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LETTERS FROM THE FRONTLINE

First Human Liver Transplantation Using a Marginal Allograft Resuscitated by Normothermic Machine Perfusion

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TO THE EDITOR:

Liver transplantation (LT) is plagued by lack of suitable donor organs, and the current waiting list mortality in the United Kingdom approaches 20%. This situation remains unchanged despite progressive expansion of the use of donors previously considered to be too high risk for transplant. Primary nonfunction (PNF) in LT recipients remains the main obstacle to further increasing the use of marginal grafts. The increasing reliance on marginal extended criteria donor livers from older donors, with higher body mass indices or ischemia times is not without consequence. The potential benefit of using these grafts is often offset by post-transplant complications including PNF, delayed graft function, and ischemia-type biliary strictures that are especially seen in recipients of donation after cardiac death (DCD) livers.[1] Therefore, organ discard rates have been increasing, most commonly for degree of graft steatosis, inadequate perfusion, or prolonged warm and/or cold ischemia time.[2]

Ischaemia/reperfusion injury is the fundamental cause of graft damage following static cold storage. The detrimental effect of cold storage is greatest in marginal organs, with PNF representing the worst end of the spectrum of graft dysfunction. The actual risk for a particular graft is difficult to predict though the reported incidence for grafts from extended criteria donors, including those from DCD with cold ischemia time exceeding 8 hours, could be as high as 30%. Recipients of DCD grafts are more likely to experience PNF and delayed graft function, and incidence of ischemia-type biliary strictures remains a problem.

Over the last decade, machine perfusion technology has been investigated to improve the quality of marginal liver grafts based on the encouraging experimental data and results from the clinical experiences. The most profound impact of normothermic machine liver perfusion (NMLP) is derived from this technology's unique ability to assess viability during storage.[3] On the basis of our preclinical perfusion experiments on discarded livers, we developed a viability testing protocol for livers deemed not transplantable then subjected to NMLP. Here, we present the first report of transplantation of a graft salvaged by postischemia NMLP with a follow-up period of 15 months.

MATERIALS AND METHODS

A liver graft offer from a 29-year-old male DCD donor was initially accepted for transplantation by our center within the same allocation region. Withdrawal of life support was performed in accordance with the UK standard practice, in the intensive care unit, without administration of heparin before withdrawal. Oxygen saturation fell quickly and was not measureable within minutes of treatment withdrawal, heralding the onset of donor warm ischemia. However, cardiac death did not occur until 1 hour and 49 minutes later, and this exceeded the current UK recommended criteria of liver graft use from donors with warm ischemia time of <30 minutes. Standard organ procurement followed, however, using University of Wisconsin perfusion solution containing heparin. The procuring surgeon described the graft as a nonsteatotic liver, with soft consistency and several areas of suboptimal perfusion.

Travel time was estimated to be 4 hours, so the projected cold ischemia time on arrival at the closest transplant center was 7 hours. Considering both extended warm and cold ischemia times, the graft was therefore deemed not suitable for transplant by our center and, subsequently, by all other centers upon allocating as a "fast-tracked" offer. Before the organ donation, appropriate consent was obtained from the donor's family for viability testing of a discarded liver

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCD, donation after cardiac death; LT, liver transplantation; MRCP, magnetic resonance cholangiopancreatography; NMLP, normothermic machine liver perfusion; PNF, primary nonfunction.

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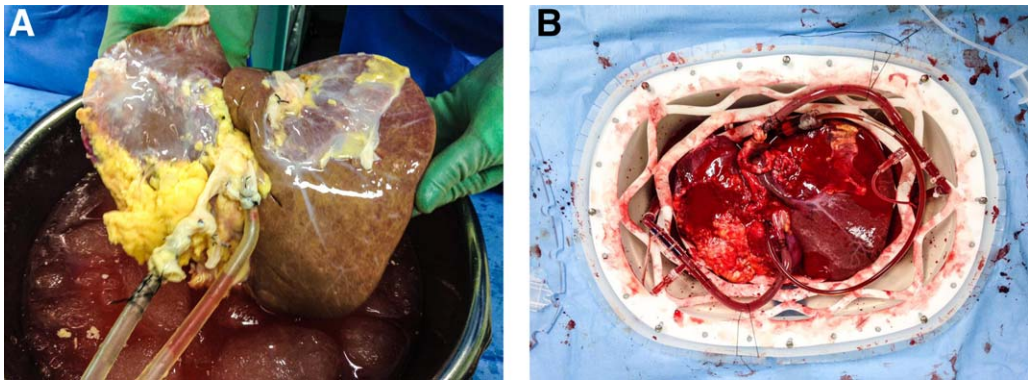

Figure 1. The liver graft on NMLP. (A) The patchy perfused graft after cold preservation and (B) homogeneously perfused liver graft perfused with NMLP.

using NMLP. The graft was therefore transported to our center using static cold storage with the aim to resuscitate. The cold ischemia time before NMLP was 422 minutes. NMLP commenced with the intent to evaluate the liver for transplantation into a human, a procedure which was approved by the Novel Therapeutics Committee. The predetermined parameters used to assess viability were monitored by a multidisciplinary team, and the hospital ethics committee granted an approval for the procedure.

