First human liver transplantation using a marginal allograft resuscitated by normothermic machine perfusion

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BRIEF COMMUNICATION

TRANSPLANTATION OF "DISCARDED" HIGH-RISK LIVER ALLOGRAFTS FOLLOWING NORMOTHERMIC EX-SITU EVALUATION

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LIST OF ABBREVIATION

NMLP, normothermic machine liver perfusion; DCD, donor/donation after circulatory death; LT, liver transplantation; SCS, static cold storage; CIT, cold ischaemic time; DBD, donor/donation after brain death; WoT, withdrawal of treatment; dWIT, donor warm ischaemic time; LFTs, liver function tests; ALT, alanine transaminase; BMI, body mass index

AUTHORS' CONTRIBUTION

HM and DFM initiated the project, were responsible for the study management, safety monitoring, and the manuscript submission. HM and RWL collected the data. RWL, HM, AS, and BTFS performed the machine perfusion, pre-clinical research and developed the liver viability criteria. HM, RWL, DAHN, SGH, SCA and DFM were

responsible for data analysis, interpretation, presentation, and the report preparation. MTPRP, PM, JRI, HC, HM, and DFM were involved in the study design, transplantation procedures, post-transplant patient management, and outpatient follow up. DAHN, SCA and SGH performed the histology assessment. All co-authors actively contributed and reviewed the final manuscript.

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The project was funded by University Hospital Birmingham Liver Charities. The Organ Assist (n=5 livers) and OrganOx Metra (n=1) devices used were on loan and neither of the two manufacturers had any role in the study design, data collection, analysis, interpretation or the manuscript preparation. The authors are employees of the University Hospital Birmingham or University of Birmingham and none of them received any payment or have any conflict of interest related to this manuscript.

ABSTRACT

The demand for liver transplantation exceeds supply with rising waiting list mortality. Utilisation of high-risk organs is low and a substantial number are discarded. We report the first series of five transplants with "discarded" livers following viability assessment by normothermic machine liver perfusion (NMLP). The testing 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 commencing NMLP. Four organs were recovered from donors after circulatory death and discarded due to prolonged donor warm ischaemic times; one liver from brain death donor was declined for very high liver function tests. The median (range) total graft preservation time was 798 (724-951) minutes. The transplant procedure was uneventful in every recipient with immediate function in all grafts. The median in-hospital stay was 10 (6-14) days. At present, all recipients are well, with normalised liver function tests at median follow-up of 6 (5-18) months. High-risk grafts viability assessment provides specific information on liver function can permit their transplantation without compromising recipient safety. This novel approach may substantially increase organ availability for liver transplantation.

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 whilst awaiting transplantation (2-5). To address this, more transplants are performed using high-risk organs, from donors with co-morbidities or relative contraindications (6-9). These organs, termed "marginal" or "extended criteria" grafts, are more susceptible to cold ischaemia, and have an increased risk of graft failure, recipient morbidity and mortality (7, 10). The devastating consequences of graft failure following LT preclude greater utilisation of high-risk livers. For example, in 2014-15, 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 6312/8144 (73%) potential donor livers were transplanted (3). Nevertheless, over the same period more than 3200 patients died or were removed from the transplant waiting list in these countries, for being too sick for transplantation (3, 11).

Normothermic machine liver perfusion (NMLP) 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 ischaemia, 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 to clinical practice recently was developed by the Oxford group, and was used for the pilot liver transplant series using standard criteria organs preserved by NMLP, completely avoiding SCS (12). Pre-clinical studies on "discarded" livers showed that bile production, in combination with maintenance of physiological pH, metabolism of lactate and stable blood flow rates, are sensitive parameters predictive of graft viability and in August 2014 our group carried out the first-in-man transplant of such a liver graft (13). Here, we present the first five recipients of NMLP treated "discarded" liver allografts.

METHODS

Study design

This series evolved from a research project of viability testing of "discarded" human allografts where NMLP 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 NMLPs, commenced with an intention to perform clinical transplantation in carefully selected and consented adults with grafts that met viability criteria.

