

ORIGINAL ARTICLE

Pilot study of a noninvasive real-time optical backscatter probe in liver transplantation

James A. Richards^{1,2,3} , Lucy V. Randle^{1,2,3}, Andrew J. Butler MChir^{1,2,3}, Jack L. Martin^{1,2,3}, Arturs Fedotovs^{1,2,3}, Susan E. Davies⁴, Christopher J.E. Watson^{1,2,4} & Paul A. Robertson⁵

1 Department of Surgery, Addenbrooke's Hospital, University of Cambridge, Cambridge, UK

2 NIHR Blood and Transplant Research Unit (BTRU) at the University of Cambridge in collaboration with Newcastle University and in partnership with NHS Blood and Transplant (NHSBT), Cambridge, UK

3 The National Institute of Health Research (NIHR) Cambridge Biomedical Research Centre, Cambridge, UK

4 Department of Pathology, Cambridge University Hospitals, Addenbrooke's Hospital, Cambridge, UK

5 Department of Engineering, Electrical Engineering Division, University of Cambridge, Cambridge, UK

Correspondence

James Richards, University of Cambridge Department of Surgery, Box 202, Level E9, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0QQ, UK.
Tel.: 01223 216108;
fax: 01223 762523;
e-mail: jamesrichards@doctors.net.uk

The views expressed are those of the authors and not necessarily those of the NIHR, the Department of Health and Social Care or NHSBT.

SUMMARY

Transplantation of severely steatotic donor livers is associated with early allograft dysfunction and poorer graft survival. Histology remains the gold standard diagnostic of donor steatosis despite the lack of consensus definition and its subjective nature. In this prospective observational study of liver transplant patients, we demonstrate the feasibility of using a handheld optical backscatter probe to assess the degree of hepatic steatosis and correlate the backscatter readings with clinical outcomes. The probe is placed on the surface of the liver and emits red and near infrared light from the tip of the device and measures the amount of backscatter of light from liver tissue via two photodiodes. Measurement of optical backscatter (Mantel–Cox $P < 0.0001$) and histopathological scoring of macrovesicular steatosis (Mantel–Cox $P = 0.046$) were predictive of 5-year graft survival. Recipients with early allograft dysfunction defined according to both Olthoff ($P = 0.0067$) and MEAF score ($P = 0.0097$) had significantly higher backscatter levels from the donor organ. Backscatter was predictive of graft loss (AUC 0.75, $P = 0.0045$). This study demonstrates the feasibility of real-time measurement of optical backscatter in donor livers. Early results indicate readings correlate with steatosis and may give insight to graft outcomes such as early allograft dysfunction and graft loss.

Transplant International 2021; 34: 709–720

Key words

deceased donation, fatty liver disease, hepatic steatosis, liver transplantation outcomes, medical device

Received: 5 October 2020; Revision requested: 30 October 2020; Accepted: 15 January 2021; Published online: 4 March 2021

Introduction

Demand for liver transplantation continues to rise with the increase in liver failure seen in the UK [1] being mirrored globally, with over a million people dying of

cirrhosis worldwide every year [2]. Liver transplantation remains the only effective treatment for end-stage disease, providing an average of 17–22 years of additional life [1,3,4]. Despite rationing access to the UK waiting list [5], 11% of patients listed in 2016/17 had died and 7%

had been removed from the waiting list within 2 years [6]. This figure may increase given the organ shortages occurring currently as a consequence of COVID-19.

The shortfall in donor organs has led to an increase in the use of extended criteria (or marginal) grafts, which are associated with higher rates of early allograft dysfunction and primary nonfunction (PNF) [7,8]. While the concept of these extended criteria livers for transplantation is less well defined than in kidney transplantation, donor factors include graft steatosis, donation after circulatory death (DCD), prolonged ICU stay and older age. The decision about whether to accept a more marginal graft for implantation for a given recipient has to be offset against an increased waiting list mortality from waiting longer for a more optimal graft [9]. Although the visual appearance of the liver (a coarse proxy for steatosis) is known to be associated with poorer outcome [8], predictive models for graft failure have erred away from its inclusion due to its subjective nature and the limitations of any categorical descriptions [8,10].

Transplantation of severely steatotic donor livers is associated with a higher incidence of postoperative complications, early allograft dysfunction (EAD), primary nonfunction (PNF), prolonged ICU stay as well as poorer 1-year graft survival [11–15]. At present, there is no consensus definition of what significant donor steatosis is or how to best quantify it [12]. While donor BMI is a useful predictor of steatosis [16], the gold standard remains histopathological evaluation [17], although this is subjective and is open to significant inter-observer bias [18] with the data typically only available postimplantation. Further, not every transplant centre has 24-hour access to pathological services and its benefits have to be balanced against the increase in cold ischaemia time resulting from tissue processing and analysis in a liver where minimising storage time is paramount. While frozen section at the time of retrieval may help to reduce this inherent delay, it overestimates the extent of steatosis [19] and, in our experience, may misinterpret transient vacuolation as significant fat. Together these practical difficulties and limitations have restricted its routine use [12,20,21].

Preprocurement ultrasound is unable to accurately or reliably predict the degree of steatosis [22], and while cross-sectional imaging by MRI or CT may allow for more objective quantification of hepatic steatosis [12,23,24], it is difficult to envisage that this will be widely available or cost-effective for the assessment of grafts in such a time-constrained situation. This leaves a surgeon's assessment of steatosis using a combination of

visual inspection and palpation, which is unreliable and open to significant bias [22]. An accurate and reproducible real-time test for assessing the degree of steatosis in a donor organ is essential to facilitate safe transplantation, aid research into new models for predicting PNF and EAD accurately, and facilitate informed discussions with patients about risk.

We have previously demonstrated in a preclinical study using optical spectroscopy techniques that backscatter of red and near infrared light from immediately beneath the liver surface showed a correlation coefficient of 0.85 in humans when referenced to clinical haematoxylin and eosin (H&E)-stained biopsies [25]. This led us to develop a portable handheld device which, when placed against the surface of the donor liver, allowed us to evaluate the degree of hepatic steatosis. Here, we report on the pilot study correlating the device's readings with liver transplant outcomes.

Materials and methods

This is a prospective observational cohort study of consecutive patients undergoing liver only transplantation at Addenbrooke's Hospital, Cambridge between August 2011 and May 2014 were recruited to participate in this study; split liver transplants were excluded. All consenting patients who underwent transplantation of a liver alone within the study period were included in the study. Outcome data were collected along with other factors that might predict EAD/PNF such as donor type, donor age and ischaemic time. EAD was defined using both the binary Olthoff criteria [26] and the continuous Model of Early Allograft Function (MEAF) scale [27,28]. Primary nonfunction (PNF) was defined as poor graft function necessitating retransplantation or culminating in death within 14 days, excluding rejection and vascular thrombosis. Cold ischaemic time was defined as the time between commencement of cold perfusion in the donor and reperfusion in the recipient.

The implanting surgeon, blinded to the optical backscatter readouts, was asked to grade the degree of steatosis as none, mild, moderate or severe based on visual inspection and palpation during preimplantation benchwork; these categories form part of the returns used by the National Health Service Blood and Transplant organisation in the UK.

