The Case for Normothermic Machine Perfusion in Liver Transplantation

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Abstract

In recent years there has been growing interest in normothermic machine perfusion as a preservation method in liver transplantation. In most countries, due to a donor organ shortage, an unacceptable number of patients die whilst awaiting transplantation. In an attempt to increase the number of donor organs available, transplant teams are implanting a greater number of high-risk livers; including those from donors after circulatory death, older donors and with steatosis. Normothermic machine perfusion maintains the liver *ex* vivo on a circuit by providing oxygen and nutrition at 37^{o}C. This permits extended preservation times, the ability

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1) Abbreviations: AST, aspartate aminotransferase; ATP, adenosine triphosphate; COPE, Consortium for Organ Preservation in Europe; DBD, donor after brain-stem death; DCD, donor after circulatory death; EAD, early allograft dysfunction; IRI, ischaemia reperfusion injury; NMP, normothermic machine perfusion; SCS, static cold storage
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to perform liver viability assessment and the potential for liver-directed therapeutic interventions during preservation. It is hoped that this technology may facilitate the enhanced preservation of marginal livers with improved post-transplant outcomes by reducing ischaemia reperfusion injury. Clinical trials have demonstrated its short-term superiority over cold storage in terms of early biochemical liver function and it is anticipated that it may result in increased organ utilisation, helping to reduce the number of waiting list deaths. However, further studies are required to demonstrate longer-term efficacy and the impact on biliary complications as well as further knowledge to exploit and maximise the potential of this exciting new technology.

Introduction

Preserving organs under physiological conditions is not a new concept. In 1935, Alexis Carell and Charles Lindbergh developed a perfusion chamber for the normothermic preservation of organs and demonstrated their viability when perfused with oxygenated serum at 37^{o}C for several days (1). However, due to its complexity, the technique was not widely adopted. Later, in the 1960s, several groups explored machine perfusion as a method of dynamic liver preservation but with complex logistical considerations and the advent of simple and effective static cold storage solutions (3), interest waned. Now, some 50 years later, the transplant community has seen a resurgence in enthusiasm for machine perfusion of organs. This is largely due to the need to expand the donor pool by successfully transplanting marginal organs. In this review article, we will focus on normothermic machine perfusion (NMP) and its role in liver preservation and transplantation.

The Need for New Technology

At present, liver transplantation's potential as a life-saving treatment option for patients with end-stage liver disease is limited by a shortage of available donor organs. A paucity of livers in the deceased donor pool results in a concerning number of patients, around 15-20% in the US and UK (4, 5), dying whilst on the waiting list. Changes in donor demographics compound the issue; with improvements in road safety and medical care leading to a highly desirable reduction of "ideal" younger donors, and the necessary move to older donors with more advanced co-morbidities (6). The world-wide obesity epidemic results in a greater number of donors with incidental hepatic steatosis (7), further contributing to the number of marginal livers in the donor pool. The number of livers from donors after circulatory death (DCD) is

also increasing; particularly in the UK, where 42% of the donor pool and 21% of transplants are made up of DCD livers (8). Unfortunately, graft loss and recipient mortality have been shown to be almost twice as high with DCD livers (9) and their use, therefore, remains limited. In order to bridge the gap between supply and demand for livers, more marginal livers are being transplanted with the accepted risk of increased complications such as primary non-function and ischaemic cholangiopathy (10, 11). The need to safely and reliably transplant more livers without compromising the outcome provides a powerful incentive for innovation, and thus the resurgence in interest in NMP, which has rapidly come to the fore in recent years.

Mechanism of Action

Static cold storage (SCS) is the standard-of-care preservation method in liver transplantation. The aim is to maintain structural and functional integrity of the liver so as to promote function at reperfusion. This is achieved through cooling to 4^{o}C with preservation solution which decreases cellular energy consumption by reducing the metabolic demand of the tissue. The composition of the preservation solution aims to reduce cell swelling and lysis associated with cooling. A primary objective of preservation is to attenuate ischaemia-reperfusion injury (IRI) which results from the efflux of accumulated metabolic products formed during ischaemia, resulting in a profound inflammatory immune response on reperfusion, causing hepatocellular injury. For a low-risk liver, SCS is an adequate preservation method as these livers are able to tolerate the modest levels of IRI with satisfactory post-transplant outcomes and survival rates. However, marginal livers tolerate ischaemia poorly, experiencing a more severe inflammatory response and reperfusion injury resulting in poor short and long-term outcomes.

