Role of Machine Perfusion in Liver Transplantation

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KEYWORDS

• Liver transplantation • Surgical techniques • Post-transplant • Machine perfusion • Liver

KEY POINTS

- One of the strategies to increase the number of available livers for transplantation is to improve organ utilization through the use of marginal organs, including elderly, overweight, or organs donated after circulatory death.
- The utilization of these "marginal" organs was associated with an increased risk of early allograft dysfunction, primary nonfunction, ischemic-type biliary complications, or even re-transplantation.
- One of the most promising strategies is the utilization of ex vivo machine perfusion.
- Although tremendous progress has been made over the past years in the fields of procurement, preservation, surgical techniques, and post-transplant immunosuppression, the mortality on the waiting list remains high due to an ever-increasing shortage of suitable donor organs.

INTRODUCTION

Liver transplantation stands as the sole curative treatment for individuals suffering from end-stage liver disease. Although tremendous progress has been made over the past years in the fields of procurement, preservation, surgical techniques, and post-transplant immunosuppression, the mortality on the waiting list remains high due to an ever-increasing shortage of suitable donor organs.[1] This current shortage of organs led to an increase in the utilization of extended criteria donors (ECDs) for transplantation, which has the potential to address the unmet needs in liver transplantation. Unfortunately, ECD livers have a higher likelihood of developing early allograft

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dysfunction (EAD), primary nonfunction (PNF), or serious late-onset complications such as ischemic cholangiopathy (IC).[2] In the recent years, ex vivo liver machine perfusion before transplantation has emerged as an attractive approach. Using machine perfusion, the rate of complications observed with donation after circulatory death (DCD) livers was found to be comparable to that of livers obtained following brainstem death (donation after brain death [DBD]).[3,4] In addition to graft preservation, machine perfusion offers an opportunity to assess the organ viability, increase organ sharing, and treat or repair livers historically considered unsuitable for transplantation. Moreover, studies have also reported positive impact of machine perfusion on graft rejection[5] and the recurrence of hepatocellular carcinoma.[6] The latter highlights potential broader therapeutic effects machine perfusion. Although machine perfusion has not yet surpassed the advantages of the simple and inexpensive static cold storage (SCS) (in particular for standard or DBD organs), the rapid increase in the utilization of ECD livers for transplantation has led to a significant interest in the use and development of machine perfusion technologies. These advancements are expected to revolutionize the field in the near future. In this review, the authors discuss the role of machine perfusion in liver transplantation with a particular focus on its clinical application.

MACHINE PERFUSION

General Principles of Machine Perfusion

In the current practice of organ transplantation, the process of procurement and storage of organs ultimately leads to ischemia–reperfusion (IR) injury. The extent of this injury varies depending on the duration of both cold and warm ischemia times. Compared with standard criteria donor livers, ECD livers, which include DCD livers, livers with severe steatosis, elderly livers, or livers infected with hepatitis C are at a greater risk of IR injury, clinically manifest as EAD or PNF.[7] The mechanisms suggested to underlie the benefits of machine perfusion include the suppression of IR injury, providing continous nutritional supplementation to the graft, and improvement in the clearance of deleterious metabolites during perfusion.[8,9] Machine perfusion of liver transplants can be categorized into three types based on the target temperature.[10] (1) Hypothermic machine perfusion (HMP), which typically involves temperatures of 4°C to 12°C HMP can be used to perfuse the portal vein only or both portal and arterial systems. In addition, HMP can be performed with or without the use of oxygen. HMP with oxygen is known as hypothermic oxygenated machine perfusion (HOPE). (2) Normothermic machine perfusion (NMP), which mimics the human body temperature (34–37°C) and requires the use of an oxygen carrier (blood or artificial hemoglobin). (3) Subnormothermic machine perfusion (SNMP), an intermediate approach between HMP and NMP. SNMP is performed at temperatures between 20°C and 22°C. The purpose of SNMP is to reduce the oxygen demand to a level that does not require the use of an oxygen carrier while still maintaining metabolic activity and the ability to assess liver function. In addition to the temperature-based categorization, machine perfusion of liver transplants can further be classified into ex situ machine perfusion and in situ machine perfusion. Ex situ machine perfusion refers to the perfusion procedure that takes place after the liver has been recovered from the donor's body. In this approach, the liver is transported to a specialized perfusion system where it is perfused with the desired solution and maintained under controlled conditions. Ex situ machine perfusion allows for extended preservation and assessment of the liver outside of the donor's body before transplantation. On the other hand, in situ machine perfusion, also known as normothermic regional perfusion

