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## Organ reconditioning and machine perfusion in transplantation

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## Original Research

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EDITORIAL
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# Organ Reconditioning and Machine Perfusion in Transplantation

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**Editorial on the Special Issue**

**Organ Reconditioning and Machine Perfusion in Transplantation**

Society's self-sufficiency in terms of organ replacement is still far away from being achieved, given the large disparity between transplant demand and donor organ availability. In the attempt to reduce this discrepancy and expand the donor pool, the transplant community has progressively increased the utilisation of extended-criteria donors (ECD) such as older donors, donors with comorbidities and donation after circulatory death (DCD). The main challenge preventing a wider utilisation of ECD and DCD allografts is the higher susceptibility to the ischemia-reperfusion injury (IRI) (1), an unavoidable part of the transplantation process. For this reason, in the last decades, there has been an exponential development on organ reconditioning strategies, in order to enable graft resuscitation and viability assessment prior to implantation (2).

*Ex-vivo* perfusion techniques are usually classified according to the perfusate temperature, hypothermic (<10°C) or normothermic (37°C), with roller or centrifugal pumps used to generate pressure-controlled pulsatile or continuous flow within the organ, *via* connection to the renal inflow (artery) and outflow (vein) (3). Given the variety of combinations of different parameters and settings (temperature, oxygen, nutrient and/or drug delivery, *in situ*/*ex-situ*), machine perfusion (MP) is considered a promising way to expand the criteria of transplantation by optimising its preservation modalities, potential of organ viability assessment and potentially decreasing the rate and gravity of postoperative complications (4).

As the commitment of Transplant International is to be the premier journal publishing the key basic science and clinical developments in organ replacement medicine (5), we aimed to develop a special issue reflecting the current state of the art on MP in transplantation. This topic collection is dedicated to Professor Paolo Muiesan (6), as a tribute to his career accomplishments in the field of dynamic organ preservation.

In the present issue, we touched upon the whole spectrum of thoracic and abdominal transplant surgery: from heart preservation (Qin et al.), whose valuable contribution was key to allow the first cardiac xenograft to happen (7), to normothermic lung perfusion, enriched by precise measurement of exhaled endogenous CO (Brenckmann et al.), to provide an additional early marker in lung grafts evaluation, moving toward the current trends in preservation solutions for pancreas (Ferrer-Fàbrega et al.), for normothermic kidney and hypothermic combined kidney-liver technologies (Fard et al.; Chang et al.), and describing the first proof-of-concept pilot study for liver viability assessment, *via* hyperspectral imaging (Fodor et al.).

Indeed, viability assessment stays at the core of the present topic collection, in fact the prediction of future organ functionality before transplantation remains the main challenge to pursue on the

Editorial

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decision to implant a non-standard criteria graft. This leads roughly to a 20% liver and kidney discard, based on the transplant provider's judgement, rather than following objectively identifiable scores, which are currently not available for most of the areas discussed. Thus, the development of MP platforms, particularly under normothermia, where more physiological conditions could be reproduced, embraces the potential to overcome this subjective evaluation (Verstraeten and Jochmans). There are in fact novel biomarkers and therapeutic strategies currently under investigation, mainly including pH and electrolytes (Dingfelder et al.), that promise to acquire reliability in view of their association with outcomes after implantation.

In more detail, normothermic MP of liver grafts builds on improvements in preoperative management, surgical technique (8), and postoperative care that successfully allowed to increase the safe use of non-standard criteria grafts, through objective assessment of both hepatocyte and cholangiocyte function (Hann et al.). Machine preservation technology is proved to minimize ischemic biliary complications, too (9, Hunt et al.). It has been recently published the clinical success of an extended time window of up to 10 days of storage prior to implantation, unveiling new horizons in research and clinical utility, namely to convert an urgent and highly demanding surgery into an elective procedure (10).

Dynamic organ perfusion technology represents a significant advancement in graft preservation techniques and the transplant community must seek to continue to incorporate future developments to ensure the benefits of organ transplantation are maximized. Yet, there are also downsides to be considered, specifically to increase MP cost-effectiveness. In the present special issue, a hospital-based health technology assessment of MP in adult liver transplantation using standard cold storage as a comparator, and within the perspective of a national health system-based hospital practice and disease-related group reimbursement policy was presented (De Simone and Ghinolfi). Results showed that the choice of the most appropriate costing model and resource-use items are crucial and require broad consensus across the healthcare professionals involved in transplant programs. The decision on which types of cost to include depends on several key factors, such as the perspective to be adopted, the form of economic evaluation (e.g., cost-effectiveness versus cost utility versus cost opportunity), the quantitative importance of the type of cost, along the entire transplant continuum, whether the cost can be attributed to the intervention and the time horizon of the economic evaluation (perioperative versus early-term versus long-term versus life-long). Furthermore, there is also a non-uniform MP practice among experts in the field highlighting the need for more focused research (11).

To this regard, it is worth mentioning that some techniques are not competitive, but rather complementary, such as abdominal normothermic regional perfusion preceding dual hypothermic oxygenated MP for controlled DCD liver transplantation. As highlighted in one of the included manuscripts (Patrono et al.), this combination allowed to achieve recipient outcomes comparable to livers retrieved from donors after brain death.

The encouraging results from DCD organs utilised after normothermic regional perfusion are also shown by the Edinburgh experience herein presented (Hunt et al.). Their implementation model overcame the barriers associated with the adoption of a new technique, leading to standard clinical practice *via* an iterative process of refinement, training, staffing and operative techniques. Using this approach, the authors achieved a four-fold increase in trained surgical staff and a six-fold increase in competent senior organ preservation practitioners in 12 months. The 61 DCD liver transplants undertaken exhibited no primary non-function or ischaemic cholangiopathy with up to 8 years of follow-up.

In conclusion, we hope this special issue will become a state of the art collection on the use of dynamic organ preservation. The possibility to inspect organ function and to limit the ischemic injury, its consequences in terms of primary or delayed graft function (Hunter et al.) and early dysfunction are key for the future of transplantation, to improve graft and patient survivals.

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REVIEW
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# Machine Perfusion for Human Heart Preservation: A Systematic Review

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Currently, static cold storage (SCS) of hearts from donations after brainstem death remains the standard clinically. However, machine perfusion (MP) is considered an approach for donor organ management to extend the donor pool and/or increase the utilization rate. This review summarizes and critically assesses the available clinical data on MP in heart transplantation. We searched Medline (PubMed), Cochrane, Embase, and clinicaltrials.gov, along with reference lists of the included publications and identified 40 publications, including 18 articles, 17 conference abstracts, and five ongoing clinical trials. Two types of MP were used: hypothermic MP (HMP) and normothermic MP (NMP). Three studies evaluated HMP, and 32 evaluated NMP. Independent of the system, MP resulted in clinical outcomes comparable to traditional SCS. However, NMP seemed especially beneficial for high-risk cases and donation after circulatory death (DCD) hearts. Based on currently available data, MP is non-inferior to standard SCS. Additionally, single-centre studies suggest that NMP could preserve the hearts from donors outside standard acceptability criteria and DCD hearts with comparable results to SCS. Finally, HMP is theoretically safer and simpler to use than NMP. If a machine malfunction or user error occurs, NMP, which perfuses a beating heart, would have a narrower margin of safety. However, further well-designed studies need to be conducted to draw clear conclusions.

**Keywords: review, heart transplantation, machine perfusion, heart preservation, donor**

## INTRODUCTION

Heart transplantation is the most effective method used to treat end-stage heart disease. Currently, static cold storage (SCS) of hearts from donations after brainstem death (DBD) remains the standard practice. SCS combines cardioplegia and hypothermia, which can significantly reduce the energy demand of the donor heart (1). However, despite decades of effort, the cold ischemia time has been limited to 4–6 h. Prolonged cold ischemia and ischemia-reperfusion injury (IRI) have been recognized as significant causes of post-transplant graft failure. According to the International Society for Heart and Lung Transplantation registration, the survival rate decreases as the ischemic time increases (2). The continuous shortage of donor hearts has always been a major limiting factor for heart transplantation (3).

Machine perfusion (MP) is considered an ideal approach for donor organ management to extend the donor pool and/or increase the utilization rate. Perfusion can supply the metabolic need of the myocardium, thus minimizing irreversible ischemic cell injury and death. Several heart perfusion systems, which are either hypothermic MP (HMP) or normothermic MP (NMP), have successfully preserved animal and/or human hearts (4). The longest reported successful human heart preservation time was 16 h with NMP (5). Currently, there is only one commercially available

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perfusion system for clinical use, the organ care system (OCS), and one recently tested system, the non-ischemic heart preservation system (NIHP) (6, 7). Another approach to extend the donor pool is to utilize organs from donation after circulatory death (DCD) (4, 8). For these donor hearts, MP can provide a platform to resuscitate, preserve, assess and even possibly recondition the cardiac function prior to planned transplantation.

Well-designed machine perfusion can theoretically expand the donor pool in different ways. A prolonged safe preservation time allows to utilize remote donor hearts and functional assessment allows to utilize some of the DCD and high-risk donor hearts. Pediatric heart transplantation may have an extra benefit since pediatric donor shortage is even worse, and long transport time occurs more frequently.

Despite the growing number of human donor hearts preserved with MP, it remains controversial whether MP is superior to SCS. In this systematic review, we summarize and critically assess all available clinical data on MP of adult donor hearts, highlighting its therapeutic potential as well as the current limitations and shortcomings.

# METHODS

## Search Strategy and Data Sources

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines. The literature search consisted of two parts: searching for published studies and searching for ongoing clinical trials (inception to 27 June 2020). Published studies were searched in the Medline (PubMed), Cochrane, and Embase databases. The following searching terms were used in combination with AND or OR: heart transplantation, organ perfusion, *ex vivo* perfusion, *ex vivo* reperfusion, heart perfusion, cardiac perfusion, non-ischemic heart preservation, perfusion preservation, antegrade perfusion, and machine perfusion. Ongoing clinical trials were searched in clinicaltrials.gov using the term of heart transplantation for condition or disease in combination with preservation or perfusion for other terms. Only original publications in English were considered. All questions regarding the literature search and article selection were resolved by discussion between two independent reviewers. All references listed in the selected articles were screened for any further publications that were not identified in the initial search.

## Inclusion and Exclusion Criteria

Articles reporting the outcome of MP in donor hearts during primary adult heart transplantation were included. Reports that met any of the following criteria were excluded: 1) irrelevant topics, 2) duplicated data, 3) non-English language, 4) not transplanted, 5) not human, 6) pediatric, or 7) reviews, editorials, and letters to the editor.

# RESULTS

The initial search yielded 3,446 potentially relevant records. **Figure 1** shows a flowchart of the study selection process. Screening resulted in 39 relevant studies. One additional study was identified from the screening of reference lists in the included publications. Ultimately, 40 studies were included in this review: 18 papers (6, 7, 9-24), 17 conference abstracts (5, 25–40), and five ongoing clinical trials (41-45). Three studies reported multicenter data (7, 25, 40), and three were randomized controlled studies (7, 12, 13).

In clinical practice, two types of MP have been used to preserve donor hearts: HMP and NMP. The system temperature was controlled below 10°C during HMP, in contrast to 34°C during NMP. We identified three non-randomized, single-centre studies that used in-house designed HMP systems (**Table 1**) (6, 9, 11). Wicomb et al. demonstrated the first system for HMP of the human heart (9). In this study, four hearts were perfused with an oxygen- and carbon dioxide-bubbled crystalloid cardioplegic solution at a pressure of 8–10 cm $H_2O$. All four hearts were transplanted after a total preservation time of 6, 7, 12, or 15 h. Only one patient survived after 16 months with normal heart function (9). Hill et al. reported successful heart transplantation with HMP using a colloid cardioplegic solution to perfuse eight hearts with a low flow rate (17 ml per 100 g per hour) for 221 min. For comparison, 13 hearts were preserved with cardiosol (185 min) and 50 hearts with modified St. Thomas solution (187 min). The 7-year survival rate was 70% in the St. Thomas solution group and 100% in the other two groups (11). In the third study, Nilsson et al. preserved six hearts using NIHP with a perfusion pressure of 20 mm Hg at 8°C. The perfusate comprised a hyperoncotic cardioplegic nutrition solution supplemented with hormones and erythrocytes. These six NIHP transplantations were compared with 25 SCS transplantations during the same period. The median total preservation time was longer for the NIHP group (223 min; IQR, 202–263) than for the SCS group (194 min; IQR, 164–223). The primary outcome showed a 100% event-free 6-month survival rate for NIHP recipients, compared to 72% for SCS recipients. Furthermore, creatine kinase-muscle/brain, assessed 6 h after ending perfusion, was 76 ng/ml for NIHP compared with 138 ng/ml for the SCS recipients (non-significant), indicating less myocardial damage when using the NIHP method (6).

The only NMP system for clinical heart transplantation is currently the OCS. With the OCS, oxygenated donor blood is used to perfuse coronary arteries at a temperature of 34°C with a perfusion pressure of 60–90 mmHg. Lactate concentration is monitored to verify that adequate perfusion is achieved and if it is above 5 mmol/L, the heart is discarded (7). In the PROCEED II trial, five donor hearts were discarded, four because of rising lactate concentrations and one because of technical issues (7).