Source of "discarded" human livers

Based on donor history and laboratory results, the livers (except donor four with a progressively rising liver tests) 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 (14). All grafts were initially preserved in University of Wisconsin preservation fluid at 4^{o}C.

On arrival at the transplanting centre, 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 centres and then offered for use in our pilot study by the NHSBT co-ordinating office. Ethical approval for the study was granted by University Hospital Birmingham NHS Foundation Trust Novel Therapeutics and NHSBT Ethics Committees.

To ensure safety, risks were minimised by excluding livers with a significant pre-existing disease, and all grafts in this study met the following inclusion criteria: maximum donor age of 65 years, cold ischaemic times (CIT) less than 16 hours for livers from donors after brain death (DBD), or less than 10 hours from donors after circulatory death (DCD), donor warm ischaemic time (systolic blood pressure less than 50mmHg to aortic perfusion) in DCD organs less than 60 minutes, absence of hepatitis B, hepatitis C, or human immunodeficiency virus infection, and a macroscopic appearance without fibrosis or cirrhosis.

Clinical protocol for "discarded" liver grafts viability testing

Graft preparation was analogous to the standard back-table procedure, and the portal vein was dissected and cannulated. The coeliac trunk branches were ligated and the hepatic artery was dissected to the gastroduodenal artery. If present, accessory left and/or right arteries were preserved and an iliac artery interposition graft was attached to the aortic patch. The arterial cannula was placed in a way it did not reach the vessel area used subsequently to perform the anastomosis during the graft implantation procedure.

NMLP was then commenced, using Liver Assist (Organ Assist, the Netherlands) or OrganOx Metra (OrganOx, UK) devices. Organ viability was assessed within three hours of perfusion. A viable graft had to produce bile or the perfusate lactate level had to be less than 2·5 mmol/L, 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 minute respectively, and 3) homogeneous graft perfusion with soft consistency of the parenchyma.

Histology

Menghini liver biopsies were obtained at three time points: 1) preNMLP, 2) at the end of NMLP, and 3) following reperfusion of the implanted liver. The cut end of the common bile duct was obtained post-NMLP. All biopsies were placed in formal saline and processed by standard procedures to a paraffin block. Sections stained with Haematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) were examined for the per cent of large droplet (ld) and small droplet (sd) macrovesicular steatosis (MS), hepatocyte necrosis and glycogen depletion. Preservation-reperfusion injury in post-reperfusion biopsies was graded based on these features together with neutrophil infiltration. 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 (15).

Transplant recipients

The recipients were patients listed for transplantation at Queen Elizabeth Hospital (Birmingham, UK). All patients received an explanation about the principles of NMLP 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 patient's additional consent to accept the "resuscitated" graft. Recipients considered for this study had low surgical perioperative risk as assessed by the multi-disciplinary team during the listing process. Patients with hepatocellular carcinoma, with a high risk of waiting list dropout due to tumour progression, were regarded as favourable recipients.

Liver transplant procedure and patients follow up

The grafts were implanted with the vena cava preserving technique. After completing the native liver hepatectomy, the NMLP was stopped and the graft was flushed with 2 litres 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, post-transplant laboratory results and patient recovery course were collected. Following discharge from the hospital, patients were reviewed on the outpatient clinic with weekly (1^{st} month) and the fortnightly (2^{nd} to 3^{rd} month) frequency.

Funding source

The project was funded by University Hospital Birmingham Liver Charities. The Organ Assist (n=5 livers) and OrganOx Metra (n=1) devices used were on loan and neither of the two manufacturers had any role in the study design, data collection, analysis, interpretation or the manuscript preparation. The authors are employees of the University Hospital Birmingham or University of Birmingham and none of them received any payment or have any conflict of interest related to this manuscript.

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 SCS time was 422 (387-474) minutes. 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 discard

Donor one (DCD) was a 29-year-old diabetic male admitted with cardiac arrest, having marginally elevated liver function tests (LFTs). Aortic *in-situ* perfusion was commenced 112 minutes following withdrawal of treatment (WoT), with a patchy graft appearance. Liver was rejected due to prolonged donor warm ischaemic time (dWIT) and poor perfusion.

Donor two (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 UI/L and multiple cardiac arrests. The liver was rejected based on history and LFTs.