(NRP), involves perfusing the liver with a preservation solution, whereas it is still inside the donor's body, before organ recovery. This technique allows for the assessment and treatment of the liver in its native environment, providing a more realistic representation of the organ's function and response to perfusion. In situ machine perfusion, or NRP, might also offers the advantage of maintaining near physiological conditions during the perfusion process. Finally, machine perfusion can be initiated at the donor hospital using a portable device, allowing for limited ischemic time, immediate preservation, and assessment of the liver graft.[3] Alternatively, the liver graft can be transferred on ice and machine perfusion started at the recipient site. This is known as end-ischemic perfusion. Finally, combinations of different therapies/types of machine perfusion techniques have gained popularity in recent years. These combinations involve using different perfusion modalities, such as incorporating hypothermic, normothermic, or subnormothermic perfusion at various stages of the transplantation process.[11,12] An overview of clinical devices available is presented in **Table 1**.

Hypothermic Machine Perfusion

Until very recently, the transplantation field heavily relied on SCS as the primary method for preserving livers intended for transplantation. However, despite its ability to slow down metabolism, SCS does not completely suspend cellular metabolism, resulting in the continued consumption of cellular energy stores during storage. Moreover, SCS is characterized by the absence of flow, whereas pulsatile preservation was shown to upregulate nitric oxide (NO) production by the vascular endothelium and faciliate the clearance of debris and toxic metabolites.[13] HMP works by providing a continuous flow to the portal circulation with or without hepatic artery perfusion. The role of oxygen will be discussed later (see below). The perfusates commonly used during liver HMP are based on the original or modified University of Wisconsin (UW) solution or UW machine perfusion solution. Perfusion studies have also been conducted using IGL-1 (Institut Georges Lopez), Celsior solution, or HTK (Histidine-Tryptophan-Ketoglutarate) solutions, but there is no direct comparison available. Lower potassium concentrations decrease vascular resistance during hypothermia, whereas the presence of starch increases viscosity. Therefore, solutions with low potassium and without starch seem advantageous.[14,15] In a pig model, single portal perfusion, the perfusate reaches all hepatocytes within less than 1 minute after initiating low-pressure cold perfusion. Considering the low oxygen demand at 4°C, supplying oxygen through single portal perfusion seems to be adequate, at least for hepatocytes, endothelial cells, Kupffer cells, and intrahepatic interlobular biliary branches.[16] Consistently, studies on discarded human livers undergoing HMP showed no significant difference in perfusion quality between singular perfusion of the portal vein or the hepatic artery alone compared with dual perfusion.[17] Another aspect of HMP is optimal perfusion pressure, aimed at maximizing perfusion while minimizing endothelial injury. Liver sinusoids are highly sensitive to endothelial shear stress. Previous studies in rat livers have indicated that reducing the portal perfusion pressure to 4 mm Hg allows for complete perfusion without endothelial injury, whereas perfusion at 8 mm Hg leads to endothelial damage.[17,18] In human livers, a low portal perfusion pressure around 3 mm Hg ensures complete perfusion without any signs of sinusoidal impairment.[18] Finally, it should be noted that hypothermia does not completely halt aerobic metabolism, and even at 4°C, the oxygen demand of the liver may exceed the available supply. Although dissolved oxygen may be adequate at standard DBD livers, DCD livers exposed to warm ischemia before organ procurement, have higher oxygen demands.[19,20] The optimal level of oxygenation also remains unclear, and high O_2 during HMP might increase oxidative stress.[21] Of

Table 1
Perfusion devices

Manufacture	OrganOx	Xvivo	TransMedics	Organ Recovery System	Bridge to Life
Name	Metra	Liver Assist	OCS liver	Lifeport Liver	Vitasmart
Type	NMP	HOPE & NMP	NMP	HMP	HMP & HOPE
Perfusion Temperature	37°C	12–38°C	34°C	4°C	4°C
Arterial Flow/ Pressure	Pressure controled (artery)	Pressure controlled. Artery and portal flows independent	Flow controlled. Total distributed to arterial and portal cannula	Continuous Flow	Continuous Flow
Portal Flow/ Pressure	Not adjustable	Pressure controlled. Artery and portal flows independent	Flow controlled. Total distributed to arterial and portal cannula	Continuous Flow	Continuous Flow
Transportable	Yes	No. Battery operated for 20-min	Yes	Yes	Yes

interest, the rate of oxygen consumption during HMP rapidly decreases within the first hour and is eventually arrested after 90 minutes of perfusion,[15] despite sufficient levels of substrate (adenosine diphosphate [ADP], oxygen). Currently, there is no randomized trial evaluating HMP without oxygen.