Perfusion with third-party red cell–based fluid at 37 °C using the Liver Assist device (Organ Assist, Groningen, the Netherlands) was done per protocol. This perfusion device provides pulsatile flow through the hepatic artery and nonpulsatile low-pressure flow through the portal vein. The device has an open circuit, meaning the hepatic venous outflow drains into a recirculating reservoir. During the perfusion, hepatic arterial flow, portal venous flow, perfusate blood gases, and bile production were assessed every 30 minutes. After only 3 minutes of NMLP, the graft was pink with homogeneous perfusion (Fig. 1).

The initial arterial and portal venous flow rates were 110 mL/minute and 210 mL/minute, respectively. Flow rates increased rapidly, reaching 727 mL/minute in the hepatic artery and 1090 mL/minute in the portal vein after 1.5 hours of NMLP (Fig. 2A). Flow rates at the end of NMLP were 531 mL/minute for the artery and 1070 mL/minute for the portal vein. Arterial and portal venous pressures were maintained within physiological thresholds (Fig. 2B). Resistances in the artery and portal vein fell as expected and remained low. The initial reservoir temperature was 20 °C, reaching 35 °C after 34 minutes of NMLP.

The initial lactate of 13.3 mmol/L decreased rapidly (Fig. 2C). Bile production commenced approximately 30 minutes after the start of perfusion (Fig. 2D). In total, 27 g of bile was excreted. Histologic assessment demonstrated maintenance of architectural structure on hematoxylin-eosin staining (Fig. 3A-D). Periodic acid–Schiff staining highlighted considerable replenishment of hepatocyte glycogen stores from 30% before NMLP up to 80% after NMLP (Fig. 3E-H). This was accompanied by a reduction in perfusate glucose concentration from a maximum of 24.3 mmol/L to 11.4 mmol/L without the administration of exogenous insulin (data not shown).

In accordance with our protocol for viability testing and transplantation of discarded liver grafts, the organ function was assessed by a multidisciplinary team consisting of 2 transplant surgeons and an anesthetist, all of whom must agree before proceeding with transplantation. Our criteria for transplantability of reconditioned livers are assessed after 2 hours of warm perfusion. At that time, the lactate must be below 2 mmol/L, and the graft must be making bile. After 2 hours of NMLP, the lactate level was 1.2 mmol/L, and bile was being produced; therefore, the decision was made to transplant the liver.

The selected recipient was a 47-year-old male with end-stage liver disease from alcohol use complicated by hepatic encephalopathy. The Model for End-Stage Liver Disease score was 17. The recipient was informed about the NMLP resuscitation, graft appearance, and the novelty of the intervention. Informed consent was obtained. The LT was performed with a vena cava–preserving (modified piggyback) technique with the creation of a temporary portocaval shunt. The NMLP continued throughout the recipient hepatectomy. The graft was then disconnected from the perfusion device and flushed with 2 L of histidine tryptophan ketoglutarate solution (Custodiol, Koehler Chemie, Bensheim, Germany). The liver was reperfused with an artery-first technique, as is our protocol with marginal liver grafts. The duration of NMLP was 416 minutes, and the cumulative time from the donor aortic cross-clamp to reperfusion in the recipient was 13 hours and 58 minutes.

RESULTS

There was no evidence of postreperfusion syndrome or fibrinolysis. The total operative time was 5 hours and 3 minutes, and the recipient required 5 units of packed red cells and 6 units of fresh frozen plasma. The patient's recovery was uneventful. The peak posttransplant alanine aminotransferase (ALT) was 1215 IU/L, on the first postoperative day, and the creatinine remained in the physiologic range (Fig. 4A,B, respectively). The

