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Mergental, Hynek; Afford, Simon; Mirza, Darius; Laing, Richard; Hubscher, Stefan; Perera, Mapatunage Thamara; Muiesan, Paolo; Isaac, J R; Smith, A; Stephenson, Barnaby; Cilliers, Hentie; Neil, D A H

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Transplantation of Declined Liver Allografts Following Normothermic *Ex-Situ* Evaluation

H. Mergental[1,2,†], M. T. P. R. Perera[1,†], R. W. Laing[2], P. Muiesan[1], J. R. Isaac[1], A. Smith[1], B. T. F. Stephenson[2], H. Cilliers[1], D. A. H. Neil[1], S. G. Hübscher[1], S. C. Afford[2] and D. F. Mirza[1,2,*]

[1]*Liver Unit, Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK*
[2]*National Institute for Health Research (NIHR) Birmingham Liver Biomedical Research Unit and Centre for Liver Research, Institute of Immunology and Immunotherapy, Institute for Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*
**Corresponding author: Darius F. Mirza, darius.mirza@uhb.nhs.uk*
[†]*These authors contributed equally to the study.*

The demand for liver transplantation (LT) exceeds supply, with rising waiting list mortality. Utilization of high-risk organs is low and a substantial number of procured livers are discarded. We report the first series of five transplants with rejected livers following viability assessment by normothermic machine perfusion of the liver (NMP-L). The evaluation protocol consisted of perfusate lactate, bile production, vascular flows, and liver appearance. All livers were exposed to a variable period of static cold storage prior to commencing NMP-L. Four organs were recovered from donors after circulatory death and rejected due to prolonged donor warm ischemic times; one liver from a brain-death donor was declined for high liver function tests (LFTs). The median (range) total graft preservation time was 798 (range 724–951) min. The transplant procedure was uneventful in every recipient, with immediate function in all grafts. The median in-hospital stay was 10 (range 6–14) days. At present, all recipients are well, with normalized LFTs at median follow-up of 7 (range 6–19) months. Viability assessment of high-risk grafts using NMP-L provides specific information on liver function and can permit their transplantation while minimizing the recipient risk of primary graft nonfunction. This novel approach may increase organ availability for LT.

Abbreviations: ALT, alanine transaminase; CIT, cold ischemic time; DBD, donor/donation after brain death; DCD, donor/donation after circulatory death; dWIT, donor warm ischemic time; ld, large droplet; ITU, intensive therapy unit; LFTs, liver function tests; LT, liver transplantation; MS, macrovesicular steatosis; NHS, National Health Service; NMP-L, normothermic machine perfusion of the liver; SCS, static cold storage; sd, small droplet

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Introduction

Deaths from liver disease have soared by 40% in the last decade, killing 11 000 a year in England at an average age of 59 years (1). Liver transplantation (LT) is highly successful in treating end-stage disease, but access is restricted by the number of available organs and approximately 20% of patients die while awaiting transplantation (2–5). To address this, more transplants are performed using high-risk organs, from donors with comorbidities or relative contraindications (6–9). These organs, termed "marginal" or "extended criteria" grafts, are more susceptible to cold ischemia, and have an increased risk of graft failure, and recipient morbidity and mortality (7,10). The devastating consequences of graft failure following LT preclude greater utilization of high-risk livers. For example, in 2014–2015, of 1282 identified UK donors, only 924 (72%) livers were deemed suitable for retrieval and 812 (63%) were subsequently transplanted (2). Data from the United States are similar, and the latest report of the Organ Procurement and Transplant Network showed that only 6312 out of 8144 (78%) potential donor livers were transplanted (3). Over the same period, in these two countries combined more than 3200 patients died or were removed from the transplant waiting list for being too sick for transplantation (3,11).

Normothermic machine perfusion of the liver (NMP-L) is a novel technique, substituting the detrimental effect of static cold storage (SCS) by preserving the organs in near-physiological conditions, with oxygen and nutrients

at 37°C. The preserved metabolic activity at normothermia not only prevents further graft damage caused by ischemia, but allows *ex-situ* monitoring of liver function by permitting objective assessment of liver biochemistry, blood flow, and bile production. The complexity of dual—arterial and portal—liver inflow has proved technically challenging. The first machine introduced into clinical practice was recently developed by the Oxford group, and was used for the pilot liver transplant series using standard criteria organs preserved by NMP-L, completely avoiding SCS (12). Our preclinical studies on discarded livers showed that perfusate lactate clearance in combination with bile production and stable blood flow rates are sensitive parameters predictive of graft viability, and in August 2014 our group carried out the first-in-human transplant of such a liver graft (13,14). Here, we present the first five recipients of NMP-L-treated rejected liver allografts.

Methods

Study design

This series evolved from a research project of viability testing of rejected human livers where NMP-L-based viability criteria were established and a perfusion fluid was developed to facilitate resuscitation of high-risk organs. After defining viability criteria, we obtained approval from the hospital ethics and novel therapeutic committees in June 2014 to perform a pilot series of five clinical transplants. Here we present the results of six consecutive NMP-Ls, commenced with an intention to perform clinical transplantation in carefully selected and consented adults with grafts that met viability criteria.