In a retrospective study, HMP with UW reduced the length of hospitalization and peak aspartate aminotransferase (AST) serum levels compared with SCS.[19] Subsequently, the same group showed that HMP was associated with fewer biliary complications, compared with SCS in ECD livers initially declined by the originating United Network for Organ Sharing region. HMP did not affect patient survival in this study[22] (**Table 2**).

Hypothermic Oxygenated Machine Perfusion

HMP with oxygen supplementation can be delivered via the portal vein only (HOPE) or both the hepatic artery and portal vein (dual HOPE [DHOPE]). These techniques aim to safely preserve the liver and enhance mitochondrial recovery and function. In ECD livers, oxygenation was suggested to restore endothelial cell viability,[23] demonstrated by increased nitric oxide (NO) levels and lower thrombomodulin. In addition, it was shown in rats that HOPE not only protects against IR injury but also dampens the immune response.[24] Finally, oxygen-rich perfusion allows for intracellular ATP regeneration and reduces lactate production via glycolysis.[25] In the first comparison of SCS versus HOPE, 25 DCD livers underwent end-ischemic HOPE solely via the portal vein, compared to 50 DCD liver transplantations after SCS.[26] HOPE was associated with a reduction in graft injury (peak alanine transaminase [ALT]), IC, biliary complications and improved 1-year graft survival (90% vs 69%, $P = 0.035$). Of importance, HOPE-perfused DCD livers achieved similar results as control DBD livers in all investigated endpoints. Subsequently, Rijn and colleagues,[27] compared 20 control patient with matched 10 DCD liver grafts, were treated with end-ischemic DHOPE. DHOPE was associated with increased ATP content and a twofold reduction in peak serum alanine transaminase (ALT) and bilirubin levels. IC and graft survival were similar in both groups.[27] Of interest, the 5-year outcomes of HOPE-treated DCD liver transplants were similar to those of DBD primary transplants and superior to those of untreated DCD liver transplants, despite much higher risk.[28] This led to two much larger RCT comparing HOPE to SCS that enrolled 160[29] and 177[30] livers, respectively. In the study led by R Porte,[29] HOPE reduced the rate of IC (6% vs 18%) and EAD occurred (26% vs 40%) in DCD livers. Similarly, the Zurich team reported a significant reduction in graft loss in DBD liver treated with HOPE compared with SCS.[30] Overall, HOPE is an effective strategy that has the potential to enable the safe utilization of extended DCD liver grafts, thereby expanding the pool of available livers for transplantation (**Table 2**).

Subnormothermic machine perfusion

SNMP has been developed, as a convenient, intermediate approach between blood-based NMP (see below) and HMP. SNMP offers the advantage to lower metabolic demands at sub-physiological temperatures while still providing adequate metabolism for viability testing and improving graft function. In addition, the utilization of acellular perfusate during SNMP minimize the presence of leukocytes, platelets, and cytokines that could potentially lead to adverse outcomes upon reperfusion/transplantation. Previously, our team demonstrated that 3 hours acellular SNMP improved DCD graft survival post-transplantation in rats compared to SCS.[31] In a porcine model, a 3-hour SNMP using an albumin-based perfusate (Steen solution) supplemented with leukocyte-depleted washed erythrocytes reduced serum ALP and bilirubin levels

Table 2
Clinical studies

RCT		Donor Type	Intervention Arm (#)	Control Arm (#)	Primary End Points	Secondary End Points	Main Outcomes	References
NMP								
No	2016	DBD and DCD	NMP (20)	SCS (40)	30-d graft survival	Biochemical measures of liver function, patient and graft survival, and graft function at 6-mo	Meadian peak AST after transplant was lower in the NMP group	PMID: 26752191
Yes	2017	DBD and DCD	NMP (120)	SCS (100)	Peak AST witihn 7-d	Organ discard rate, post-reperfusion syndrome, PNF, EAD, length of hospital/ICU stay, renal replacement therapy, IC by MRCP at 6 mo after transplant, graft/patient survival at 1-y	A 50% lower level of graft injury and 50% reduction in organ discard rate and 54% longer preservation time, but the comparable patient and graft survival	PMID: 29670285
Yes	2018	Elder than 70 DBD	NMP (10)	SCS (10)	Graft and patient survival at 6-mo posttransplantation	Liver and bile duct biopsies; IRI by means of peak transaminases within 7-d after surgery; and incidence of biliary complications at 6-mo	Older liver grafts after NMP is associated with histologic evidence of reduced IRI	PMID: 30362649
No	2019	DBD and DCD	NMP back to base (26)	NMP local (17)	The safety and efficacy of NMP for liver preservation applied in a back-to-base strategy	Opatient/graft survival at 90-d and 6-mo, LFTs, incidence of EAD, IC at 6 mo	The back-to-base approach was safe, did not compromise the overall benefit of NMP	PMID: 30938039