Donor three (DCD) was a 49-year-old female with body mass index (BMI) 45 kg/m^2 with a history of hypertension, depression with two paracetamol suicide attempts, deep vein thrombosis with an infected chronic ulcer. The liver was rejected due to the prolonged dWIT in combination with high BMI suggesting significant steatosis.

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

Donor five (DCD) was a 46-year-old male who collapsed with 40 minutes cardiac

arrest. He was a known heavy drinker and the admission ALT was 1297 UI/L. The graft was rejected due to its large size of 2486g and abnormal LFTs.

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

Viability testing

All but one graft 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 two hours to the median level 1.5 mmol/L. The median NMLP time was 332 (318-564) minutes. The total preservation time of the transplanted livers was 798 (724-951) minutes.

Graft 2 did not meet viability criteria, showing initially a rapid lactate clearance with levels decreasing from 11.4 mmol/L to 2.1 mmol/L within two hours of perfusion. The liver had aberrant arterial anatomy, with an accessory right hepatic artery rising from superior mesenteric artery. Despite a presence of back flow bleeding from the artery stump after graft connection to the device, there was noticeable colour difference on the liver surface after 90 minutes of perfusion, prompting arterial reconstruction. Following re-established inflow via the accessory artery, lactate levels rose and did not normalise within the three hour time frame, and the liver was "discarded". Details of the NMLP parameters, graft function, and transplantation procedure are provided in Table 1.

Histological findings

No significant large droplet steatosis was seen in these livers, with the majority (4/6)

also having neglible sdMS and two having mild (<33%) sdMS (Figure 2A,B). Hepatocyte necrosis (Figure 2C,D) of more than just a few cells was present in one liver which was transplanted (30% increasing to 50% post-transplant), and in the one which did not reach transplant criteria (15% hepatocyte loss from necrosis at an earlier time point). In 4/5 of the transplanted livers glycogen stores appeared to be replenished during NMLP (Figure 2E,F). The injury post-transplant varied from mild to severe.

Bile duct injury (Figure 3) was generally mild: there were only mild epithelial changes in deep peribiliary glands. One post-NMLP bile duct biopsy showed mild and two moderate stromal nuclear loss. Minimal arteriolar necrosis was only seen in one of the post-NMLP biopsies. Thrombosis was not seen. The detailed findings are provided in Table 2.

Patient outcomes

The median recipient age was 56 (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 (2-6) days, with one early readmission, in a patient who developed acute coronary syndrome 8 days following surgery, requiring percutaneous coronary angioplasty with stent insertion. The median in-hospital stay was 10 (6-15) days. To date, all patients are well, with normalised liver tests at a median follow-up of 6 (5-18) months. The recipient demographics and outcome details are provided in Table 3.

DISCUSSION

The consequences of transplanting a liver, which fails to function, are potentially dire. NMLP 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 "discarded" liver allografts transplanted following successful assessment and resuscitation by NMLP. This pilot study shows that a substantial proportion of high-risk donor livers might be transplanted by subjecting them to viability testing during NMLP, 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. On-going medical advancement, ranging from the improved management of intracranial vascular malformations to the vast improvements in road traffic safety, has had an impact on decreasing the availability of DBD organ donors. National and international regulatory bodies have proposed strategies and have 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 (16).

Machine perfusion technology has shown promising results in preserving cardiothoracic and abdominal organs (17-22). 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 (18).

Normothermic perfusion replicating near-physiological conditions *ex-vivo* has for long time been regarded as the optimal machine perfusion strategy. It has required advanced technology that was previously not available. Several groups have successfully pursued simpler hypothermic machine perfusion (HMP) (19, 23, 24). The early adoption of HMP was also facilitated by the negligible risk of graft loss related to potential device malfunction. Clinical trials of hypothermic machine perfusion of kidneys have demonstrated improved results in renal transplantation (21, 25). Numerous teams have reported encouraging outcomes following hypothermic liver perfusion, however the first reported high-risk graft series demonstrated a high incidence of biliary complications and also observed primary non-function (19, 26).