Source of rejected human livers

Based on donor history and laboratory results, the livers (except donor 4 with progressively rising liver function tests [LFTs]) were initially accepted and procured by one of the teams from the UK National Organ Retrieval Service, using a nationally agreed surgical protocol, with the intention of transplantation (15). All grafts were initially preserved in University of Wisconsin preservation fluid at 4°C.

On arrival at the transplanting center, each liver was assessed and deemed unsuitable by the consultant surgeon. The liver was then offered to and turned down by all UK liver transplant centers and then offered for use in our pilot study by the National Health Service Blood and Transplant (NHSBT) coordinating office. Ethical approval for the study was granted by the University Hospital Birmingham NHS Foundation Trust Novel Therapeutics and NHSBT Ethics Committees.

To ensure safety, risks were minimized by excluding livers with a significant pre-existing disease, and all grafts in this study met the following inclusion criteria: cold ischemic times (CIT) less than 16 h for livers from donors after brain death (DBD), or less than 10 h from donors after circulatory death (DCD), donor warm ischemic time (dWIT; defined as the interval between systolic blood pressure less than 50 mmHg or oxygen saturation less than 70% to aortic perfusion) in DCD organs less than 60 min, absence of hepatitis B, hepatitis C, or human immunodeficiency virus infection, and a macroscopic appearance without fibrosis or cirrhosis. Following a review of the protocol after the unsuccessful second perfusion, an additional criterion of maximum donor age of 65 years was added.

We were offered about 15 livers for machine perfusion research over the study period but utilized only a proportion of these due to the limited availability of personnel to perform the perfusions.

Clinical protocol for liver viability testing

Graft preparation was analogous to the standard back-table procedure, and the portal vein was dissected and cannulated. The celiac trunk branches were ligated and the hepatic artery was dissected to the gastroduodenal artery. We routinely attached an iliac artery interposition graft to the aortic patch to facilitate the insertion of the arterial perfusion cannula.

The perfusion fluid was based on 3 units of the donor liver specific blood group, Rhesus-negative, packed red blood cells, supplemented with 1000 mL human albumin solution 5%, 30 mL sodium bicarbonate 8.4%, and 10 mL calcium gluconate 10%. The circuit was loaded with 10 000 IU heparin, 500 mg vancomycin, and 60 mg gentamicin prior to connecting the liver, with the continuous infusion of epoprostenol (8 μg/h).

NMP-L was then commenced, using two different devices. Livers from donors 1 to 5 were perfused with Liver Assist (Organ Assist, Groningen, the Netherlands). This device provides a pulsatile arterial and continuous nonpulsatile portal flow via two independent rotary pump circuits. The liver from donor 6 was perfused with the OrganOx Metra device (OrganOx, Oxford, UK) delivering continuous nonpulsatile arterial and portal flows powered by one rotary pump. Organ viability was assessed within 3 h of perfusion. In a viable liver the perfusate lactate level had to be less than 2.5 mmol/L or the liver had to produce bile, in combination with at least two of the following three criteria: (1) perfusate pH greater than 7.30, (2) stable arterial flow of more than 150 mL and portal venous flow more than 500 mL per min, and (3) homogeneous graft perfusion with soft consistency of the parenchyma.

Histology

Menghini liver biopsies were obtained at three time points: (1) pre-NMP-L, (2) at the end of NMP-L, and (3) following reperfusion of the implanted liver. The cut end of the common bile duct was obtained post-NMP-L. All biopsies were placed in 10% formalin and processed by standard procedures to a paraffin block. Sections stained with hematoxylin and eosin and periodic acid–Schiff were examined for the percentage of large droplet (ld) and small droplet (sd) macrovesicular steatosis (MS), hepatocyte necrosis, and glycogen depletion. Preservation–reperfusion injury in postreperfusion biopsies was graded based on these features together with neutrophil infiltration. The grading system used has been developed in-house over many years by correlation with peak postoperative transaminases (unpublished data) and evolved from examination of sequential findings prior to retrieval, during cold storage, and following reperfusion (16). Bile duct biopsies were assessed for loss of the lining epithelium, epithelial damage in superficial and deep peribiliary glands, stromal necrosis, arteriolar necrosis, and thrombosis according to previously published criteria (17). Histological assessments were all performed after graft implantation and therefore did not impact on decisions concerning viability assessment.

Transplant recipients

The recipients were patients listed for transplantation at Queen Elizabeth Hospital Birmingham, UK. All patients received an explanation about the principles of NMP-L during consenting for LT. When a recovered viable liver graft became available, the consultant surgeon familiar with the project re-explained the procedure in detail and obtained patients' additional consent to accept the graft. Recipients considered for this study had low surgical perioperative risk as assessed by the multidisciplinary team during the listing process. Patients with hepatocellular carcinoma, with a high risk of waiting list dropout due to tumor progression, were regarded as favorable recipients.

