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## Review article

## Normothermic perfusion and outcomes after liver transplantation

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## ABSTRACT

Ischemia has been a persistent and largely unavoidable element in solid organ transplantation, contributing to graft deterioration and adverse post-transplant outcomes. In liver transplantation, where available organs arise with greater frequency from marginal donors (i.e., ones that are older, obese, and/or declared dead following cardiac arrest through the donation after circulatory death process), there is increasing interest using dynamic perfusion strategies to limit, assess, and even reverse the adverse effects of ischemia in these grafts. Normothermic perfusion, in particular, is used to restore the flow of oxygen and other metabolic substrates at physiological temperatures. It may be used in liver transplantation both in situ following cardiac arrest in donation after circulatory death donors or during part or all of the ex situ preservation phase. This review article addresses issues relevant to use of normothermic perfusion strategies in liver transplantation, including technical and logistical aspects associated with establishing and maintaining normothermic perfusion in its different forms and clinical outcomes that have been reported to date.

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## Contents

|  |   |
|--|---|
| 1. Introduction . . . . .  | 0 |
| 2. In situ normothermic regional perfusion. . . . .  | 0 |
| 2.1. Beneficial effects of NRP. . . . .  | 0 |
| 2.2. Technical aspects of performing NRP . . . . .   | 0 |
| 2.3. Ethical and legal issues associated with the use of NRP in DCD . . . . .                  | 0 |
| 2.4. Clinical outcomes following the application of NRP in DCD liver transplantation . . . . . | 0 |
| 3. Ex situ hepatic Normothermic machine perfusion . . . . .                                    | 0 |
| 3.1. Beneficial effects of hepatic NMP. . . . .  | 0 |
| 3.2. Technical aspects of performing hepatic NMP . . . . .                                     | 0 |
| 3.3. Perfusate during hepatic NMP . . . . .  | 0 |
| 3.4. Clinical outcomes following the application of NMP in liver transplantation . . . . .     | 0 |
| 4. Alternative strategies to normothermic perfusion in “high-risk” livers . . . . .            | 0 |
| 5. Summary & future directions. . . . .  | 0 |
| Funding. . . . .   | 0 |

**Abbreviations:** ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ATP, Adenosine triphosphate; BO<sub>2</sub>, Oxygen binding capacity of hemoglobin; cDCD, Controlled donation after circulatory death; CF, Continuous flow; CiO<sub>2</sub>, Inflow oxygen content; CIT, Cold ischemia time; CoO<sub>2</sub>, Outflow oxygen content; DBD, Donation after brain death; DCD, Donation after circulatory death; DO<sub>2</sub>, Oxygen delivery; DWIT, Donor warm ischemia time; EAD, Early allograft dysfunction; HAF, Hepatic artery flow; HAP, Hepatic arterial pressure; [Hb], Concentration of effective hemoglobin; HOPE, Hypothermic oxygenated machine perfusion; IFLT, Ischemia-free liver transplantation; IQR, Interquartile range; ITBL, Ischemic type biliary lesions; NMP, Normothermic machine perfusion; NR, Not reported; NRP, Normothermic regional perfusion; PF, Pulsatile flow; PiO<sub>2</sub>, Inflow partial pressure of oxygen; PNF, Primary non-function; PoO<sub>2</sub>, Outflow partial pressure of oxygen; PVP, Portal venous pressure; RA, Rescue allocation; SA, Standard allocation; SCS, Static cold storage; SiO<sub>2</sub>, Inflow oxygen saturation; SoO<sub>2</sub>, Outflow oxygen saturation; SRR, Super rapid recovery; VO<sub>2</sub>, Oxygen extraction; uDCD, Uncontrolled donation after circulatory death.

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| Acknowledgments . . . . .                   | 0 |
| References . . . . .                        | 0 |

## 1. Introduction

The composition of the pool of organ donors has shifted progressively, in particular in developed Western countries over the past 20 years. Whereas donors were once largely young and previously healthy individuals declared dead secondary to traumatic brain injury, they are now much older (in some countries, not infrequently up to 80 and on occasion even 90 years of age) and present more comorbidities and/or are declared dead following cardiac arrest through the donation after circulatory death (DCD) process. While static cold storage (SCS) is simple and relatively inexpensive and remains the most common form of preservation in organ transplantation, the ever-increasing pool of suboptimal donors and organs has prompted renewed interest in dynamic preservation modalities, including *in situ* normothermic regional perfusion (NRP) and *ex situ* normothermic machine perfusion (NMP), to restore the flow of warm, oxygenated blood following a period of ischemia and prior to reperfusion at transplantation.

The aim of the current review article is to discuss potential benefits associated with the use these normothermic perfusion strategies and the impact they have had to date on clinical liver transplant outcomes.

## 2. *In situ* normothermic regional perfusion

Donation after circulatory death donors are an increasingly more common source of organs for transplantation and represent a great if not majority portion of the donor pool in some countries (30% in Belgium, approximately 40% in the United Kingdom, and over 50% in The Netherlands) [1]. While DCD donors may be classified among four or five categories depending on conditions surrounding arrest, category III controlled DCD donors (arrest following intentional withdrawal of life support in ventilated patients not meeting brain death criteria, cDCD) and, to a lesser extent, category II uncontrolled DCD donors (sudden cardiac arrest followed by unsuccessful resuscitation maneuvers, uDCD) comprise essentially all DCD donors that are used for transplantation globally [2]. The period of warm ischemia surrounding cardiac arrest in these donors provokes organ injury, and DCD in general yields fewer organs per donor and ones of inferior quality when compared with donation after brain death (DBD) [3]. For this reason, there has been increasing interest in forgoing super rapid recovery (SRR) following the declaration of death and instead using NRP to temporarily restore oxygenated blood flow in the abdominal and more recently thoracic organs prior to cold preservation.