NMP maintains the liver *ex vivo* in a fully functioning state, providing it with oxygen and nutrients at 37^{o}C. Several NMP circuits have been described in detail (12-16); these function on the principle of physiological preservation which maintains cellular metabolism throughout the preservation period. Principal components include: blood reservoir; pump(s) (some circuits comprise two pumps, for portal venous and hepatic arterial flow); an oxygenator; and, a heat exchanger. A comparison of the devices which are either commercially available or used in clinical trials is shown in table 1. NMP's superiority over SCS in terms of synthetic and metabolic liver function and post-transplant survival has been shown in large animal models (13, 14) through evidence of *ex vivo* bile production, reduction of markers of cell injury and post-transplant survival. However, the precise mechanism(s) underpinning the beneficial effects of NMP have not yet been fully elucidated. It is likely that perfusion helps maintain a

healthy endothelium and replenish adenosine triphosphate (ATP). The importance of increasing hepatic ATP in human liver transplantation has previously been shown, with a direct correlation between high hepatic ATP content and good post-transplant outcomes (17). NMP's role in ATP regeneration has been confirmed in murine experiments where rapid ATP recovery following initiation of NMP has been demonstrated (18, 19) as well as mitochondrial ATPase activity (20). More recently, human studies have demonstrated histological evidence of glycogen repletion during NMP (21). Glycogen is essential to maintain hepatocellular integrity and function by supplying glucose for ATP generation. Once glycogen is consumed, ATP depletion ensues, leading to irreversible cell injury and necrosis (22). In order to further exploit and maximise NMP's potential, a sound understanding of the injury and repair pathways affected during preservation will be important. This may be aided by a multiplatform –omics approach in combination with computational biology (23).

Clinical Experience of NMP

In recent years, clinical experience of NMP has grown and the body of evidence supporting the beneficial impact of NMP in liver transplantation has increased. The first NMP livers were transplanted as part of a phase one study undertaken by Ravikumar *et al* which demonstrated the safety and feasibility of NMP from retrieval to transplantation, including transportation (24). This was the first study to report post-transplant outcomes from livers preserved by NMP. As well as safety and feasibility (the primary purpose) this trial demonstrated a significant biochemical benefit in the form of a halving of peak serum aspartate aminotransferase (AST) when compared to matched controls preserved via SCS. The peak serum AST in the first 7-days post-transplant is a validated surrogate marker for graft and patient survival (25-27). Two further pilot studies were subsequently performed by Selzner *et al* (28) and Bral *et al* (29) which once again demonstrated the safety and feasibility of the technology and compared post-transplant outcomes to cold-stored controls. Selzner and colleagues demonstrated a non-significant reduction in peak serum AST in the NMP-preserved livers compared to retrospective SCS controls but this finding was not achieved by Bral and colleagues, in whose study there was a non-significant increase in the median peak serum AST in the NMP cohort. It is important to note that none of these studies was either randomised or powered to demonstrate a difference in outcomes. Across these three studies, only one graft was lost during preservation. This was due to an occult twist in the portal vein which was obscured within the hepatic hilum, compromising perfusion of the liver (29). A summary of published studies from

livers transplanted following NMP is shown in table 2. A phase 3 multi-centre randomised controlled trial to compare the efficacy of NMP versus SCS in liver transplantation was recently conducted by the Consortium for Organ Preservation in Europe (COPE; www.cope-eu.org). A total of 222 livers were transplanted as part of the study (121 NMP, 101 SCS) with the primary endpoint of difference in peak serum AST between the two treatment arms. Data from a published abstract (30) demonstrates that the study achieved its primary endpoint with a significant reduction in peak serum AST in the NMP group (974 IU/L SCS vs 485IU/L NMP; $p<0.001$). There was also a significant reduction in early allograft dysfunction (EAD), another surrogate marker of graft outcome, in livers preserved via NMP (29.9% SCS vs 12.6% NMP; $p=0.002$). Another notable finding was that of a reduced rate of liver discard in organs randomised to NMP and there was a significant difference between the two groups (32 SCS vs 16 NMP; $p=0.01$). Although, to date, no trial has shown an improvement in patient or graft survival, or reduction in biliary complications, it is notable that trials with much larger numbers and longer-term follow-up would be required to test these outcomes. Even if the sole benefit of NMP were shown to be improved organ utilisation with superior early biochemical function when compared to SCS, this would have an enormous impact on reducing the number of deaths on the waiting list.