The probe

In previously published work, we have described in detail the principles of and technology underpinning

red and near infrared light backscatter measurements to assess hepatic steatosis [25]. Briefly, a custom-made diffuse reflectance (DF) optical fibre probe attached to a spectrometer was used to measure tissue absorbance and backscatter by the liver.

For this work, a compact and portable handheld probe was developed (Medicines & Healthcare products Regulatory Agency (MHRA) approval CI/2011/0004). The probe emits red and near infrared light from the tip of the device via two light-emitting diodes (LEDs) linked via optical fibres (see Figure S1) and measures the amount of backscatter of light from approximately 2mm into the liver tissue via two photodiodes, also coupled via optical fibres to the tip of the device. Measurements are taken by placing the device against the surface of the liver, with the amount of backscatter represented on a digital display in arbitrary units. The readings are automatically logged to memory in the device, with a timestamp, for later upload to a PC. To ensure a sterile measurement, the tip of the device includes a disposable cap, which is attached to the probe before use.

The probe was placed against the surface of the liver and readings were taken from 4 pre-specified sites (2 on the right lobe, 2 on the left) from each donor liver during retrieval, pre-implantation benchwork and following reperfusion. The mean reading from the 4 sites was used in the analysis. The absorption largely reflects blood within the liver, while scattering is specific for the size, density and cellular constituents of tissue. We have previously shown that differences in scatter between livers of different patients correlates with differences in the lipid content (steatosis) [25], but equally other cellular processes may affect backscatter.

Histopathology

Pre-implantation core biopsies of the donor livers were taken on the backtable. These biopsies were immediately split with half being snap frozen, stored at -80°C to subsequently allow frozen sections to be cut for oil red O staining and the remainder formalin-fixed and processed to paraffin with sections cut and stained with haematoxylin and eosin (H&E). As previously described [25,29], the extent of steatosis and reperfusion injury were scored by a histopathologist with a special interest in liver disease, blinded to the macroscopic description from the surgeon and optical backscatter results. Macrovesicular steatosis was further subdivided as either large or small droplet in line with others [30,31]. Large droplet macrovesicular steatosis was characterised by a single large fat droplet

in hepatocyte cytoplasm, displacing the nucleus to the edge of the cell. This was quantified based on the percentage of large droplet fat occupying the surface area of the parenchyma and given a score 0 to 3 (Table S1). This was subdivided as none or mild (score 0 to 1) or moderate to severe (score greater than or equal to 2). Small droplet macrovesicular steatosis (termed vacuolation by ourselves) consists of multiple small and tiny lipid droplets in the cytoplasm, all being less than size of the nucleus, which retained its central position; this was graded using a score 0 to 2 depending on their extent (Table S1) and termed none to mild (score 0 to 1) or moderate to severe (score 2). Total Fat score was a sum of the small and large droplet scores with none to mild (score 0 to 2) or moderate to severe (score greater than or equal to 3). Microvesicular steatosis refers to the intracytoplasmic accumulation of tiny vesicles within hepatocytes and is a result of severe mitochondrial dysfunction [32]. Given that it highly unlikely that a liver from an affected patient would even be considered as a donor liver [30] and is typically seen in $< 1\%$ of transplanted livers [33], we did not formally score the degree of microvesicular steatosis.

Statistics

Groups were analysed with the aid of Prism 8 for Mac OSX (Graphpad Software, La Jolla, USA); statistical methods are referred to specifically in the results section. Briefly, transplant characteristics were compared using Fisher's exact test, chi-squared or the Mann-Whitney test, as appropriate. Concordance was correlated using the methods described by Lin [34]. Unadjusted graft and patient survival were displayed using Kaplan-Meier plots and curves compared by Mantel-Cox analysis.

Ethical approval

Prospective ethical approval was granted for the project by the Regional Ethics Committee (Ref: 10/H0308/94). The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. No organs from executed prisoners were used in this study.

Results

Of 97 patients (77 (79%) DBD, 20 (21%) DCD) underwent liver only transplantation at Addenbrooke's

Hospital in the study period and consented to the study. No patients had undergone any form of machine perfusion in this study. Donor and recipient demographics and outcomes are summarised in Supplemental Tables 2 and 3.

The overall 1- and 5-year patient survival was 91.7% and 86.2%, respectively, while the 1- and 5-year death-censored graft survival was 92.6% and 89.2% in this mixed cohort of DCD and DBD grafts (Figure S2). The

1- and 5-year graft and patient survival data are summarised in Table 1.

Backscatter readings

There was excellent concordance between optical readings taken in the donor, on the backtable and postreperfusion with concordance greater than 0.85 (data summarised in Table 2).

Table 1. One and five-year graft and patient survival. Mantel–Cox comparison of survival was performed.

| | Graft Survival | | Mantel–Cox | Patient Survival | | Mantel–Cox |
|---|----------------|-------|-------------------------|------------------|-------|-------------------------|
| | 1 | 5 | | 1 | 5 | |
| Overall | 92.6% | 89.2% | | 94.5% | 86.2% | |
| <i>Donor type</i> | | | | | | |
| DBD | 94.4% | 89.9% | <i>P</i> = 0.81 | 93.2% | 86.3% | <i>P</i> = 0.99 |
| DCD | 86.4% | 86.4% | | 87.0% | 82.4% | |
| <i>Donor BMI</i> | | | | | | |
| <30 kg/m ² | 92.4% | 91.0% | <i>P</i> = 0.74 | 92.6% | 87.4% | <i>P</i> = 0.26 |
| >30 kg/m ² | 92.3% | 84.7% | | 85.7% | 78.6% | |
| <i>Large droplet macrovesicular steatosis</i> | | | | | | |
| None to mild (score 0 to 1) | 92.5% | 89.7% | <i>P</i> = 0.24 | 90.2% | 83.9% | <i>P</i> = 0.70 |
| Moderate to severe (score ≥ 2) | 85.7% | 71.4% | | 100% | 85.7% | |
| <i>Small droplet macrovesicular steatosis</i> | | | | | | |
| None to mild (score 0 to 1) | 93.7% | 90.2% | <i>P</i> = 0.041 | 92.7% | 85.0% | <i>P</i> = 0.046 |
| Moderate to severe (score ≥ 2) | 78.0% | 78.0% | | 85.1% | 80.0% | |
| <i>Total fat score</i> | | | | | | |
| None to mild (score 0 to 2) | 95.3% | 93.5% | <i>P</i> = 0.046 | 93.8% | 87.4% | <i>P</i> = 0.098 |
| Moderate to severe (score ≥ 3) | 87.0% | 80.3% | | 84.4% | 81.3% | |
| <i>Reperfusion Injury</i> | | | | | | |
| 0 | 91.7% | 91.7% | <i>P</i> = 0.68 | 100% | 91.7% | <i>P</i> = 0.76 |
| 1 | 89.9% | 85.7% | | 90.4% | 86.4% | |
| 2 | 95.2% | 89.6% | | 85.7% | 80.7% | |
| 3 | 100% | 100% | | 100% | 100% | |
| <i>Olthoff</i> | | | | | | |
| No | 94.5% | 91.5% | <i>P</i> = 0.28 | 94.5% | 85.9% | <i>P</i> = 0.70 |
| Yes | 86.1% | 81.5% | | 83.3% | 79.1% | |
| <i>MEAF</i> | | | | | | |
| <4 | 96.7% | 96.7% | <i>P</i> = 0.072 | 100% | 89.3% | <i>P</i> = 0.55 |
| 4–7 | 88.2% | 88.2% | | 90.1% | 88.3% | |
| 7–9 | 100% | 83.0% | | 85.7% | 71.1% | |
| >9 | 50% | 50% | | 66.7% | 66.7% | |
| <i>Surgeon Assessment</i> | | | | | | |
| Healthy | 93.8% | 92.1% | <i>P</i> = 0.29 | 91.2% | 86.5% | <i>P</i> = 0.44 |
| Suboptimal | 93.0% | 85.5% | | 89.7% | 82.6% | |
| <i>Surgeon Assessment of Steatosis</i> | | | | | | |
| None | 96.0% | 96.0% | <i>P</i> = 0.11 | 92.1% | 90.0% | <i>P</i> = 0.24 |
| Mild | 93.1% | 86.2% | | 90.0% | 83.3% | |
| Moderate | 86.7% | 72.7% | | 86.7% | 72.7% | |
| <i>Post-transplant Acute Kidney Injury</i> | | | | | | |
| No AKI | 97.7% | 90.3% | <i>P</i> = 0.37 | 93.1% | 90.7% | <i>P</i> = 0.29 |
| AKI | 90.2% | 81.9% | | 86.8% | 80.9% | |