Twenty-one publications, including eight papers (7, 10, 12–16, 21) and 13 conference abstracts (5, 25–35, 40) presented results from using the OCS at transplantation of DBD hearts with or without a control group (**Tables 2**, **3**). Three of these studies were randomized (**Table 2**). The only randomized and multicenter study, PROCEED II, which recruited 130 patients from 10 heart transplant centres in the United States and Europe, showed no significant differences in the primary endpoint (30-day patient and graft survival) or secondary endpoints. However, the mean

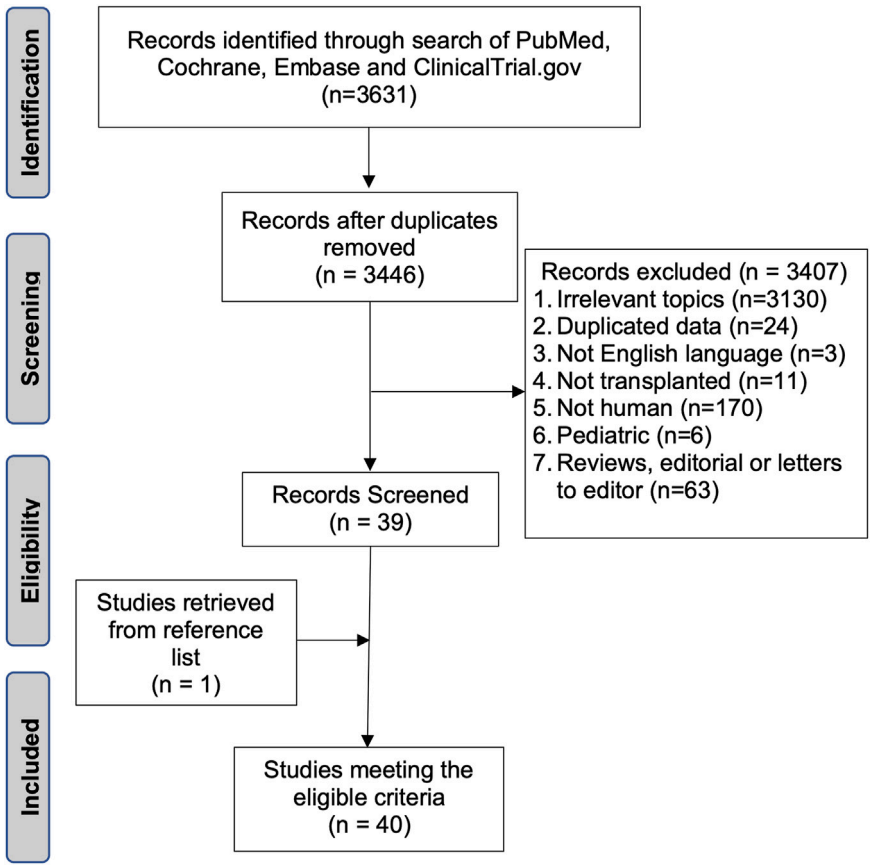


**FIGURE 1 |** Flowchart of the search strategy.

**TABLE 1 |** Hypothermic machine perfusion.

| Study | Number of patients | Temperature (°C) | Perfusate | Outcome | Publication type |
|---|---|---|---|---|---|
| Wicomb et al., 1984 (9) | HMP = 4 | 4–10 | Crystalloid cardioplegic solution | Total preservation time 12, 7, 15, and 6 h. One patient survived over 16 months | Single-center |
| Hill et al., 1997 (11) | HMP = 8, SCS = 12 | Ice-cooling | Colloid cardioplegic solution | 7-year survival rate 100% in both the HMP and the SCS groups | Single-center |
| Nilsson et al., 2020 (6) | HMP = 6, SCS = 25 | 8 | Albumin-rich solution with erythrocytes | 6-month event-free survival rate 100% in the HMP group and 72% in the SCS group | Single-center |

*HMP, hypothermic machine perfusion; SCS, static cold storage.*

total out-of-body time was significantly longer in the OCS group than in the control group (324 vs. 195 min) (7). The other two randomized studies reported data from single institutional heart transplant candidates, previously enrolled in the PROCEED II study and subsequently followed for an additional one and 2 years (12, 13). There were no significant differences between the OCS and SCS groups regarding changes in intimal thickness for the left main and left anterior descending coronary arteries (13). Chan et al. followed the recipient for 2 years and did not find any significant differences in patient survival, freedom from non-fatal major cardiac events, or cardiac allograft vasculopathy (12).

Thirteen studies (5, 14, 16, 21, 25, 26, 29–33, 35, 40) used the OCS in high-risk cases. High risk was defined as an adverse donor/recipient profile, including an estimated ischemic time longer than 4 h, left ventricular ejection fraction less than 50%, left ventricular hypertrophy, donor cardiac arrest, alcohol/drug abuse, coronary artery disease, recipient mechanical circulatory support, and/or elevated pulmonary vascular resistance.

In nine publications, the OCS was compared with SCS (**Table 2**) (14, 15, 25–31). The results of three of these studies favored OCS perfusion (27, 29, 31), including two studies that used the OCS for high-risk cases (29, 31). The other six studies

**TABLE 2 |** Studies of normothermic machine perfusion for hearts from donation after brainstem death with static cold storage as the control group.

| Study | Number of patients | Total preservation time (min) | Outcomes | Publication type | Risk case |
|---|---|---|---|---|---|
| Ardehali et al., 2015 (7) | OCS = 67, SCS = 63 | OCS = 324, SCS = 195 | No difference in 30-day survival rate and SAE between groups | Multi-center, randomized, article | No |
| Chan et al., 2017 (12) | OCS = 19, SCS = 19 | OCS = 361, SCS = 207 | 2-year patient survival rate: 72.2% in OCS group, 81.6% in SCS group ($p = 0.38$) | Single-center, randomized, article | No |
| Sato et al., 2019 (13) | OCS = 5, SCS = 13 | OCS = 362, SCS = 183 | ΔMIT ≥0.5 mm with no significant difference between groups. From baseline to 1 year post-transplant, ΔMIT, maximal intimal area, and percent stenosis were similar between groups | Single-center, randomized, article | No |
| Botta et al., 2017 (26) | OCS = 7, SCS = 95 | OCS = 296, SCS = 187 | No significant difference in CK-MB post- transplant | Conference abstract | Yes |
| Falk et al., 2019 (27) | OCS = 16, SCS = 24 | Not reported | OCS perfusion reduces IRI at the cytokine and endothelial level in recipient blood immediately after transplantation | Conference abstract | Not mentioned |
| Fujita et al., 2018 (28) | OCS = 29, SCS = 169 | Not reported | Survival rate similar between groups | Conference abstract | Not mentioned |
| Garcia et al., 2015 (29) | OCS = 15, SCS = 15 | OCS = 373, SCS = 204 | 30-day survival rate: 100% in OCS group and 73.3% in SCS group ($p = 0.03$) | Conference abstract | Yes |
| Jain et al., 2017 (14) | OCS = 1, SCS = 1 | OCS = 495, SCS = 412 | Total cost of OCS transplantation significantly less than SCS transplantation | Article | Yes |
| Koerner et al., 2014 (15) | OCS = 29, SCS = 130 | OCS = 313, SCS: not reported | No significant difference in cumulative survival rates at 30 days, 1 year, and 2 years | Article | No |
| Rojas et al., 2020 (30) | OCS = 49, SCS = 48 | OCS = 402, SCS = 225 | No significant difference in 30-day, 1-year, and 2-year survival rate | Conference abstract | Yes |
| Sponga et al., 2019 (31) | OCS = 17, SCS = 70 | Not reported | Improved 30-day, 1-year, and 5-year survival rate in the OCS group | Conference abstract | Yes |
| Sponga et al., 2020 (25) | OCS = 44, SCS = 21 | OCS = 428, SCS = 223 | No significant difference in 30-day mortality | Conference abstract | Yes |

*IRI, ischemia-reperfusion injury; MIT, maximal intimal thickness; NS, not significant; OCS, organ care system; SAE, serious adverse events; SCS, static cold storage.*

**TABLE 3 |** Non-randomized studies of normothermic machine perfusion for hearts from donation after brainstem death, without control group.

| Study | Number of patients | Total preservation time (min) | Outcomes | Publication type | Risk case |
|---|---|---|---|---|---|
| Ayan Mukash et al., 2019 (32) | 47 | Not reported | Kaplan-Meier survival estimates 91%, 85%, and 80% at 3 months, 6 months, and 1 year | Conference abstract | Yes |
| Garcia et al., 2016 (33) | 60 | Not reported | Survival rate similar between regular donor group ($n = 24$) and extended criteria donor group ($n = 36$) | Conference abstract | Yes |
| Garcia et al., 2014 (16) | 26 | 371 | Survival rate 100% at 1 month and 96% at follow-up of 257 days | Article | Yes |
| Kaliyev et al., 2019 (10) | 43 | 344 | 30-day survival 100% | Article | Not mentioned |
| Koerner et al., 2012 (34) | 13 | Not reported | 1- and 2-year survival rate 89% | Conference abstract | Not mentioned |
| Nurmykhametova et al., 2018 (5) | 1 | 960 | Total out-of-body time 16 h, longest out-body time to date | Conference abstract | Yes |
| Rojas et al., 2020 (40) | 76 | 382 | Survival rate 92.1% and 82.9% at 30 days and 1 year | Conference abstract | Yes |
| Stamp et al., 2015 (21) | 1 | 611 | Total out-of-body time 10 h | Article | Yes |
| Yeter et al., 2014 (35) | 21 | 388 | Freedom from cardiac-related death 95% at 30 days and 6 months, 87% at 1 and 4 years | Conference abstract | Yes |

did not find any significant difference in the primary outcomes (14, 15, 25, 26, 28, 30). The total preservation time was reported in five studies, and it was significantly longer in the OCS groups (14, 25, 26, 29, 30).

Botta et al. compared day-0/day-1 CK-MB levels between an OCS group and an SCS group and did not find any significant difference (26). Falk et al. compared IRI between the OCS and SCS groups by measuring interleukin (IL)-6, IL-8, IL-18, angiopoietin-2, and insulin-like growth factor-binding protein-1 immediately after and 24 h after heart transplant (27). The results showed that OCS preservation significantly reduced all these proteins. Seven studies compared short- and long-term patient survival rates and found no significant difference between the groups (14, 15, 25, 28-31).

One case report reported two long-distance heart transplantations, with or without the OCS. Although both patients remained well at 6 months with normal cardiac function, the patient who received the SCS-preserved heart

**TABLE 4 |** Studies of normothermic machine perfusion for hearts from donation after circulatory death.

| Study | Number of patients | Outcomes | Publication type |
|---|---|---|---|
| Chew et al., 2017 (36) | DCD = 12, MBD = 12 | All hearts retrieved with DPP, comparable survival rate between OCS-preserved DCD hearts and OCS-preserved MBD hearts | Conference abstract |
| Chew et al., 2019 (22) | DCD = 23, DBD = 94 | All DCD hearts retrieved with DPP, comparable survival rate between OCS-preserved DCD hearts and SCS-preserved DBD hearts | Paper |
| Dhital et al., 2015 (23) | DCD = 3 | All hearts retrieved with DPP, survival to date: 77, 91, and 176 days | Article |
| Garcia et al., 2016 (17) | DCD = 2 | Both hearts retrieved with DPP, survival to date: 290 and 291 days | Article |
| Mehta et al., 2019 (18) | DCD = 7 | All hearts retrieved with DPP, 90-day survival rate 86% | Article |
| Messer et al., 2016 (20) | DCD = 9 | 8 hearts retrieved with TA-NRP + OCS; all patients survived during follow-up (range, 48–297 days) | Article |
| Messer et al., 2017 (24) | DCD = 26, DBD = 26 | DCD hearts retrieved with DPP or TA-NRP, comparable results of the OCS-preserved DCD hearts and the SCS-preserved DBD hearts | Article |
| Messer et al., 2019 (37) | DCD = 50, DBD = 50 | DCD hearts retrieved with DPP or TA-NRP, comparable results in 30-day survival | Conference abstract |
| Mohite et al., 2019 (19) | DCD = 1 | Heart retrieved with DPP, alive to date at 5 months | Article |
| Page et al., 2017 (38) | DCD = 20, DBD = not reported | Biopsies within first month after transplantation showed significantly lower positive C4d rate in OCS-preserved DCD hearts suggesting a lower IRI rate. During first year, acute cellular rejection (2R) was lower in DCD than DBD group | Conference abstract |
| Page et al., 2018 (39) | DCD = 31, DBD = 31 | DCD hearts retrieved with DPP or TA-NRP, comparable results | Conference abstract |

*DBD, donation after brainstem death; DCD, donation after circulatory death; DPP, direct procurement and perfusion; IRI, ischemia reperfusion injury; MBD, marginal brain dead; TA-NRP, normothermic regional perfusion; OCS, organ care system; SCS, static cold storage.*

had a longer hospital stay (50 vs. 12 days) and a higher cost (AU$ 234,160 vs. 56,658) compared with the OCS recipient (14). In nine publications, only the OCS was studied (**Table 3**) (5, 10, 16, 21, 32–35, 40). In general, the OCS preserved heart function well, resulting in a satisfactory postoperative survival rate for the recipients. Two case reports presented successful transplantations after 10 and 16 h preservation time (5, 21). In one study, hearts from both standard criteria donors and marginal donors (outside standard acceptability criteria) were preserved with the OCS, and no significant differences in 1-month, 1-year, and 2-year survival rates were found. However, there was an increased requirement for extracorporeal membrane oxygenation (ECMO) support in the standard criteria donor group (33% vs. 11%) (33).

The OCS was used for DCD hearts in 11 studies (**Table 4**) (17–20, 22–24, 36–39). In clinical practice, DCD hearts are retrieved with either direct procurement and perfusion (DPP) (17–19, 22–24, 36, 37, 39) or thoracoabdominal normothermic regional perfusion (TA-NRP) (20, 24, 37, 39). For DPP, after confirmation of death, a cardioplegic flush is applied. Thereafter, the heart is excised and transported in a beating state using an OCS. For TA-NRP, after confirmation of death, cardiac resuscitation is achieved with the help of an external pump. After weaning from the TA-NRP, cardiac functional assessment is performed using a pulmonary artery flotation catheter and transesophageal echocardiogram. Four studies reported comparable results between the OCS-preserved DCD hearts and the SCS-preserved DBD hearts (22, 24, 37, 39). However, two hearts were discarded after OCS preservation owing to machine failure (22). One study reported a 100% 3-month survival rate in both OCS-preserved DCD hearts and OCS-preserved marginal brain donor hearts (36). One study compared post-transplant biopsies for C4d and acute rejection episodes. The results suggested a lower IRI rate and similar patterns of cellular rejection for the OCS-preserved DCD hearts compared with the regular DBD transplantation (38). The other five publications presented successful DCD heart transplantations using OCS (17–20, 23). Messer et al. also compared the DPP plus OCS with TA-NRP plus OCS for DCD hearts and found no significant difference in 30- and 90-day survival rates (24, 37).