### 2.1. Beneficial effects of NRP

During warm ischemia, adenosine triphosphate (ATP) degradation leads to the progressive accumulation of xanthine and hypoxanthine, important sources of superoxide radicals at organ reperfusion [4]. A period of post-ischemic NRP in DCD donors is useful to restore cellular energy substrates, [5], reduce levels of nucleotide degradation products [6], improve the concentrations of endogenous antioxidants [7], and even stimulate processes of cellular repair prior to graft recovery [8]. An experimental study demonstrates that by blocking the A2 receptors of adenosine, the beneficial effects of NRP are abolished, indicating that NRP mediates its effects in great part through adenosine [9]. Post-ischemic NRP may also be useful to reduce the vasoconstrictive effects of cold graft washout with the SCS solution [10] and offers an

opportunity to assess liver injury by evaluating the evolution of hepatic transaminase levels and lactate clearance in the perfusate [11–13].

### 2.2. Technical aspects of performing NRP

In uDCD, cannulation for the establishment of abdominal NRP is performed post-mortem after death is declared in the emergency department. In cDCD, in contrast, cannulation for abdominal NRP may be performed either prior to the withdrawal of life support (pre-mortem cannulation, which is typical in Spain and has also been performed in the United States) [14,15] or following the declaration of death. Pre-mortem cannulation may be performed in a variety of settings (intensive care unit, radiology suite, operating room). Post-mortem cannulation in cDCD, on the other hand, is most often done in open abdomen in the operating room (the case in the United Kingdom and the Netherlands), though the use of femoral artery and vein catheters or guidewires placed prior to withdrawal of care to access and thereby cannulate the femoral vasculature following the declaration of death is permitted by law in France, Italy, Norway, and Switzerland [16,17].

For uDCD donors and cDCD donors with pre-mortem vessel cannulation or preparation, access to unilateral femoral vessels is achieved via open femoral cutdown and isolation of the femoral artery and vein or percutaneously using Seldinger technique [12,18]. If the entire cannulation procedure is performed prior to the withdrawal of ventilatory support, the potential cDCD donor is heparinized, and cannulae are left clamped and connected to the tubing of the primed NRP circuit. The contralateral femoral artery may also be accessed for placement of an aortic occlusion balloon catheter, which is left deflated in the case of cDCD and advanced into the supraceliac aorta under radiographic control. Following the withdrawal of life support and the declaration of death in cDCD, the supraceliac aorta is occluded, and the abdominal NRP circuit is initiated. Proper positioning of the balloon excluding the aortic arch vessels is confirmed by chest radiograph and absence of flow measured in a left radial arterial catheter.

For cDCD donors undergoing open post-mortem cannulation, once death has been declared, the surgical team performs midline laparotomy to cannulate the abdominal aorta immediately proximal to and the infrarenal inferior vena cava immediately distal to their respective bifurcations. Cannulae are connected to the tubing of the primed NRP circuit, the supraceliac aorta is clamped, and NRP is initiated.

In general, NRP is run for a minimum of 1 h and a maximum of 4 h to allow adequate reconditioning of the abdominal organs and recovery of energy substrates without provoking additional end-organ injury [5,6,8,9,19,20]. Different centers use different criteria to assess the adequacy of a DCD liver undergoing NRP (Table 1). This assessment is largely based on factors related to the length of the initial warm ischemic insult and the evolution of hepatic transaminases and occasionally lactate levels during NRP. Some centers also rely on the results of hepatic biopsy to rule out moderate-to-severe macrosteatosis and/or fibrosis.

### 2.3. Ethical and legal issues associated with the use of NRP in DCD

There are ethical concerns surrounding the use of NRP in DCD, and laws vary from one country to another regarding whether or not NRP may be applied in DCD and, if so, how and when.

In uDCD, cardiac arrest is sudden and unexpected, and death is declared based on the irreversible loss of cardio-respiratory function (demonstrated after prolonged efforts to reverse it have failed). Death

**Table 1**

Donor and preservation conditions, acceptance criteria, and clinical outcomes using normothermic regional perfusion in DCD liver transplantation. In controlled/expected donation after circulatory determination of death (cDCD) donors, cardiac arrest was provoked by the intentional removal of life support; in uncontrolled/unexpected donation after circulatory determination of death (uDCD) donors, sudden (typically extrahospitalary) cardiac arrest was followed by unsuccessful resuscitation and intrahospitalary declaration of death. Numerical figures are reported as mean  $\pm$  standard deviation or median [25–75% interquartile range], unless otherwise specified.