In bold purely to highlight they are statistically significant.

Table 2. Concordance of measurements taken in the donor, on the backtable and postreperfusion. Lin's concordance [34] was calculated using data using from all patients within the study ($n = 97$).

| Readings | Lin's concordance coefficient | 95% confidence interval |
|--|-------------------------------|-------------------------|
| <i>Reading in donor versus on backtable</i> | | |
| Red | 0.85 | 0.82–0.87 |
| Infrared | 0.96 | 0.95–0.97 |
| <i>Reading in donor versus postreperfusion in recipient</i> | | |
| Red | 0.91 | 0.88–0.93 |
| Infrared | 0.85 | 0.80–0.89 |
| <i>Reading in on backtable versus postreperfusion in recipient</i> | | |
| Red | 0.85 | 0.78–0.89 |
| Infrared | 0.88 | 0.82–0.92 |

There were no grafts transplanted with a histological large droplet macrovesicular steatosis score of 3 in this study. While there was no significant difference in survival between patient or graft survival depending upon the severity of large droplet macrovesicular steatosis (Table 1 and Fig. 1a, Mantel–Cox $P = 0.70$ and $P = 0.24$), there was a significant decrease in survival of patients receiving allografts with more severe small droplet macrovesicular steatosis (Fig. 1b, $P = 0.046$ and $P = 0.041$). A higher total macrovesicular steatosis score in the donor organ was associated with poorer graft survival ($P = 0.046$), but not patient survival ($P = 0.098$).

Backscatter readings were significantly higher in grafts with more extensive large droplet ($P < 0.0001$) and small droplet macrovesicular steatosis ($P = 0.0001$) (Fig. 1). Oil Red O staining also strongly correlated with backscatter readings (Pearson's Rank 0.53 (95% CI 0.35–0.67, $R^2 = 0.28$, $P < 0.0001$). In general, increased severity of steatosis as judged by the surgeon was associated with an increase in both the large ($P = 0.0002$) and small droplet macrovesicular steatosis histological score ($P = 0.0007$, Figure S3), although the overall concordance between the surgeon and histology was relatively poor (coefficient 0.41).

Graft survival and early allograft dysfunction

In those grafts with EAD defined according to Olthoff criteria, the 1-year graft survival was 86.5% compared 94.5% (Fig. 2a); the backscatter reading was significantly greater in these livers ($P = 0.0067$).

When looking at survival by MEAF groupings [28], those with a score less than 4 had a 100% 1-year patient and 96.7% 1-year graft survival compared to patient survival of 66.7% and 50%, respectively, for those with a score greater than 9 (Fig. 2b). A higher MEAF score

was associated with a significantly greater backscatter ($P = 0.0097$) (Fig. 2b). Increased backscatter readings subsequently correlated with increased MEAF scores (Pearson's $r = 0.33$ (95% CI 0.14 to 0.49), $R^2 = 0.11$, $P = 0.0011$) (Fig. 2c); this was particularly true in organs from DCD donors ($r = 0.77$ (95% CI 0.50 to 0.90), $R^2 = 0.59$, $P < 0.0001$) compared to DBD ($r = 0.23$ (95% CI 0.0019 to 0.43), $R^2 = 0.52$, $P = 0.048$).

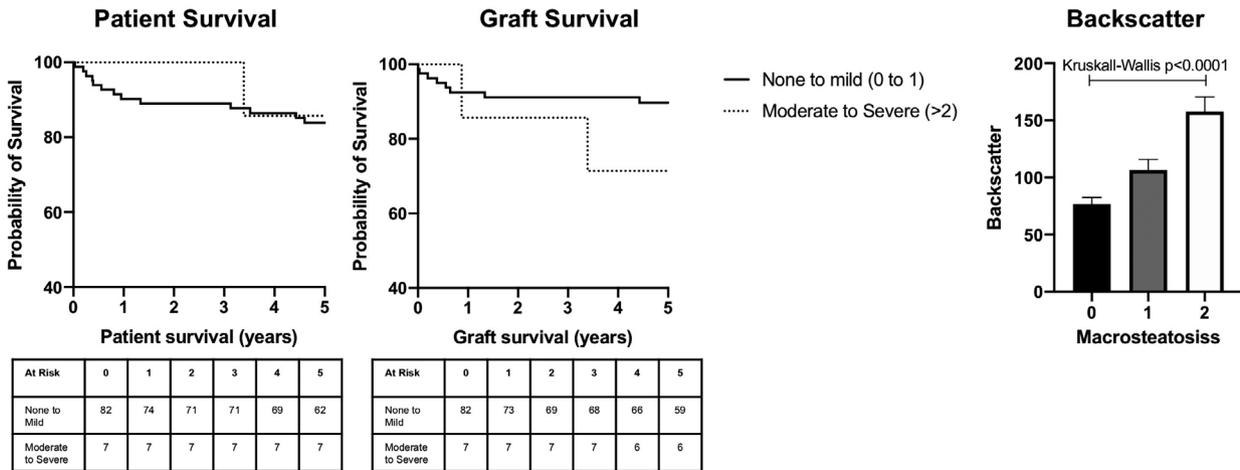
On inspection by the surgeon, 45/97 (46%) were visually macrosteatotic (Table S3). Surgeon assessment of grafts failed to accurately predict differences in 1-year graft (93.8%) or patient (91.1%) survival in those deemed 'healthy' compared to 93.0% and 93.0% in those deemed 'suboptimal' (Fig. 3a). There was a significant difference between the backscatter readings between 'suboptimal' and 'healthy' livers (Mann–Whitney $P = 0.0004$) (Fig. 3a). No grafts deemed to be severely steatotic by the implanting surgeon were implanted during this study. One-year graft survival for those deemed to not be fatty was 96.0% compared to 93.1% (mildly steatotic) and 86.7% (moderately steatotic), with corresponding 1-year patient survival of 92.1%, 90.0% and 86.7% (Fig. 3b). The backscatter reading was significantly higher in the moderately steatotic livers compared to those with minimal or no steatosis ($P = 0.0054$) (Fig. 3b).