Five clinical trials are currently recruiting patients (**Table 5**) (41–45). Among these trials, three have a randomized design (42, 43, 45) and four are multicenter studies (41, 42, 44, 45). All ongoing clinical trials use patient/graft survival as the primary endpoint and patient/graft survival in a different time frame and/or graft function as secondary endpoints.

## DISCUSSION

Despite encouraging results, considerable challenges still need to be overcome before sound conclusions can be drawn regarding MP for heart preservation. Existing literature in this field is limited. Most of the studies were non-randomized and retrospective, and half of the publications were conference abstracts. The total number of transplantations using MP was low, especially for HMP. A clear advantage of MP has not been observed in randomized controlled studies. Although NMP has shown its superiority in high-risk cases in non-randomized single-centre studies, high-quality clinical trials still need to be conducted.

**TABLE 5 |** Ongoing clinical trials.

| NCT number | Institution | Study phase/ design | Starting date–estimated primary completion date | Estimated number of enrolled patients | Study arms | Outcome measures (time frame) |
|---|---|---|---|---|---|---|
| NCT03687723 (41) | Hannover Medical School, Hannover, Germany | Multicenter, observational | October 2016–December 2021 | 60 | Clinical use of OCS | Primary outcome: patient survival (12 months); secondary outcomes: patient and graft survival (30 days) |
| NCT03991923 (42) | UZ Leuven, Leuven, Flemish Brabant, Belgium, etc., total eight centers in Europe | Multicenter, randomized | July 2020–July 2021 | 202 | NIHP, STS | Primary outcome: mortality and graft dysfunction (30 days); secondary outcomes: mortality and graft dysfunction (time frame 12 months) |
| NCT04066127 (43) | Skane University Hospital Lund, Skane, Sweden | Randomized | June 2020–December 2022 | 66 | NIHP, STS | Primary outcome: survival free of acute cellular rejection and re-transplantation (12 months); secondary outcomes: I/R-tissue injury, early allograft dysfunction, and health status |
| NCT03835754 (44) | Cedars-Sinai, Stanford University, Yale New Haven Hospital, etc., total 12 centers from United States | Multicenter | June 2019–November 2020 | 48 | Clinical use of OCS, high risk donors | Primary outcome: patient survival (30 days), absence of severe PGD (24 h post heart transplant); secondary outcome: patient and graft survival (30 days), incidence of severe PGD and donor heart utilization rate (24 h post-transplant) |
| NCT03831048 (45) | Stanford University, Yale New Haven Hospital, Mayo Clinic, etc., total 16 centers from United States | Multicenter, randomized | December 2019–August 2021 | 212 | DCD donors: OCS, SCS | Primary outcome: survival (6 months); secondary outcome: utilization rate (within 24 h post-transplant) |

*DCD, donation after circulatory death; NIHP, non-ischemic hypothermic preservation; OCS, organ care system; PGD, primary graft dysfunction; SCS, static cold storage.*

Several publications have concluded that the effectiveness of the OCS seems to be more prominent in high-risk cases and for DCD hearts (5, 16, 46). One explanation could be that the OCS provided a platform for the functional assessment of donor hearts. During perfusion, perfusion parameters such as lactate production could be evaluated, and visual assessment could be performed. Only hearts that meet predefined criteria proceed to transplantation. However, as the only biomarker, serum lactate levels in the perfusate might not be reliable One study reported that five DCD hearts with a perfusate lactate concentration >5 mmol/L had been transplanted with a good outcome (22). As an alternative, TA-NRP can also assess DCD heart function *in situ* (24). During TA-NRP, donor hears can be assessed in a physiologic condition. With the help of a Swan-Ganz catheter and echocardiography, functional assessment can theoretically be better done during TA-NRP than OCS. In one study, two successful DCD heart transplantations were performed after TA-NRP and SCS preservation (37). However, whether the same result can be repeated for more significant number of candidates still needs to be confirmed.

MP may reduce acute graft rejection. A porcine heart study showed that NIHP could significantly reduce donor heart immunogenicity via loss of resident leukocytes, reducing recipient T cell recruitment up to 48 h following transplantation in the absence of immunosuppression (47). No clinical study has addressed on this topic so far. However, if this is confirmed clinically, all the transplantations can benefit from MP.

Ischemia is the main reason a donor heart can only be preserved within a few hours. The principle of the MP is to avoid ischemia. Both preclinical (46) and clinical (5, 21) studies have shown that successful transplantations after more than 10 h of MP preservation can be achieved. A prolonged preservation time would theoretically benefit the transplantation teams and reduce transplantation costs.

Literature on pediatric heart transplantation has been excluded in this review. As far as we know, no MP has been used for clinical pediatric heart transplantation so far. However, due to donor shortage, pediatric transplantations more often involve distant retrieval and complex operations. A MP system for pediatric donor hearts would be extra beneficial.

The perfusion technique and perfusate are the two keys to successful preservation. In Wicomb et al.'s study of HMP (9), only one of the four recipients survived over 16 months. Because the study was performed before 1982, many factors might have played roles in the low survival rate, such as the operative technique, perioperative care, etc. Among other factors, the combination of inadequate perfusion and lack of colloid in the perfusate might also have played a specific role. In pilot studies of porcine heart preserved using HMP, we observed that the albumin concentration in the perfusate was positively related to the myocardial water content (48, 49). The feasibility and effectiveness of this method have been shown in a clinical study (6). In contrast to this albumin-rich hyperoncotic and hyperkalemic solution supplemented with erythrocytes, the

OCS uses diluted whole blood. This can theoretically provide all the necessary nutrients for the heart. However, some donor blood components may have adverse effects, such as pharmacological substances, metabolites, and platelets.

MP could theoretically cause hemolysis, especially at higher pressures and extended preservation times. An animal study showed no hemolysis occurred after 24 h of porcine heart perfusion with the NIHP system (49). With a higher perfusion pressure and flow, the OCS has a higher risk of hemolysis. However, we have not seen any reports about this in clinical trials. Apart from hemolysis, prolonged MP time, especially with NMP, would also lead to metabolite accumulation in the perfusate. However, with post-transplant ECMO support, successful transplantations have been reported after 10 and 16 h of total preservation time with the OCS (5, 21).

In addition to better clinical outcomes, safety and simplicity are crucially important for MP. HMP is theoretically safer and simpler to use than NMP. If a machine malfunction or user error occurs, NMP, which perfuses a beating heart, would have a narrower margin of safety. It was reported that two hearts were discarded after using the OCS owing to machine failure in one DCD study (22). In PROCEED II, five donor hearts were discarded after OCS preservation, despite these hearts being appropriate for transplantation at harvest. However, whether the OCS caused this effect was unclear (7, 50).

Using MP leads to a longer preservation time (129 min longer in the OCS group and 29 min longer in the NIHP group than in the SCS group) (6, 7). Moreover, MP requires additional surgical and technical support, proprietary equipment, appropriate transport, and additional costs. However, it may reduce the length of stay in the intensive care unit or hospital, postoperative mechanical support, and need for reoperation. Therefore, the total cost and labor demand may be reduced (14).

A challenge emerged during literature collection because the same data on MP transplantation has been used repeatedly in different conference abstracts and papers. Such examples can be found in publications from the groups of Rojas S., et al, Nilsson J., et al, Yeter R., et al, Chew, H., et al and García Sáez, D., et al. When the same data have been used in a series of publications, we included only the latest the publications and when only part of the data has been used with different study design, we included all these publications to avoid missing data (16, 33). Consequently, this may jeopardize the objectiveness of this review. Fortunately, the conclusions of these publications have been consistent, and the impact is theoretically minimal.

In summary, the machine perfusion in the form of either HMP or NMP, has emerged a potentially beneficial method for heart preservation. Based on the currently available data, when preserving a regular human donor heart, MP seems to yield clinical outcomes comparable to traditional SCS. However, HMP seems especially beneficial for high-risk cases and DCD hearts. Compared to NMP, HMP seems to be less complex, which may make it more feasible and safer, and this is an excellent advantage for the transportation of donor hearts. In future studies, we believe it's important address the efficiency of MP for donor hearts with isolated risk factors, such as prolonged preservation time, hearts from higher age donors, or low ejection fraction. Additionally, it is also essential to develop an ideal perfusion medium for different types of MP and a system for pediatric transplantation considering the more significant donor shortage.

## AUTHOR CONTRIBUTIONS

GQ: Study design; GQ and JN: Study conduction; GQ and JN: Data analysis; GQ, VJ, TS, SS, and JN: Paper writing; JN: Fund collection.

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## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Continuous Endogenous Exhaled CO Monitoring by Laser Spectrometer in Human EVLP Before Lung Transplantation

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Endogenous production of carbon monoxide (CO) is affected by inflammatory phenomena and ischemia-reperfusion injury. Precise measurement of exhaled endogenous CO (eCO) is possible thanks to a laser spectrometer (ProCeas® from AP2E company). We assessed eCO levels of human lung grafts during the normothermic *Ex-Vivo* Lung Perfusion (EVLP). ProCeas® was connected in bypass to the ventilation circuit. The surgical team took the decision to transplant the lungs without knowing eCO values. We compared eCO between accepted and rejected grafts. EVLP parameters and recipient outcomes were also compared with eCO values. Over 7 months, eCO was analyzed in 21 consecutive EVLP grafts. Two pairs of lungs were rejected by the surgical team. In these two cases, there was a tendency for higher eCO values (0.358 ± 0.52 ppm) compared to transplanted lungs (0.240 ± 0.76 ppm). During the EVLP procedure, eCO was correlated with glucose consumption and lactate production. However, there was no association of eCO neither with edema formation nor with the $PO_2/FiO_2$ ratio per EVLP. Regarding post-operative data, every patient transplanted with grafts exhaling high eCO levels (>0.235 ppm) during EVLP presented a Primary Graft Dysfunction score of 3 within the 72 h post-transplantation. There was also a tendency for a longer stay in ICU for recipients with grafts exhaling high eCO levels during EVLP. eCO can be continuously monitored during EVLP. It could serve as an additional and early marker in the evaluation of the lung grafts providing relevant information for post-operative resuscitation care.

**Keywords: lung transplant, ischemia-reperfusion, *ex-vivo* lung perfusion, lung inflammation, carbon monoxide, spectroscopy, cavity enhanced laser absorption spectroscopy, gasotransmitters**

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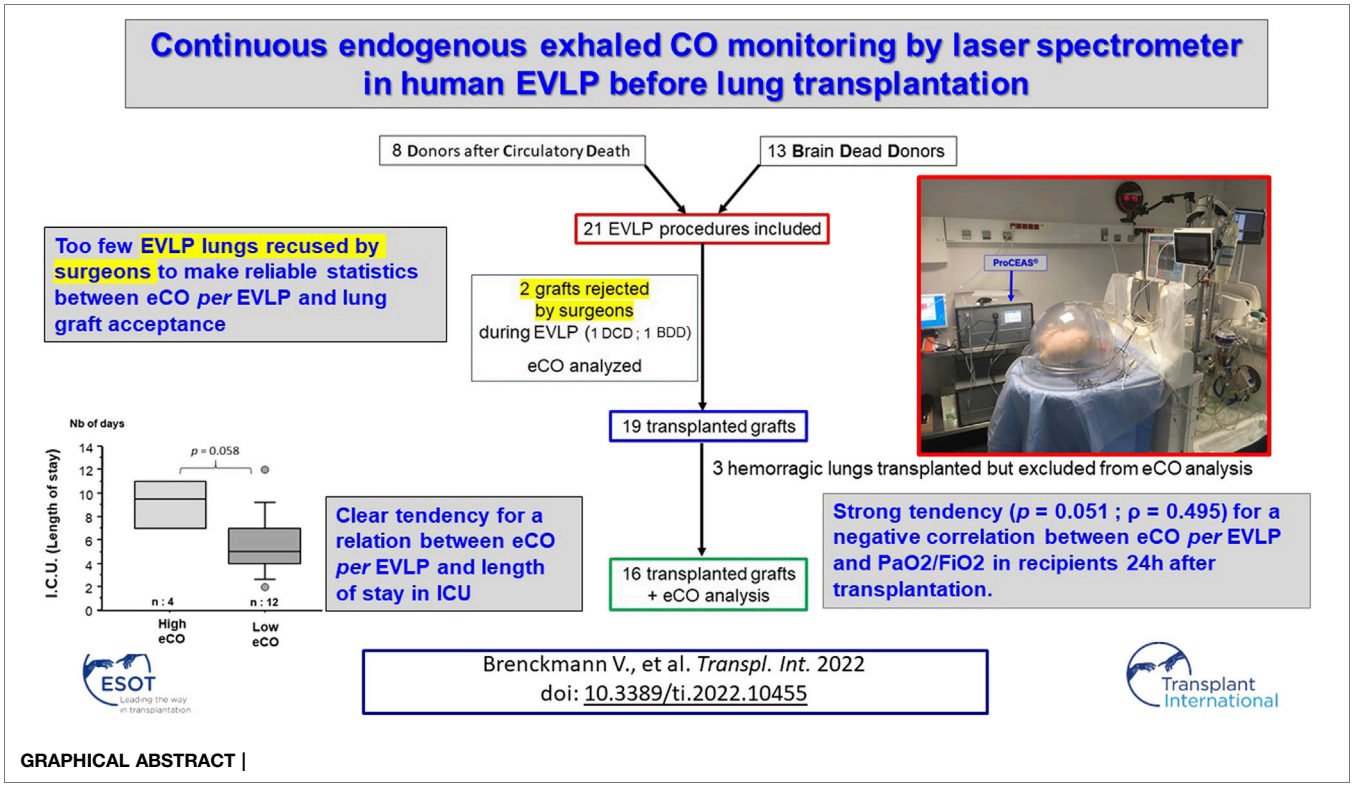
**Abbreviations:** BDD, brain dead donors; CO, carbon monoxide; eCO, endogenous exhaled carbon monoxide; DCD, donor after circulatory death; ECMO, extra corporeal membrane oxygenation; EVLP, *ex-vivo* lung perfusion; HO, heme oxygenase; IL, interleukine; ISHLT, International Society for Heart and Lung Transplantation; OF-CEAS, optical-Feedback cavity-enhanced absorption spectroscopy; PGD, primary graft dysfunction; ppm, parts per million; TNF, tumoral necrosis factor.