| Study                           | Country | N                 | Donor age (y)    | Cannulation | DWIT <sup>a</sup> (min) | NRP (h)             | CIT (h)              | Acceptance criteria     |   |   | EAD (%) | PNF (%) | Overall biliary complications (%) | ITBL (%)              | One-year graft survival (%) |
|---------------------------------|---------|-------------------|------------------|-------------|-------------------------|---------------------|----------------------|-------------------------|---|---|---------|---------|-----------------------------------|-----------------------|-----------------------------|
|                                 |         |                   |                  |             |                         |                     |                      | DWIT                    | Biochemistry                              | Biopsy  |         |         |                                   |                       |                             |
| Controlled DCD                  |         |                   |                  |             |                         |                     |                      |                         |   |   |         |         |                                   |                       |                             |
| Hesseimer et al. [39]           | Spain   | 95 <sup>b</sup>   | 57 [45–65]       | Pre-mortem  | 18 [13–23]              | 2.0 [1.3–2.3]       | 5.3 [4.4–6.1]        | FWIT $\ll$ 30'          | AST/ALT stable & $\ll$ 4 $\times$ ULN     | –   | 22      | 2       | 8                                 | 2                     | 88                          |
| Ruiz et al. 2019 [38]           | Spain   | 46 <sup>b</sup>   | 58 [27–76]       | Pre-mortem  | NR (FWIT 10 [6–22])     | 2.1 [1.4–2.7]       | 4.7 [2.5–6.8]        | FWIT $\ll$ 30'          | AST/ALT stable & $\ll$ 4 $\times$ ULN     | –   | 23      | 0       | 2                                 | 0                     | 100                         |
| Watson et al. [40]              | UK      | 43                | 41 [33–57]       | Post-mortem | 30 [26–36]              | 2.1 [1.7–2.2]       | 6.4 [5.1–8.4]        | –                       | ALT stable & $\ll$ 500 IU/L               | –   | 12      | 0       | 7                                 | 0                     | 98 (death-censored)         |
| Rojas-Pena et al. [15]          | USA     | 13                | 37 $\pm$ 3       | Pre-mortem  | NR                      | 1.4 $\pm$ 0.1       | NR                   | TWIT $\ll$ 90'          | –   | –   | NR      | 8       | NR                                | 8                     | 86                          |
| Hagness et al. [18]             | Norway  | 8                 | 50 (range 23–63) | Post-mortem | 29 (range 16–96)        | 1.6 (range 1.2–3.7) | 7.1 (range 3.4–9.6)  | FWIT $\ll$ 30'          | Lactate declining                         | –   | 0       | 0       | 25                                | 0 (13% recurrent PSC) | 100                         |
| Uncontrolled DCD                |         |                   |                  |             |                         |                     |                      |                         |   |   |         |         |                                   |                       |                             |
| Jimenez-Romero et al. 2019 [80] | Spain   | 75                | 42 $\pm$ 10      | Post-mortem | 130 $\pm$ 22            | NR                  | 6.4 $\pm$ 1.4        | Arrest-to-CPR $\ll$ 15' | AST/ALT $\ll$ 4 $\times$ ULN              | $\ll$ 30% macrosteatosis<br>No fibrosis             | NR      | 8       | 31                                | 16                    | 73                          |
| Hesseimer et al. [39]           | Spain   | 43                | 46 [27–57]       | Post-mortem | 107 [102–131]           | 3.3 [3.1–3.8]       | 6.3 [5.5–7.2]        | Arrest-to-CPR $\ll$ 15' | AST/ALT $\ll$ 4 $\times$ ULN              | –   | 42      | 9       | 16                                | 12                    | 74                          |
| De Carlis et al. [13]           | Italy   | 20 (incl. 6 cDCD) | 51 [46–61]       | Post-mortem | 125 [72–143]            | 5.9 [5.1–7.2]       | 8 [6–9] <sup>c</sup> | TWIT $\ll$ 150'         | ALT $\leq$ 1000 IU/L<br>Lactate declining | $\leq$ 30% macrosteatosis<br>Minimal-to-no fibrosis | 24      | 10      | 20                                | 10                    | 85 (death-censored)         |
| Savvier et al. [32]             | France  | 13                | 37 $\pm$ 3       | Post-mortem | 137 $\pm$ 13            | 4.2 $\pm$ 0.6       | 5.8 $\pm$ 0.5        | Arrest-to-CPR $\ll$ 15' | ALT $\ll$ 200 IU/L                        | $\ll$ 20% macrosteatosis                            | 54      | 23      | 15                                | 8                     | 69                          |
|                                 |         |                   |                  |             |                         |                     |                      | TWIT $\ll$ 150'         |   |   |         |         |                                   |                       |                             |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; cDCD, controlled donation after circulatory death; CIT, cold ischemia time; CPR, cardiopulmonary resuscitation; DCD, donation after circulatory death; DWIT, donor warm ischemia time; EAD, early allograft dysfunction; FWIT, functional warm ischemia time; ITBL, ischemic type biliary lesions; NRP, normothermic regional perfusion; PNF, primary non-function; PSC, primary sclerosing cholangitis; TWIT, total warm ischemia time.

<sup>a</sup> Total warm ischemic times for transplanted DCD liver grafts

<sup>b</sup> Include some of the same transplants

<sup>c</sup> Includes a period of hypothermic oxygenated machine perfusion.

is usually declared in the emergency room by a team entirely independent of that responsible for organ recovery and preservation. More often than not, potential uDCD donors are declared dead prior to the arrival of next-of-kin. Based on a consequentialist ethical standpoint and the principles of utility and donor autonomy, certain countries, including Spain and France, allow cannulation maneuvers to commence in this setting, even in cases where first-person consent may not have yet been obtained [14,21]. The will of the potential donor regarding donation is always subsequently investigated in the context a family interview, where information regarding the circumstances of the arrest, the outcome of resuscitation maneuvers, and the measures taken related to the donation process is relayed. Next-of-kin then decide, taking into consideration the potential donor's wishes, whether to proceed with donation or abort the process. Throughout this process, it should be clear that NRP is organ maintenance and not therapy. While the technology employed is similar, terms such as "extracorporeal membrane oxygenation/ECMO" and "extracorporeal life support/ECLS" should not be used in relation to organ donation. Such terminology is confusing, especially considering the fact that it is used to describe therapeutic maneuvers that may be used to recover patients suffering sudden cardiac arrest more commonly occurring inside a hospital.