Backscatter readings > 100 units were associated with worse graft survival (Mantel–Cox, $P < 0.0001$, Fig. 4), but not poorer patient survival ($P = 0.17$) (summarised in Table 3).

Acute kidney injury after liver transplantation

The development of acute kidney injury after liver transplantation was associated with a reduction in 1-year graft survival from 97.7% to 90.2% and patient

(a) Large droplet Macrosteatosis



(b) Small Droplet Macrosteatosis

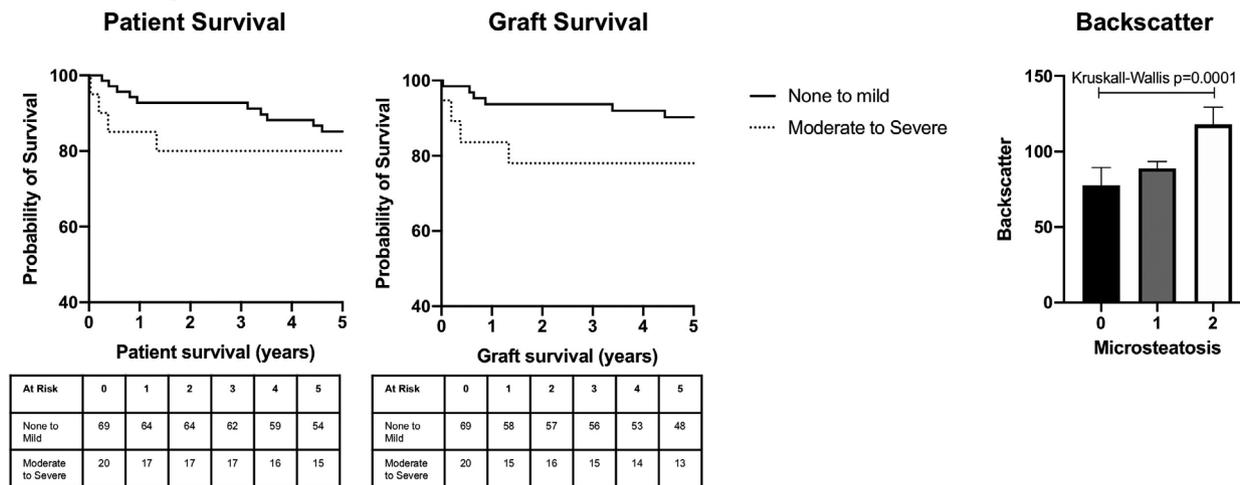


Figure 1 Histological scoring and survival. Patient and graft survival by histological evaluation of large and small droplet macrovesicular steatosis are displayed in Kaplan–Meier plots. There was no difference in survival between patient or graft survival depending upon the severity of large droplet macrovesicular steatosis in the donor organ (Mantel–Cox $P = 0.70$ and $P = 0.24$) (a). However, there was a significant decrease in survival patients receiving allografts with more severe small droplet macrovesicular steatosis (Mantel–Cox $P = 0.046$ and $P = 0.041$) (b). Increased backscatter readings were seen with higher scores of large (Kruskal–Wallis $P < 0.0001$) and small droplet macrovesicular steatosis ($P = 0.0001$).

survival of 93.1% to 86.8% (see Table 1 and Figure S4). There was not a significantly increased MEAF score between the 2 groups ($P = 0.09$), but the backscatter reading was significantly higher ($P = 0.0027$).

Predicting graft loss

Using a multiple logistic regression model of donor factors known pre-implantation (donor age, donor BMI, donor type and backscatter reading), it was possible to generate a model to predict graft loss (Logit[P (graft loss)] = (donor age x 0.05853) + (donor BMI x -0.01520) + (Donor type (0 = DBD, 1 = DCD) x

-0.01323) + (Backscatter x 0.009219) - 5.817). In spite of the limitations of performing this sort of analysis using only 97 separate data points, the area under the curve analysis showed the model to perform reasonably well (AUC 0.75 (95% CI 0.60-0.90), $P = 0.0072$) (Fig. 5). Multiple logistic regression looking at the same donor factors (but excluding backscatter), performed less well (AUC 0.66 (95% CI 0.50-0.82), $P = 0.093$). Simple logistic regression to look at graft loss, using only backscatter on its own performed similarly at predicting graft loss (AUC 0.75 (0.58-0.91), $P = 0.0045$) (Fig. 5). The odds ratio for graft loss was 1.004 (95% CI 1.00-1.01) for every unit increase in backscatter.

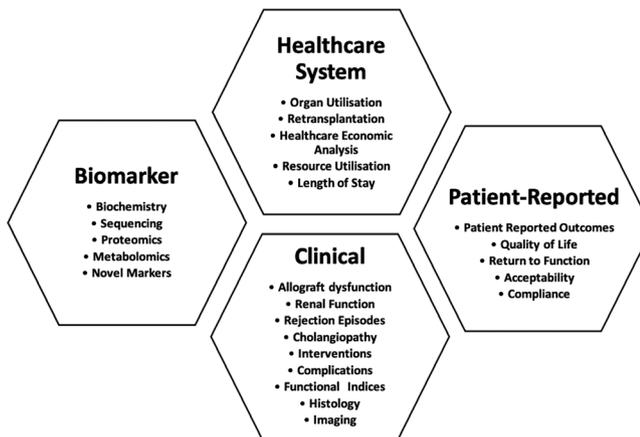


Figure 2 Early Allograft Dysfunction by Olthoff Criteria and MEAF grouping. In those grafts with EAD defined according to Olthoff criteria, the 1-year graft survival was 86.5% compared to those that did not meet the criteria 94.5% (a); this difference was not statistically significant (summarised in Table 1) although this may be related to sample size. The backscatter readings were significantly higher in those livers ($P = 0.0067$) meeting the Olthoff Criteria (a). When looking at survival by MEAF grouping, those with a score less than 4 had a 100% 1-year patient and 96.7% 1-year graft survival compared to patient survival of 66.7% and 50%, respectively, for those with a score greater than 9 (b). Increased MEAF was associated with increased graft or patient survival (Mantel–Cox $P = 0.031$ and $P = 0.55$, respectively). A higher MEAF score was associated with a significantly greater backscatter (Kruskal–Wallis $P = 0.0097$) (b). Increased backscatter readings subsequently correlated with increased MEAF scores (Pearson’s $r = 0.33$ (95% CI 0.14 to 0.49), $R^2 = 0.11$, $P = 0.0011$) (c); this was particularly true in organs from DCD donors ($r = 0.77$ (95% CI 0.50 to 0.90), $R^2 = 0.59$, $P < 0.0001$) compared to DBD ($r = 0.23$ (95% CI 0.0019 to 0.43), $R^2 = 0.52$, $P = 0.048$).