**GRAPHICAL ABSTRACT |**

## INTRODUCTION

In an attempt to compensate for the lack of pulmonary grafts, *Ex-Vivo* Lung Perfusion techniques (EVLP) have been developed. These techniques allow a second chance to be offered in the case of certain grafts that do not initially meet all criteria for being procured for transplantation. EVLP lungs derive from either marginal donors or from donors who have undergone a controlled cardiac arrest after interruption of life-sustaining therapies (Donor after Circulatory Death (DCD) Class III of Maastricht classification). French legislation requests that lung grafts harvested from cardiac DCD donors should be evaluated by EVLP before transplantation. EVLP allows an optimization and a new evaluation of the grafts. Above all, *via* these procedures, an increase in the number of available grafts has been observed (+25% in specialized teams) with comparable long-term outcomes in recipients (1).

The lungs are subject to inflammatory phenomena, particularly to ischemia-reperfusion injury responsible for a runaway inflammatory cascade (2, 3). This will lead to lesions of the capillary-alveolar membrane up to a Primary Graft Dysfunction (PGD) associating pulmonary edema and alteration of gas exchange capacities in the recipients (4). It would be of great interest to be able to detect early severe inflammatory phenomena during an EVLP procedure, ideally by a real-time and non-invasive monitoring. Some research teams have tested the prognostic performance of dosing inflammatory proteins (cytokines) in perfusion fluids (5–7) and more specifically during the EVLP procedures (8). Overall, elevated cytokine concentrations seem to be associated with lung damage and poor recipient outcomes. Unfortunately, the real-time assay of cytokines does not seem realistic at the present time. For this reason, we aimed to monitor the production of carbon monoxide (CO) which is a biomarker of inflammation (9).

Inflammatory cytokines, *via* the MAP kinase pathway, stimulate the transcription and production of Heme-oxygenase type 1 (HO-1) (10). This enzyme will catalyze the breakdown of heme. The latter protein, present in large quantities in red blood cells, also exists in every tissue of the body, especially in the lungs (11). The breakdown of heme results in the production of biliverdin which will yield bilirubin, iron, and CO. Endogenous CO is evacuated through the respiratory tract (eCO). Many studies have already shown a correlation between pathological conditions and eCO production. For example, eCO is increased during sepsis (12), or inflammatory respiratory pathologies including allergic rhinitis, asthma, and bronchiectasis (13). More specifically, after lung transplantation, high eCO levels appear to be associated with the recruitment of alveolar neutrophils (14) and later with post-transplant bronchiolitis obliterans syndrome (13).

OF-CEAS (Optical Feedback—Cavity Enhanced Absorption Spectroscopy) is a sensitive gas concentration measurement technique based on absorption spectroscopy, on which the ProCeas® instruments by the AP2E company are based. It allows very precise and real-time detection of CO with a very short response time. Its mode of operation has already been described in detail in previous publications(15, 16). Relying on

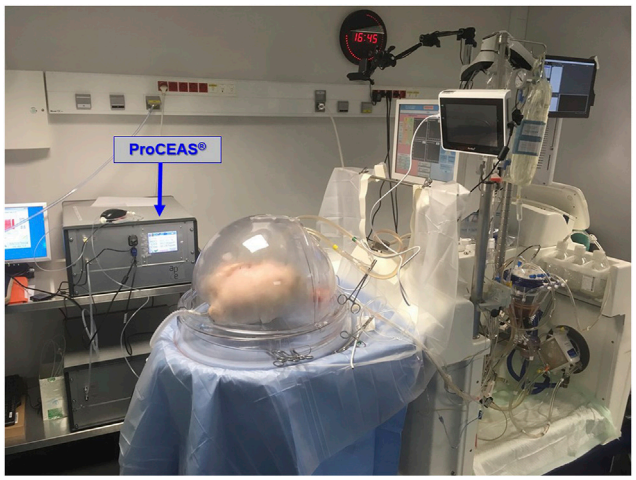



**FIGURE 1 |** Overview of the setup of EVLP preparation with the XPS™ device on the right and the ProCEAS® in the background, monitoring eCO in real-time. In the front plane is the ventilated and perfused *ex-vivo* lung.

healthy human volunteers, we have shown *via* the use of an OF-CEAS analyzer that breathing pure oxygen quadruples CO levels compared to ambient air conditions, whether or not the subject is a smoker (17). Additionally, our team also worked on an EVLP pig model. We proved that EVLP lungs are a source of eCO production (18), and that eCO concentrations are higher in lungs exposed to severe ischemia-reperfusion injury (19).

The main objective of this monocentric prospective study was to test, for the first time, eCO measurement by ProCeas® in an EVLP with human lungs during a real transplantation procedure in patients. We aimed to see if eCO might be an early biomarker to decide if an EVLP lung could be transplanted or not. Moreover, we compared eCO values with other evaluation parameters during EVLP procedure. We also compared EVLP eCO values with post-operative outcomes collected in transplanted recipients during the early post-operative period.

# METHODS

## Design of the Study

This prospective monocentric, simple blind study of feasibility took place at Foch Hospital, Suresnes, France. Over a seven-month period (December 2018 to June 2019) twenty-one pairs of lung grafts, rejected by every French transplantation centers for a standard transplantation, were included in the protocol allowing eCO measurement during an EVLP procedure.

## Prototype "Medical ProCEAS®"

Briefly, laser spectroscopy measurements of very low gas concentrations (less than 1 ppm) require a large light absorption path. Similar to some other spectroscopy techniques, OF-CEAS exploits a resonant optical cavity that allows an effective optical absorption path length of several kilometers while the cavity is only 1 m long (folded in two arms) and its volume about 20 $cm^3$. In this way, very sensitive measurements with compact instruments and a small sampling volume can be obtained. Additionally, OF-CEAS provides absolute concentration measurements with sufficient accuracy exempting any periodic calibration with certified gas mixtures. In contrast to the somewhat complex physics underlying OF-CEAS, its optical layout consists of few basic optical elements allowing for a compact and robust device. In this study, we use an OF-CEAS analyzer prototype specially derived, for the purpose of the study, from a commercial device (ProCEAS®) developed by the AP2E company (Aix-en-Provence, France) one of the partners of the research consortium. This instrument, including electronics for laser control and data acquisition and a vacuum pump for sample gas circulation, measures approximately 50 × 60 × 60 cm, is transportable on a trolley and is perfectly adapted to a medical environment (**Figure 1**). It also connects easily with a common sterile sampling line for the ventilation on standard airway filters.

To ensure that ProCEAS® meets the constraints of safety and hygiene for its deployment in an operating room, a risk analysis has been carried out by a specialized and independent company (SurgiQual institute™; Meylan; France).

## *Ex-Vivo* Lung Perfusion Techniques

The EVLP procedures were performed by the surgical transplant team of Foch Hospital (Suresnes; France), who perfectly masters this technique (20). Briefly, the EVLP device was an XVIVO Perfusion System (XPS shown in **Figure 1**, from XVIVO Perfusion AB, Göteborg, Sweden). This perfusion technique follows the "Toronto's protocol" from the Canadian team with the greatest experience, to date, on clinical EVLP transplantation (21). The perfusion fluid was acellular (Steen® solution without red blood cells) with a target of maximum perfusate flow rate of 40% of the estimated donor cardiac output. Once the lungs reached 32°C, they started to be gently ventilated with ambient air ($FiO_2$ 21%) at a frequency between 7 and 10 cycles/min, with a Positive End Expiratory Pressure (PEEP) of 5 cmH2O, and a tidal volume progressively increased up to 7 ml $kg^{-1}$ of ideal weight. When lung perfusion and ventilation were established at their target levels (approximately 30–45 min from the beginning of the perfusion), the evaluation phase began from this steady state (**Figure 2**). Recruitment maneuvers were performed every hour, during which lungs were ventilated with 100% $FiO_2$, first at PEEP 10 cmH2O for 10 min, and then at PEEP 5 cmH2O for others 5 min. Insufflation pressure never exceeded 25 cmH2O. Blood gases were analyzed at the end of each recruitment phase. During the inter-recruitment phases, $FiO_2$ was set down to 21% and PEEP remained at 5 cmH2O. The lungs all benefited from a bronchoscopy. The decision to transplant the lungs or not was taken by the surgeon without knowing the eCO values measured by ProCEAS®.

## Data Collection

ProCEAS® was connected to the airways from the beginning of the procedure, and data were recorded continuously to the end of EVLP (**Figure 1**). ProCEAS® sampled small quantities of gas (200 ml $min^{-1}$) extracted from the ventilation circuit. The eCO values were given by the difference between the maximum values (end of expiratory phase) and the minimum values (end of inspiratory phase). This made it possible to measure the

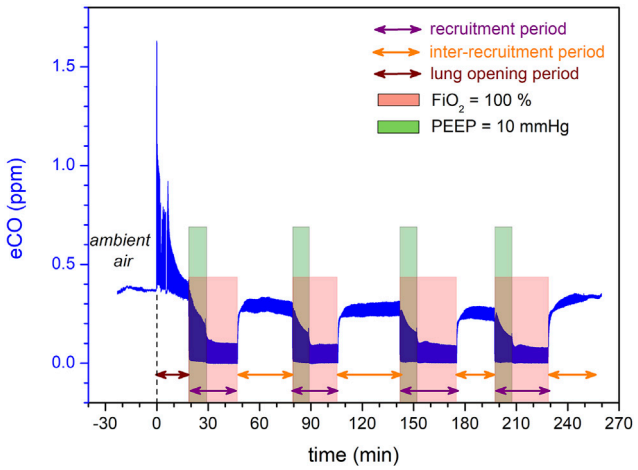



**FIGURE 2 |** CO endogenous production monitored in real -time during *ex-vivo* lung perfusion. Time origin corresponds to the ventilation start. Before the connection to the lung, the analyzer measures CO concentration in ambient air. A strong release of eCO is observed at the beginning of the lung opening period (wine arrow). During inter-recruitment periods (orange arrows), lungs are ventilated with a $FiO_2$ of 21% and a PEEP of 5 cmH2O. During each recruitment period (purple arrow), the $FiO_2$ is increased to 100% (pink box), while the PEEP is increased to 10 cmH2O only during the first 10 min (green box) and then set back to 5 cmH2O.

production of eCO regardless of its content in the ambient air used by the ventilator when $FiO_2$ was set to 21%.

ECO data were averaged over a period of 5 min following each recruitment maneuver.

Hemodynamic parameters and pulmonary mechanics (compliance, pulmonary pressure, and vascular resistance) were recorded by the XPS™ device. The quantifications of the perfusion fluids were conducted manually.

Samples of perfusate fluid were collected after each recruitment maneuver to measure blood gases, glucose, and lactate levels.

At the very end of the procedure, a sample of perfusate was taken for later cytokines measurements. The following cytokines (Interleukine (IL) 1-β, 6, 8, 10 and Tumor Necrosis Factor α (TNFα) were assayed by the MagPix device and Xponent software (Luminex Corp), using a porcine cytokine/chemokine magnetic bead panel (Milliplex MAP kit, Millipore Corp.), according to manufacturers' instructions.

## Ethics

For the ProCEAS® prototype, which is not a medical device, a general risk management approach was carried out by the SurgiQual Institute™ (http://www.surgiqual-institute.com/; Meylan, France), a company expert in Medical Device Development Regulation. The aim was to identify and reduce the safety and functional risks inherent to the design, as well to the use of the device in the context of the clinical investigation. This risk analysis was performed according to the standard ISO 14971 (dedicated to medical devices) and has concluded to a favorable benefit/risk ratio for the patients (and users). ProCEAS® measured eCO by sampling exhaled gas in a non-invasive manner. All eCO measurements were done on grafts initially rejected for transplantation by all French centers. For this study, EVLP procedures on these rejected grafts and eCO measurements were performed exclusively at Foch hospital (Suresnes, France). Surgeons could not access the measured eCO values during the EVLP, not to be influenced in their decision to requalify or not the EVLP lungs for a possible transplantation in a patient. The retrospective analysis of data from patients transplanted with EVLP lungs at Foch hospital (Suresnes; France) has been approved by the ethical committee of the French Society of Thoracic and Cardio-Vascular Surgery (approval reference: CERC-SFCTCV-2020-05-06-05-SAED).

## Statistics

The experimentation constituted a feasibility study without prior hypothesis of the eCO threshold used to get a power of the study and calculate a number of patients to include.

Our goal was to include about twenty consecutive procedures for this first study, whether the lungs were transplanted or not.

The comparisons between groups are presented in medians and interquartile ranges (box plots). Due to the relatively small number of included experiments, we verified that the distribution of the data did not differ significantly from a Gaussian distribution by a Kolmogorov-Smirnov test. In the same way, we checked the homogeneity of variances by Barlett's test. The correlation between different parameters was performed by a simple linear regression and tested statistically by variance analysis (ANOVA). The comparison of the data between groups was made by a nonparametric test (Mann Withney U test). For the analysis of distribution between groups we used an exact Fischer test. We also performed a ROC curve with the hope to determine a clinically relevant eCO threshold to predict the evolution of the transplanted lungs. Differences with a $p < 0.05$ were considered as significant. These analyses were carried out using Statview software except for the ROC curve obtained with R version 3.6.1 (R Foundation for Statistical Computing).