In contrast to uDCD, where cardiac function has been lost irreversibly, the no-touch period of asystole that is used to declare death in cDCD does not necessarily reflect an irreversible loss of cardiac or, for that matter, neurological function. No-touch periods in Europe currently range from 5 min in 12 countries to 10 min in three countries, 20 min in Italy, and 30 min in Russia [17]. Given that the acceptance of human death is ultimately based in all circumstances (including DCD) on the irreversible loss of all functions of the brain and brainstem, it is clear that 5 min of no-touch may be enough time to rule out return of spontaneous circulation [22] but not enough for brain death to develop under all circumstances. Therefore, the declaration of death and the ability to initiate organ preservation maneuvers after 5 min of cardiorespiratory arrest in cDCD, in particular, are predicated on a condition of "permanence": that permanent loss of circulation to the brain and brainstem will not be reversed and will inevitably lead to irreversible loss of circulation (i.e., brain death) [23]. In spite of the fact that it is limited to the abdomen and occasionally the chest, some authors feel that establishment of NRP negates the condition of permanence and the diagnosis of death [24,25].

While views vary according to region and ethos, it is undeniable that clear and effective measures have to always be enacted to ensure lack of flow to the aortic arch vessels during NRP, thereby maintaining the permanence of circulatory arrest in the brain and brainstem and allowing brain death to progress [26]. With pre-mortem cannulation, positioning of the aortic occlusion balloon in the supradiaphragmatic aorta distal to the left subclavian artery is confirmed radiographically prior to withdrawal of care. As additional measure, the aortic occlusion balloon may be briefly inflated for a few seconds prior to ventilatory withdrawal, in order to ensure disappearance of femoral arterial pressure and simultaneous maintenance of a normal pressure waveform in the left radial arterial line. In doing so, the minimum filling volume needed to entirely block the supradiaphragmatic aorta may be recorded [27]. Once NRP is initiated, adequate occlusion is confirmed through the use of a left radial artery catheter demonstrating absence of flow.

#### 2.4. Clinical outcomes following the application of NRP in DCD liver transplantation

The cells of the liver, in particular those lining the biliary tree, are particularly sensitive to warm ischemia, and initial experiences with DCD liver transplantation described high rates of graft dysfunction and non-function and non-anastomotic biliary strictures/ischemic type biliary lesions (ITBL) in up to 50% of cases [28]. While complication rates have improved with experience, the rate of post-transplant ITBL remains higher among recipients of DCD versus DBD grafts: 16% versus

3%, according to two meta-analyses [29,30]. The clinical relevance of ITBL lies in the fact that up to 70% of patients with ITBL require retransplantation or die [31].

After an initial period where different donor maintenance techniques were used, including rapid *in situ* cold preservation, simultaneous chest and abdominal compressions, and total body cooling, NRP has come to be the "gold standard" and primary means by which uDCD livers are recovered for transplantation. Using NRP, even livers with extensive pre-recovery warm ischemic periods of up to 2.5 h have been successfully transplanted, with biliary complication and graft survival rates comparable to those seen using cDCD livers that have suffered considerably less warm ischemia [11,12,32–35] (Table 1).

In spite of its relative success in the setting of uDCD, the application of NRP in cDCD remains more limited. The great majority of cDCD livers that are transplanted in the world today are still recovered with rapid *in situ* cold preservation, and reports on the use of NRP in cDCD liver transplantation have been, until recently, anecdotal [13,15,16,36–38]. In the past year, however, two larger multicenter studies have come out describing the benefits that may be achieved with post-mortem NRP in cDCD liver transplantation. First, a Spanish national study compared the results of 95 cDCD liver transplants performed with post-mortem NRP with those of 117 cDCD liver transplants performed with SRR. Median donor age in the study was relatively high (57 years [25–75% interquartile range, IQR, 45–65] NRP, 56 years [25–75% IQR 47–64] SRR). With a median follow-up of 20 months, the use of post-mortem NRP appeared to significantly reduce rates of postoperative biliary complications (overall 8% NRP vs. 31% SRR,  $P < .001$ ; ITBL 2% NRP vs. 13% SRR,  $P = .008$ ) and graft loss (12% NRP vs. 24% SRR,  $P = .008$ ) [39]. Similarly, a combined experience from centers in Cambridge and Edinburgh in the United Kingdom compared the results of 43 cDCD liver transplants performed with post-mortem NRP with those of a contemporary cohort of 187 cDCD liver transplants performed with SRR. Median donor age was less for cDCD livers with NRP versus those with SRR: 41 years (25–75% IQR 33–57) vs. 54 years (25–75% IQR 38–63), respectively. Reported rates of anastomotic biliary strictures were 7% NRP vs. 27% SRR ( $P = .004$ ), ITBL 0% NRP vs. 27% SRR ( $P < .001$ ), and 90-day graft loss 2% NRP vs. 10% SRR ( $P = .102$ ) [40]. Considered together, the results of these two studies are remarkably consistent and provide a rather clear indication that the use of post-mortem NRP in cDCD liver transplantation can help reduce rates of biliary complications, ITBL, and graft loss, and allow for the successful transplantation of cDCD livers even from donors of advanced age.

### 3. Ex situ hepatic Normothermic machine perfusion

During ischemia, cellular energy stores are progressively depleted, leading to sodium accumulation, loss of the transmembrane electrochemical gradient, and cell swelling [41]. Hypoxia also triggers a switch from aerobic to anaerobic metabolism and the development of lactic acidosis. Severe acidosis activates phospholipases and proteases, ultimately leading to cellular damage and death. Static cold storage is used to slow the activity of catabolic enzymes during ischemia. Hypothermia, however, leads to local vasoconstriction and dysfunctional regulation of cations (calcium and potassium, in particular), independent of cellular ischemia [42]. In endothelial cells, actin disassembly provokes cell rounding and detachment. Cell adhesion molecules are also expressed on endothelial cell surfaces [43].