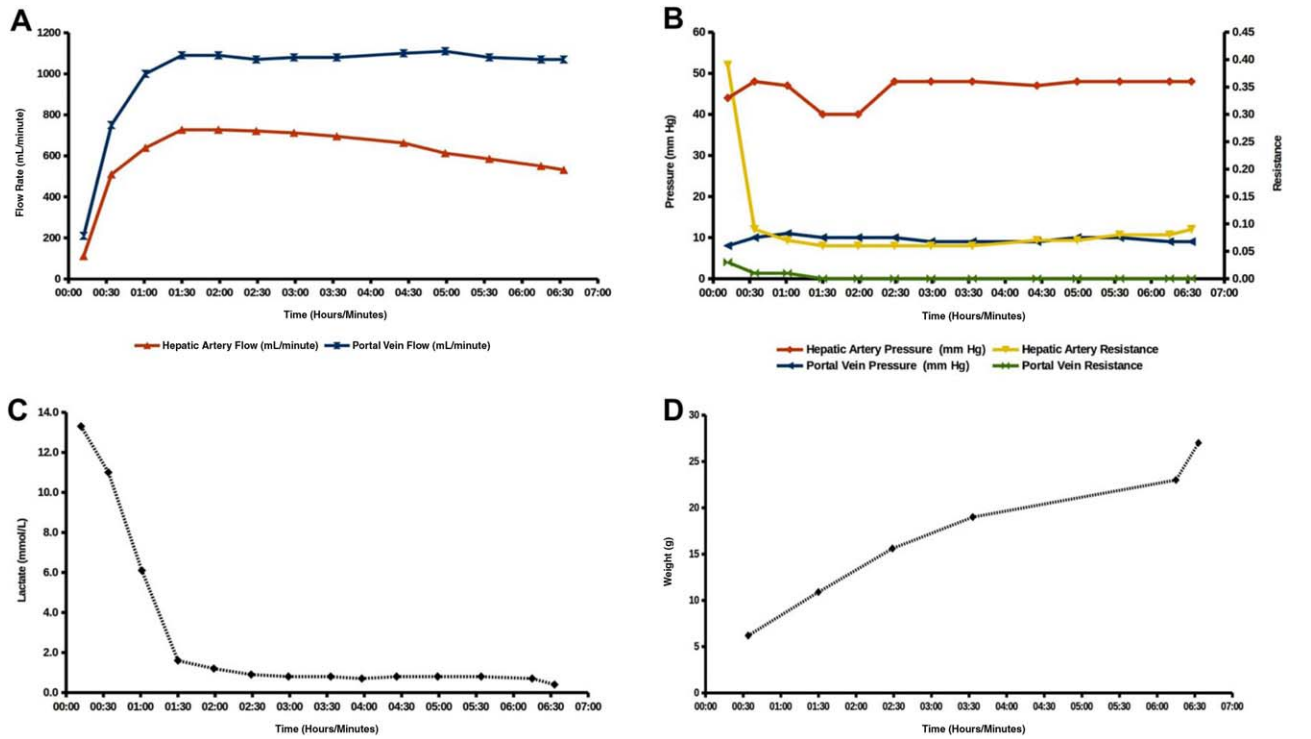

Figure 2. Monitoring liver graft during the normothermic resuscitation using mechanistic parameters and lactate clearance/bile production. (A) Flow parameters, hepatic artery flow (red) and portal vein flow (blue). (B) Hepatic artery pressure (red), hepatic artery resistance (yellow), portal vein pressure (blue), and portal vein resistance (green). (C) Lactate concentration in the arterial end of the circuit. (D) Cumulative bile production.

patient was discharged home on postoperative day 10. Since the first postoperative month, his aspartate aminotransferase (AST), ALT, bilirubin, and alkaline phosphatase (ALP) have remained normal. Per study protocol, the recipient underwent magnetic resonance cholangiopancreatography (MRCP) at 6 months after the transplantation, which showed a mild anastomotic biliary narrowing that has remained clinically silent (Fig. 4C). With 15 months of follow-up, the recipient has normal liver and kidney function, without any evidence of intrahepatic biliary ischemia.

DISCUSSION

We present the world's first case of successful normothermic resuscitation of a liver graft initially deemed too high risk that was rejected for transplantation. We aim to highlight the innovation this new technique presents, and the profound impact it could have on organ utilization.

Liver viability testing may transform the graft selection by providing objective assessment of graft function after procurement. The acceptance criteria for organ usage vary widely among regions depending largely on the local organ availability and waiting-list mortality. The ability to use extended criteria liver grafts in the United Kingdom is significant because of the center-based liver allocation system. This allocation system allows the matching of high-risk organs to low-risk recipients. Applying NMLP technology for viability testing of marginal grafts could finally allow real-time assessment of objective data about liver function. In the United Kingdom, there is potential to increase graft availability by 10%-15% by incorporating such an approach.