No	2019	DBD and DCD	Post static cold storage and NMP (31)	Continuous NMP (104)	30-d graft survival	LFTs, incidence of EAD, post-reperfusion syndrome, adverse events, length of hospital/ICU stay, biliary complication, 12-mo graft survival	Applying NMP after SCS is feasible and safe with a 30-d graft survival rate of 94%	PMID: 31206217
No	2020	DBD and DCD	NMP(31)		Feasibility of NMP in discarded organ recovery and achievement of successful transplantation	LFTs, incidence of PNF, EAD, length of hospital/ICU stay, incidence of vascular complications, biliary complications by MRCP at 6-mo, 90-d graft survival	Viability testing with NMP is feasible and enabled successful transplantation of 71% of discarded livers, with 100% 90-d patient and graft survival	PMID: 32546694
Yes	2022	DBD and DCD	NMP (151)	SCS (142)	The incidence of EAD, PNF, graft related significant events	The ability of NMP to monitor donor liver function, incidenc of IC/bile leak, reperfusion syndrome, and histology of the graft	Reduction in the incidence of EAD (18% vs 31%) and IC (1.3% and 2.6% vs 8.5% and 9.9% at 6 and 12-mo after transplant) in the NMP group	PMID: 34985503
No	2022	DBD and DCD	NMP DCD (123)	NMP DBD (80)	To identify perfusion variables relate to EAD/PNF		Perfusate ALT and lactate at 2-h, the amount of supplementary bicarbonate required to keep the perfusate pH > 7.2 in the first 4-h, and peak bile pH were associated with early graft function	PMID: 36044364

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RCT		Donor Type	Intervention Arm (#)	Control Arm (#)	Primary End Points	Secondary End Points	Main Outcomes	References
No	2022	DCD	NMP(34)	NRP (157)	Liver utilization rate, 30-d and 12 and 24-mo patient and graft survival, incidence of biliary complications and EAD, and peak transaminase levels		Both NMP and NRP achieved similar results for recipients after brain death livers	PMID: 35662403
No	2022	DCD	NMP(67)/NRP (69)	SCS (97)	The incidence of EAD, PNF, model for early allograft function, biliary complications, postoperative AKI, chronic kidney injury at 6-mo, length of hospital/ICU stay, hepatic artery thrombosis, surgical complication rates within 30 d, patient/ graft survival at 6-mo, 1 and 3-y		NRP and NMP were associated with better early liver function compared to SCS, whereas NRP was associated with superior preservation of the biliary system	PMID: 35258511
Yes	2023	DBD	NMP(32)	SCS (33)	The incidence of early allograft dysfunction	Complications related to graft IRI	Ischemia free liver transplant decreased the incidence of EAD, reperfusion syndrome, IC	PMID: 37086919

No	2023	DBD and DCD	NMP(79)	SCS (386)	Graft survival	Mortality rate, the incidence of EAD, vascular/biliary complications, length of hospital/ICU stay	NMP could extend the total preservation time of livers without increasing complications	PMID: 37086951
No	2023	DBD and DCD	NMP (165)	SCS (4270)	Length of hospital/ICU stay, rates of PNF and graft failure, graft survival		NMP mitigated donor risk factors, which were relative contraindications for transplant in elderly liver recipients	PMID: 36906889
NRP								
No	2012	DBD and uncontrolled DCD	Potential DCD(400), transplant (34)	DBD (538)	Feasibility of NRP for uncontrolled DCD		NRP expands donor criteria albeit <10% applicability	PMID: 22070538
No	2014	Controlled DCD	NRP (37)		Feasibility of NRP for controlled DCD		13 out of 37 (61.9%) livers were utilized	PMID: 24825520
No	2019	Controlled DCD	NRP (70)	Non-NRP(187)	EAD and cholangiopathy		A reduction in EAD, 30-d graft loss, the incidence of IC, an anastomotic strictures in the NRP group. A multivariable analysis showed that NRP had a protective effect on IC	PMID: 30589499
No	2019	Controlled DCD	NRP (95)	Non-NRP (117)	PNF, EAD and IC		Incidence of overall biliary complications, IC, and graft loss were lower in the NRP group with 0.14, 0.11, and 0.39 odds ratio, respectively	PMID: 30582980