Table 1: Donor demographics, liver characteristics, and machine perfusion data

Donor number	Donor 1 (transplant 1)	Donor 2 (discarded)	Donor 3 (transplant 2)	Donor 4 (transplant 3)	Donor 5 (transplant 4)	Donor 6 (transplant 5)
Donor information						
Age	29	69	49	49	46	51
Donor type	DCD	DBD	DCD	DBD	DCD	DCD
Sex	Male	Male	Female	Female	Male	Female
Height (cm)	173	174	169	161	179	165
Body weight (kg)	75	94	130	52	90	90
Body mass index (kg/m^2)	25	31	45	20	28	33
Premorbid cardiac arrest (downtime minutes)	Yes (58)	Yes (multiple)	Yes (35)	No	Yes (40)	No
Peak ALT (IU/L)	137	2264[1]	52	997[2]	1297[3]	49
Days on ventilator	8	27	2	7	6	2
Comorbidities and history	Diabetes mellitus (type 1)	Bladder cancer (recent surgery) hypertension	Paracetamol overdoses, DVT hypertension	Suprasellar meningioma (recent surgery)	Alcohol misuse	Diabetes mellitus (type 2) hypertension
Cause of death	Hypoxic brain injury	Hypoxic brain injury	Hypoxic brain injury	Intracranial hemorrhage	Hypoxic brain injury	Intracranial hemorrhage
Liver characteristics						
Liver weight (g)	1997	2400	1943	1382	2486	2522
Donor warm ischemic time (min)	109	NA	36	NA	31	19
Cold ischemic time (min)	422	518	406	387	453	474
Donor risk index	2.31	1.97	2.36	1.83	2.25	3.03
Graft offering[4]	Fast-track	Full offer	Full offer	Fast-track	Fast-track	Fast-track
Retrieval team and location[5]	Regional[6]	Regional	Regional	Extra-zonal	Extra-zonal	Extra-zonal
Reason for initial rejection	Long dWIT, poor liver flush	High LFTs, biopsy findings	Long dWIT, donor history, BMI	High LFTs, macroscopic appearance	Long dWIT, macroscopic appearance	Macroscopic appearance
Machine perfusion parameters						
Perfusion device	Organ Assist	Organ Assist	Organ Assist	Organ Assist	Organ Assist	OrganOx Metra
Lactate (mmol/L)						
Highest	13.3	11.4	5.5	13.1	12.4	13.9
Lowest	0.7	2.1	1.4	2.2	1.2	0.9
Last	0.7	4.5	1.4	2.4	1.2	2.8
Total bile production (g)	23.2	6.1	0.0	18.5	11.3	0.0
Mean arterial flow (mL/min)	558	491	476	623	654	360
Mean portal vein flow (L/min)	0.8	0.7	1.2	1.5	1.3	1.1

Table 1. Continued

Donor number	Donor 1 (transplant 1)	Donor 2 (discarded)	Donor 3 (transplant 2)	Donor 4 (transplant 3)	Donor 5 (transplant 4)	Donor 6 (transplant 5)
Mean liver mass perfusion (mL/g/min)	0.68	0.49	0.88	1.54	0.78	0.36
Perfusion time (min)	416	255	318	564	345	305
Total preservation time (min)	838	773	724	951	798	779
Transplanted	Yes	No	Yes	Yes	Yes	Yes
Operation lactate levels (mmol/L)						
Lactate peak/end of surgery	7.0/4.5	NA	4.3/3.0	4.0/2.9	5.0/3.3	3.6/1.4

ALT, alanine transaminase; DBD, donor after brain death; DCD, donor after circulatory death; DVT, deep vein thrombosis; dWIT, donor warm ischemic time; LFTs, liver function tests; NA, not applicable; UHB, University Hospitals Birmingham.
[1]ALT 2264 IU/L post cardiac arrest, reducing to 883 IU/L at time of retrieval.
[2]ALT progressively rising to 997 IU/L at the time of retrieval.
[3]ALT 1297 IU/L post cardiac arrest, reducing to 257 IU/L at time of retrieval.
[4]Fast-track offers denotes the liver was offered following refusal by other teams, often after it was procured and inspected by the retrieval team.
[5]Regional liver procurements were performed by the UHB team, with the expected travel time back to the hospital less than 3 h; extra-zonal procurements were performed by other teams, with the expected shipment time greater than 3 h.
[6]Expected travel time greater than 4 h.

LT procedure and patient follow-up

The grafts were implanted with the vena cava–preserving technique. After completing the native liver hepatectomy, the NMP-L was stopped and the graft was flushed with 2 L of cold histidine–tryptophan–ketoglutarate solution, vascular and bile duct cannulas were removed, and bile duct and liver biopsies were taken. The graft was immediately implanted and reperfused in the standard manner. The perioperative data, posttransplant laboratory results, and details of the patient's recovery course were collected. Following discharge from the hospital, patients were reviewed in the outpatient clinic with weekly (first month) and then every 2 weeks (second to third month) frequency.

Results

The median donor age was 49 (range 29–54) years. Four livers were recovered from DCD and two from DBD donors. There was an even split between the liver offers initially accepted and retrieved by our team versus other teams. The median CIT was 422 (387–474) min. Five out of six livers met the viability criteria and were used for transplantation. The detailed demographics and graft characteristics are provided in Table 1.

Donor history details and reasons for initial graft rejection

Donor 1 (DCD) was a 29-year-old diabetic male admitted with cardiac arrest, having elevated LFTs. The dWIT was 109 min and the graft appearance was patchy. The liver was rejected due to prolonged dWIT and poor perfusion.

Donor 2 (DBD) was a 69-year-old male ventilated for 27 days following surgery for ascending aorta dissection, with a peak alanine transaminase (ALT) of 2264 IU/L and multiple cardiac arrests. The liver was rejected based on history and LFTs.