The devastating consequences of primary graft non-function in cardiothoracic and liver transplantation preclude further extension of organ acceptance criteria. The utilisation 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, allowing patients to be kept alive until a lower risk donor becomes available. In contrast, the constant growth in demand for liver transplants has extended utilisation of marginal livers to 70-80%, often compromising post-transplant outcomes and patients' safety (7, 10).

The limits in the utilisation of high-risk livers have been explored in countries such as the UK, where these organs can be allocated to lower risk recipients (27, 28). The

protocol presented here may transform use of high-risk livers worldwide. Diminishing the risk of primary non-function 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 (29).

In this series, livers were declined by all the UK transplant units, after which NMLP commenced with a variable period of static cold storage. Still, five out of six tested grafts were viable. Recovering 80% of the unutilised organs would allow over 2000 additional liver transplantations in the UK and US alone. Although we envisage that viability testing will transform the organ selection and acceptance process and shift boundaries in using high-risk organs, our observation focuses only on feasibility and short-term outcome. Five months follow up of the last included transplant in this series, in combination with normal LFTs in all included patients is likely to exclude any early clinically relevant form of ischaemic type biliary complication, one of the main problems in recipients of DCD livers (30). The histology of the post-NMLP common bile duct biopsy is also not suspicious for the development of ischaemic bile duct lesions with less than 50% epithelial necrosis of deep peribiliary glands, no thrombi and minimal arteriolar necrosis (15). Other potential limitations could be the additional costs and challenges of wider implementation of NMLP technology and expertise, but this may be justified by the increases in transplant activity and improved organ utilisation. In addition, our study shows the feasibility to perform NMLP following SCS and inspection at the transplant centre, with logistical and financial advantages, and may allow targeting livers that would benefit most from NMLP.

This report demonstrates that a substantial proportion of currently "discarded" liver allografts might be salvaged by subjecting them to NMLP and viability testing. Use of this technology can transform the utilisation of high-risk organs and may improve access to treatment for thousands of patients awaiting liver transplantation globally.

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Table 1. Donor demographics, graft 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)
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
Bodyweight (kg)	75	94	130	52	90	90
Body mass index (kg/m^2)	25	31	45	20	28	33
Pre-morbid cardiac arrest (downtime minutes)	Yes (58)	Yes (multiple)	Yes (35)	No	Yes (40)	No
Liver function tests	Elevated	Very high[1]	Normal	Very high[2]	Very high[3]	Normal
Days on ventilator	8	5	2	7	6	2
Co-morbidities 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 haemorrhage	Hypoxic brain injury	Intracranial haemorrhage
Liver weight (g)	1997	2400	1943	1382	2486	2522
Donor warm ischaemic time (minutes)	109	NA	36	NA	31	19
Cold ischaemic time (minutes)	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 discard	Long dWIT, poor liver flush	High LFTs, biopsy findings	Long dWIT, donor history, BMI	High LFTs, macroscopic appearance	Long dWIT, macroscopic appearance	Macroscopic appearance
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)	656	549	529	682	772	360
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
Lactate peak / end of surgery (mmol/L)	7.0 / 4.5	NA	4.3 / 3.0	4.0 / 2.9	5.0 / 3.3	3.6 / 1.4

Abbreviation
ALT=alanine transferase. BMI=body mass index. DVT=deep vein thrombosis. UHB=University Hospitals Birmingham; DBD=donor after brain death. DCD=donor after circulatory death. NA=not applicable. dWIT=donor warm ischaemic time. LFTs=liver function tests.

Note
[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 hours, extra-zonal procurements were performed by other teams, with the expected shipment time greater than 3 hours. [6] expected travel time greater than 4 hours.