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RCT		Donor Type	Intervention Arm (#)	Control Arm (#)	Primary End Points	Secondary End Points	Main Outcomes	References
No	2020	DBD and controlled DCD	NRP (50)	DBD (100)	Death noncensored/ censored graft survival, patient survival, PNF, EAD, AKI, biliary complications, IC		Similar results in the incidence of EAD, AKI, arterial and biliary complications and 2-y graft and patient survival were obtained between controlled DCD and DBD liver transplants	PMID: 32639402
No	2021	DBD and controlled DCD	NRP (144)	DBD (447)	RBC transfusion, 1-y graft/patient survival		No differences in the number of RBC units transfused, graft and patient survival between controlled DCD and DBD liver transplants	PMID: 34848373
No	2021	DBD and controlled DCD	NRP (100)	DBD (200)	Overall and death-censored graft survival	Biliary complications, incidence of postreperfusion syndrome, ALT peak, EAD and AKI	No differences in ALT peaks, the incidence of EAD and the 1 and 3-y graft survival between controlled DCD and DBD liver transplants	PMID: 34455694
No	2022	Controlled DCD	NRP (545)	Non-NRP (258)	Biliary complications, IC, graft/patient survival		Incidence of overall biliary complications, IC, and patient death were lower in the NRP group with 0.30, 0.112, and 0.54 odds ratio, respectively	PMID: 34856070

HMP								
No	2010	DBD	HMP (20)	SCS (20)	The incidence of PNF, EAD and patient and graft survival at 1 mo and 1 y	Biliary and vascular complications and LFTs and renal function	Lower incidence of EAD with low LFTs and short hospitalization were observed in the HMP group	PMID: 19958323
Yes	2015	DCD	HOPE (25)	SCS (50)	The incidence and severity of biliary complications within 1 y after transplantation	Liver ischemia reperfusion injury and function as well as graft survival	HOPE provided low ALT peak and incidence of IC, and higher 1-y graft survival	PMID: 26583664
No	2015	ECD	HMP (31)	SCS (30)	The incidences of PNF, EAD and vascular complications, graft and patient survival at 1-y	The incidence of biliary complications, AKI, hospital length of stay, LFTs and complications including reoperations	Lower incidence of IC and shorter hospitalization were observed in the HMP group.	PMID: 25521639
No	2017	DCD	DHOPE (10)	SCS (20)	Graft survival at 6-mo	Graft and patient survival rates at 1-y, the safety of machine perfusion, microbiological testing of perfusate and post transplant complications	Twofold lower in ALT and Bil 1-wk after transplant and none of the DHOPE livers required retransplantation due to IC	PMID: 28394402
No	2018	DCD	DHOPE (10)	SCS (9)	To dermine K/Na shifts during machine perfusion to reperfusion		Potassium level in recipients who received DHOPE preserved livers decreased after reperfusion Requirement of noradrenaline was less in the DHOPE group	PMID: 30411502

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RCT		Donor Type	Intervention Arm (#)	Control Arm (#)	Primary End Points	Secondary End Points	Main Outcomes	References
No	2019	DBD and DCD	HOPE (50)	SCS DCD (50) and SCS DBD (50)	Post transplant complications, and non-tumor related patient death or graft loss	Intraoperative parameters, lactate clearance at the end of transplant, complications, length of hospital/ICU stay	5 y graft survival in the HOPE group was equivalent with that of the DBD cohort and superior to the SCS DCD group	PMID: 30342115
No	2020	ECD DBD	HOPE (10)	SCS (30)	The incidence of EAD	1, 3, and 12-mo graft and patient survival and length of hopitalization	Peak AST within 7-d and INR on post operative day 7 were significantly lower in the HOPE group	PMID: 32269237
Yes	2021	DCD	DHOPE (78)	SCS (78)	The incidence of nonanastomotic biliary strictures within 6-mo after transplantation	Intraoperative postreperfusion syndrome, PNF, EAD, thrombosis of the hepatic artery or portal vein, anastomotic biliary stricture or leakage, and renal replacement therapy within 6-mo after transplant	Reduction in the incidence of IC (OR 0.35), postreperfusion syndrome (OR 0.43), and EAD (OR 0.61) in the DHOPE group.	PMID: 33626248
No	2021	ECD DBD	HOPE (25)	SCS (69)	The incidence of EAD	Determined by intraoperative/ biological/ postoperative parameters and economic impact frrom the hospital's perspective	HOPE improved AST/ ALT/lactate/Cr after transplant and reduced ICU and hospital stay	PMID: 33237618