The Role of NMP in Increasing Organ Utilisation

Over the last 10 years in the UK, the number of livers retrieved but not transplanted has doubled from 8.2 to 16.6% (8). The situation is somewhat worse in the US, with the Organ Procurement and Transplantation Network reporting that only 78% of potential donor livers were transplanted (31). Livers are discarded based on reported donor characteristics as well as the gross appearance of the organ (32-34). However, the lack of validated, objective predictors of function inevitably results in organs being turned down that would have functioned if transplanted. As well as reducing IRI and promoting liver regeneration (35), the potential to perform a viability assessment on the liver *ex vivo* will undoubtedly increase the number of transplanted livers as surgeons can make an objective assessment of a liver's function during preservation, with the potential to predict post-transplant outcomes. Mergental *et al* recently reported the normothermic reconditioning of 6 livers which had been rejected by all UK liver transplant centres (21). Five of these livers were subsequently transplanted as they demonstrated *ex vivo* function; specifically lactate clearance, bile production, acid/base homeostasis, stable flow dynamics as well as healthy graft appearance and consistency. At a

median 7 (range 6-19) months follow-up, all recipients remained well with functioning grafts. Watson *et al* (36) also published their experience of transplanting livers which had been declined by other UK liver transplant centres, following NMP and assessing organ quality and function. They observed that preserving livers at physiological oxygen tensions resulted in a reduced incidence of post-reperfusion syndrome and suggested the importance of biliary pH in predicting post-transplant cholangiopathy. The Birmingham group is undertaking a further clinical trial to assess more formally the benefit of NMP in liver grafts declined by other centres. A summary of all registered on-going NMP clinical trials is shown in table 3.

There is some evidence that NMP is particularly beneficial in high-risk sub-groups such as DCD and steatotic livers. Brockmann *et al* (13) investigated extended preservation times (20 hours) in both DBD and DCD (with 40 min warm ischaemia) porcine models and demonstrated markedly superior outcomes compared with SCS. It is notable that no difference in outcome was seen in DBD livers preserved for only 5 hours, suggesting that the main benefit of NMP might be in high-risk livers. Furthermore, in the COPE RCT, the improvement in early biochemical function observed with NMP livers was greater in the DCD cohort ($p = 0.02$) (30). These data suggest that NMP has a role in reversing energy depletion and the immediate effects of warm ischaemia; this may be a particularly important finding when only around 5-10% of DCD livers are transplanted in the US and Euro-Transplant regions. It has also been reported in porcine and rodent models of hepatic steatosis that NMP can maintain physiological function compared to lean livers and that the fat content of the liver can be reduced through the use of specific de-fatting agents administered during NMP (37, 38).

As well as enhanced preservation and functional liver assessment contributing to increased utilisation, improved logistics might also result in more livers being transplanted. This is attributable to increased preservation times of up to 24hrs on the NMP device which can facilitate a more structured and organized approach to transplantation by improving utilisation of the operating room and elective lists, arranging appropriate staffing levels and facilitating the pre-operative preparation of the recipient.

The Future of NMP

As experience with this novel technology increases, new questions will be posed. These will refine the way this technology is used and enhance its potential. NMP is logistically more challenging and costlier than SCS, particularly when used for the whole preservation period,

where dedicated personnel may be required to attend the donor hospital and different arrangements made for transportation. Indeed, Selzner *et al* acknowledged that the use of NMP prolonged the organ retrieval process by 2hrs because of back-bench preparation of the liver, cannulation, and connection to the device (28). The majority of NMP livers have been transplanted following perfusion for the entire preservation period from the donor to the recipient. However, it would be logistically more straight-forward and less expensive if livers could be placed on the NMP circuit at the recipient hospital, following a period of SCS, termed post-SCS-NMP (pSCS-NMP) (39). The safety and feasibility of this approach has recently been tested by the Oxford Group in a clinical trial setting with outcomes compared to the NMP and SCS cohorts from the COPE RCT; the detailed results of this study are awaited (table 3). Further studies will be needed to explore: longer-term outcomes; late biliary complications; outcomes in specific high-risk groups; viability biomarkers; optimum and maximum perfusion duration; perfusate composition; and liver-directed therapeutic interventions during NMP. These challenges provide exciting clinical and academic opportunities for those working with this novel technology.