Temperature is the principal factor distinguishing different machine perfusion techniques, and the way these techniques can best be implemented in the clinical setting is yet to be defined. Liver perfusion was pioneered by Guarrera et al.,[4] who recently published the results of a series of patients transplanted with very marginal grafts preserved by hypothermic perfusion with successful outcomes with low incidence of PNF (3%) and early allograft dysfunction. In parallel, the Zurich group introduced into clinical practice hypothermic oxygenated machine perfusion.[5] The advantage of cold perfusion is its simplicity and resultant low risk of device failure–related graft loss. NMLP is technically more challenging, and therefore, carries a small risk of graft loss related to the device malfunction. The overwhelming advantage of NMLP is that it is the only machine perfusion technique that can evaluate graft viability and provide a real-time indication of the organ transplantability.

The presented 15-month follow-up data might be too short to eliminate the presence of asymptomatic nonanastomotic biliary strictures, which can occur in DCD grafts that may present very late.[1] The presented

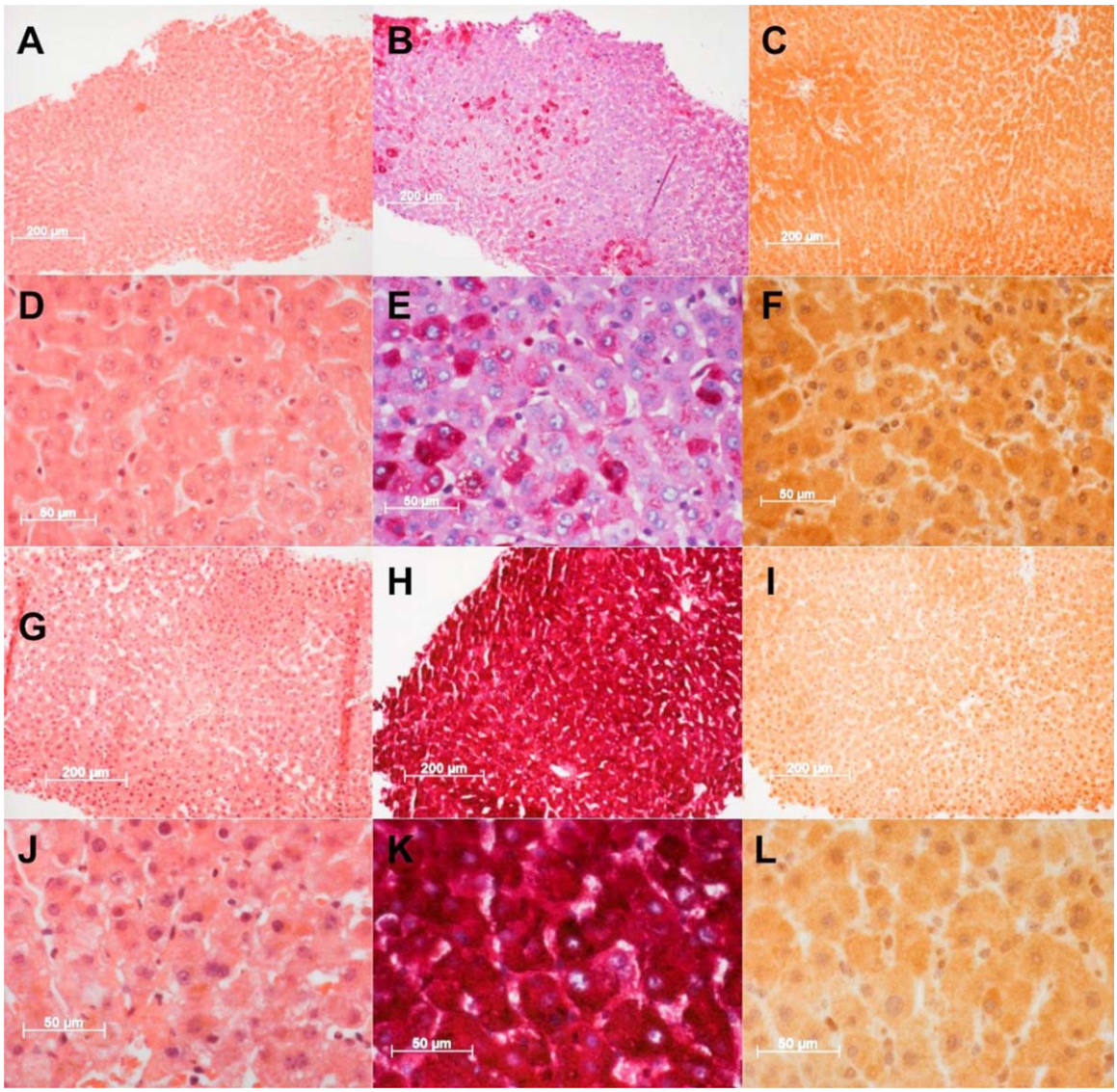

Figure 3. Histology from 16-gauge biopsy samples. (A-F) Before the NMLP and (G-L) at the end of NMLP but before implantation. (A,D,G,J) Hematoxylin-eosin staining to demonstrate tissue architecture; (B,E,H,K) periodic acid–Schiff staining to detect polysaccharide content, including glycogen stores; (C,F,I,L) complement degradation product 4d immunostaining to demonstrate necrotic hepatocytes. (A-C, G-I) At × 10 magnification; (D-F, J-L) at × 40 magnification.

patient's case highlights the potential benefits of NMLP, though these need to be validated by adequately powered clinical trials.