#### 3.1. Beneficial effects of hepatic NMP

Unlike SCS, NMP is dynamic and provides a continuous supply of oxygen and other metabolic precursors at physiological temperatures (35–38 °C). Liver transplantation studies in pigs have shown excellent post-reperfusion function and survival among grafts preserved with NMP, including ones with significant previous warm ischemic damage (up to 90 min) that universally failed when SCS was applied [44–46].

In human studies, NMP has been shown to lead to the repletion of glycogen and, thereby, graft energy stores [47]. The results of a recently published report on genetic and histological changes appreciated in human livers undergoing NMP have also demonstrated upregulated expression of genes implicated in processes of cell growth and repair and less inflammation, neutrophil infiltration, and programmed and unprogrammed cell death [48]. Given that the liver is fully metabolically active, NMP additionally offers the best opportunity to assess graft viability prior to reperfusion *in vivo* [49]. The difficulty lies in providing sufficient oxygen and other necessary substrates to prevent graft deterioration during the *ex situ* period. As well, concerns have been raised regarding whether performing NMP directly following SCS might actually trigger ischemia-reperfusion injury, in particular among grafts of more marginal/suboptimal quality.

### 3.2. Technical aspects of performing hepatic NMP

Currently, the primary devices for *ex situ* hepatic NMP are the Liver Assist (Organ Assist, B.V., Groningen, The Netherlands), OrganOx metra® (OrganOx Ltd., Oxford, UK), and OCS™ Liver (TransMedics®, Andover, Massachusetts, USA). All three devices provide inflow via canulae to the portal vein and hepatic artery, with graft outflow returning to one or more pumps via tubing. Continuous flow to the portal vein may be provided by a dedicated pump (Liver Assist, OCS™ Liver) or by a gravity bag (OrganOx metra®), while flow to the hepatic artery may be either pulsatile (Liver Assist, OCS™ Liver) or continuous (OrganOx metra®). In addition to cannulating liver inflow and the bile duct to recover and analyze bile production, the liver outflow is cannulated in the OrganOx metra® device, though this is not the case in either the Liver Assist or OCS™ Liver, where the effluent flows freely from the suprahepatic veins into the graft receptacle. While a closed circuit might be considered more sterile due to lack of contact between the perfusate and ambient air, cannulating the inferior vena cava runs the risk of provoking outflow resistance and hepatic congestion and impeding graft inflow. As well, when the perfusion circuit is closed and all the effluent is recovered, it is more difficult to perform rapid cold perfusion to avoid graft loss in the case of technical malfunction during NMP [50,51].

### 3.3. Perfusate during hepatic NMP

The perfusate used in hepatic NMP is typically composed of a crystalloid or colloid solution, an oxygen carrier, calcium, broad-spectrum antibiotics, insulin, and heparin [52–55]. Depending on the length of perfusion, metabolic substrates, including glucose or parenteral nutrition, trace elements, and multivitamins, may also be added. Some groups have also added plasma and/or albumin as colloids/“volume expanders”, though this is likely unnecessary since the liver itself should produce clotting factors and albumin during NMP [56].

Given high metabolic requirements present at 35–37 °C, a specific oxygen carrier in the NMP perfusate is required [57]. Most commonly, a solution based on red blood cells is used. Cell-free solutions based on hemoglobin have also been employed for hepatic NMP [58,59] and can be particularly useful when the same organ undergoes continuous perfusion at varying temperatures. At low temperatures, red cell membranes are stiff, and cells lyse. Acellular hemoglobin-based oxygen carriers, such as bovine-derived HBOC-201 (Hemopure, HbO2 Therapeutics LLC, Souderton, Pennsylvania, USA), on the other hand, are temperature-stable and offer a similar oxygen dissociation profile to hemoglobin in red cells, permitting liver rewarming to be performed progressively without necessitating a change in the perfusion solution [60].

The oxygen delivery and dissociation characteristics of hemoglobin have been extensively studied (Table 2). The concentration of effective hemoglobin and the oxygen saturation and rate of graft inflow are all fundamental elements in maintaining adequate oxygen delivery during NMP. It is also essential that the membrane oxygenator be able to

maintain enough forward flow while still allowing for adequate gas exchange to occur. In order to avoid progressive liver injury due to oxygen debt during NMP, oxygen delivery needs to exceed the critical threshold beyond which oxygen extraction is not delivery-dependent (Fig. 1). Aside from the aforementioned measures, serial checks of the outflow oxygen saturation and lactate levels can be useful to confirm that oxygen uptake by the graft on the device is satisfactory.

### 3.4. Clinical outcomes following the application of NMP in liver transplantation

The use of NMP in human livers considered acceptable for transplantation has been evaluated in several clinical pilot studies and one large randomized multicenter trial (Table 3). In the latter, results were compared between transplants performed with NMP ( $N = 121$ ) and SCS ( $N = 101$ ). The primary endpoint was to detect a significant difference in peak serum aspartate aminotransferase after graft reperfusion, which was lower in NMP livers versus those undergoing SCS by an average of 500 IU/L. No difference in any major post-transplant outcome measure was detected, though the study was not designed nor powered to do so [51].

Given that it restores near-physiological conditions and, most importantly, bile production, NMP has also been used to test and recover of marginal livers for transplantation that might otherwise be rejected (Table 3). Two separate experiences from the United Kingdom (one published and the other presented thus far only in abstract form, for which reason the latter is not included in the table) have each transplanted initially discarded livers after evaluating their function during NMP. Watson and colleagues described 47 perfusions (28 performed for viability assessment and 19 initially performed for research purposes) that resulted in transplantation of 22 liver grafts (6 DBD, 16 cDCD), including the use of two research livers. Outcomes of these transplants included 5% PNF, 18% ITBL, and 86% 6-month graft survival [61]. More recently, the Viability Testing and Transplantation of mArginal Livers (“VITTAL”) trial evaluated livers rejected by all transplant centers in the United Kingdom and meeting one or more “high-risk” criteria [62]. Grafts clearing lactate within 4 h of initiating NMP and meeting at least two of an additional set of criteria related to hepatic perfusion and hepatocellular function were considered transplantable. Among 31 livers that underwent NMP viability assessment, 22 (12 DBD, 10 DCD) were transplanted into low-risk recipients. There was no PNF, and 90-day patient survival (primary study outcome) was 100%. However, the rate of clinically significant ITBL was 30% among DCD livers, indicating need for further refinement of the NMP technique and/or selection criteria for these grafts.