Discussion

Here we demonstrate that real-time measurements of backscatter of red and near infrared light from the liver whilst in the donor, on the backtable and after implantation in the recipient is a feasible approach to assessing in real-time the degree of hepatic steatosis in the setting of liver transplantation. As we had previously seen in a preclinical study of murine and human liver specimens [25], backscatter strongly correlated with the extent of hepatic steatosis as determined by Oil Red O staining (Pearson’s Rank 0.53, $P < 0.0001$) and as scored by a transplant histopathologist (Fig. 2). While increased severity of steatosis as judged by the surgeon was associated with an increase in both the large and small droplet macrovesicular steatosis histological score (Figure S3), the overall concordance between the surgeon and histology was relatively poor (coefficient 0.41). This probe, therefore, may help to overcome the

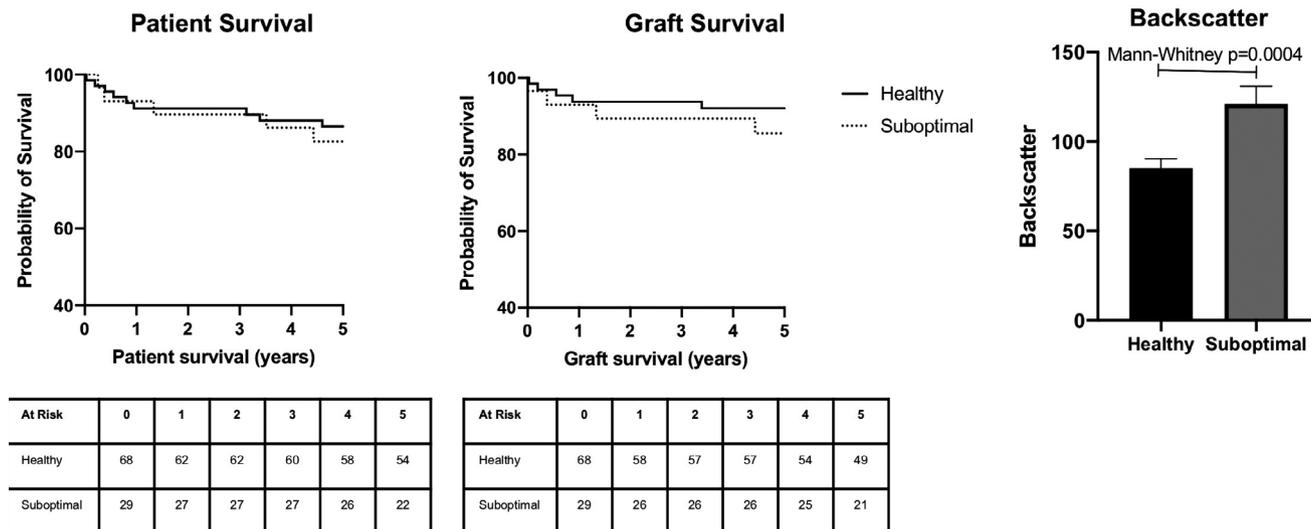
inherent problem of inter-observer bias seen when relying on arbitrary macroscopic inspection by a surgeon or microscopic evaluation by a histopathologist. While both may remain important within an individual centre, they prevent standardisation of reporting the degree of steatosis in research and across clinical trials, where heterogeneity in approach and inter-observer bias can make outcomes difficult to interpret [18,22].

As well as correlating with the extent of steatosis, measurement of optical backscatter correlated with early allograft dysfunction according to both the Olthoff and MEAF parameters (Fig. 2) as well as with acute kidney injury post-liver transplantation (Figure S4). More complex multiple logistic regression analysis of this data is limited by the sample size, however, we demonstrated in principle how backscatter could be incorporated into a predictive algorithm utilising in this case donor factors identified at the time of procurement/implantation looking at graft failure as the endpoint. We demonstrated that backscatter measurements were predictive of graft loss (Fig. 5). Further evaluation will require large numbers from a multi-centre study to validate or refute these findings and incorporate both donor and recipient factors as well as other novel readouts into a highly predictive algorithm that will help quantify risk the of a given allograft to a particular recipient. Increased backscatter was not predictive of patient survival, in part due to the ready availability of early retransplantation at that time in the UK, and also potentially the small sample size.

While we utilised a categorical scoring system for assessing the extent of steatosis, others have recently developed a digital algorithm to quantify steatosis in tissue sections, which may make histological evaluation in future clinical studies more sensitive [35], though its usefulness in preimplantation decision-making may be limited by its inherent retrospective nature, and also the time taken to prepare and scan a sample.

Evers *et al* have also previously demonstrated good concordance with the histological quantification of fat by a similar approach in the context of liver resection surgery [36]. Fibroscan, CT and MRI have also been used successfully as a noninvasive tools for quantifying fat in the field of nonalcoholic fatty liver disease [37], but their role may be limited in the setting of liver transplantation by their portability, availability 24 hours per day across all potential donor hospitals, national laws about pre-mortem interventions in donors and cost. Other groups have also demonstrated the effectiveness of analysis of smartphone photographs and digital analysis software to assess the extent of macrovesicular

(a) Healthy versus suboptimal grafts



(b) Surgeon Assessment of steatosis

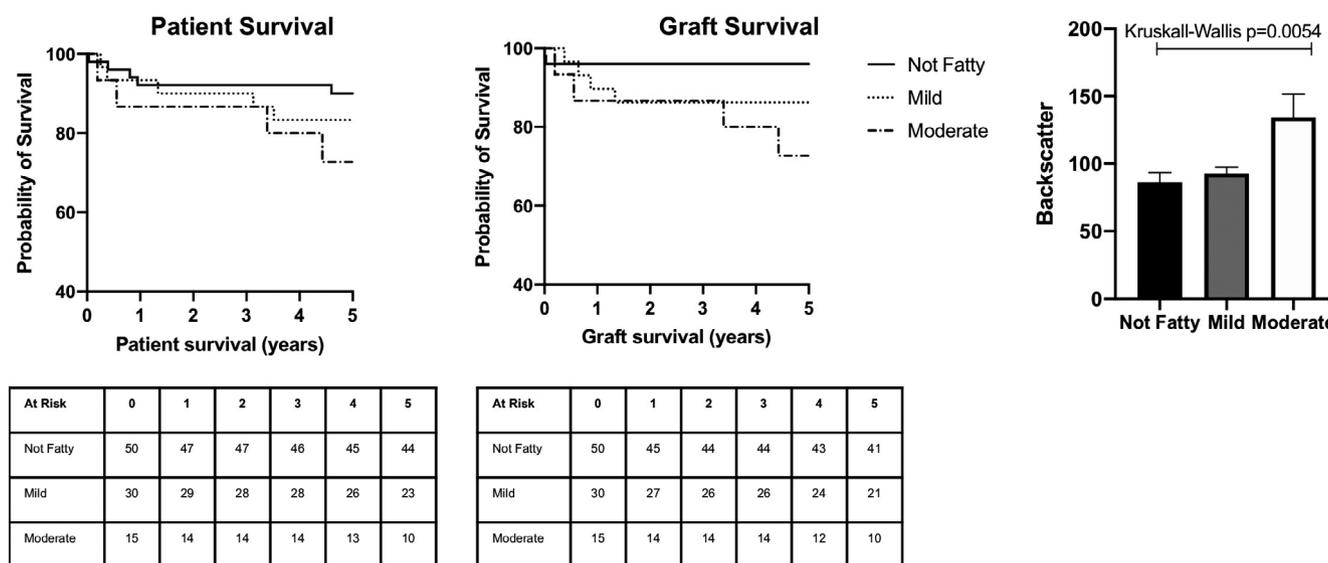


Figure 3 Backscatter by surgeon assessment. Surgeon assessment of grafts did not predict differences in 1-year graft (93.8%) or patient (91.1%) survival in those deemed ‘healthy’ compared to those that were ‘suboptimal’ 93.0% and 93.0%, respectively (a). There was a significant difference between the backscatter readings between ‘suboptimal’ and ‘healthy’ livers (Mann–Whitney $P = 0.0004$) (a). No grafts deemed to be severely steatotic by the implanting surgeon were implanted during this study. 1-year graft survival for those deemed to not be fatty was 96% compared to 93.1% (mildly steatotic) and 86.7% (moderately steatotic), with corresponding 1-year patient survival of 92.1%, 90.0% and 86.7% (b). The backscatter reading was significantly higher in the moderately steatotic livers compared to those with minimal or no fat (Kruskal–Wallis $P = 0.0054$) (b).