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-NMLP	nil	NA	NA	nil	<5%	nil
Post-NMLP	nil	nil	nil	<5%	<5%	nil
Post-reperfusion	nil	NA	nil	<5%	<5%	nil
Small droplet macrovesicular steatosis[2]						
Pre-NMLP	<5%	NA	NA	20%	20%	<5%
Post-NMLP	<5%	30%	<5%	<5%	20%	nil
Post-reperfusion	nil	NA	10%	<5%	25%	10%
Necrosis[3]						
Pre-NMLP	nil	NA	NA	5%	nil	nil
Post-NMLP	1%	15% (old)	5%	nil	30%	nil
Post-reperfusion	1%	NA	10%	1%	50%	5%
Glycogen depletion[4]						
Pre-NMLP	moderate-severe		moderate	minimal	severe	mild-moderate
Post-NMLP	mild	severe	mild-moderate	moderate-severe	mild	nil
Post-reperfusion	moderate	NA	moderate-severe	moderate	moderate-severe	moderate-severe
Post-reperfusion injury	mild	NA	moderate	moderate	severe	moderate-severe

Abbreviation
NMLP=normothermic machine liver perfusion. NA=not applicable / available

Note
[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 which are necrotic.
[4]Glycogen depletion is graded as mild -up to 20% of non-necrotic 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.

Table 3. Recipient demographic and outcomes

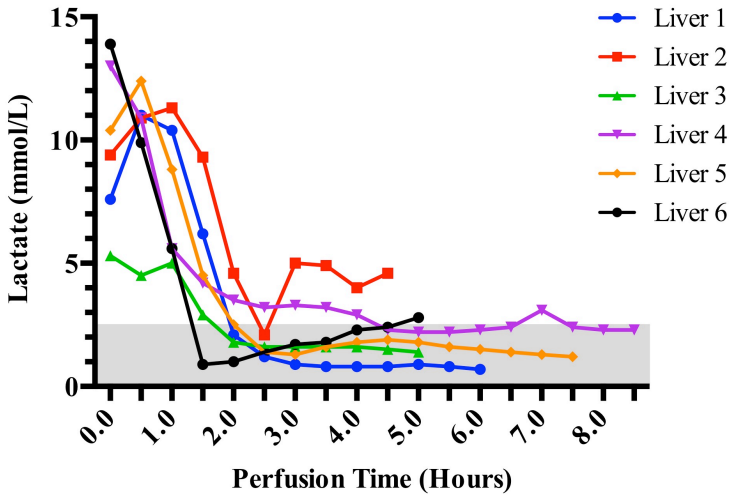
	Recipient 1 (donor 1)	Recipient 2 (donor 3)	Recipient 3 (donor 4)	Recipient 4 (donor 5)	Recipient 5 (donor 6)
Age at transplant (years)	46	56	66	65	56
Sex	Male	Male	Male	Male	Female
Primary etiology	Alcohol	NAFLD	Alcohol and NAFLD	Haemo-chromatosis	Alcohol
Indication for transplant	Encephalopathy	Refractory ascites	HCC	HCC	Refractory ascites
MELD at LT	17	9	7	7	8
UKELD at OLTx	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
Early complications	nil	nil	nil	Myocardial requiring PCI and stent	nil
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

Abbreviation
HCC=hepatocellular carcinoma. MELD=model for end-stage liver disease. UKELD=UK model for end-stage liver disease score. NAFLD=non-alcoholic fatty liver disease; LT=liver transplantation. ALP=alkaline phosphatase. ALT=alanine transferase. AST=aspartate transferase; Bili=Bilirubin. ITU=intensive treatment unit. LFT's=liver function tests. PCI=percutaneous coronary intervention.

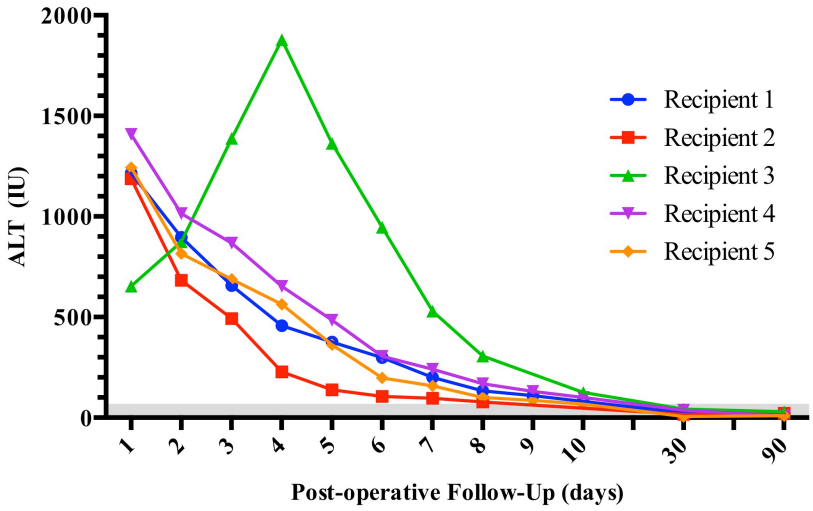
Note
[1] Early allograft dysfunction consists of presence one or more of the following variables: (1) bilirubin 10 mg/dL on postoperative day 7; (2) INR 1·6 on postoperative day 7; (3) aminotransferase level (alanine aminotransferase [ALT] or aspartate aminotransferase [AST]) >2000 IU/mL within the first 7 postoperative days (Olthoff *et al*, Liver Transplantation, 2010)