Abbreviations: AKI, acute kidney injury; ALT, alanine transaminase; AST, aspartate transaminase; DBD, donation after brain death; DCD, donation after circulatory death; DHOPE, dual hypothermic oxygenated perfusion; EAD, early allograft dysfunction; HMP, hypothermic machine perfusion; HOPE, hypothermic oxygenated perfusion; IC, ischemic cholangiopathy; ICU, intensive care unit; IRI, ichemia reperfusion injury; LFTs, liver function tests; NMP, normothermic machine perfusion; NRP, normothermic regional perfusion; PNF, primary non function; RBC, red blood cells; RCT, randomized control trial; SCS, static cold storage.

compared with SCS. Graft survival was similar.[32] In discarded human grafts, SNMP improved energy status, TCA cycle intermediate, and lactate clearance.[33] Of interest, this study further demonstrated that steatotic human livers with 3 hours SNMP resulted in higher ATP stores compared with NMP. However, SNMP was associated with lower antioxidative capacity (assessed by glutathione, and N-acetylcysteine level).[34] In summary, SNMP effectively supports human liver ex vivo with minimal injury, and stable, if not improved, metabolic activity. Moreover, SNMP can be performed using an acellular perfusate, thus avoiding the need for complex machinery required for perfusion at normothermic temperature. To date, no clinical trial has examined the benefits of liver SNMP.

Normothermic machine perfusion

NMP creates an ex situ environment that closely mimics physiological conditions by using a perfusate containing red blood cells as an oxygen carrier and essential substrates. Additional components of the perfusate include insulin and steroids (dexamethasone) to support glucose metabolism and reduce inflammation, respectively. This approach allows for the maintenance of metabolic homeostasis in the liver graft during preservation and assessment. One of the significant advantages of NMP is the ability to evaluate the function of the liver graft before transplantation. Several parameters are assessed to determine the viability and functionality of the liver during NMP. These assessments include (1) Homogeneous perfusion on visual inspection: The perfusion of the liver graft should appear even and consistent throughout the parenchyma. (2) Adequate portal and arterial flow, as well as resistance to assess the vascular integrity and functionality of the liver graft. (3) Lactate clearance: Lactate levels in the perfusate are measured to evaluate the metabolic activity of the liver graft. A slow or fluctuating decrease in lactate levels during NMP may indicate insufficient or compromised perfusion. Such deviations from the expected downward trend should be thoroughly investigated to identify the underlying issue and appropriate measures to optimize perfusion and metabolic activity should be taken. (4) Bile production: The presence of bile production is an indicator of hepatocyte function. Monitoring bile production during NMP provides insights into the functionality of the biliary system and liver secretory function. In general, a steady decrease in lactate levels below 2.5 mmol/L and the presence of new bile production soon after initiating NMP are considered favorable markers of viability.[35,36] Based on the evaluation of these parameters, transplant teams can make informed decisions regarding the suitability of the liver graft for transplantation.[35]

The optimal initiation location for NMP, whether local or back-to-base, is unknown. Although back-to-base NMP is associated with longer cold ischemia time (CIT) compared with local NMP, both strategies were associated with similar lactate clearance. Importantly, there was no difference in the incidence of EAD, PNF, or graft and patient survival[37] between the two approaches. These results are also supported by a study that showed the feasibility of a 6-hour SCS followed by 8.5 hours NMP.[38] Of importance, the initiation of NMP at the donor sites carries a risk of additional warm ischemia if some areas of the liver where to be non/mal-perfused. It is crucial to closely monitor the liver graft during NMP, identify promptly any non-perfused areas, and take appropriate measures to ensure homogenous to minimize potential graft damage.

The Liver Assist machine is designed to support both HMP and NMP. It is not transportable, meaning it is typically used within the hospital or transplantation center. The Metra machine and Organ Care System (OCS) are only designed for NMP and are transportable machine, allowing for the initiation of perfusion at the donor site. Currently, three randomized clinical trial (RCT) evaluated NMP in the context of liver

transplantation (**Table 2**). In the UK NMP study, using the Metra (OrganOx, London, UK) was associated with a 50% lower level of graft injury, a 50% reduction in organ discard rate despite 54% longer preservation time compared with SCS. Patient and graft survival were similar.[39] In a recent study using the OCS, our team found that NMP significantly decreased the incidence of EAD compared with SCS (18% vs 31%). In addition, the development of IC, assessed by magnetic resonance cholangiopancreatography (MRCP) at 6 and 12 months post-transplant was reduced. Importantly, the OCS Liver resulted in significantly higher use of DCD livers.[3] In the most recent RCT, recipients of livers from donors after brain death (DBD) were randomly assigned to receive either an ischemia-free liver transplant using combined in situ and ex vivo NMP with the Liver Assist machine or a "conventional" transplant. Here, NMP ischemia-free liver transplant group had lower rates of EAD and post-reperfusion syndrome compared with the conventional transplant group.[40] These findings are consistent with other RCTs discussed previously. In addition to its clinical effectiveness, the introduction of NMP also seems to be a cost-effective approach in the current health care system.[41]