steatosis [38,39]. Novel biomarkers for fatty liver disease are also currently being investigated [40], but would need to be validated in the context of organ donors.

While this study utilises a custom-built prototype, it is likely that a commercially available device could be developed and would prove to be cost-effective while not causing an increase in cold ischaemia (in contrast

to biopsy examination) and could be utilised in centres who do not have 24-hour access to a transplant histopathologist. It also avoids the potential risk of bleeding or bile leak from the biopsy site.

While declining the offer of a steatotic liver has been shown to increase an individual’s waiting list mortality [41], the unpredictable response of steatotic

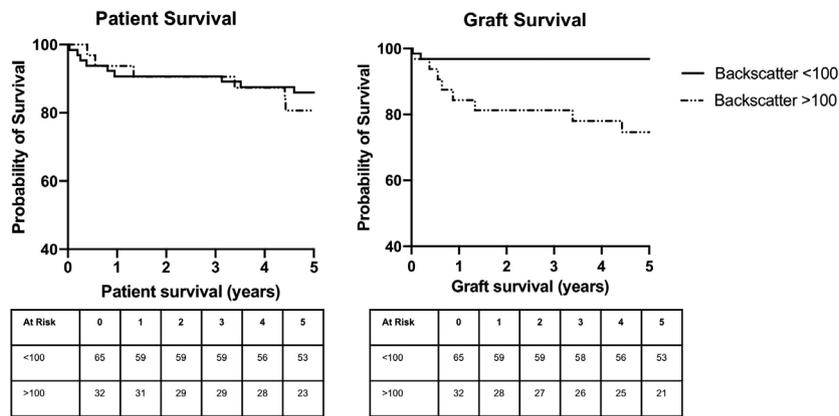


Figure 4 Backscatter readings and survival. Kaplan–Meier plots of graft and patient survival comparing survival in cohorts of patients backscatter readings greater or less than 100. Backscatter readings > 100 were associated with worse graft (Mantel–Cox, $P < 0.0001$), but not patient survival ($P = 0.085$) (summarised in Table 3).

Table 3. Backscatter and graft and patient survival.

| | Graft Survival | | Mantel–Cox | Patient Survival | | Mantel–Cox |
|-----------------------------|----------------|-------|-----------------------------------|------------------|-------|-------------|
| | 1 | 5 | | 1 | 5 | |
| <i>Backscatter Readings</i> | | | | | | |
| Less than 100 | 96.9% | 96.9% | | 96.9% | 96.9% | |
| Greater than 100 | 84.4% | 74.9% | $P < 0.0001$ | 84.3% | 74.6% | $P = 0.085$ |

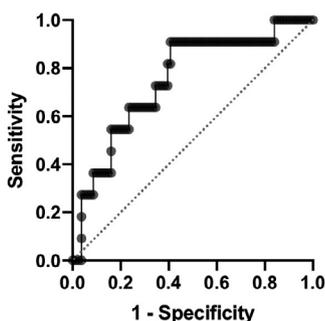
In bold purely to highlight they are statistically significant.

livers to reperfusion, with an increased severity of ischaemia-reperfusion injury (IRI) and subsequently increased rates of PNF, EAD and post-liver transplant acute kidney injury mean that there is an understandable reluctance to routinely transplant such livers [21,42]. As the demand for organ transplantation continues to remain high and the epidemic of obesity in the west is resulting in higher rates of steatosis in donor organs [43,44], we will inevitably need to implant more steatotic livers in the future. Others have shown that one possible solution to overcoming the excess risk of a steatotic organ is to allocate steatotic organs to ‘preferred recipients’ (defined as first-time recipients with a MELD 15–34, without primary biliary cirrhosis and not on life support prior to transplantation), as these recipients have no significant increase in mortality or graft loss when receiving steatotic compared to nonsteatotic livers [45]. While their study was necessarily performed retrospectively on biopsies using registry data, one could envisage that backscatter in the donor or on the backtable could be used prospectively to guide these decisions, without the need to wait for biopsy results. The continuous nature and spread of the potential backscatter data is also

such that the allocation process could be less dichotomous and identify a greater range of potential recipients that would benefit from a given organ and help identify risk in each individual.

As well as matching the ‘high risk’ organ to the ‘low risk’ recipient, another strategy to mitigate the excess risk of steatotic livers is to utilise novel technology or therapeutics to identify which livers are safe to implant and identify those which need some other intervention, such as *ex situ* machine perfusion [46–50], that is personalising or targeting therapy for a given donor liver. Assessment of backscatter seems to be one such potential objective strategy to stratify risk and rather than being used in isolation, we envisage that optical backscatter measurements would be incorporated in a more complex model utilising all available data on the donor and recipient (Fig. 6) to really inform the patient and surgeon about personalised risk, especially when coupled with better modelling of individual waiting list mortality. With the advent of machine perfusion, these readings could be used by the retrieval centre and/or national organ allocation service to stratify organs into ‘safe to transplant’, ‘not safe to transplant’, ‘needs further viability testing’ [48] and in the future an

(a) Multiple logistic regression model (including Donor BMI, donor age, donor type and backscatter) of graft loss



(b) Simple logistic regression model of backscatter and graft loss

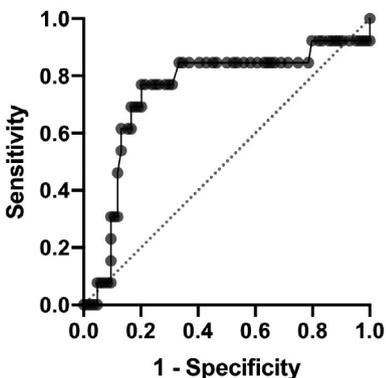


Figure 5 Logistic regression analysis of graft loss. Multiple logistic regression modelling of factors known preimplantation (donor age, donor BMI, donor type and backscatter reading) was performed and used to generate model to predict graft loss ($\text{Logit}[P(\text{graft loss})] = (\text{donor age} \times 0.05853) + (\text{donor BMI} \times -0.01520) + (\text{Donor type } (0 = \text{DBD}, 1 = \text{DCD}) \times -0.01323) + (\text{Backscatter} \times 0.009219) - 5.817$). Receiver operating characteristic (ROC) curves of this analysis were plotted (a), and area under the curve (AUC) analysis showed the model to perform reasonably well (AUC 0.75 (95% CI 0.60–0.90), $P = 0.0072$). Simple logistic regression to look at graft loss, using only backscatter alone was also performed and ROC curve was plotted (b). This performed similarly at predicting graft loss (AUC 0.75 (95% CI 0.58–0.91), $P = 0.0045$).

additional arm that would recommend directed therapy (see Fig. 6). Furthermore, this technology may help validate the effectiveness of *ex situ* ‘defatting’ strategies in a given liver undergoing machine perfusion that are currently being developed [51,52], without the need for serial biopsies.