Donor 3 (DCD) was a 49-year-old female with BMI 45 kg/m^2 with a history of hypertension, depression with two suicide attempts with paracetamol overdose, and deep vein thrombosis with an infected chronic leg ulcer. The liver was rejected due to the prolonged dWIT (36 min) in combination with high BMI.

Donor 4 (DBD) was a 54-year-old female with an intracranial bleed postresection of a suprasellar meningioma. Because of rising LFTs (ALT 997 IU/L on day of donation), the liver was not accepted.

Donor 5 (DCD) was a 46-year-old male who collapsed with a cardiac arrest of 40-min duration. He was a known heavy drinker and the admission ALT was 1297 IU/L. The graft was rejected due to its large size (2486 g) and abnormal LFTs.

Donor 6 (DCD) was a 51-year-old male with an intracranial hemorrhage, diabetes on metformin, and BMI 33 kg/m^2. The liver was rejected due to large size (2522 g) and steatotic appearance on macroscopic assessment.

Viability evaluation

All but one liver met defined criteria for viability and showed signs of function as assessed by the perfusate lactate clearance and bile production. The median starting lactate level was 9.9 mmol/L that decreased in 2 h to the median level 1.5 mmol/L (Figure 1A). The median NMP-L time was 332 (318–564) min. The total preservation time of the transplanted livers was 798 (724–951) min.

The donor 2 liver did not meet viability criteria, despite initially showing a rapid lactate clearance with levels decreasing from 11.4 to 2.1 mmol/L within the first 2 h of perfusion. The liver had aberrant arterial anatomy, with an accessory right hepatic artery rising from the superior mesenteric artery. Despite a presence of back-flow bleeding from the artery stump after graft connection to the device, there was noticeable color difference on the liver surface after 90 min of perfusion, prompting arterial reconstruction. Following re-established inflow via the accessory artery, lactate levels rose and did not normalize within the 3-h time frame, and the liver was discarded. This event suggests that any vascular reconstruction for aberrant arterial anatomy should be performed prior to commencing the perfusion.

The donor 6 graft function recovery occurred soon after starting NMP-L, with fluctuations and increase of lactate levels during the later perfusion course. In terms of the decision-making, we consider the key lactate reading as being the one taken at the time of graft viability assessment (the point when lactate drops below 2.5 mmol/L, or the reading taken at 3 h after commencing NMP-L). Although we continued to measure the parameters every 30 min thereafter, these values do not have any impact on the transplantation procedure, as the recipient operation is already in progress. Details of the NMP-L parameters, graft function, and transplantation procedure are provided in Table 1.

Histological findings

No significant ld steatosis was seen in these livers, with the majority (four of six) also having negligible sdMS and two having mild (<33%) sdMS (Figures 2A and B). Hepatocyte necrosis (Figures 2C and D) of more than just a few cells was present in one liver that was transplanted (30% increasing to 50% posttransplant), and in the one that did not reach transplant criteria (15% hepatocyte loss from necrosis at an earlier time point). In four of five of the transplanted livers, glycogen stores appeared to be replenished during NMP-L (Figures 2E and F). The injury posttransplant varied from mild to severe.

Bile duct injury (Figure 3) was generally mild: there were only mild epithelial changes in deep peribiliary glands in the livers transplanted. One post-NMP-L bile duct biopsy showed mild, two moderate, and three severe stromal