# RESULTS

We included 21 bi-pulmonary graft procedures from December 2018 to July 2019. The characteristics of donors and recipients are summarized in **Tables 1**, **2**. Raw data of the study are available, as a single Table, in **Supplementary Materials**.

During the EVLP procedure, only 2 grafts were rejected for transplantation by the surgical team. These two rejected lungs had absorbed large amounts of perfusion fluid during the procedure; their compliance had decreased significantly and their $PO_2/FiO_2$ ratio was less than 350 mmHg.

eCO measurement was possible in all of the 21 EVLP procedures and, as expected, did not perturb their progress. The ProCeas allows monitoring in real-time eCO during the entire EVLP as plotted in **Figure 2**, where four recruitments periods, spaced by inter-recruitment periods, were performed. The thickness of the eCO trace in this figure corresponds to the concentration oscillations during inhalation and exhalation phases, as shown in the zoom-in **Figure 3**. Indeed, the short response time of the analyzer allows the respiratory cycles to be resolved.

**TABLE 1 |** Donor history of the 21 included EVLP lung grafts.

| N° | Age | Height (cm) | Gender | Reason for EVLP | Anamnesis | Heart failure | Rescucitation duration before procurement (day) | Tracheal intubation duration before procurement (day) | X Ray | Bronchoscopy | $PaO_2/FiO_2$ (mmHg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 66 | 175 | M | BDD | Post-traumatic brain hemorrhage | No | 7 | 6 | Effusion and atelectasis | Purulent secretions | 310 |
| 2 | 57 | 165 | F | BDD | Spontaneous hemorrhagic stroke | No | 6 | 6 | Atelectasiss and inhalation | Purulent secretions | 327 |
| 3 | 48 | 171 | M | DCD | Spontaneous hemorrhagic stroke | No | 40 | 40 | Atelectasis | Purulent secretions | 522 |
| 4 | 61 | 164 | M | DCD | STEMI and Heart failure | Yes | 5 | 5 | Effusion and atelectasis, Inhalation | | 346 |
| 5 | 47 | 183 | M | DCD | Heart failure | Yes | 5 | 5 | Contusions and pneumothorax | | 360 |
| 6 | 66 | 170 | M | BDD | Spontaneous hemorrhagic stroke | No | 4 | 4 | Atelectasis and condensations | | 318 |
| 7 | 59 | 200 | M | DCD | Spontaneous hemorrhagic stroke | No | 7 | 7 | Condensations and inhalation | | 418 |
| 8 | 50 | 166 | | BDD | Heart failure | Yes | 2 | 2 | Effusion and atelectasis | Purulent secretions | 322 |
| 9 | 52 | 170 | M | BDD | Spontaneous hemorrhagic stroke | Yes | 2 | 2 | Contusions and inhalation | Blood | 300 |
| 10 | 53 | 165 | F | BDD | Post-traumatic brain hemorrhage | No | 1 | 1 | Condensations | Purulent secretions | 388 |
| 11 | 41 | 180 | M | BDD | Spontaneous hemorrhagic stroke | No | 1 | 1 | Inhalation | Blood | 330 |
| 12 | 34 | 168 | F | DCD | Spontaneous hemorrhagic stroke | No | 5 | 5 | Atelectasis | | 480 |
| 13 | 34 | 183 | M | BDD | Spontaneous hemorrhagic stroke | No | 4 | 4 | Effusion | Purulent secretions | 266 |
| 14 | 31 | 187 | M | BDD | Hanging | Yes | 1 | 1 | Condensations | Blood | 404 |
| 15 | 64 | 158 | F | DCD | Spontaneous hemorrhagic stroke | Yes | 1 | 1 | | | 528 |
| 16 | 15 | 185 | M | BDD | Heart failure | Yes | 14 | 14 | Contusions and inhalation | Purulent secretions | 353 |
| 17 | 21 | 178 | M | BDD | Post-traumatic brain hemorrhage | Yes | 4 | 4 | Atelectasiss and inhalation | Purulent secretions | 375 |
| 18 | 28 | 182 | M | BDD | Post-traumatic brain hemorrhage | Yes | 4 | 4 | Contusions and inhalation | | 401 |
| 19 | 53 | 167 | F | BDD | Spontaneous hemorrhagic stroke | Yes | 1 | 1 | Inhalation | Purulent secretions | 277 |
| 20 | 66 | 146 | F | DCD | Heart failure | Yes | 10 | 10 | Effusion and atelectasis | Purulent secretions | 362 |
| 21 | 61 | 162 | F | DCD | Drowing and Heart failure | Yes | 7 | 7 | Effusion and atelectasis | | 297 |

*M, male; F, female; BDD, brain dead donors; DCD, donor after circulatory geath. In dark grey, the two non-accepted lungs for transplant after EVLP. The lungs 9, 11, and 14 had macroscopical blood in their respiratory tract. They were transplanted to recipients by surgeons but we excluded them from the eCO analysis.*

Three grafts had, from the beginning of the EVLP procedure, extremely high eCO values compared to other lungs. The only difference we observed in their donors' medical history is that blood had been found in the respiratory tract during bronchoscopy. No other difference was found during EVLP procedures. These three lungs have been transplanted in the same way by the surgical team

**TABLE 2 |** Medical outcomes of the 19 recipients transplanted with EVLP grafts.

| N° | Indication | Age | Resuscitation duration after Tx (day) | Total Hospitalization duration (day) | $PaO_2/FiO_2$ at H0 (mmHg) | PGD at H0 | $PaO_2/FiO_2$ at 24 h (mmHg) | PGD at 24 h | $PaO_2/FiO_2$ 48 h (mmHg) | PGD at 48 h | $PaO_2/FiO_2$ 72 h (mmHg) | PGD at 72 h | DPG 3 during first 72 h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | AAT | 55 | 4 | 24 | 206 | 2 | 440 | 1 | 413 | 1 | 380 | 1 | No |
| 2 | CF | 35 | 4 | 25 | 314 | 1 | 328 | 1 | 360 | 1 | 314 | 1 | No |
| 3 | CF | 20 | 4 | 17 | 476 | 1 | 366 | 1 | 541 | 1 | 490 | 1 | No |
| 4 | COPD | 53 | 12 | 40 | 114 | 3 | 166 | 3 | 204 | 2 | 171 | 3 | Yes |
| 5 | CF | 48 | 5 | 35 | 194 | 3 | 519 | 1 | 419 | 1 | 350 | 1 | Yes |
| 8 | CF | 36 | 7 | 24 | 89 | 3 | 359 | 1 | 329 | 1 | 428 | 1 | Yes |
| 9 | COPD | 57 | 12 | 33 | 151 | 3 | 168 | 3 | 190 | 3 | 283 | 2 | Yes |
| 10 | CF | 37 | 8 | 23 | 97 | 3 | 466 | 1 | 271 | 2 | 366 | 1 | Yes |
| 11 | CF | 33 | 4 | 26 | 312 | 1 | 400 | 1 | 271 | 1 | 324 | 1 | No |
| 12 | CF | 21 | 6 | 23 | 283 | 2 | 346 | 1 | 391 | 1 | 289 | 2 | No |
| 13 | CF | 39 | 7 | 22 | 392 | 1 | 388 | 1 | 367 | 1 | 304 | 1 | No |
| 14 | CF | 34 | 3 | 28 | 246 | 2 | 316 | 2 | 444 | 1 | 280 | 2 | No |
| 15 | CF | 20 | 2 | 23 | 437 | 1 | 437 | 1 | 357 | 1 | 440 | 1 | No |
| 16 | CF | 48 | 3 | 29 | 182 | 3 | 248 | 2 | 412 | 1 | 370 | 1 | Yes |
| 17 | COPD | 59 | 6 | 42 | 90 | 3 | 337 | 1 | 382 | 1 | 353 | 1 | Yes |
| 18 | COPD | 64 | 10 | 31 | 181 | 3 | 418 | 1 | 370 | 1 | 436 | 1 | Yes |
| 19 | Fibrosis | 42 | 9 | 26 | ECMO | 3 | ECMO | 3 | ECMO | 3 | ECMO | 3 | Yes |
| 20 | Alveolar proteinosis | 22 | 12 | 37 | ECMO | 3 | ECMO | 3 | ECMO | 3 | ECMO | 3 | Yes |
| 21 | Cystic pneumonia | 43 | 4 | 22 | 309 | 1 | 345 | 1 | 310 | 1 | 309 | 1 | No |

*AAT, Alpha-1 antitrypsin deficiency; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; PGD at HO, primary graft dysfunction score at admission intensive care unit etc.*

*The lungs 9, 11 and 14 (highlighted in gray), were transplanted to recipient but excluded from the eCO analysis (macroscopical blood in their respiratory tract). A subgroup analysis comparing clinical outcomes of patients transplanted with these hemorrhagic lungs versus other patients transplanted with non-hemorrhagic lungs did not show any statistical difference in the above clinical outcomes (nonparametric Mann Withney U test).*

*The lungs 6 and 7 were non-accepted for transplant after EVLP and are not presented in this table.*

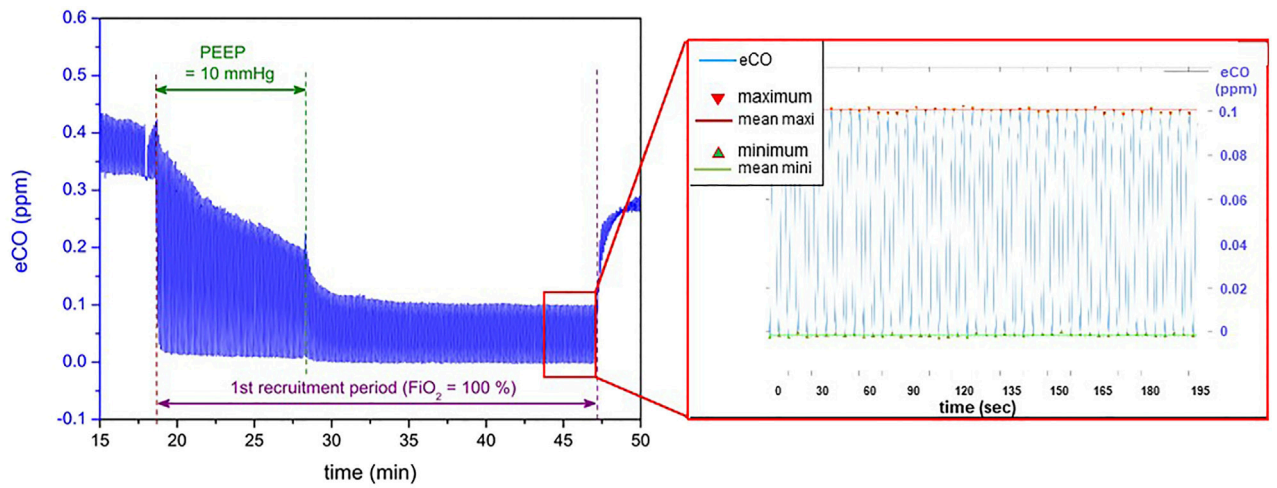



**FIGURE 3 |** Zooms-in of eCO measurements presented in **Figure 2** (Data from one EVLP procedure, displayed as example) Left: entire first recruitment period. Right: zoom on the last minutes of the recruitment phase. The respiratory cycles can be distinguished, allowing the identification of maximum (red) and minimum (green) CO values to derive the lungs' endogenous CO production.

with good clinical results in recipients. A subgroup analysis comparing clinical outcomes of patients transplanted with these hemorrhagic lungs versus other patients transplanted with non-hemorrhagic lungs did not show any statistical difference (**Table 2**).

Because of the very high concentrations of CO, which we assume to occur due to the presence of blood in the airways, we decided to exclude these three "hemorrhagic" paired grafts from the eCO data analysis. They were excluded *a priori* (prior to

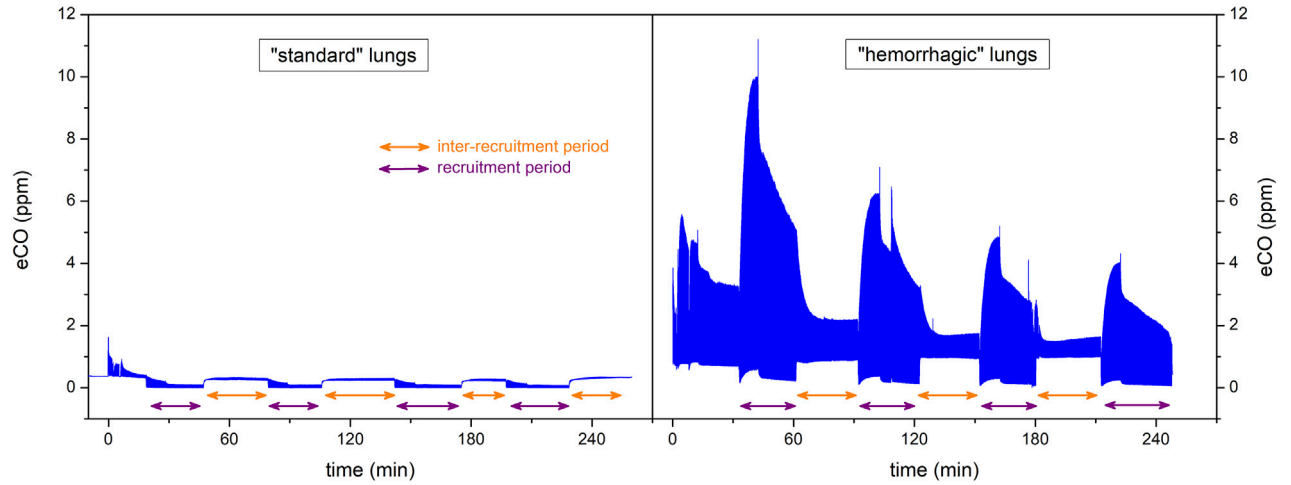



**FIGURE 4 |** Data from two EVLP procedures displayed as examples of eCO monitoring of "standard" lungs (left) and eCO monitoring of "hemorrhagic" lungs (right). Among the total of 21 EVLP procedures that we monitored, only 3 pairs of lungs had an "hemorrhagic profile" like the one shown on the right part of this figure. Although these 3 pairs of lungs were transplanted by the surgeons without problems for the recipients, we decided to exclude a priori these 3 pairs of lungs from the eCO data analysis.