In conclusion, the data from this pilot study are promising, but needs more extensive validation alongside other novel noninvasive real-time approaches to generate robust data to support their further use and/or generate more accurate predictive models. If further validated, measuring optical backscatter in donor livers may have a role in the safe allocation of livers for

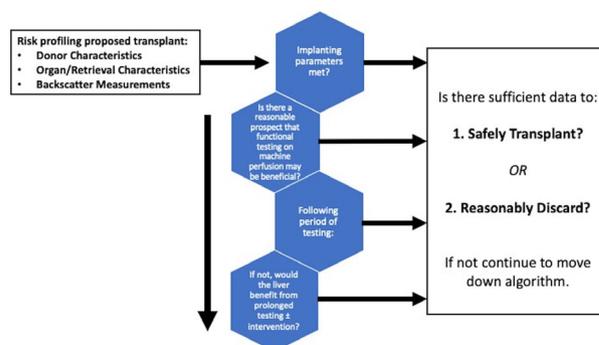


Figure 6 Hypothesised future decision-making algorithm.

transplantation and inform discussions between clinicians and patients about the risk of a given donor organ [53]. In addition, it may allow increased utilisation by helping to determine a subset of livers requiring specific pre-treatments before transplantation, such as targeted drug therapy or defatting during *ex situ* perfusion [54,55].

Authorship

JR, LR, AB, CW and PR have made a substantial contribution to the conception, data collection, analysis and writing of this manuscript. PR designed and produced the probe. SD performed histological analyses and aided with analysis and writing of this manuscript. JM and AF made a significant contribution to the data collection.

Funding

We would like to acknowledge support from the Evelyn Trust who funded this study as well as the National Institute for Health Research (NIHR) Blood and Transplant Research Unit (NIHR BTRU) in Organ Donation and Transplantation at the University of Cambridge in collaboration with Newcastle University and in partnership with NHS Blood and Transplant (NHSBT). JR was also supported by a NIHR Academic Clinical Lectureship. The Human Research Tissue Bank is supported by the NIHR Cambridge Biomedical Research Centre. The University of Cambridge has received salary support in respect of CJEW from the NHS in the East of England through the Clinical Academic Reserve.

Conflict of interest

While working on this study, LR was wholly employed by the University of Cambridge / Cambridge University

Hospitals. She has subsequently moved to work for the machine perfusion device company OrganOx, who have no commercial or other interest in this body of work and as such LR feels that there is no significant conflict of interest to declare. The remaining authors have no other conflicts of interest to declare.

Acknowledgements

We would like to specifically acknowledge the contribution of Diane Walters and all staff at The Human Research Tissue Bank in Cambridge, whose contribution enabled this study to happen successfully.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Diffuse reflectance (DF) optical fibre probe.

Figure S2. Overall Graft and Patient survival.

Figure S3. Histological Scoring and Surgeon's Assessment of steatosis.

Figure S4. Histological Scoring and Surgeon's Assessment of steatosis.

Table S1. Histological Scoring.

Table S2. Donor demographics.

Table S3. Recipient details.

REFERENCES

- Leon DA, McCambridge J. Liver cirrhosis mortality rates in Britain from 1950 to 2002: an analysis of routine data. *Lancet* 2006; **367**: 52.
- Byass P. The global burden of liver disease: a challenge for methods and for public health. *BMC Med* 2014; **12**: 159.
- Barber K, Blackwell J, Collett D, Neuberger J. Life expectancy of adult liver allograft recipients in the UK. *Gut* 2007; **56**: 279.
- Abt PL, Desai NM, Crawford MD, et al. Survival following liver transplantation from non-heart-beating donors. *Ann Surg* 2004; **239**: 87.
- Neuberger J, James O. Guidelines for selection of patients for liver transplantation in the era of donor-organ shortage. *Lancet* 1999; **354**: 1636.
- NHSBT. ANNUAL REPORT ON LIVER TRANSPLANTATION 2018/19 2019 [Available from: <https://nhsbt.blob.core.windows.net/umbraco-assets-corp/16782/nhsbt-liver-transplantation-annual-report-2018-19.pdf>]
- Kim WR, Lake JR, Smith JM, et al. Liver. *Am J Transplant* 2016; **16**(Suppl 2): 69.
- Collett D, Friend PJ, Watson CJ. Factors Associated With Short- and Long-term Liver Graft Survival in the United Kingdom: Development of a UK Donor Liver Index. *Transplantation* 2017; **101**: 786.
- Taylor R, Allen E, Richards JA, et al. Survival advantage for patients accepting the offer of a circulatory death liver transplant. *J Hepatol* 2019; **70**: 855.
- Feng S, Goodrich NP, Bragg-Gresham JL, et al. Characteristics Associated with Liver Graft Failure: The Concept of a Donor Risk Index. *Am J Transplant* 2006; **6**: 783.
- Spitzer AL, Lao OB, Dick AA, et al. The biopsied donor liver: incorporating macrosteatosis into high-risk donor assessment. *Liver Transpl* 2010; **16**: 874.
- McCormack L, Dutkowski P, El-Badry AM, Clavien P-A. Liver transplantation using fatty livers: Always feasible? *J Hepatol* 2011; **54**: 1055.
- McCormack L, Petrowsky H, Jochum W, Mullhaupt B, Weber M, Clavien PA. Use of severely steatotic grafts in liver transplantation: a matched case-control study. *Ann Surg*. 2007; **246**: 940.
- Li J, Liu B, Yan LN, et al. Reversal of graft steatosis after liver transplantation: prospective study. *Transplant Proc*. 2009; **41**: 3560.
- Doyle MBM. Short- and Long-term Outcomes After Steatotic Liver Transplantation. *Arch Surg* 2010; **145**: 7.
- Rinella ME, Alonso E, Rao S, et al. Body mass index as a predictor of hepatic steatosis in living liver donors. *Liver Transpl* 2001; **7**: 409.
- Silva MA. Putting Objectivity Into Assessment of Steatosis. *Transplantation* 2009; **88**: 620.
- El-Badry AM, Breitenstein S, Jochum W, et al. Assessment of Hepatic Steatosis by Expert Pathologists. *Ann Surg* 2009; **250**: 691.
- Lo IJ, Lefkowitz JH, Feirt N, et al. Utility of liver allograft biopsy obtained at procurement. *Liver Transpl* 2008; **14**: 639.
- McCormack L, Petrowsky H, Jochum W, Mullhaupt B, Weber M, Clavien P-A. Use of Severely Steatotic Grafts in Liver Transplantation. *Ann Surg* 2007; **246**: 940.
- Imber C. Current practice regarding the use of fatty livers: A Trans-Atlantic survey. *Liver Transpl* 2002; **8**: 545.
- Rey JW, Wirges U, Dienes HP, Fries JWU. Hepatic Steatosis in Organ Donors: Disparity Between Surgery and Histology? *Transpl Proc* 2009; **41**: 2557.
- Nickkholgh A, Weitz J, Encke J, et al. Utilization of extended donor criteria in liver transplantation: a comprehensive review of the literature. *Nephrol Dial Transplant*. 2007; **22**: viii29.
- Raptis DA, Fischer MA, Graf R, et al. MRI: the new reference standard in quantifying hepatic steatosis? *Gut* 2012; **61**: 117.
- McLaughlin BL, Wells AC, Virtue S, et al. Electrical and optical spectroscopy for quantitative screening of hepatic steatosis in donor livers. *Phys Med Biol* 2010; **55**: 6867.
- Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* 2010; **16**: 943.
- Pareja E, Cortes M, Hervas D, et al. A score model for the continuous grading of early allograft dysfunction severity. *Liver Transpl* 2015; **21**: 38.
- Richards JA, Sherif AE, Butler AJ, et al. (2020). Model for early allograft function is predictive of early graft loss