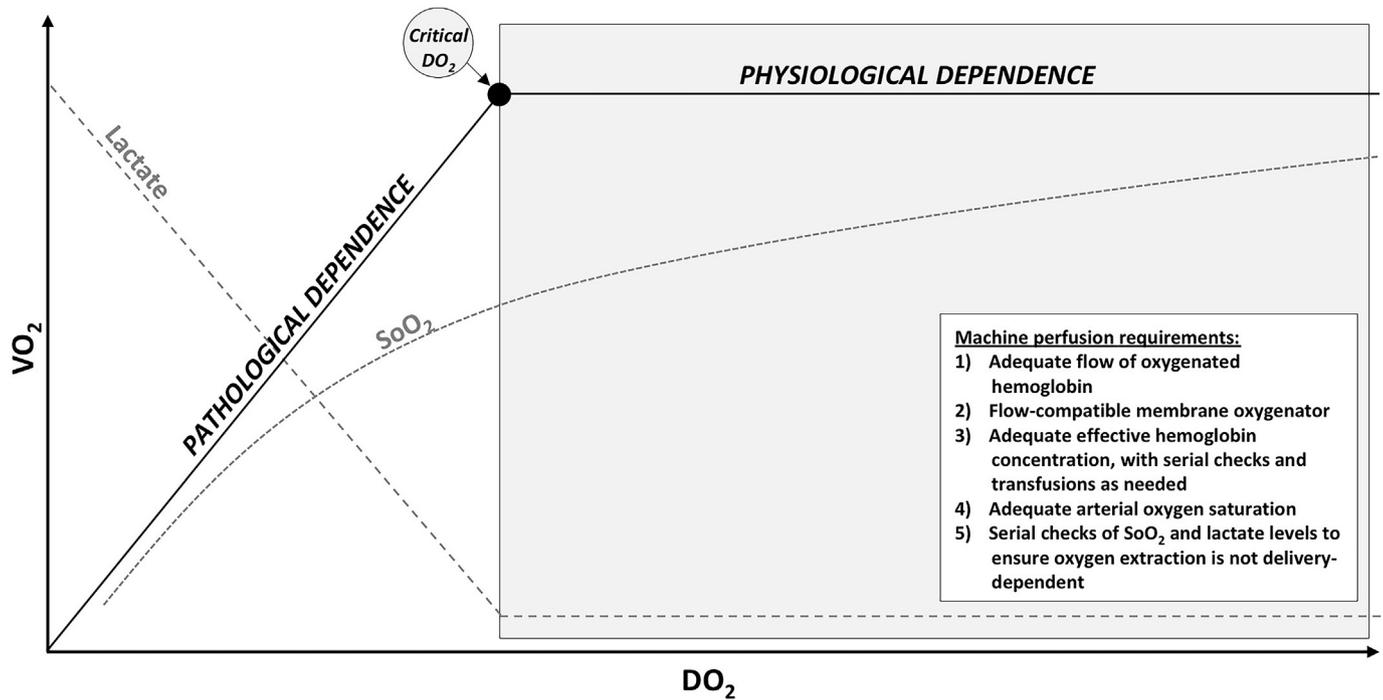
Another promising application of NMP– ischemia-free liver transplantation (IFLT) – has been described by First Affiliated Hospital in Guangzhou, China. To date, this group has performed 42 cases of IFLT, including using 85–95% macrosteatotic grafts [63]. Analysis of perfusate and biopsies from IFLT allografts has demonstrated absence of typical

**Table 2**

These formulae describe the oxygen delivery and dissociation characteristics of hemoglobin. Based on these formulae, it is clear that the concentration of effective hemoglobin, oxygen saturation, and rate of inflow of the perfusate are all critical in maintaining adequate oxygen delivery during *ex situ* liver MP.

$$\begin{aligned} &\text{Oxygen delivery and dissociation characteristics of Hemoglobin} \\ &\text{Oxygen delivery (DO}_2\text{)} = \text{Flow} \times \text{CiO}_2 \\ &\text{CiO}_2 = (\text{BO}_2 \times [\text{Hb}] \times \text{SiO}_2) + (\text{PiO}_2 \times 0.003 \text{ mL O}_2/100 \text{ mL blood/mmHg}) \\ &\text{Oxygen extraction (VO}_2\text{)} = \text{Flow} \times (\text{CiO}_2 - \text{CoO}_2) \\ &\text{CoO}_2 = (\text{BO}_2 \times [\text{Hb}] \times \text{SoO}_2) + (\text{PoO}_2 \times 0.003 \text{ mL O}_2/100 \text{ mL blood/mmHg}) \end{aligned}$$

BO<sub>2</sub>, oxygen binding capacity of hemoglobin (1.39 mL/g for hemoglobin in red cells at physiological temperatures, 1.26 mg/dL for acellular Hemopure); CiO<sub>2</sub>, inflow oxygen content; CoO<sub>2</sub>, outflow oxygen content; [Hb], concentration of effective hemoglobin; PiO<sub>2</sub>, inflow partial pressure of oxygen; PoO<sub>2</sub>, outflow partial pressure of oxygen; SiO<sub>2</sub>, inflow oxygen saturation; SoO<sub>2</sub>, outflow oxygen saturation.