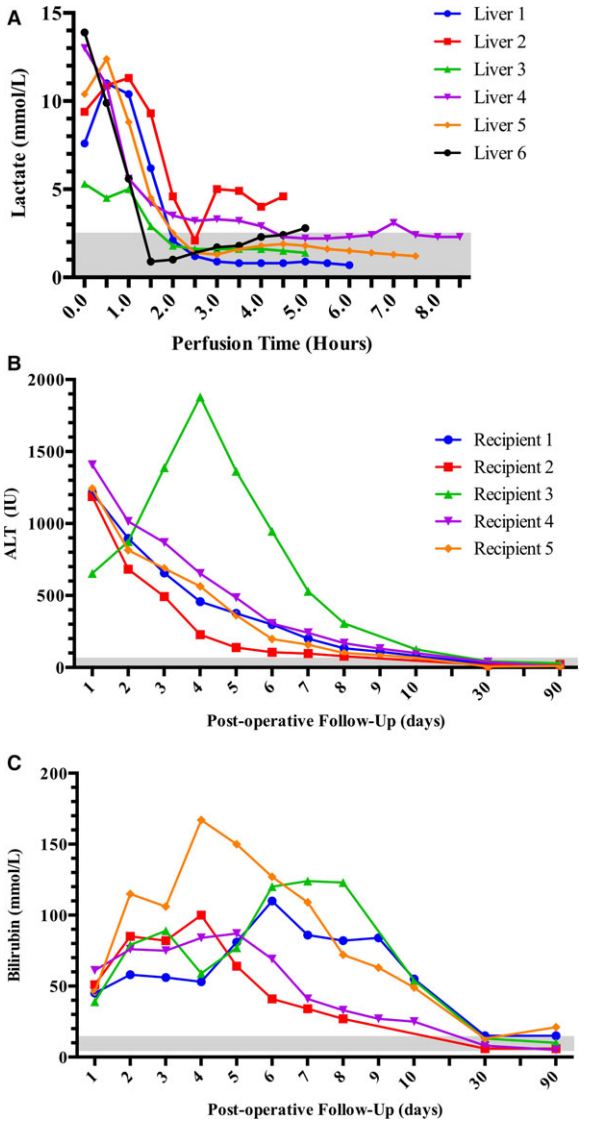

Figure 1: Viability assessment by the perfusate lactate clearance and the posttransplant liver function tests. Panel (A) shows the lactate clearance during the normothermic perfusion. All livers demonstrated metabolic activity, and perfusate lactate levels dropped below 3.0 mmol/L. In liver number 2 the lactate levels fell to 2.1 mmol/L, but started to rise again after 150 min. The organ failed to meet the viability criteria and was not used for transplantation. Panel (B) shows the posttransplant changes in the ALT levels; the enzyme is often used as a surrogate marker for preservation-related liver injury. The initial posttransplant levels were similar in all livers, with progressive improvement within the first posttransplant week. In all recipients the ALT levels were normal within the first month after transplantation. Panel (C) demonstrates a similar improvement pattern with bilirubin levels. In recipient number 1, bilirubin levels slightly increased later during follow-up and the magnetic cholangiography performed at 6 months posttransplant revealed a mild anastomotic biliary stricture. The bilirubin level normalized with conservative management of ursodeoxycholic acid medication. ALT, alanine transaminase.

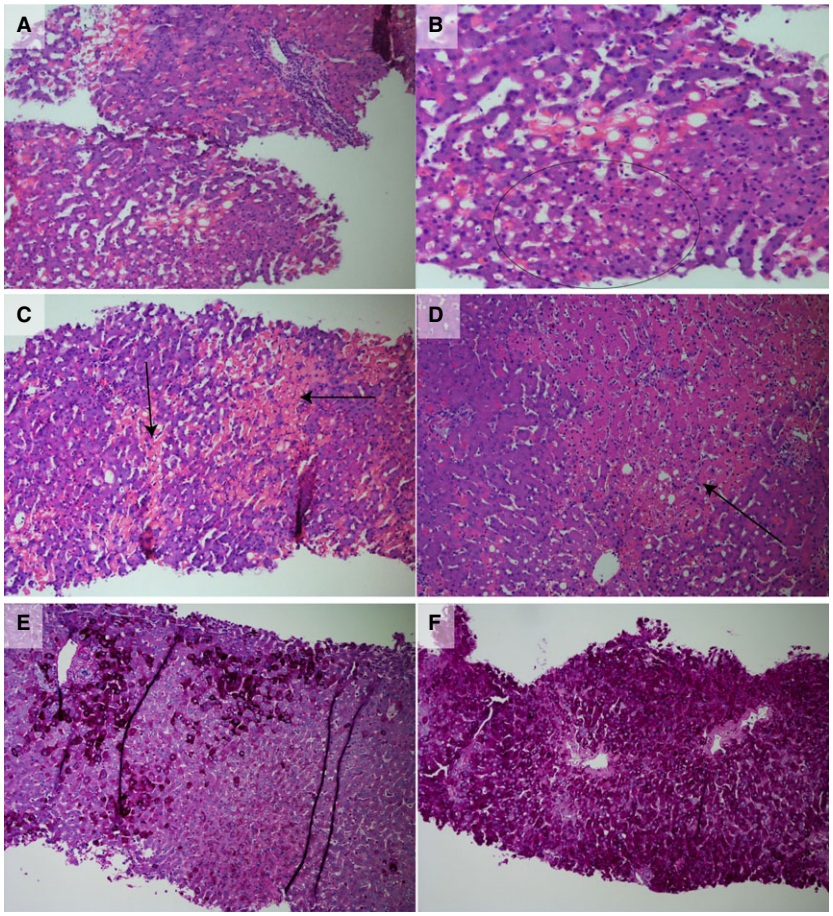

Figure 2: Histological findings in liver biopsies. Panels (A) and (B) show pre-NMP-L H&E-stained biopsies from liver number 5. Panel (A) shows negligible large droplet macrovesicular steatosis (10× objective). Panel (B) is a higher magnification showing small droplet macrovesicular steatosis involving roughly 20–30% of the hepatocytes. This is seen within the circled area as tiny white holes in the hepatocytes. This type of steatosis, often referred to as microvesicular steatosis, is not considered to be important in determining the amount of fat in an assessment for transplantation. None of the livers had more than 5% large droplet steatosis, the type that determines suitability for transplantation (20× objective). Panels (C) and (D) demonstrate areas of necrosis seen as the pale pink hepatocytes (arrows) in post-NMP-L biopsies from liver number 5. Panel (C) shows approximately 30% necrosis in the preimplantation biopsy. Panel (D) shows an increase in the number of necrotic hepatocytes in the postreperfusion biopsy, approximating to 50% of the liver parenchyma. This liver showed the most necrosis in this presented series; this degree of necrosis is considered unfavorable by currently used assessment standards. The additional information provided by the functional assessment using the normothermic perfusion confirmed the liver viability and the graft was successfully transplanted with immediate intraoperative recovery of the function and good patient recovery (both sections H&E, 10× objective). Panels (E) and (F) are PAS-stained sections of biopsies from liver number 1 in which glycogen in hepatocytes stains dark pink. Panel (E) shows the pre-NMP-L biopsy with moderate glycogen depletion. Panel (F) shows the post-NMP-L biopsy with increased glycogen content, now amounting to only mild depletion (both 10× objective). H&E, hematoxylin and eosin; NMP-L, normothermic machine perfusion of the liver; PAS, periodic acid–Schiff.

nuclear loss. Mild arteriolar necrosis was seen in three of the post-NMP-L biopsies. Thrombosis was not seen. The detailed findings are provided in Table 2.

