| Wilson's disease | 20 | 1.6 |
| Primary graft nonfunction | 20 | 1.6 |
| Other | 20 | 1.6 |
| Biliary complication | 16 | 1.3 |
| Chronic rejection | 13 | 1.0 |
| Budd–Chiari syndrome | 12 | 0.9 |
| Familial adenomatous polyposis | 11 | 0.9 |
| Hepato-cellular carcinoma (noncirrhotic) | 8 | 0.6 |
| Other malignancy | 8 | 0.6 |
| Nonthrombotic infarction | 6 | 0.5 |
| Ductopenic rejection | 3 | 0.2 |
| Polycystic disease | 1 | 0.1 |
| Biliary atresia | 1 | 0.1 |

**Table 4.** AST levels on the 1st, 3rd and 7th post-operative day.

| | Day 1 | Day 3 | Day 7 |
|---|---|---|---|
| Mean (IU) | 1006 | 614 | 120 |
| Median (IU) | 651 | 272 | 61 |
| Std Deviation | 1560 | 1215 | 248 |

**Table 5.** Pathology and period of liver specific graft failure.

| Pathology of graft failure | 1–3 months | 3 months – 5 years |
|---|---|---|
| Extra-hepatic cause | 95 | 157 |
| Vascular occlusion | 56 | 5 |
| Nonthrombotic infarction | 23 | 2 |
| Primary graft nonfunction | 23 | 0 |
| Acute rejection | 1 | 0 |
| Chronic rejection | 3 | 25 |
| Ductopenic rejection | 0 | 14 |
| Biliary complications | 4 | 16 |
| Others | 1 | 2 |

**Table 6.** Binary logistic regression model showing correlation between day 3 AST levels (*) and early but not late graft loss.

| | Grafts lost 0–3 months | Grafts lost 3 months–5 years |
|---|---|---|
| MELD | 0.000 | 0.903 |
| AST day 1 | 0.634 | 0.935 |
| ALT day 1 | 0.351 | 0.468 |
| AST day 3 | 0.002* | 0.703* |
| ALT day 3 | 0.622 | 0.804 |
| AST day 7 | <0.001 | 0.402 |
| ALT day 7 | 0.005 | 0.901 |
| Rec gender | 0.137 | 0.657 |
| Rec age | 0.732 | 0.965 |
| Don age | 0.447 | 0.287 |
| CIT | 0.014 | 0.625 |
| Reperfusion Time | 0.434 | 0.243 |


 

### AST on the 3rd post-operative day and clinical outcome measures

Having identified AST levels on the 3rd post-operative day as the earliest transaminases to correlate with early graft loss, a ROC curve was performed to identify the AST level on the 3rd post-operative day at which the risk of developing liver specific graft failure at 3 months became significant as it yielded a stronger AUC than total graft loss (Fig. 1). The area under the curve was 0.739 (0.663–0.814) with a *P* value of <0.001. An AST of below 106.5iU was identified as the best fit to predict graft survival with a negative likelihood ratio of 0.13 and negative predictive valve of 99.45%. An AST level of above 2744.5iU was identified as the best fit to predict graft failure with a positive likelihood ratio of 13.1 and a positive predictive value of 34.62%. To prove that it is not only extreme values that correlate with graft failure, the central group was further divided. An AST level of 1213iU was chosen as the positive likelihood ratio of this value was 4.1 identifying it as a moderate predictor of graft failure.

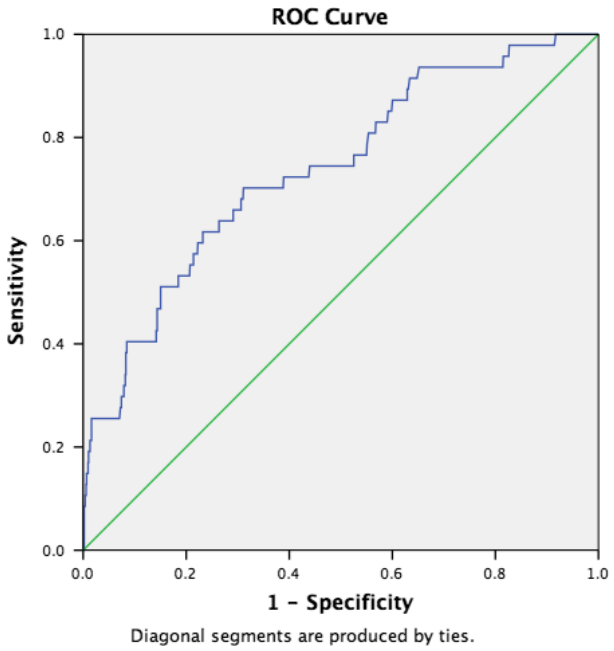


**Figure 1** ROC curve of AST levels on the 3rd post-operative day against liver specific graft loss at 3 months. AUC 0.739 95% CI −0.663 to 0.814.