- in donation after circulatory death liver transplantation. *Clinical Transplantation*, **34**. <http://dx.doi.org/10.1111/ctr.13982>
29. Ali JM, Davies SE, Brais RJ, et al. Analysis of ischemia/reperfusion injury in time-zero biopsies predicts liver allograft outcomes. *Liver Transpl* 2015; **21**: 487.
 30. Brunt EM. Surgical assessment of significant steatosis in donor livers: The beginning of the end for frozen-section analysis? *Liver Transpl* 2013; **19**: 360.
 31. Yersiz H, Lee C, Kaldas FM, et al. Assessment of hepatic steatosis by transplant surgeon and expert pathologist: A prospective, double-blind evaluation of 201 donor livers. *Liver Transpl* 2013; **19**: 437.
 32. Day CP, Saksena S. Non-alcoholic steatohepatitis: definitions and pathogenesis. *J Gastroenterol Hepatol*. 2002; **17**(Suppl 3): S377.
 33. Yersiz H, Lee C, Kaldas FM, et al. Assessment of hepatic steatosis by transplant surgeon and expert pathologist: a prospective, double-blind evaluation of 201 donor livers. *Liver Transpl*. 2013; **19**: 437.
 34. Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 1989; **45**: 255.
 35. Munsterman ID, van Erp M, Weijers G, et al. A Novel Automatic Digital Algorithm that Accurately Quantifies Steatosis in NAFLD on Histopathological Whole-Slide Images. *Cytometry B Clin Cytom* 2019; **96**: 521.
 36. Evers DJ, Westerkamp AC, Spliethoff JW, et al. Diffuse reflectance spectroscopy: toward real-time quantification of steatosis in liver. *Transpl Int* 2015; **28**: 465.
 37. Lv S, Jiang S, Liu S, Dong Q, Xin Y, Xuan S. Noninvasive Quantitative Detection Methods of Liver Fat Content in Nonalcoholic Fatty Liver Disease. *J Clin Transl Hepatol* 2018; **6**: 217.
 38. Cesaretti M, Poté N, Cauchy F, et al. Noninvasive assessment of liver steatosis in deceased donors: A pilot study. *Liver Transpl* 2018; **24**: 551.
 39. Cesaretti M, Brustia R, Goumard C, et al. Use of Artificial Intelligence as an Innovative Method for Liver Graft Macrosteatosis Assessment. *Liver Transpl* 2020; **26**: 1224.
 40. Ladaru A, Balanescu P, Stan M, Codreanu I, Anca IA. Candidate proteomic biomarkers for non-alcoholic fatty liver disease (steatosis and non-alcoholic steatohepatitis) discovered with mass-spectrometry: a systematic review. *Biomarkers* 2016; **21**: 102.
 41. Jackson KR, Bowring MG, Holscher C, et al. Outcomes after declining a steatotic donor liver for liver transplant candidates in the United States. *Transplantation* 2019.
 42. Escartin A, Castro E, Dopazo C, Bueno J, Bilbao I, Margarit C. Analysis of Discarded Livers for Transplantation. *Transpl Proc* 2005; **37**: 3859.
 43. Moosburner S, Gassner JMGV, Nösser M, et al. Prevalence of Steatosis Hepatis in the Eurotransplant Region: Impact on Graft Acceptance Rates. *HPB Surg* 2018; **2018**: 1.
 44. Jackson KR, Long J, Philosophe B, Garonzik-Wang J. Liver Transplantation Using Steatotic Grafts. *Clin Liv Dis* 2019; **14**: 191.
 45. Jackson KR, Motter JD, Haugen CE, et al. (2020). Minimizing risks of liver transplantation with steatotic donor livers by preferred recipient matching. *Transplantation*, **104**: 1604–1611. <http://dx.doi.org/10.1097/tp.0000000000003052>.
 46. Watson CJ, Kosmoliaptis V, Randle LV, et al. Normothermic perfusion in the assessment and preservation of declined livers prior to transplantation: hyperoxia and vasoplegia - important lessons from the first 12 cases. *Transplantation* 2017; **101**: 1084.
 47. Watson CJE, Jochmans I. From, "Gut Feeling" to Objectivity: Machine Preservation of the Liver as a Tool to Assess Organ Viability. *Curr Transplant Rep* 2018; **5**: 72.
 48. Watson CJE, Kosmoliaptis V, Pley C, et al. Observations on the ex situ perfusion of livers for transplantation. *Am J Transplant* 2018; **18**: 2005.
 49. Nostedt JJ, Skubleny DT, Shapiro AMJ, Campbell S, Freed DH, Bigam DL. Normothermic Ex Vivo Machine Perfusion for Liver Grafts Recovered from Donors after Circulatory Death: A Systematic Review and Meta-Analysis. *HPB Surg* 2018; **2018**: 1.
 50. Detelich D, Markmann JF. The dawn of liver perfusion machines. *Curr Opin Organ Transplant*. 2018; **23**: 151.
 51. Nagrath D, Xu H, Tanimura Y, et al. Metabolic preconditioning of donor organs: defatting fatty livers by normothermic perfusion ex vivo. *Metab Eng* 2009; **11**: 274.
 52. Ceresa CDL, Nasralla D, Jassem W. Normothermic Machine Preservation of the Liver: State of the Art. *Curr Transplant Rep* 2018; **5**: 104.
 53. Kamran S, Conti F, Pomey MP, Baron G, Calmus Y, Vidal-Trecan G. Patients' preferences in transplantation from marginal donors: results of a discrete choice experiment. *Transpl Int* 2017; **30**: 589.
 54. Dengu F, Abbas SH, Ebeling G, Nasralla D. Normothermic Machine Perfusion (NMP) of the Liver as a Platform for Therapeutic Interventions during Ex-Vivo Liver Preservation: A Review. *Journal of Clin Med* 2020; **9**: 4.
 55. Aoudjehane L, Gautheron J, Le Goff W, et al. Novel defatting strategies reduce lipid accumulation in primary human culture models of liver steatosis. *Dis Model Mech* 2020; **13**: 4.