There are several important surgical considerations to review during NMP. To avoid kinking of the portal vein, it is preferable to recover a long segment. Sufficient dissection below the confluence of the superior mesenteric vein and splenic vein can help achieve this goal The hepatic arteries are perfused through the celiac trunk. Similarly, obtaining a long vessel will prevent any tension and prevent potential dissection. Of note, when the right hepatic artery is replaced, or when an hepatic artery arise separately, it should be reconstructed with the common hepatic artery to create one conduit for machine perfusion. There are several methods to achieve this. Ideally, it is the best to have a reconstructed artery so that it is ready for implantation without additional procedures. We believe it is ideal to perform the anastomosis between the splenic artery (SPA) and the replaced right hepatic artery with or without the superior mesenteric artery (SMA) cuff or the gastroduodenal artery and the replaced right hepatic artery. In addition, the aortic segment, with the celiac artery and the SMA, can be anastomosed and perfused from the SPA. Finally, the bile duct cannula should be secured and position to avoid kinking. A pediatric feeding tube can be used for cannulation in small bile ducts.

Normothermic regional perfusion

NRP refers to donor in situ normothermic perfusion. Similar to NMP, NRP aims to provide oxygenated flow and nutrients to mitigate warm ischemic damage, before organ procurement, using extra corporeal membrane oxygenation and pump. This approach can also potentially increase the number of DCD organs. NRP also enables the evaluation of each organ before procurements.[42] During DCD procurement, the donor warm ischemic time (DWIT) is an important consideration. Total DWIT encompasses the period from withdrawal of life support to cold preservation flush, whereas asystolic DWIT refers to the period from circulatory arrest to the flush. To determine clinically significant warm ischemia, functional DWIT is often used during DCD procurements. The duration of functional DWIT varies depending on the country or organ procurement organization. Typically, it starts when certain thresholds are met, such as a drop in SpO2 (peripheral oxygen saturation <80%–70%) or systolic/mean arterial pressure below specific values (eg, <60–50 mm Hg) and continues until the initiation of cold systemic perfusion.[43] Under these circumstances, NRP aims to minimize functional DWIT. In a cohort of Maastricht type 2 (DCD, suffering sudden and unexpected cardiac arrest), NRP allowed a recovery of 34 additional livers in 8 years (9%). In those, 1-year recipient and graft survivals were 82% and 70%, respectively (median follow-

up 24 months). Although less than 10% livers were transplantable, these reports indicate that NRP is effective in expanding the donor population.[44] The potential of NRP for uncontrolled DCD was also described in a report from Spain where only one out of 10 (10%) uncontrolled DCD liver transplants developed IC.[45] According to a retrospective cohort study involving 803 controlled DCD liver transplants in Spain, NRP was superior to SCS followed by super-rapid recovery. The study demonstrated significant reductions in overall biliary complications, IC, and patient death in the NRP group compared with SCS.[46] Moreover, several studies have supported that the outcomes of liver transplantation after NRP are comparable to those of traditional DBD livers.[46–49] It has been debated whether NRP is superior to NMP. In a study from the Netherlands,[50] NRP was used to salvage 20 variable livers out of 43 donors initially deemed unsuitable for transplantation. The results showed a 95% 1-year graft and patient survival rate, along with a comparable 11% incidence of IC, indicating the effectiveness of NRP in rescuing discarded livers. An international observational study from Europe, using propensity score matching, found similar graft and patient survival, rate of EAD, and IC when comparing NRP to NMP. In this study, NRP livers had higher peak AST levels.[51] However, a single-center retrospective study suggested that NRP reduces the incidence of IC compared with NMP.[52] Overall, NRP seems to be a reasonable approach to improve outcomes in DCD liver transplant, provided that ethical issues surrounding NRP are properly addressed.[53]