Figure 1

A


Liver 1
Liver 2
Liver 3
Liver 4
Liver 5
Liver 6
Lactate (mmol/L)
15
10
5
0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Perfusion Time (Hours)

B


Recipient 1
Recipient 2
Recipient 3
Recipient 4
Recipient 5
ALT (IU)
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
10
30
90
Post-operative Follow-Up (days)

C

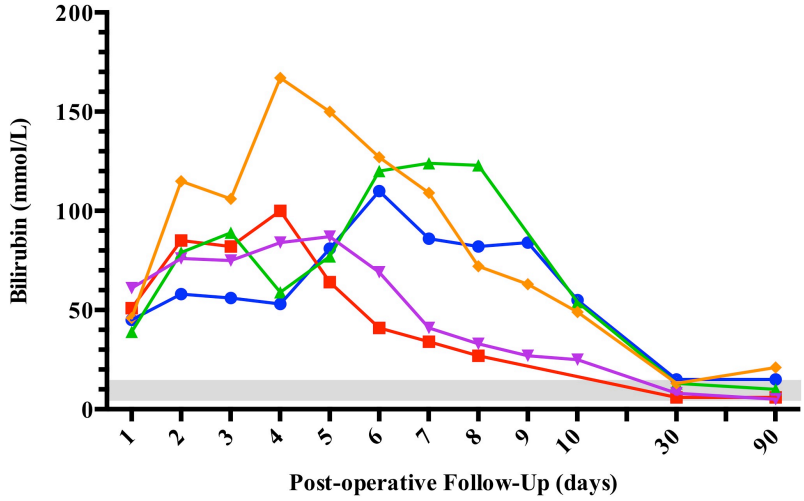

Bilirubin (mmol/L)
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
30
90
Post-operative Follow-Up (days)