Patient outcomes

The median recipient age was 56 (range 47–66) years. The transplantation procedure was uneventful for every recipient, with immediate function recovery in all grafts. The median intensive therapy unit (ITU) stay was 3 (range 2–6) days, with one early ITU readmission in a patient who developed acute coronary syndrome 8 days following surgery, requiring percutaneous coronary intervention with stent insertion. The median in-hospital stay was 10 (range 6–15) days. To date, all patients are well, with normalized liver tests at a median follow-up of 7 (range 6–19) months. Recipient 3 (donor 4 liver) showed a different posttransplant ALT profile compared to the other recipients, and this may be related to the severe preretrieval injury as documented by the progressively rising ALT (peak 997 IU/L) within 24 h prior to donation

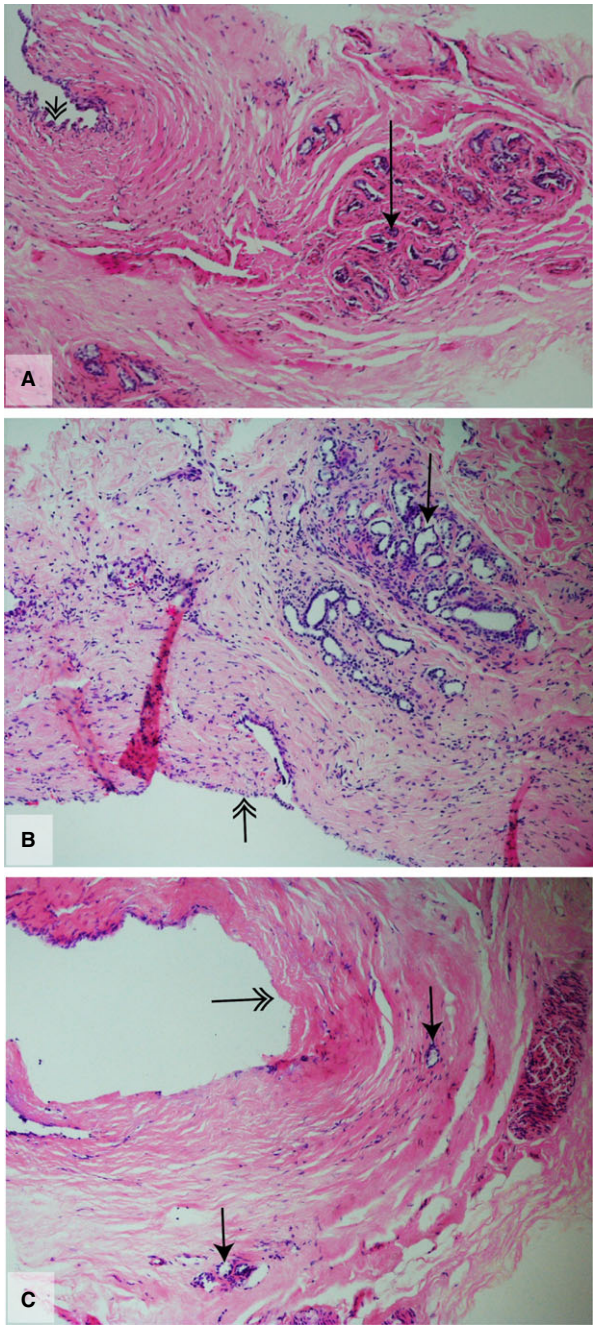

Figure 3: Bile duct histology. This figure demonstrates H&E-stained sections of bile duct. The double arrowhead shows the surface epithelial lining and the single arrowhead points to a deep peribiliary plexus. Panel (A) shows the surface epithelium is intact in this part of the bile duct with relatively mild changes to the deep peribiliary glands in liver number 6. Panel (B) displays partial surface epithelial loss with well-preserved peribiliary glands in liver 4. Panel (C) shows another fragment of bile duct from liver 6 in which there is moderately extensive loss of surface epithelium, with stromal nuclear loss deep to the double arrowhead; the deep peribiliary glands in this area look moderately injured (all 10× objective). H&E, hematoxylin and eosin.

(Figures 1B and C). This might also explain the different pattern of its lactate clearance, and in this particular case the viability criteria were met by bile production rather than lactate level at 3 h.

The recipient demographics and outcome details are provided in Table 3.

Discussion

The consequences of transplanting a liver that fails to function are potentially dire. NMP-L offers the opportunity to assess and improve the quality of high-risk livers deemed unsuitable for transplantation. To our knowledge, this report describes the first patient series of "rejected" liver allografts transplanted following successful assessment and resuscitation by NMP-L. This pilot study shows that a proportion of high-risk donor livers might be transplanted by subjecting them to viability testing during NMP-L, without compromising patient safety in a cohort of low-risk recipients.