**Fig. 1.** Relationship between oxygen delivery and extraction during ex situ machine perfusion of the liver. DO<sub>2</sub>, oxygen delivery; SoO<sub>2</sub>, outflow oxygen saturation; VO<sub>2</sub>, oxygen extraction.

histological changes associated with ischemia-reperfusion injury, stable metabolism throughout preservation and transplantation, minimal changes in gene transcription, and minimal-to-no inflammation [64]. Upon graft reperfusion, recipients of IFTL grafts have maintained stable hemodynamic parameters and core body temperatures. Post-

operatively, there was only one case of early allograft dysfunction in this group's series (2%), a noteworthy finding, given typical rates of 40–50% in Chinese centers. As well, there was only one patient that required renal replacement therapy, and no recipient developed ITBL [65].

**Table 3**

Results of clinical series published to date detailing the use of NMP during the ex situ phase of preservation in human liver transplantation. Numerical figures are reported as mean ± standard deviation or median [25–75% interquartile range], unless otherwise specified.

| Study  | Graft type                 | DWIT <sup>a</sup> (min) | N   | CIT (h)       | Perfusion time (h)         | PVP (mmHg)          | HAP (mmHg)       | HAF                    | EAD (%) | PNF (%) | Overall biliary complications (%) | ITBL (%)         | 6-mo. graft survival |
|--|----------------------------|-------------------------|-----|---------------|----------------------------|---------------------|------------------|------------------------|---------|---------|-----------------------------------|------------------|----------------------|
| Livers accepted for transplantation prior to NMP                   |                            |                         |     |               |                            |                     |                  |                        |         |         |                                   |                  |                      |
| Nasralla et al. [51]   | DBD (N = 87), DCD (N = 34) | 9–93                    | 121 | 2.1 [1.8–2.4] | 9.1 [6.2–11.8]             | NR                  | NR               | CF                     | 10      | 0.8     | NR                                | 0.8 <sup>b</sup> | 95% <sup>c</sup>     |
| Ravikumar et al. 2016 [79]   | DBD (N = 16), DCD (N = 4)  | 14–31                   | 20  | NR            | 9.3 [3.5–18.5]             | NR                  | 60–75            | CF                     | 15      | 0       | 20                                | 0                | 100%                 |
| Ghinolfi et al. 2018 [80]  | DBD                        |                         | 10  | 4.1 [3.4–4.5] | 4.2 [3.3–4.7]              | NR                  | NR               | PF                     | 20      | 0       | 10                                | 10               | 90%                  |
| Selzner et al. 2016 [81]   | DBD (N = 8), DCD (N = 2)   | 28–30                   | 10  | NR            | 9.8 [3.7–12.2]             | NR                  | NR               | CF                     | NR      | 0       | 0 <sup>d</sup>                    | 0 <sup>d</sup>   | NR                   |
| Bral et al. 2017 [50]  | DBD (N = 6), DCD (N = 3)   | 16–23                   | 9   | 3.1 [1.6–4.9] | 11.5 [3.3–22.5]            | NR                  | NR               | CF                     | 56      | 0       | 0                                 | 0                | 80% <sup>e</sup>     |
| Livers accepted for transplantation after NMP viability assessment |                            |                         |     |               |                            |                     |                  |                        |         |         |                                   |                  |                      |
| Watson et al. [61]   | DBD (N = 6), DCD (N = 16)  | 16–160                  | 22  | 6.4 [5.5–7.4] | NR                         | 4–6 start, 8–10 end | 30 start, 60 end | PF                     | 5       | 5       | NR                                | 18               | 86%                  |
| de Vries et al. 2019 [60]  | DCD                        | 23–35                   | 5   | 4.6 [4.0–4.9] | 8.2 [7.4–8.6] <sup>f</sup> | 11                  | 70               | PF                     | 0       | 0       | NR                                | 0                | 100%                 |
| Mergental et al. [47]  | DBD (N = 1), DCD (N = 4)   | 19–109                  | 5   | 7.0 [6.5–7.9] | 5.8 [5.1–9.4]              | NR                  | NR               | PF (N = 4), CF (N = 1) | 0       | 0       | 0                                 | 0                | 100%                 |

CF, continuous flow; CIT, cold ischemia time; DBD, donation after brain death; DCD, donation after circulatory death; DWIT, donor warm ischemia time; EAD, early allograft dysfunction; HAF, hepatic artery flow; HAP, hepatic arterial pressure; ITBL, ischemic type biliary lesions; NMP, normothermic machine perfusion; NR, not reported; PF, pulsatile flow; PNF, primary non-function; PVP, portal venous pressure.

<sup>a</sup> Donor warm ischemic time describes the range of the total warm ischemic times for transplanted DCD liver grafts.

<sup>b</sup> According to the authors, there was only one clinically relevant case of ITBL, though cholangiographic imaging performed in 81 recipients demonstrated a 9% rate of non-anastomotic biliary strictures.

<sup>c</sup> One-year graft survival.

<sup>d</sup> Follow-up to 3 months.

<sup>e</sup> Based on intention-to-treat and including one graft that was lost during NMP due to twisting of the portal vein.

<sup>f</sup> Total ex situ perfusion time, including an initial period of dual hypothermic oxygenated machine perfusion followed by approximately an hour of controlled re-warming; duration of NMP only 6.2 h [5.4–6.6].