Combination Therapy

The ideal duration of perfusion, timing, and perfusate composition in NMP and NRP is still under intense research. There is no consensus on a standardized protocol, and it may vary depending on the specific clinical scenario. Combining therapies and using different approaches may be necessary to achieve optimal outcomes. R. Porte's team showed that a 1 hour of HOPE, followed by stepwise increase in temperature, and subsequent NMP led to a 20% increase in transplantable livers with favorable outcomes.[54,55] The mechanism proposed was an improvement in ATP store during HOPE and adequate viability assessment during NMP. Finally, a study using a 1:2 propensity score matching method, the effectiveness of NRP and HOPE interventions in DCD liver showed that these interventions increased the number of livers suitable for implantation by 59.5%. Importantly, the combination of NRP and HOPE resulted in outcomes equivalent to DBD liver transplant in terms of 1-year patient and graft survival and incidence of IC. It is noteworthy that in this study, DCD procurements was characterized by a 20 minutes asystole prior to the recovery. Despite the prolonged warm ischemic time, NRP and HOPE resulted in improving the outcomes of DCD liver transplant.[56]

Molecular Markers of Viability

The improvement of organ quality through liver perfusion is directly linked to the development of a reliable methodology to assess organ viability before implantation. In fact, a critical barrier in expanding the number of available livers for transplantation is that there are no reliable tests for viability.[57,58] The viability decision is often based on texture of organ and quality of perfusion at retrieval[59] and in some cases histology.[60] Routine blood gas or biochemical analysis has been incorporated into organ preservation for a long time, with measurements taken from either the cold storage solution or machine perfusates.[60] However, most of these markers lack specificity, as they are adapted from clinical practices for patients with liver diseases, such as pH, lactate, or liver transaminases. However, in a study of human discarded liver, viability defined as lactate clearance to levels $\leq$2.5 mmol/L within 4 hour of perfusion enabled successful

transplantation of 71% of discarded livers ($n = 22$), with 100% 90-day patient and graft survival.[35] Composite/multiparameter index has also been developed, which incorporates not only lactate clearance but also factors pH maintenance, bile production, vascular flow patterns, and liver macroscopic appearance.[61] Such comprehensive index might provide a more holistic assessment of liver viability during NMP ensuring a thorough evaluation of organ function and suitability for transplantation. Throughout perfusion modalities, various samples can be obtained, including perfusates, bile, and tissues. These samples offer opportunities for a wide range of tests, such as quantification of microRNA, mitochondrial DNA, Damage-associated molecular patterns and cytokines, as well as metabolomic, proteomic, genomic analyses, and ATP quantification.[60–62] However, the clinical implementation of these modalities is constrained by the extended time required to obtain results or the necessity for tissue biopsies. Evidence from our laboratory showed in rats and humans that preservation of energy status was of utmost importance during the peri-transplant period. In this context, the ratios of ATP/ADP/adenosine monophosphate (AMP) prior to and following reperfusion correlated with graft function.[33,63,64] In a rat study, we measured various metabolites during NMP, which was used to develop an ischemia index. This index was able to discriminate livers that experienced previous ischemia with a sensibility and specificity of over 0.98.[65] A consistent metabolomic analysis of perfusate has identified a specific protein called flavinmononucleotid (FMNH2). Under normal physiological conditions, flavin mononucleotide (FMN) is tightly bound to mitochondrial complex I.[66] However, during ischemia and subsequent reoxygenation, FMN is released into the perfusate, which was associated with degree of mitochondrial injury as well as post-transplant in rodent and human.[67] During HOPE, if the concentration of perfusate FMN at 30 minutes are below 8800 arbitrary units, the liver is generally accepted for transplant. Interestingly, this threshold was applied for both DBD and ECD livers of all types. Altogether, these are promising approaches to improve graft assessment during machine perfusion and increase organ utilization.

THE FUTURE OF MACHINE PERFUSION: CONCLUSION

Machine perfusion is a revolutionary technology that has the potential to significantly enhance the safety and efficacy of liver transplantation. During the past decade, there has been a significant research interest in optimizing organ preservation using machine perfusion, especially for DCD and ECD organs. Both hypothermic and NMP techniques offer the ability to restore and preserve energy stores while minimizing the adverse effects of IR injury after transplantation. Standardizing machine perfusion protocols remains a major challenge and should continue to be an area of intense research. Recent clinical trials[3,29,39] have highlighted the potential benefits of machine perfusion in reducing the risk of IC and EAD. Future work will also help determine the long-term efficacy of machine perfusion and develop viability markers to aid in matching each organ to the appropriate recipient.

CLINICS CARE POINTS

- Machine perfusion has the potential to enhance the safety, efficacy, and number of liver available for transplantation.
- HOPE reduces nonanastomotic biliary strictures following DCD liver transplantation.

- NMP reduces posttransplant early liver allograft dysfunction and ischemic biliary complications.

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DISCLOSURE

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