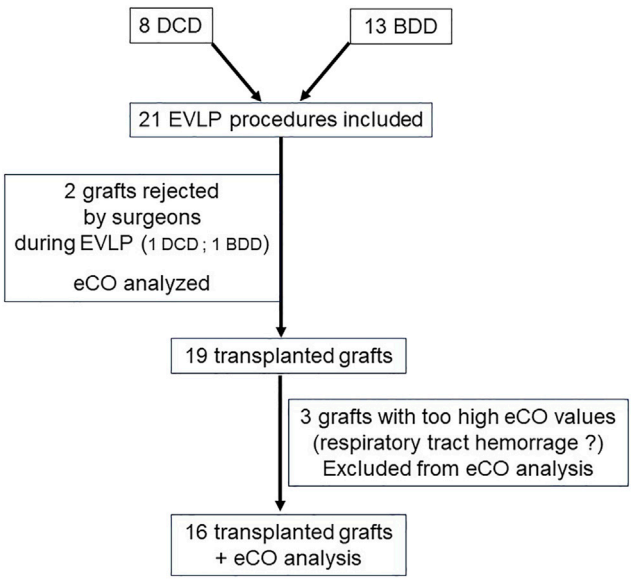



**FIGURE 5 |** Flow chart. Abbreviations: DCD: Donor after Circulatory Death, BDD: Brain Dead Donors.

commencement of the data analysis). An example of the eCO measurements of these hemorrhagic lungs is given in **Figure 4**.

There was no difference in eCO levels depending on the origin of the marginal grafts (Brain Dead Donors or DCD), for this reason we did not make a subgroup for the analyses.

The analysis flow chart is presented in **Figure 5**.

## Acceptance of Grafts

**Table 3** displays eCO levels in the 18 lungs where eCO could finally be analyzed. At the end of the 1st recruitment maneuver, eCO value in the two non-accepted lungs for transplant was 0.358 (±0.051) ppm. In the 16 other transplanted lungs, eCO value was 0.240 (±0.076) ppm. There was a tendency towards higher eCO levels in non-accepted lungs ($p = 0.068$).

## Parameters Tested During the *Ex-Vivo* Lung Perfusion

There was a tendency for higher vascular resistance for lungs with high eCO levels ($p = 0.062$). However, there was no association between eCO levels and gas exchange capacities, pulmonary compliances, nor a decrease in perfusion fluid in the EVLP reservoir (as a reflection of pulmonary edema formation).

There was a positive correlation between eCO and glucose consumption estimated by the difference in glucose levels in two perfusate samples taken 1 hour apart ($p = 0.042$). ECO was also correlated with lactate levels in perfusate ($p = 0.035$) (**Figure 6**).

## Short-Term Outcomes of the Recipients

There was a strong tendency ($p = 0.051$; $\rho = 0.495$) for a negative correlation between eCO after the first recruitment maneuver of the EVLP and $PaO_2/FiO_2$ ratio in recipients 24 h after transplantation.

A Receiver Operating Characteristic (ROC) curve has been drawn to assess the value of eCO and to predict an alteration in post-operative gas exchange ($PaO_2/FiO_2$ < 300 mmHg, 24 h after transplantation). The Area Under the Curve (AUC) was 0.668 and the most relevant threshold of eCO was 0.235 ppm (Youden index [Se + Sp-1] = 0.444). Using this threshold of 0.235 ppm, lung grafts were separated into two groups: lungs exhaling high eCO levels (>0.235 ppm) and those exhaling low eCO levels (≤0.235 ppm) after the first recruitment maneuver of the EVLP. Every patient who received grafts exhaling high eCO levels during the EVLP had a PGD score of 3 within 72 h.

**TABLE 3 |** eCO at each recruitment phase in the 18 EVLP where eCO could finally be analyzed.

| N° | eCO end 1st recruitement | eCO end 2nd recruitement | eCO end 3rd recruitement | eCO end 4th recruitement |
|---|---|---|---|---|
| 1 | 0.139 | 0.115 | 0.101 | |
| 2 | 0.102 | 0.094 | 0.089 | 0.078 |
| 3 | 0.047 | 0.065 | 0.062 | |
| 4 | 0.114 | 0.104 | | |
| 5 | 0.278 | 0.256 | | |
| 6 | 0.306 | 0.411 | | |
| 7 | 0.409 | 0.19 | | |
| 8 | 0.218 | 0.229 | 0.165 | |
| 10 | 0.081 | 0.102 | 0.101 | 0.091 |
| 12 | 0.041 | 0.046 | 0.041 | 0.04 |
| 13 | 0.125 | 0.116 | 0.117 | |
| 15 | 0.235 | 0.2 | 0.167 | |
| 16 | 0.163 | 0.194 | 0.164 | |
| 17 | 0.159 | 0.143 | 0.105 | 0.091 |
| 18 | 1.338 | 1.084 | 0.94 | |
| 19 | 0.363 | 0.313 | 0.305 | |
| 20 | 0.27 | 0.294 | 0.105 | 0.242 |
| 21 | 0.163 | 0.13 | 0.112 | 0.096 |

*In grey, the two non-accepted lungs for transplant after EVLP, eCO values of the 3 excluded procedures are not shown, eCO end 1st R = mean of the eCO during the 5 last minutes of the first recruitment phase etc. Values given in ppm.*
*The lungs 9, 11, and 14, excluded from the eCO analysis (macroscopical blood in their respiratory tract), are not presented in this table.*
*At the end of 1st recruitment: eCO value in the two non-accepted lungs for transplant was 0.358 (±0.051) ppm. In the 16 other transplanted lungs, eCO value was 0.240 (±0.076) ppm. There was a tendency towards higher eCO levels in non-accepted lungs (p = 0.068).*

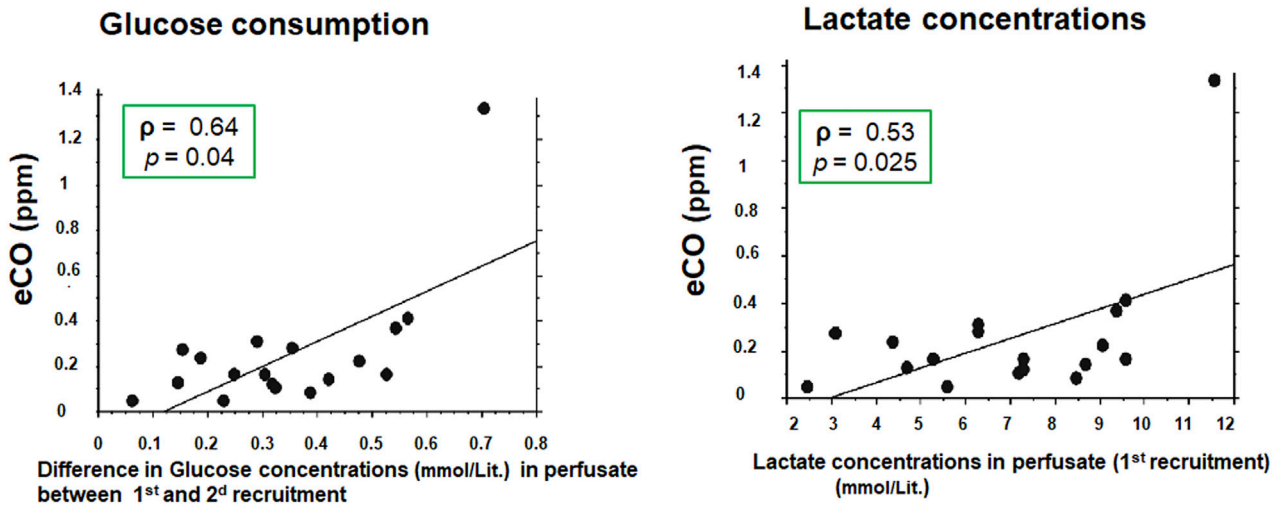



**FIGURE 6 |** eCO and metabolism. Glucose consumption is estimated as the difference in glucose concentration in perfusate between the two first recruitment maneuvers. When removing the outlier point (lung #18), the correlation is still significant ($p < 0.05$; $\rho = 0.49$) between eCO and glucose consumption, but not anymore with lactate concentration.

Conversely, only one patient with a low eCO level graft had a PGD score at 3 in 72 h.

There was a trend towards a longer stay in intensive care unit for patients who received grafts with high eCO levels during EVLP ($p = 0.058$) (**Figure 7**). All patients transplanted with an EVLP lung were alive at 30 days.

## Inflammatory Proteins

There was no significant correlation between cytokines concentrations in perfusion fluid and eCO levels during the EVLP. Nevertheless, there was a positive correlation between TNF-$\alpha$ glucose consumption ($p = 0.033$), and lactate production ($p = 0.049$) as well as between IL-1β and $PaO_2/FiO_2$ ratio in recipients 24 h after transplantation ($p = 0.039$), duration of the mechanical ventilation ($p = 0.002$) and length of time in ICU ($p = 0.004$).

# DISCUSSION

Our study confirms the feasibility and the accuracy of OF-CEAS non-invasive continuous monitoring of exhaled CO in EVLP lung

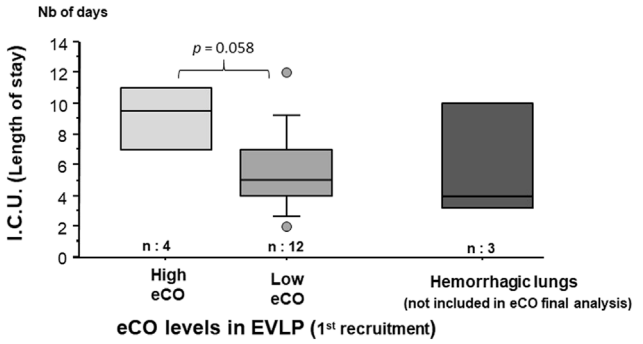



**FIGURE 7 |** eCO and length of stay in ICU. Patients receiving lungs exhaling "high" eCO levels (>0.235 ppm) at 1st EVLP evaluation, have a trend to stay longer in ICU.

grafts. In our series, despite the very low number (2) of lungs rejected for transplantation after the EVLP, eCO shows interesting trends for a future marker of lung graft status. A significant amount of blood into airways may, however, interfere with the eCO measurement.

## Limitations of the Study

This was a single-center study, the first objective of which was to assess the feasibility of eCO measurement by ProCEAS® in EVLP applied to human lung. The number of EVLP procedures performed over a seven- month period of observation is the highest published in Europe at that time. However, statistically speaking, this is a relatively small number, and only two grafts were recused for transplantation after the EVLP. Therefore, our observations about the eCO as an early marker of acceptance for the graft or as a prognostic marker for the receivers need to be interpreted with caution. The next step, to increase the statistical power of the evaluation of eCO for EVLP, will be to set up a multicenter study which will probably have to be international, given the few centers mastering the EVLP technique with a sufficient rate of procedures. Another limitation of the technique is in case of presence of blood in the airways. The degradation of the heme contained in large quantities in the blood is responsible for the production of CO in large quantities and has been widely described in the literature (22, 23). This probably explains the extremely high CO levels observed in grafts for which blood was visualized by bronchoscopy.

## Influence of Ventilatory Parameters on the eCO Measurements

The ventilator of the XPS™ device uses mixed gases (medical $O_2$ from operating room gas supply and ambient air). Except during recruitment maneuvers where lungs were ventilated with pure oxygen, the EVLP procedure was performed all the time at 21% $FiO_2$ using ambient air. Ambient air contains CO from air pollution and the breathing of nursing staff. Therefore, at 21% $FiO_2$, the minimum of eCO, measured during the inspiratory phase reflected the CO contained in ambient air (**Figure 2**). This intermittent intake of external CO explains the staircase aspect of the eCO curves. However, the cycle-to-cycle measurement of eCO made it possible to overcome these variations in the baseline (**Figure 3**). The increase to 100% $FiO_2$ (with pure oxygen without any CO) at the beginning of each recruitment phase showed a drop near zero of the eCO bottom line (inspiratory phase), while displaying a huge peak of eCO at the expiratory phase. As an increased PEEP to 10 cmH2O was applied at the same moment for recruitment, it is difficult to figure out the respective influence of each setting on eCO variation. Probably the highest PEEP led to a transient increase of eCO due to a better alveolar recruitment, allowing the elimination of CO, having no influence, however, on its production. On the other hand, a high concentration of oxygen may have been responsible for a competitive effect on CO adsorption on heme or other metalloproteins, as previously described (17, 24, 25) and may explain a part of the increase in eCO.

While lowering PEEP back to 5 cmH2O at the end of the recruitment phase, eCO dropped to lower values but reached a very stable phase (**Figure 3**) which probably represents the balance between newly recruited alveoli and previously aerated areas. The evaluation phase (blood gases, hemodynamics, eCO measurement averaged over 1 minute) took place at this steady state (left graph in **Figure 3**). The return in ambient air containing CO (end of the evaluation phase) led to an increase in eCO bottom line (inspiratory phase) and an irregular eCO exhalation in the expiratory phase. This probably reflects the end of hyperoxia effect on CO adsorption on metalloproteins.