The patient's safety is of paramount importance when physicians introduce a novel technique to the clinical practice. In our unit, the viability testing protocol and the institutional project approval was granted for a small, closely monitored recipient series of carefully selected discarded grafts. We considered a nonsteatotic DCD graft from a young donor to be an acceptable risk–benefit balance for the first clinical use of this technique. Furthermore, our group was also involved in the first human safety and feasibility study of continuous NMLP (ORGANOX study; in press) and currently a major contributor to the randomized European multicenter trial investigating the benefits of continued NMLP versus static cold preservation (consortium of organ preservation in Europe [COPE] trial). The procedure described herein was performed in the background of this experience; undoubtedly such experience helped us in this approach.

In conclusion, this first report proves the clinical feasibility of liver viability testing by NMLP on a liver that was deemed unsuitable for transplant by all the centers in the United Kingdom. With the progressive graft shortage and increasing waiting-list mortality, this technique may revolutionize the use of extended criteria grafts by providing objective functional parameters in real-time during storage to determine transplantability. NMLP may salvage discarded grafts, and minimize the risk of early graft dysfunction and PNF when transplanting marginal livers. Although the recipient in this case has excellent liver function at 10

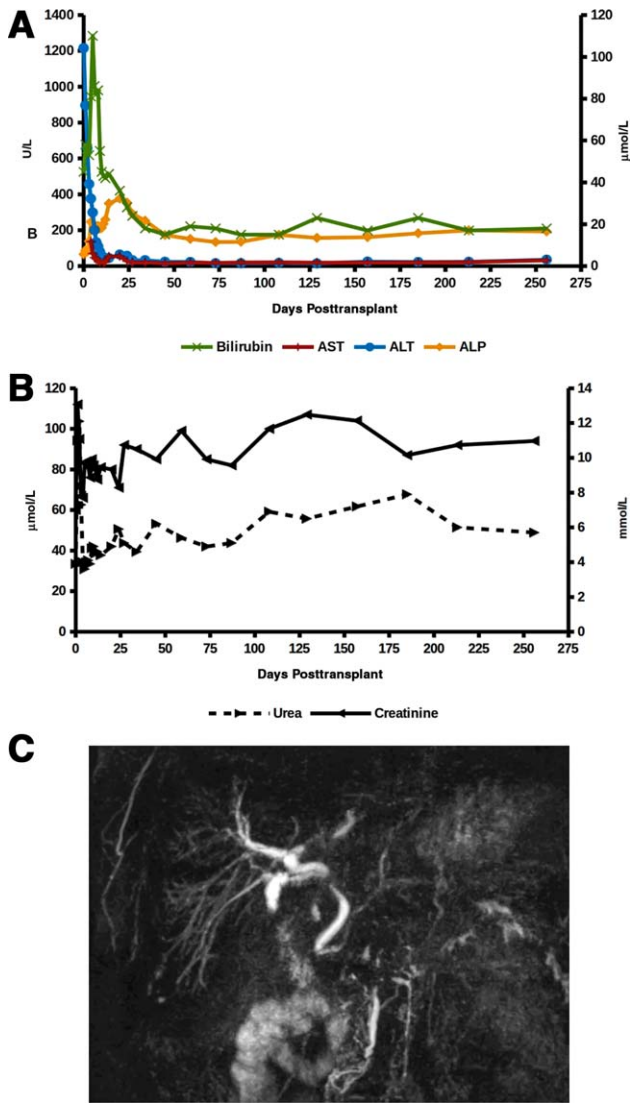

Figure 4. Postoperative biochemistry: (A) liver function, (B) renal functions of the patient with (C) follow-up MRCP.

months, a prospective randomized trial is warranted to better define longterm outcomes and effects on organ utilization rates.

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