#### 4. Alternative strategies to normothermic perfusion in “high-risk” livers

While there are widely recognized donor and graft risk factors for an adverse outcome following liver transplantation (e.g., advanced donor age, macrosteatosis  $\gg 30\%$ , and prolonged warm and/or cold ischemia), there is no universally accepted definition for a “high risk” liver or an “extended criteria” graft. That is to say, there is still a lot of subjectivity when it comes to rejecting an offer or discarding a liver for transplantation. That said, two studies published in the last year address the issue of livers transplanted directly without the use of any perfusion technology and following at least one prior rejection of the liver offer. In one study from the United Kingdom, the reason that livers were rejected was for logistical reasons in almost a quarter of cases included, and donor/graft quality was the reason in less than half [66]. Another single-center French study evaluated livers previously rejected five times and transplanted into – by and large – low-risk recipients. They compared outcomes of these “rescue allocation” (RA) transplants ( $N = 33$ ) with those of standard allocation (SA) grafts ( $N = 321$ ) [67]. For the RA transplants, mean donor age was  $63 \pm 17$  years and cold ischemia time  $7.9 \pm 2.2$  h; 15% of grafts had  $\gg 20\%$  macrosteatosis. While the mean donor risk index [68] among RA transplants was higher than for SA transplants, the BAR score (which reflects a combination of donor, graft, and recipient risk factors for an adverse post-transplant outcome and ranges from 0 to 27) [69] was lower among RA vs. SA transplants:  $5.5 \pm 2.9$  vs.  $9.2 \pm 5.5$  ( $P \leq .001$ ). In spite of initially healthier recipients, outcomes for RA transplants were inferior: hepatic artery thrombosis 15% vs. 3% ( $P = .001$ ), re-transplantation 18% vs. 5% ( $P = .002$ ), and graft survival 65% vs. 83% ( $P = .022$ ), with a median follow-up of 23 months. While one-year patient survival was improved among RA recipients versus patients from the same center awaiting an extended criteria liver (81% vs. 44%,  $P = .004$ ), RA liver outcomes were inferior to those of not only SA transplantation but also recently published benchmarks, where  $\leq 11\%$  one-year graft loss and  $\leq 9\%$  patient death were established as the goals for DBD liver transplantation (using a population with risk – as assessed by BAR score – of 4) [70].

Hypothermic machine perfusion is another alternative to normothermic perfusion to try and recruit more high-risk livers for transplantation. Hypothermic oxygenated machine perfusion (HOPE), in particular, may be used to restore cellular energy levels and improve the state of parenchymal- and non parenchymal-cell mitochondria prior the oxidative burst at graft reperfusion [71,72]. By performing a relatively brief period (1–2 h) of end-ischemic HOPE, some groups have observed acceptable post-transplantation graft survival using cDCD livers, including some with relatively prolonged pre-recovery periods of donor warm ischemia, though they have also observed higher rates of overall biliary complications (24–30%) and ITBL (8–10%) compared with cDCD livers of a similar donor profile recovered with NRP [73,74].

Another strategy is to combine both HOPE and NMP in high-risk livers. Discarded human liver studies have shown that an initial brief period of HOPE leads to improvements in liver ATP content, nitric oxide production, portal vein flow, lactate clearance, bile production, and bile bicarbonate and bilirubin levels during NMP when compared with livers undergoing NMP immediately following SCS [75–77]. In a trial that is ongoing ([www.trialregister.nl;NTR5972](http://www.trialregister.nl;NTR5972)), the University Medical Center Groningen is evaluating the strategy of one hour of dual portal and arterial HOPE followed by one hour of progressive rewarming and finally NMP viability assessment. They have reported that among 16 cDCD livers initially declined for transplantation that were perfused in this manner and assessed for perfusate lactate and pH, bile production, and biliary pH and bicarbonate, 11 were ultimately transplanted (69%). All livers cleared lactate, but the five cases that were not transplanted did not produce alkaline bile. Six-month graft and patient survival rates were 100%. With regards to biliary complications, the rate of anastomotic biliary strictures was 18%, and there was one case of ITBL (9%)

that arose in a graft that did not actively alkalinize bile relative to the perfusate [78].

#### 5. Summary & future directions

Over the past decade, the important rise in the use of in situ and ex situ normothermic perfusion has cemented the role of these strategies as essential for the recovery and preservation of suboptimal livers for transplantation. Normothermic regional perfusion is now considered necessary by groups performing uDCD liver transplantation to not only limit warm ischemia but also reverse ischemic injury while donor evaluation and consent processes are underway. Based on the results of two multicenter level 2 studies, outcomes with cDCD livers may also be improved when a period of post-ischemic NRP is applied following the declaration of death and preceding cold preservation. With respect to the use of ex situ normothermic perfusion, while livers accepted for transplantation outright do not, as of now, appear to derive any benefit, livers of marginal quality can be assessed during a period of NMP performed either directly following SCS or after an initial period of HOPE. Finally, for severely steatotic livers, in particular, recovery, preservation, and transplantation performed under continuous normothermic perfusion may be a logistically complex but at the same time necessary process to be able to successfully utilize grafts that would almost certainly fail following conventional transplantation performed with SCS.

Going forward, as the use of normothermic perfusion in liver transplantation expands, there is still need for ongoing investigation into markers measured during not only ex situ NMP but also in situ NRP capable of accurately predicting immediate hepatocellular function as well as irreversible biliary injury, the ultimate manifestation of which might not appear until months after transplantation. It also remains to be determined whether prolonged periods of ex situ NMP, which, until now, have only been investigated in the context of preclinical and discarded human liver studies, can actually maintain livers in a sufficient state of viability for subsequent successful transplantation. Finally, and above all, future trials on normothermic perfusion in human liver transplantation need to include clinically relevant endpoints (e.g., biliary complications and graft loss) that can justify the use of these more complex and costly preservation techniques over the relatively simple and inexpensive standard that is cold storage.

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#### Declaration of competing interest

The authors have no conflicts of interest to declare.

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