## Comparison With Other Markers During the *Ex-Vivo* Lung Perfusion

Our data showed a correlation between eCO and both glucose consumption as well as lactate levels in the perfusate. Valenza et al. have already shown that the consumption of glucose during an EVLP in pig lungs was correlated with pulmonary edema (26). Effros et al. demonstrated that fluid reabsorption in edematous rat lungs increased glucose consumption (27). The stimulation of alveolar fluid clearance, by β-Adrenergic agonist infusion, increased glucose consumption in an EVLP pig lung model (28). In our series, it is conceivable that injured lungs struggled against the development of alveolar edema by activating the Na/K/ATPase pump system thus increasing alveolar fluid clearance. The energy cost of Na-K pump activity in an anaerobic environment could explain the increased glucose consumption and lactates levels in injured lungs exhaling high concentrations of eCO and expressing high levels of pro-inflammatory cytokine TNF-α.

The lack of correlation between eCO levels and $PO_2/FiO_2$ during the EVLP may be due to a delay between gas exchange alterations compared to the onset of inflammatory ischemia-reperfusion lesions. Indeed, Yeun et al. have shown, in a porcine EVLP model, that *ex vivo* $PO_2$ may not be the first indication of lung injury in cell-free EVLP models and, taken alone, may be misleading in assessing the *ex vivo* lung (29). This argues in favor of a more reliable marker of pulmonary lesion in EVLP.

## eCO and Recipients' Outcomes

CO is a gasotransmitter known to have anti-inflammatory, anti-apoptotic, anti-proliferative, and anti-thrombotic properties by the activation of soluble guanylate cyclase (sGC) and cyclic guanosine monophosphate (cGMP) pathways (9). In pig and rodent ischemia-reperfusion models, lungs administered with exogenous CO showed less histologic damages, lower levels of inflammatory cytokines (IL6 and TNFalpha), and had much better gas exchange capacities (30–32). Therefore, grafts exhaling high eCO levels might be those with a high potentiality to react and adapt to ischemia-reperfusion injury. However, our results do not support this hypothesis and a high eCO level seems to be associated with a poorer outcome of the grafts. In our series, elevated eCO (>0.235 ppm) at the first EVLP recruitment procedure predicted a PGD of 3 within the first 72 h post-transplantation. Therefore, high eCO levels could warn about difficult short-term outcomes of the recipients and the need for sustained supportive care. Conversely, in low eCO grafts, better outcomes might be expected and, for example, earlier extubation could be considered.

## CONCLUSION

During EVLP, eCO can be measured continuously and non-invasively thanks to the ProCEAS® analyzer. It appears to be associated with the severity of the ischemia-reperfusion injury and could provide new information for early acceptance of transplants. Future multicenter studies about eCO and EVLP are necessary to provide stronger evidence.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of the French Society of Thoracic and Cardio-Vascular Surgery (approval reference: CERC-SFCTCV-2020-05-06-05-SAED). The patients/ participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

All authors designed the clinical protocol. VB and RB collected the data, performed the data analysis, and wrote the initial draft and the final manuscript. MB, KJ, DR, IV, VB, and RB worked on the ethical and regulatory part of the PROCEAS used in the protocol. ES, MG, and JDW performed EVLP and lung transplantations and participated in clinical data collection. CT carried out the assays of the different biomarkers and cytokines. IV, DR, and ES participated in data collection and signal analysis and in revising the final manuscript. All authors have read and approved the final manuscript.

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## CONFLICT OF INTEREST

Author KJ was employed by the company AP2E Company.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10455/full#supplementary-material

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# Current Trends in Organ Preservation Solutions for Pancreas Transplantation: A Single-Center Retrospective Study

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Due to the high vulnerability of the pancreas to ischemia-reperfusion injury, choices regarding preservation solution markedly affect pancreas transplant success. A retrospective single-center analysis of 380 pancreas transplants (2000–2019) was performed to correlate current preservation solutions with transplant outcomes. Early graft failure requiring transplantectomy within 30 days post-transplant occurred in 7.5% for University of Wisconsin (UW) group ($n = 267$), 10.8% of Celsior (CS) group ($n = 83$), 28.5% of Histidine-Tryptophan-Ketoglutarate (HTK) group ($n = 7$), and none for Institut Georges Lopez-1 (IGL-1) group ($n = 23$). The most common causes of technical failures in this cohort included abdominal hemorrhage (8.4%); graft pancreatitis (3.7%); fluid collections (2.6%); intestinal complications (6.6%); and vascular thrombosis (20.5%). Although IGL-1 solution provided lower surgical complication rates, no significant differences were found between studied groups. Nevertheless, HTK solution was associated with elevated pancreatitis rates. The best graft survival was achieved at 1 year using UW and IGL-1, and at 3 and 5 years using IGL-1 ($p = 0.017$). There were no significant differences in patient survival after a median follow-up of 118.4 months. In this setting therefore, IGL-1

**Abbreviations:** BMI, Body Mass Index; CIT, Cold Ischemia Time; CS Celsior; DGF, Delayed Graft Function; DM, Diabetes Mellitus; HTK, Histidine-Tryptophan-Ketoglutarate; HR, Hazard Ratio; IGL-1, Institut Georges Lopez-1; IQR, Interquartile Range; ISGPS, International Study Group for Pancreatic Surgery; LR, Log-rank Test; PAK, Pancreas After Kidney; PPASS, Pre-Procurement Pancreas Suitability Score; PTA, Pancreas Transplant Alone; PTx, Pancreas transplantation; SPK, Simultaneous Pancreas-Kidney; UW, University of Wisconsin.

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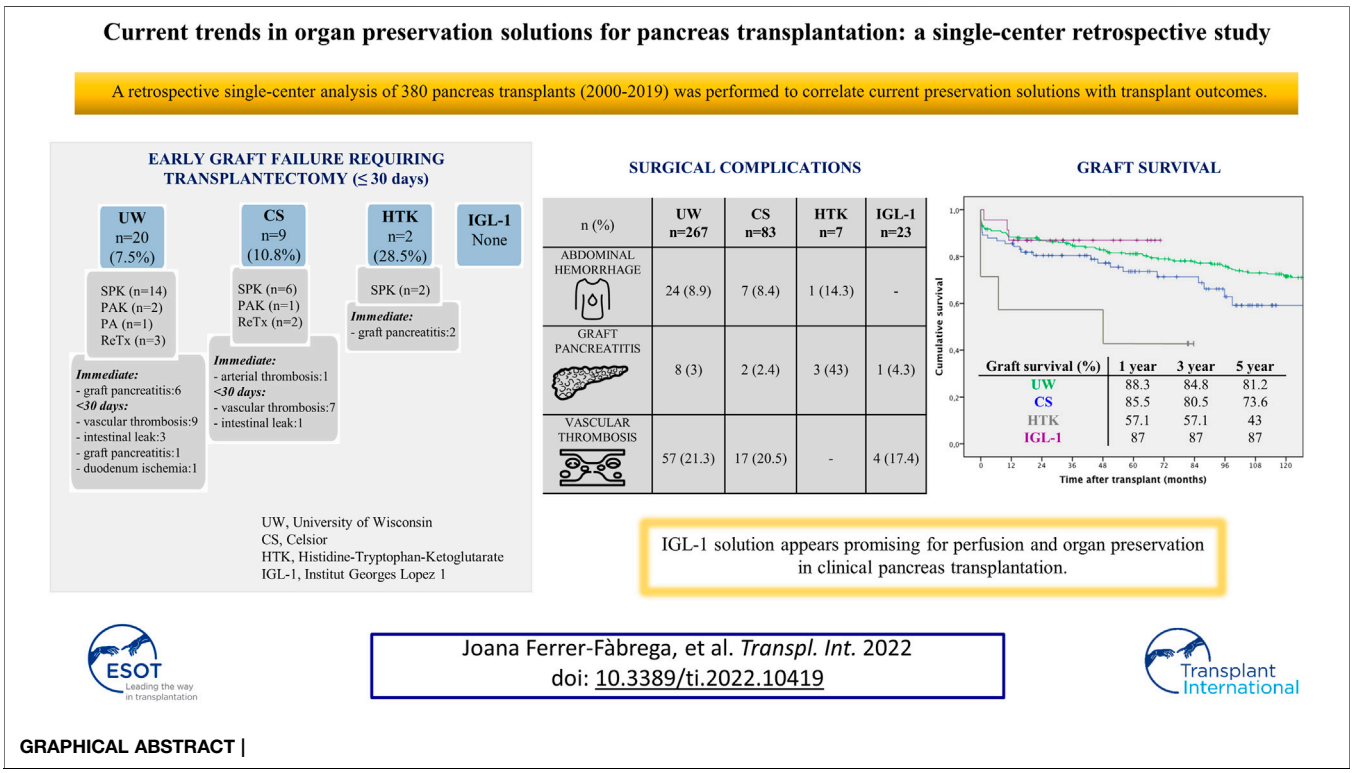
solution appears promising for perfusion and organ preservation in clinical pancreas transplantation, compared to other commonly used solutions.

**Keywords: pancreas transplantation, graft survival, preservation solution, ischemia-reperfusion, pancreatitis, postoperative outcomes**




**GRAPHICAL ABSTRACT |**

## INTRODUCTION

For patients with diabetes mellitus (DM) type 1, pancreas transplantation (PTx) is the only therapeutic option capable of normalizing blood glucose and minimizing secondary complications of diabetes, resulting in an increase in the survival and an improved quality of life (1). According to data from the International Pancreas Transplant Registry, more than 56,000 PTx's were carried out worldwide between the first operation in the 1960s and 2017 (2). In Spain, with 12 accredited centers, 2,006 PTx's have been performed since the program started in 1983 (3-5).

The maintenance of organ viability from donation to transplantation is a decisive factor for the adequate function and survival of the graft, especially in organs such as the pancreas, which is highly susceptible to ischemic damage. Preservation has become a key challenge due to the increasing use of marginal donors, in whom the functionality of the organ is most affected (6,7).

In this scenario, four preservation solutions are currently in use for pancreas transplantation. University of Wisconsin (UW) solution has been considered for organ perfusion in abdominal organ transplantation since the late 80s (8). It features a potassium concentration that mimics the intracellular medium and uses hydroxyethyl starch (HES) as the oncotic agent. In contrast, Histidine-Tryptophan-Ketoglutarate (HTK) and Celsior (CS) solutions, which were originally designed for cardiac graft protection, have the advantage of a much lower viscosity, providing more rapid cooling and better washout during organ procurement. Meanwhile, Institut Georges Lopez 1 (IGL-1) preservation solution was introduced in the early 2000s for the maintenance of abdominal organs and, although clinical experience in PTx with this solution is limited, initial results have been promising (9). Its composition resembles that of UW, with inversed potassium/sodium contents and replaced HES [with a tendency to induce red blood cell aggregation (10)] with 35-kDa molecular-weight polyethylene glycol (PEG35), a neutral, water-soluble, non-toxic polymer that acts like a colloid (11).

At present there is no universal consensus regarding the optimal preservation solution in the setting of PTx albeit UW solution continues to be recognized as the "gold standard" (12). Considering that early technical failure remains the Achilles' heel of pancreas transplantation, there is a growing need within the scientific community for new solutions with superior preservation properties and reduced side effects.

In recent years, the Pancreatic Transplant Unit at Hospital Clínic of Barcelona has routinely used IGL-1 as a preservation solution for PTx from its own donors. The aim of this study was

to compare the effectiveness of the four currently in-use preservation solutions on the outcome of PTx regarding early pancreatic graft function as well as long-term patient and graft survival. Secondly, postoperative surgical complications were also evaluated, as well as their relation with ischemia-reperfusion injury.

# MATERIAL AND METHODS

## Study Design

Five hundred ninety-one consecutive pancreas transplants were performed at the Hospital Clínic of Barcelona from 1983 through to the end of 2019. A prospectively assembled database of all pancreas transplants from January 2000 to March 2019 was reviewed, i.e. since surgical technique and immunosuppression strategies were standardized. The patient cohort included 380 patients who underwent PTx: 312 (82.1%) simultaneous pancreas-kidney (SPK); 27 (7.1%) pancreas after kidney (PAK) and 3 (0.8%) pancreas transplant alone (PTA). In addition, 38 (10%) patients received a pancreas retransplantation. Data from this cohort were stratified into four groups according to the organ preservation solution employed (UW, CS, HTK and IGL-1). UW and CS were used throughout the whole period of analysis, HTK from January to December 2013 while IGL-1 has been in use from 2014 to the present.

This study protocol was approved by our institutional review board (HCB/2020/0499) and complied with the ethical standards of the Helsinki Declaration of 1975.

## Donor Characteristics

Graft pancreas acceptance criteria was performed based on the consensus document of the National Transplant Organization described in 2005 and updated in 2018 (13). Donor analyzed characteristics included: age; gender; cause of death; body mass index (BMI); cold ischemia time (CIT); pre-procurement pancreas suitability score (PPASS); perfusion volume, and amylase/lipase levels.

During organ procurement, both abdominal aorta and portal vein cannulation (dual perfusion) were used to perfuse the organs (perfusion time 8–10 min). The perfusion volume differed depending on the surgeon criteria to obtain a clear effluent via vena cava. The standard, whole-pancreas graft included the entire pancreas and a duodenal segment.

## Recipient Characteristics

The indications for PTx were patients with DM who met the inclusion criteria according to the protocol established in our institution (14). Venous systemic drainage was performed between graft portal vein and recipient vena cava or right iliac vein. Arterial supply for the pancreatic graft was done through the anastomosis of the recipient right iliac primitive artery to the graft superior mesenteric artery or the common iliac graft artery, depending on the backtable reconstruction (15). For exocrine secretion, enteric drainage was created "side-to-side", either by duodeno-jejunostomy (from January 2000 to April 2016) or duodeno-duodenostomy anastomosis (from May 2016 to March 2019).