Patients were divided into 4 groups according to day 3 AST values (<107iU, 107–1213iU, 1213–2744iU and >2744iU). In grafts with AST levels of <107iU ($n$ = 201), the incidence of graft loss at 3 months was 9.6% and 1-year mortality was 12.2%. In grafts with AST levels of between 107 and 1213iU ($n$ = 883), the incidence of graft loss at 3 months was 12.2% and 1-year mortality was 14.4%. In grafts with AST levels of between 1213 and 2744iU ($n$ = 97), the incidence of graft loss at 3 months was 25.8% and 1-year mortality was 26.4%. In grafts with AST levels above 2744iU ($n$ = 40), the incidence of graft loss at 3 months was 57.5% and 1-year mortality was 61.1%. This incremental increase among the groups was also observed in liver specific graft loss at 3 months, 30- and 90-day mortality (Table 7).

The need for organ support and length of stay in ITU similarly increased among the groups. The incidence of renal replacement therapy (RRT) in the group of patients with an AST of less than 107iU was 13.3% and in the group of patients with AST levels of >2744iU was 58.8% (Table 7).

There was a greater incidence of proven bacteraemias and site-specific infective complications in the groups of patients with higher AST levels (Table 7).

**Table 7.** Grouped AST levels (iU) on the 3rd post-operative day and incidence of clinical morbidity and mortality.

| AST levels (iU) | <107 | 107–1213 | 1213–2744 | >2744 |
|---|---|---|---|---|
| No. in group | 201 | 882 | 98 | 41 |
| Liver specific graft loss | 0.5% | 3.2% | 7.7% | 34.6% |
| Total graft loss | 10% | 13.7% | 26.5% | 58.5% |
| 30 day mortality | 3.5% | 5.1% | 15.3% | 34.1% |
| 90 day mortality | 5.5% | 8.2% | 18.4% | 48.8% |
| 1-year mortality | 12.2% | 12.4% | 27.2% | 62.2% |
| Days in ITU (mean) | 7 | 7 | 9 | 15 |
| Days ventilated (mean) | 5 | 6 | 8 | 14 |
| Need for renal support | 13.3% | 12.4% | 23.8% | 57.1% |
| Bacteraemia | 0.5% | 4.9% | 16.3% | 12.5% |
| Intra-abdominal infection | 1% | 3.2% | 16.3% | 17.5% |
| Chest infection | 1% | 4.7% | 14.3% | 10% |
| Wound infection | 0.5% | 8.4% | 24.5% | 27.5% |

### The effect of donor and transplant variables on day 3 AST levels

Having identified AST on the 3rd post-operative day as correlating with post-transplant outcomes, linear regression models were performed to assess the impact of donor and transplant variables on AST levels on the 3rd post-operative day. Length of cold ischaemic time ($P = 0.041$) and time taken to perform the vascular anastomoses (secondary warm ischaemia) ($P < 0.001$) correlated with AST levels on the 3rd post-operative day while older age of donor ($P = 0.705$) and length of donor time spent in ITU ($P = 0.317$) did not. Type of graft (DBD/DCD/split/domino) inserted did not correlate with AST levels on the 3rd post-operative day ($P = 0.200$).

### The effect of recipient variables on day 3 AST levels

Higher pre-operative MELD scores correlated with increased AST levels on the 3rd post-operative day ($P = 0.042$). No other recipient variable correlated with AST levels on the 3rd post-operative day.

## Discussion

Using a large single centre prospective liver transplant database, serum AST and ALT levels within the first post-operative week were correlated with post-operative recovery and both short- and long-term graft and patient outcomes. AST levels on the 3rd post-operative day were found to closely correlate with the rate of post-operative complications and short-term graft and patient survival. A simple scale for grouping day 3 AST levels was found to correlate well with post-transplant outcomes and could be adopted as a surrogate endpoint for short-term graft and patient survival in clinical trials of liver transplantation.

An audit performed by the Royal College of Surgeons of England in November 2012 found that between 1994 and 2012, 7953 elective liver transplants were performed in the UK with a 90 day graft loss of 10.7% and a 5-year graft loss of 29.5%. Our centre has a 90 day graft loss of 13.1% and a 5-year graft loss of 31.2%. This data is therefore representative of liver transplantation outcomes in the UK and hence this analysis of the prognostic significance of day 3 AST levels is likely to be widely applicable.

Previous studies have correlated peak AST levels with outcome postliver transplantation and found that peak AST levels correlate with the diagnosis of primary graft nonfunction and graft loss within 7 days. However, this is the first study to identify the post-operative day on which the AST level is most predictive of outcome. AST levels on the 3rd post-operative day most accurately predict post-operative morbidity and mortality.

This analysis shows that higher AST levels in the early post-transplant period correlate with the short-term (<3 months) but not the long-term (>3 months) graft and patient survival suggesting that early graft damage is amenable to recovery. In a recent study [22] high peak transaminases were found to predict the incidence of nonanastomotic strictures (biliary complications) in DCD grafts but had no correlation with outcome in DBD grafts. This finding is backed up by these results as 97% of grafts analysed were DBD grafts and AST levels did not correlate with long-term graft loss.