Since transplantation was established as a highly successful treatment almost half a century ago, scarcity of suitable donors has become a worldwide factor limiting access to this treatment. Ongoing advancements, ranging from the improved management of intracranial vascular malformations to the vast improvements in road traffic safety, have had an impact on decreasing the availability of DBD organ donors. National and international regulatory bodies have proposed strategies and identified funding to overcome the shortage, but these are largely based on increasing the number of extended criteria organs, known to be associated with a higher risk for the recipient (18).

Machine perfusion technology has shown promising results in preserving cardiothoracic and abdominal organs (12,19–23). Although most of the reported series showed its feasibility in organs acceptable for transplantation, the technology has already demonstrated the potential to expand the donor pool. For example, the team at St Vincent's Hospital in Sydney recently reported a series of heart transplants using allografts recovered from donors after circulatory death that were previously deemed unfeasible (20).

Normothermic perfusion replicating near-physiological conditions *ex-vivo* has for a long time been regarded as the optimal machine perfusion strategy, but has required advanced technology that was previously not available. Several groups have successfully pursued simpler hypothermic machine perfusion (HMP) (21,24,25). The early adoption of HMP was also facilitated by the negligible risk of graft loss related to potential device malfunction. Clinical trials of HMP of kidneys have demonstrated improved results in renal transplantation (23,26). Numerous teams have

Table 2: Histological features on liver biopsies

	Donor 1 (transplant 1)	Donor 2 (discarded)	Donor 3 (transplant 2)	Donor 4 (transplant 3)	Donor 5 (transplant 4)	Donor 6 (transplant 5)
Large droplet macrovesicular steatosis[1]						
Pre-NMP-L	None	NA	NA	None	<5%	None
Post-NMP-L	None	None	None	<5%	<5%	None
Post-reperfusion	None	NA	None	<5%	<5%	None
Small droplet macrovesicular steatosis[2]						
Pre-NMP-L	<5%	NA	NA	20%	20%	<5%
Post-NMP-L	<5%	30%	<5%	<5%	20%	None
Post-reperfusion	None	NA	10%	<5%	25%	10%
Necrosis[3]						
Pre-NMP-L	None	NA	NA	5%	None	None
Post-NMP-L	1%	15% (old)	5%	None	30%	None
Post-reperfusion	1%	NA	10%	1%	50%	5%
Glycogen depletion[4]						
Pre-NMP-L	Moderate–severe		Moderate	Minimal	Severe	Mild–moderate
Post-NMP-L	Mild	Severe	Mild–moderate	Moderate–severe	Mild	None
Post-reperfusion	Moderate	NA	Moderate–severe	Moderate	Moderate–severe	Moderate–severe
Post-reperfusion injury	Mild	NA	Moderate	Moderate	Severe	Moderate–severe
Bile duct biopsies[5]						
Superficial epithelium	>50%	>50%	>50%	<50%	<50%	<50%
Superficial PBG	>50%	>50%	>50%	<50%	<50%	<50%
Deep PBG	<50%	>50%	<50%	<50%	<50%	<50%
Stromal nuclear loss	Severe	Severe	Severe	Mild	Moderate	Moderate
Arterial medial loss	Mild	Mild	None	None	Mild	None
Thrombi	None	None	None	None	None	None
Hemorrhage	None	None	None	None	None	None

NA, not applicable/available; NMP-L, normothermic machine perfusion of the liver; PAS, periodic acid–Schiff; PBG, peribiliary gland.

[1]Large droplet macrovesicular steatosis is defined as a single large fat droplet within the hepatocyte cytoplasm displacing the nucleus. Mild <1/3, moderate 1/3–2/3, and severe >2/3 of hepatocytes contain large droplet macrovesicular fat.

[2]Small droplet macrovesicular steatosis is defined as fat droplets, usually multiple within the cytoplasm of the hepatocyte, which do not displace the nucleus. Mild <1/3, moderate 1/3–2/3, and severe >2/3 of hepatocytes contain small droplet macrovesicular fat.

[3]Necrosis is depicted as the percent of total hepatocytes in the biopsy that are necrotic.

[4]Glycogen depletion is graded as mild—up to 20% of nonnecrotic hepatocytes do not contain PAS-positive glycogen, moderate 20–95% of hepatocytes do not contain glycogen, and severe >95% of hepatocytes do not contain glycogen.

[5]Classification grading as follows: loss of surface and peribiliary glands none – no loss, mild ≤50%, and severe >50% loss of cells; stromal nuclear loss none – no loss, mild ≤25%, moderate 25–50% loss, severe >50% loss; arterial medial loss none – no loss of nuclei from media, mild – incomplete nuclear loss in ≤50% of arteries/arterioles, moderate >50% incomplete nuclear loss, severe – complete necrosis of wall in >50% of arteries/arterioles. [op den Dries et al. (17)].