Figure 2

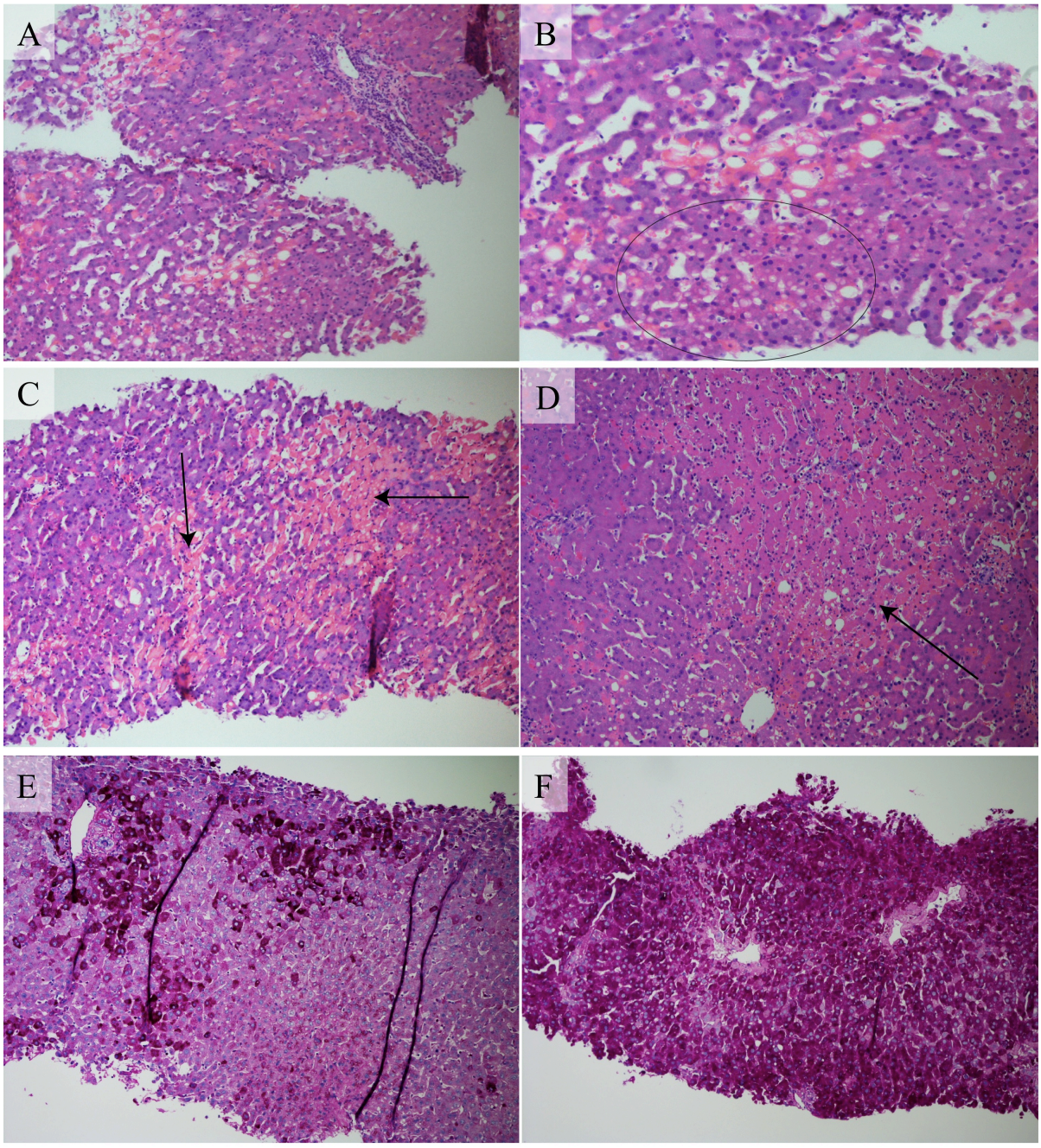


Figure 3

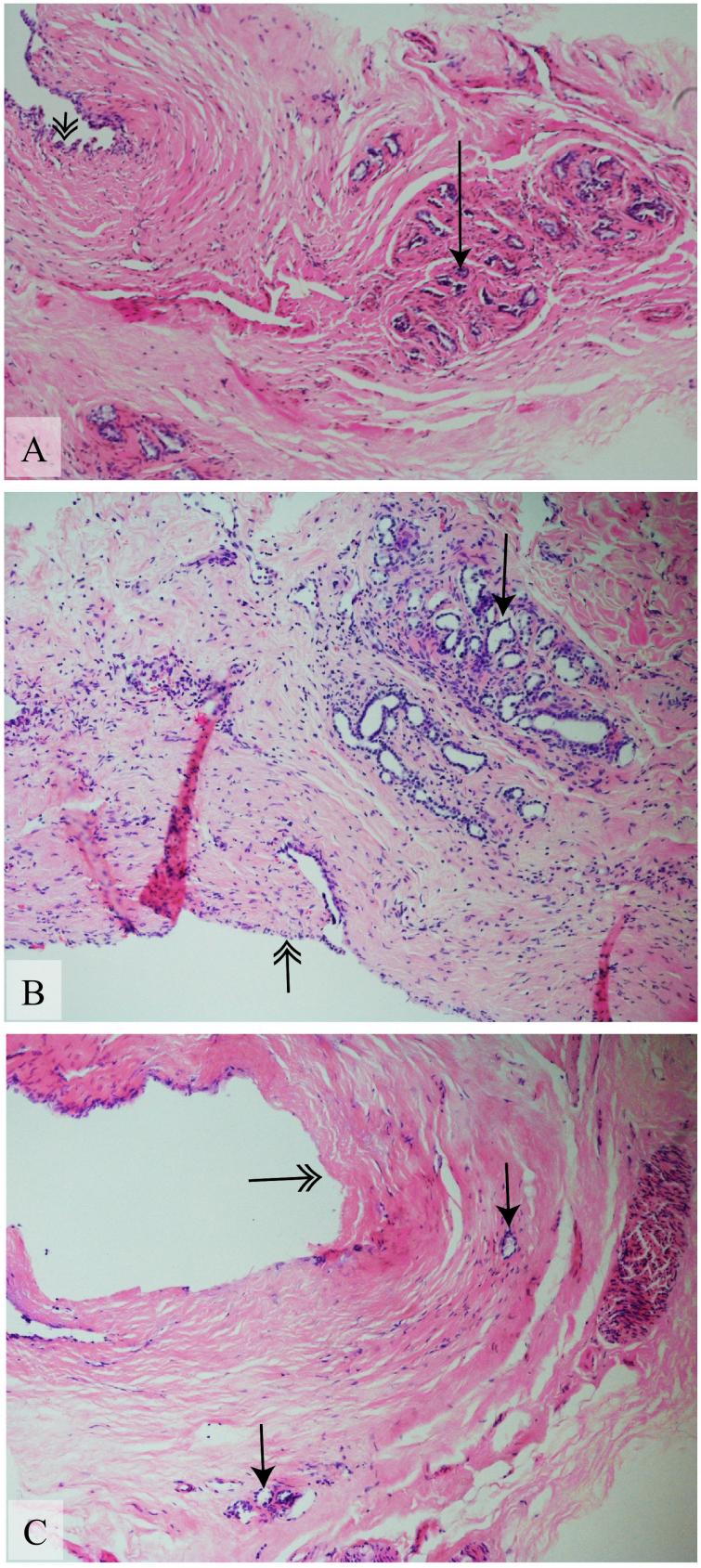


FIGURE LEGENDS

Figure 1: Viability assessment by the perfusate lactate clearance and the post-transplant 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 did not decrease below 2.5 mmol/L but started to rise after 150 minutes. The organ failed to meet the viability criteria and was not used for transplantation. Panel B shows the post-transplant changes in the ALT levels, the enzyme is often used as a surrogate marker for the preservation related liver injury. The initial post-transplant levels were similar in all livers with progressive improvement within the first post-transplant week. In all recipients the ALT levels were normal by one month following the transplantation. Panel C demonstrates similar improvement pattern if bilirubin levels. In the recipient number one bilirubin levels slightly increased later during the follow up and the magnetic cholangiography performed at 6 months post-transplant revealed mild anastomotic biliary stricture. The bilirubin level normalised with a conservative management with ursodeoxycholic acid medication.

Figure 2: Histological findings in liver biopsies

Panels A and B show pre-NMLP haematoxylin and eosin (H&E) stained biopsies from liver number 5. Panel A shows negligible large droplet macrovesicular steatosis (10x 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 which determines suitability for transplantation (20x objective). Panels C and D demonstrate areas of necrosis seen as the pale pink hepatocytes (arrows) in post-NMLP biopsies from liver number 5. Panel C shows approximately 30% necrosis in the pre-implantation biopsy. Panel D shows an increase in the number of necrotic hepatocytes in the post-reperfusion biopsy, approximating to 50% of the liver parenchyma. This liver showed the most necrosis in this presented series, this degree of necrosis is considered unfavourable 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, 10x objective). Panels E and F are Periodic Acid Schiff stained sections of biopsies from liver number 1 in which glycogen in hepatocytes stains dark pink. Panel E shows the pre-NMLP biopsy with moderate glycogen depletion. Panel F shows the post-NMLP biopsy with increased glycogen content, now amounting to only mild depletion (both 10x objective).

Figure 3: Bile duct histology

Figure demonstrates H&E stained sections of bile duct. Double arrowhead shows the surface epithelial lining and single arrowhead points at 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 10x objective).

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