It is interesting that although ALT levels on the third post-operative day were significantly higher in grafts that failed within 3 months than those that were still functioning, when analysed in the regression analysis, ALT levels were not significant until the 7th post-operative day. This may reflect the fact that although ALT is more liver specific than AST, there is more AST contained in the liver than ALT [24]. Our results show that median peak AST levels were higher than median peak ALT levels (651iU vs. 452iU). Furthermore serum AST levels reduce more rapidly than serum ALT levels [24]. In this analysis median AST levels had dropped to 272iU by day three while serum ALT levels remained elevated at 425iU and by day 7 serum ALT levels correlated with outcome. This pattern of transaminase reduction is mirrored in other studies [25] and may explain why serum AST levels are a better marker in early identification of a healthy graft without ongoing damage.

A ROC curve demonstrated that the AST value on the 3rd post-operative day was a moderate predictor of liver specific graft failure with an AUC of 0.739 (0.663–0.814). Using likelihood values, an AST level of below 106.5iU on day 3 was identified as the strongest predictor of graft survival with a NPV of 99.45% and an AST level of >2744 iU as the best predictor of graft failure with a PPV of 34.62%. Day 3 AST levels of below 106.5iU are associated with a 10% risk of graft loss from all causes and a 6% risk of mortality within 3 months. AST levels of >2744iU are associated with a 59% risk of graft loss and 49% risk of mortality within 3 months. Patients with AST levels above 2744iU are also at significantly increased risk of end organ failure and infective complications. These are post-transplant complications associated with graft preservation and IR injury and the resulting poor initial graft function. The high incidence of these complications in patients with high day 3 AST levels supports the use of day 3 AST levels as a clinically relevant surrogate endpoint of outcome in clinical trials in liver transplantation.

To obtain sufficient data to analyse graft and patient survival in a single centre study, required a long time period to be analysed, which could be viewed as a limitation of this study. During this time period some practices in liver transplantation have changed including immunosuppression

protocols, retrieval and implantation techniques and transfusion practices. However the time duration is also beneficial in that it allows AST levels to be analysed over a period of changing practice and suggests that changes over time do not alter the importance of the day 3 AST and its correlation with graft and patient survival. A subgroup analysis of AST levels in transplants performed only between 1998 and 2012 demonstrated that AST levels on the 3rd post-operative day remain significantly correlated with graft loss.

In this study, the most significant donor and transplant factors to result in raised AST levels on the 3rd day post-op were a prolonged cold ischaemic time and a longer duration of vascular reconstruction (secondary warm ischaemia) suggesting that the ischaemic damage is a key determinant of short-term graft and patient survival. The length of ischaemic time correlates more strongly with peak AST levels on the 1st rather than on the 3rd post-operative day. This would suggest that day 3 AST levels are a reflection of the graft's ability to recover from cold or warm ischaemia. It is well documented that length of cold ischaemic time correlates with outcome post-transplantation and this is in keeping with our results. Cold ischaemic time however was not the only factor that correlated with outcome. The length of time taken to perform the vascular anastomoses or secondary warm ischaemic time significantly correlated with AST levels and graft loss. To our knowledge only a limited number of studies have investigated the length of time taken to perform the vascular anastomosis with outcome in renal transplantation and found that longer anastomotic time correlates with a poorer outcome [26,27]. It is unclear if this finding reflects a more difficult implantation period, an increased likelihood of HAT and PVT or increased graft damage occurring during secondary warm ischaemia. It is likely a combination of all three factors but further work is needed to clarify this.

Interestingly although the use of a DCD graft correlated with increased peak AST levels on day 1, it was not an independent predictor of raised AST levels on the 3rd post-operative day. This may reflect the fact that the study period ended at a time period when the use of DCD grafts was limited (only 33 DCD grafts were in this study) and the donor criteria for use of DCD grafts was very restricted.

Although both the donor risk index (DRI) and the EuroTransplant Donor Risk Index (ETDRI) identify donor age as an independent risk factor for early graft loss [6,28], in this cohort, donor age had no correlation with early graft loss. This may reflect the fact that the average cold ischaemic time in this current UK patient cohort (604 min) is significantly longer than in the cohort originally used to describe the DRI (493 min) and slightly longer than the cold ischaemic time in the ET-DRI cohort (582 min). This will alter the impact of age and other variables on graft outcome as cold ischaemic time is the one of the strongest predictors of early graft failure and raised post-operative transaminases. Furthermore it is unclear if length of anastomotic time was included in these analyses which will influence the results.

Surrogate markers are routinely used in other medical disciplines to evaluate the risk/benefit of new treatment modalities. Surrogate markers need to reflect the underlying disease process and to provide an early indication of clinical benefit or harm [29]. This analysis identifies AST levels on day 3 as an effective surrogate marker of outcome following liver transplantation that could be used as a suitable endpoint in trials of liver transplantation.

## Authorship

FR: designed the study, analysed the data and wrote the manuscript. RD: contributed to the data collection and analysis of the data. PB and NT: contributed to the analysis of the data and provided statistical input. NR: manages the database and contributed to the collection of the data. BF and BD: contributed to the design of the study and revised the manuscript.

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