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TABLE 1. TECHNICAL COMPARISON OF NMP DEVICES

Device	Arterial Flow	Pressure Control	Cannulation	Oxygen Delivery Method	Preservation Duration	Battery Life	Temperature	Transportable during preservation	No. of livers transplanted using device
OrganOx *metra*® (OrganOx Ltd, Oxford, UK).	Continuous	Automated	Hepatic Artery Portal Vein IVC Closed System	On-board oxygen concentrator	24hrs	2-4hrs	Normothermic	Yes	>240
Liver Assist (Organ Assist, Groningen, the Netherlands)	Pulsatile	Manual	Hepatic Artery Portal Vein Open IVC Drainage	External gas mixer and/or cylinder	24hrs (oxygenator dependent)	20mins	Hypothermic Sub-normothermic Normothermic	No	>300 (hypothermic, sub-normothermic + normothermic)
OCS™ Liver System (Transmedics, Andover, Massachusetts)	Information not currently available								
Cleveland NMP Device (Cleveland Clinic, Ohio)	Continuous	Manual	Hepatic Artery Portal Vein Open IVC Drainage	Mixing air and pure oxygen	18hrs	30mins-4hrs	Sub-normothermic Normothermic	Yes	15

TABLE 2. PUBLISHED MANUSCRIPTS OF TRANSPLANTED NMP LIVERS

Author	**Group**	**Year Published**	**Device**	**Study Design**	**No. of livers transplanted**	**%DBD/ DCD**	**Primary Outcome(s)**
Ravikumar *et al* (24)	Oxford	2016	OrganOx *metra*®	Phase I pilot with retrospective matched controls	20	80% DBD 20% DCD	30-day patient and graft survival
Selzner *et al* (28)	Toronto	2016	OrganOx *metra*®	Phase I pilot with retrospective matched controls	10	80% DBD 20% DCD	90-day patient and graft survival
Bral *et al* (29)	Edmonton	2016	OrganOx *metra*®	Phase I pilot with retrospective matched controls	9	66.7% DBD 33.3% DCD	30-day graft survival
Mergental *et al* (21)	Birmingham	2016	Liver Assist OrganOx *metra*®	Case Series	5	20% DBD 80% DCD	
Watson *et al* (36)	Cambridge	2017	Liver Assist	Case Series	12	25% DBD 75% DCD	

TABLE 3. ONGOING REGISTERED CLINICAL NMP TRIALS

Study Title	Study Type	Estimated Enrolment	Primary Outcome(s)	Start Date	Identifier	Device	Group
Efficacy Evaluation of NMP in Liver Transplant using Very Old Donors (CEFEMA)	Randomized Pilot	30	6-month graft survival	October 2016	NCT02940600	Liver Assist (Organ Assist, Groningen, the Netherlands)	Pisa, Italy
Normothermic Liver Preservation	RCT	226	EAD	June 2016	NCT02775162	OrganOx *metra*® (OrganOx Ltd, Oxford, UK).	OrganOx Ltd., Oxford, UK NAMSA, USA
Post Static Cold Storage NMP	Phase II Safety and feasibility	30	30-day patient and graft survival	May 2017	NCT03176433	OrganOx *metra*® (OrganOx Ltd, Oxford, UK).	Oxford, UK
Normothermic Liver Preservation Trial	Phase II	50	30-day graft survival	February 2017	NCT03089840	OrganOx *metra*® (OrganOx Ltd, Oxford, UK).	Edmonton, Canada
Pilot Study to Assess Safety and Feasibility of NMP in Human Liver Transplantation	Phase I Pilot	32	EAD	July 2015	NCT02515708	Cleveland NMP Device	Cleveland, Ohio
Viability Testing and Transplantation of Marginal Livers (VITTAL)	Prospective Non-randomized	22	90-day patient survival NMP to identify the proportion of transplantable livers from currently rejected donor pool	October 2016	NCT02740608	OrganOx *metra*® (OrganOx Ltd, Oxford, UK).	Birmingham, UK
TransMedics (OCS) Liver Trial: Preserving and Assessing Donor Livers for	RCT	300	EAD SAEs	January 2016	NCT02522871	OCS™ Liver System (Transmedics, Andover, Massachusetts)	TransMedics, Andover, Massachusetts, USA

Transplantation (Liver PROTECT)							
Using Ex-vivo Normothermic Machine Perfusion with the OrganOx Metra™ Device to Store Human Livers for Transplantation	Phase I	40	Rates of: PNF Re-transplantation Recipient Death	Dec 2014	NCT02478151	OrganOx *metra*® (OrganOx Ltd, Oxford, UK).	Toronto, Canada
Work Package 2 (WP2) - Normothermic Liver Perfusion Vs Cold Storage in Liver Transplants	RCT	220	Peak Serum AST	April 2014	ISRCTN39731134	OrganOx *metra*® (OrganOx Ltd, Oxford, UK).	COPE Consortium, Oxford, UK