Table 3: Recipient demographics and outcomes

	Recipient 1 (donor 1)	Recipient 2 (donor 3)	Recipient 3 (donor 4)	Recipient 4 (donor 5)	Recipient 5 (donor 6)
Age at transplant (years)	46	56	66	65	56
Sex	Male	Male	Male	Male	Female
Primary etiology	Alcohol	NAFLD	Alcohol and NAFLD	Hemochromatosis	Alcohol
Indication for transplant	Encephalopathy	Refractory ascites	HCC	HCC	Refractory ascites
MELD at LT	17	9	7	7	8
UKELD at LT	55	49	51	47	51
Waiting list time (months)	2	6	7	1	3
ITU stay (days)	5	2	3	6	3
Early allograft dysfunction[1]	No	No	No	No	No
Renal replacement therapy	No	No	No	Yes (10 days)	No
In hospital stay (days)	12	7	6	15	10
Posttransplant complications[2]	None	None	None	Grade IVb (MI, PCI, RRT)	None
Liver function tests					
Peak ALT (IU/L)	1215	1188	1879	1408	1242
Peak bilirubin	110	100	124	87	167
At 1 month					
ALT (IU/L)	24	17	43	38	6
Bili (μmol/L)	15	6	13	8	13
ALP (IU/L)	73	113	114	178	64
At 3 months					
ALT (IU/L)	16	21	29	8	10
Bili (μmol/L)	15	6	10	5	21
ALP (IU/L)	135	103	79	63	81
Creatinine (μmol/L)					
At 1 month	90	67	78	168	62
At 3 months	82	77	98	147	92

ALP, alkaline phosphatase; ALT, alanine transferase; AST, aspartate transferase; Bili, bilirubin; HCC, hepatocellular carcinoma; ITU, intensive therapy unit; LT, liver transplantation; MELD, Mayo end-stage liver disease score; MI, myocardial infarction; NAFLD, nonalcoholic fatty liver disease; PCI, percutaneous coronary intervention; RRT, renal replacement therapy; UKELD, UK model for end-stage liver disease score.

[1]Early allograft dysfunction consists of the presence of one or more of the following variables: (1) bilirubin 10 mg/dL on postoperative day 7; (2) INR 1.6 on postoperative day 7; (3) aminotransferase level (ALT or AST) >2000 IU/L within the first 7 postoperative days; Olthoff et al (31).

[2]According to Clavien-Dindo classification; Clavien et al (32).

reported encouraging outcomes following HMP of the liver; however, the first reported high-risk graft series demonstrated a high incidence of biliary complications and also primary nonfunction was observed (21,27).

The devastating consequences of primary graft nonfunction in cardiothoracic transplantation and LT preclude further extension of organ acceptance criteria. The utilization of high-risk hearts or lungs is only 30–40%, which might relate to the use of ventricular assist devices and extracorporeal membrane oxygenation as a bridge to transplantation until a lower-risk donor becomes available. In contrast, the constant growth in demand for liver transplants has extended utilization of marginal livers to 70–80%, often compromising posttransplant outcomes and patients' safety (7,10).

The limits in the utilization of high-risk livers have been explored in countries such as the United Kingdom, where these organs can be allocated to lower-risk recipients (28,29). The protocol presented here may transform use of high-risk livers. Diminishing the risk of primary nonfunction or severe dysfunction, with their often fatal consequences, might allow further evolution of this novel approach and permit safe allocation of high-risk organs to the sickest recipients, benefiting the patients with the highest waiting list mortality (30).

In this series, the livers were declined by all the UK transplant units and NMP-L was commenced following a variable period of static cold storage, with no differences observed between the use of the two available devices, and five out of six tested grafts were viable. During the period of this pilot study, there were 149 (81 DCD, 68 DBD) livers meeting the study inclusion criteria discarded in the United Kingdom. Recovering 70% of these organs would allow over 100 additional LTs in the United Kingdom, increasing the number of available organs by 15% (unpublished data, courtesy of Sally Rushton, NHSBT). We envisage that viability testing will transform the organ selection and acceptance process, and this case series represents a promising start. The technique showed favorable outcomes in a predefined subgroup of high-risk organs. Nevertheless, the presented results must be taken cautiously and seen as a feasibility report. One limitation is that this small group of livers did not include any organs with moderate or severe ld fatty change (macrosteatosis), a key risk factor for initial graft dysfunction/primary nonfunction. Other potential limitations could be the additional costs and challenges of wider implementation of NMP-L technology and expertise, but this may be justified by the increases in transplant activity and improved organ utilization. In addition, our study shows the feasibility of performing NMP-L following SCS and inspection at the transplant center, with logistical and financial advantages, and may allow targeting of livers that would benefit most from NMP-L.

This report demonstrates that a proportion of currently rejected livers might be salvaged by subjecting them to NMP-L and viability testing. Use of this technology may transform the utilization of high-risk organs and improve access for patients to transplantation.

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Disclaimer

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

Authors' Contribution

H.M. and D.F.M. initiated the project, were responsible for the study management, safety monitoring, and the manuscript submission. H.M. and R.W.L. collected the data. R.W.L., H.M., A.S., and B.T.F.S. performed the machine perfusion, preclinical research, and developed the liver viability criteria. H.M., R.W.L., D.A.H.N., S.G.H., S.C.A., and D.F.M. were responsible for data analysis, interpretation, presentation, and the report preparation. M.T.P.R.P., P.M., J.R.I., H.C., H.M., and D.F.M. were involved in the study design, transplantation procedures, posttransplant patient management, and outpatient follow-up. D.A.H.N., S.C.A., and S.G.H. performed the histology assessment. All coauthors actively contributed and reviewed the final manuscript.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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