**TABLE 1 |** Components and function of the various preservation solutions compared in the study.

| | UW | CS | HTK | IGL-1 | Function |
|---|---|---|---|---|---|
| mOsm/L | 320 | 320 | 310 | 290 | — |
| $Na^+$ | 30 | 100 | 15 | 120 | Maintenance of osmotic balance |
| $K^+$ | 125 | 15 | 10 | 25 | Maintenance of osmotic balance |
| $Cl^-$ | — | — | 50 | — | Maintenance of osmotic balance |
| $Mg^{2+}$ | 5 | 13 | 4 | — | Maintenance of osmotic balance |
| $Ca^{2+}$ | — | 0.25 | 0.015 | 0.5 | Maintenance of osmotic balance |
| $HCO^{3-}$ | 5 | — | — | — | Buffer |
| $SO^{4-}$ | 5 | — | — | 5 | Buffer |
| $PO^{4-}$ | 25 | — | — | 25 | Buffer |
| HES (g/L) | 50 | — | — | — | Oncotic agent, impermeant |
| PEG35 (g/L) | — | — | — | 1 | Oncotic agent, impermeant |
| Mannitol | — | 60 | 30 | — | Impermeant, membrane stabilizer |
| Lactobionate | 100 | 80 | — | 100 | Impermeant, membrane stabilizer |
| Raffinose | 30 | — | — | 30 | Impermeant |
| Allopurinol | 1 | — | — | 1 | Antioxidant |
| Histidine | — | 30 | 180 | — | Antioxidant, buffer |
| Tryptophan | — | — | 2 | — | Antioxidant, membrane stabilizer |
| Glutathione | 3 | 3 | — | 3 | Antioxidant |
| Ketoglutarate | — | — | 1 | — | Energy metabolism substrate |
| Adenosine | 5 | — | — | 5 | Energy metabolism substrate |
| Glutamate | — | 20 | — | — | Energy metabolism substrate |

*Concentrations are expressed in mmol/L, unless otherwise specified.*
*HES, indicates hydroxyethyl starch; PEG35, polyethylene glycol 35 kDa; UW, University of Wisconsin; CS, Celsior; HTK, Histidine-Tryptophan-Ketoglutarate; IGL-1, Institut Georges Lopez-1.*

The demographic recipient factors included age; gender; BMI; DM type-1; time of DM evolution (DM *vintage*), and type and duration of dialysis (Dialysis *vintage*). In addition, surgical complications were defined according to the modified Clavien Dindo classification (16) as any postoperative event related to the procedure within the 90 days following the transplant. Postoperative hemorrhage was classified according to the definition of the International Study Group for Pancreatic Surgery (ISGPS) (17). As there was a lack of consensus regarding a clear definition of graft pancreatitis, it was considered the case when it was readily apparent that it had arisen intraoperatively from ischemia-reperfusion injury and its related-complications such as pancreatic abscesses, and peripancreatic fluid collections. Other entities were also considered such as sterile or infected abdominal fluid collections either diagnosed by ultrasound/abdominal computed tomography or evidenced by clinical symptoms. Intestinal complications included duodenum-related leaks and small-bowel obstruction.

Early pancreatic graft function was evaluated both by biochemical parameters (peak serum amylase and lipase levels in the first 48 h together with insulin requirements) and by clinical outcomes, including the need of transplantectomy within 30 days of transplantation.

## Immunosuppression

Routine immunosuppression in SPK and PAK consisted of different regimens administered following the institutional protocol, which varied according to the date of transplant

**TABLE 2 |** Relationship between preservation solutions and clinicopathological features of donors.

| | Total *n* = 380 | UW *n* = 267 | CS *n* = 83 | HTK *n* = 7 | IGL-1 *n* = 23 | *P* |
|---|---|---|---|---|---|---|
| Age (years) | 32 (21–40) | 30 (20–39) | 37 (29–45) | 43 (33–47) | 30 (19–39) | 0.803[a]<br>0.042[b]<br><0.001[c] |
| Gender M/F | 224 (58.9)/156 (41.1) | 164 (61.4)/103 (38.6) | 45 (54.2)/38 (45.8) | 4 (57.1)/3 (42.9) | 11 (47.8)/12 (52.2) | 0.266[a]<br>0.642[b]<br>0.251[c] |
| Cause of death | | | | | | |
| -Trauma | 197 (51.8) | 153 (57.3) | 31 (37.3) | 2 (28.6) | 11 (47.8) | 0.561[a] |
| -Anoxic damage | 21 (5.5) | 14 (5.2) | 5 (6) | - | 2 (8.7) | 0.100[b] |
| -CVA | 146 (38.4) | 90 (33.7) | 44 (53) | 4 (57.1) | 8 (34.8) | 0.012[c] |
| -Others | 16 (4.2) | 10 (3.7) | 3 (3.6) | 1 (14.3) | 2 (8.7) | |
| BMI (kg/m$^2$) | 23.4 (21.5–25.3) | 23.2 (21.3–25.2) | 23.4 (22.3 25.5) | 24.2 (23.1–27.3) | 23.6 (20.8–25.6) | 0.839[a]<br>0.418[b]<br>0.065[c] |
| Pancreas CIT (hours) | 10.1 (8–12) | 10 (8–12) | 11 (9–12.1) | 8.3 (6–10.3) | 8.2 (7.1–10.1) | 0.001[a]<br><0.001[b]<br>0.115[c] |
| Kidney CIT (hours) | 12.3 (10–14.3) | 12.3 (10–14.3) | 12.8 (10.2–14.7) | 10.8 (9.4–14.1) | 11.2 (9.9–12.8) | 0.262[a]<br>0.188[b]<br>0.600[c] |
| PPASS | 16 (14–18) | 16 (14–18) | 17 (14–18) | 17 (15–20) | 17 (14–18) | 0.637[a]<br>0.683[b]<br>0.043[c] |
| Perfusion Volume (L) | 6.8 (6.0–7.4) | 6.5 (6.0–7.0) | 6 (5–6.1) | 7 (6–7.5) | 7.5 (7–8) | 0.014[a]<br>0.002[b]<br>0.099[c] |
| Amylase (IU/L) | 84 (47–164.2) | 86 (48–172) | 73 (39–146) | 51 (39–63) | 94 (57–294) | 0.629[a]<br>0.202[b]<br>0.112[c] |
| Lipase (IU/L) | 45 (17–109) | 50 (20–126) | 22 (11–85.5) | 29 (8.2–55.7) | 33 (6–79) | 0.088[a]<br>0.820[b]<br>0.091[c] |

*Continuous variables are expressed as median (interquartile ranges) and categorical variables as frequencies (percentages).*
*Comparison of the analyzed variables have been made between UW, CS, and IGL-1, groups. For HTK, group only a descriptive analysis is displayed.*
*aIGL-1, vs. UW; bIGL-1, vs. CS; cUW, vs. CS.*
*M, indicates male; F, female; CVA, cerebrovascular accident; BMI, body mass index; CIT, cold ischemia time; PPASS, pre-procurement pancreas suitability score; UW, University of Wisconsin; CS, Celsior; HTK, Histidine-Tryptophan-Ketoglutarate; IGL-1, Institut Georges Lopez-1.*

including monoclonal antibody (OKT3), anti-interleukin-2 monoclonal antibody (basiliximab), rabbit anti-human lymphocytes polyclonal antibodies (thymoglobulin) among others, as standard induction therapy. Maintenance immunosuppression was based on triple therapy with calcineurin inhibitor (cyclosporine A until 2005 vs. tacrolimus introduced in the late 90s), mycophenolate and steroids.

## Anticoagulant Therapy and Antibiotic Prophylaxis

Our standard anticoagulation protocol included enoxaparin 20 mg every 12 h, starting 8-h post-surgery and maintained until patient discharge (in the absence of thrombotic/hemorrhagic complications), and aspirin 50 mg/d starting at 12-h post-surgery until discharge (100 mg/d).

Vancomycin plus third-generation cephalosporin (from 2000 to 2014) or ertapenem (from 2015 to 2019) were used as antibiotic prophylaxis in the perioperative period. Fungal prophylaxis with fluconazole was universally used in all recipients. Cytomegalovirus prophylaxis was provided by ganciclovir or valganciclovir, depending on glomerular filtration rates.

## Statistical Analysis

Categorical variables are expressed as frequencies (%), percentages and continuous variables such as median and interquartile range (IQR). Categorical variables were analyzed by use of Fisher's exact or $\chi^2$ test. Mann-Whitney U test or the Kruskal Wallis in the case of nonparametric distribution were used for the analysis of continuous variables. Due to the limited number of cases for HTK group, and the resulting bias that may arise in subgroup analysis, we have deemed it appropriate to

**TABLE 3 |** Relationship between preservation solutions and clinicopathological features of recipients.

| | Total *n* = 380 | UW *n* = 267 | CS *n* = 83 | HTK *n* = 7 | IGL-1 *n* = 23 | *P* |
|---|---|---|---|---|---|---|
| Age (years) | 40 (35–45) | 39 (34–44) | 42 (37–47) | 45 (33–49) | 47 (37–53) | 0.003[a]<br>0.218[b]<br>0.001[c] |
| Gender M/F | 240 (63.2)/140 (36.8) | 170 (63.7)/97 (36.3) | 55 (66.3)/28 (33.7) | 3 (42.9)/4 (57.1) | 12 (52.2)/11 (47.8) | 0.369[a]<br>0.231[b]<br>0.696[c] |
| BMI (kg/m$^2$) | 22.7 (20.9–25.6) | 22.4 (20.6–25.5) | 23 (20.7–25.7) | 22.5 (21.2–26.1) | 23.1 (21.8–25.5) | 0.198[a]<br>0.581[b]<br>0.402[c] |
| DM type | | | | | | <0.001[a] |
| -DM I | 374 (98.4) | 266 (99.6) | 80 (96.4) | 7 (100) | 21 (91.3) | 0.017[b] |
| -Others | 6 (1.6) | 1 (0.4) | 3 (3.6) | | 2 (8.7) | 0.015[c] |
| DM *vintage* (years) | 26 (21–31) | 25 (21–30) | 28 (22–33.2) | 32 (25–34) | 27 (21–37) | 0.182[a]<br>0.904[b]<br>0.020[c] |
| Dialysis *vintage* (months) | 26.5 (17.4–36.7) | 26 (18–36.7) | 26.8 (19–36.1) | 47.3 (26.4–52.3) | 24 (11.5–35.3) | 0.361[a]<br>0.322[b]<br>0.917[c] |
| Type of dialysis | | | | | | |
| -Hemodialysis | 213 (56) | 151 (56.6) | 36 (43.4) | 5 (71.4) | 12 (52.2) | 0.846[a] |
| -Peritoneal dialysis | 85 (22.4) | 63 (23.6) | 22 (26.5) | 2 (28.6) | 5 (21.7) | 0.992[b] |
| -Pre-emptive | 30 (7.9) | 20 (7.5) | 9 (10.8) | | 2 (8.7) | 0.469[c] |
| -No dialysis | 52 (13.7) | 33 (12.3) | 9 (10.8) | | 4 (17.4) | |
| Transplant type | | | | | | |
| -SPK | 312 (82.1) | 224 (83.9) | 62 (74.7) | 7 (100) | 19 (82.6) | 0.933[a] |
| -PAK | 27 (7.1) | 16 (6) | 9 (10.8) | — | 2 (8.7) | 0.847[b] |
| -PA | 3 (0.8) | 2 (0.7) | 1 (1.2) | — | — | 0.281[c] |
| -Retransplant | 38 (10) | 25 (9.4) | 11 (13.3) | — | 2 (8.7) | |
| Induction therapy | | | | | | |
| -Basiliximab | 151 (39.7) | 116 (43.5) | 32 (38.5) | 3 (42.8) | - | <0.001[a,b] |
| -Thymoglobulin | 192 (50.5) | 114 (42.7) | 51 (61.5) | 4 (47.2) | 23 (100) | 0.001[c] |
| -Others | 37 (9.8) | 37 (13.8) | — | — | — | |
| Graft reconstruction | | | | | | |
| -SA-SMA | 350 (92.1) | 249 (93.3) | 72 (86.7) | 7 (100) | 22 (95.7) | 0.823[a] |
| -"Y" iliac graft | 27 (7.1) | 15 (5.6) | 11 (13.3) | — | 1 (4.3) | 0.191[b] |
| -Others | 3 (0.8) | 3 (1.1) | — | — | — | 0.012[c] |
| Intestinal anastomosis | — | — | — | — | — | |
| -Duodeno-jejunostomy | 337 (88.7) | 256 (95.9) | 67 (80.7) | 7 (100) | 7 (30.4) | <0.001[a,b,c] |
| -Duodeno-duodenostomy | 43 (11.3) | 11 (4.1) | 16 (19.3) | — | 16 (69.6) | |
| Transplant Era | | | | | | |
| -2000–2009 | 226 (59.5) | 220 (82.4) | 6 (7.2) | — | — | <0.001[a,c] |
| -2010–2019 | 154 (40.5) | 47 (117.6) | 77 (92.7) | 7 (100) | 23 (100) | 0.336[b] |

*Continuous variables are expressed as median (interquartile ranges) and categorical variables as frequencies (percentages).*
*Comparison of the analysed variables have been made between UW, CS, and IGL-1, groups. For HTK, group only a descriptive analysis is displayed.*
*aIGL-1, vs. UW; bIGL-1, vs. CS; cUW, vs. CS.*
*M, indicates male; F, female; BMI, body mass index; DM, Diabetes Mellitus; SPK, Simultaneous Pancreas-Kidney; PAK, Pancreas After Kidney; PA, Pancreas Transplant Alone; SA-SMA, Splenic Artery - Superior Mesenteric Artery; UW, University of Wisconsin; CS, Celsior; HTK, Histidine-Tryptophan-Ketoglutarate; IGL-1, Institut Georges Lopez-1.*

provide a detailed description of the immediate post-transplant complications instead of including it for comparison with other groups.

The following variables have been included in the univariate and multivariate analysis as potential risk factors for early graft survival: donor demographics (age, gender, cause of death, body mass index, amylase and lipase values, and P-PASS); donor procurement factors (preservation solution, total perfusion volume, cold ischemia time); era of transplant (before and after 2010); recipient demographics (age, gender, body mass index, DM type, DM vintage, dialysis vintage, type of dialysis, transplant type, and induction therapy). Other factors related to

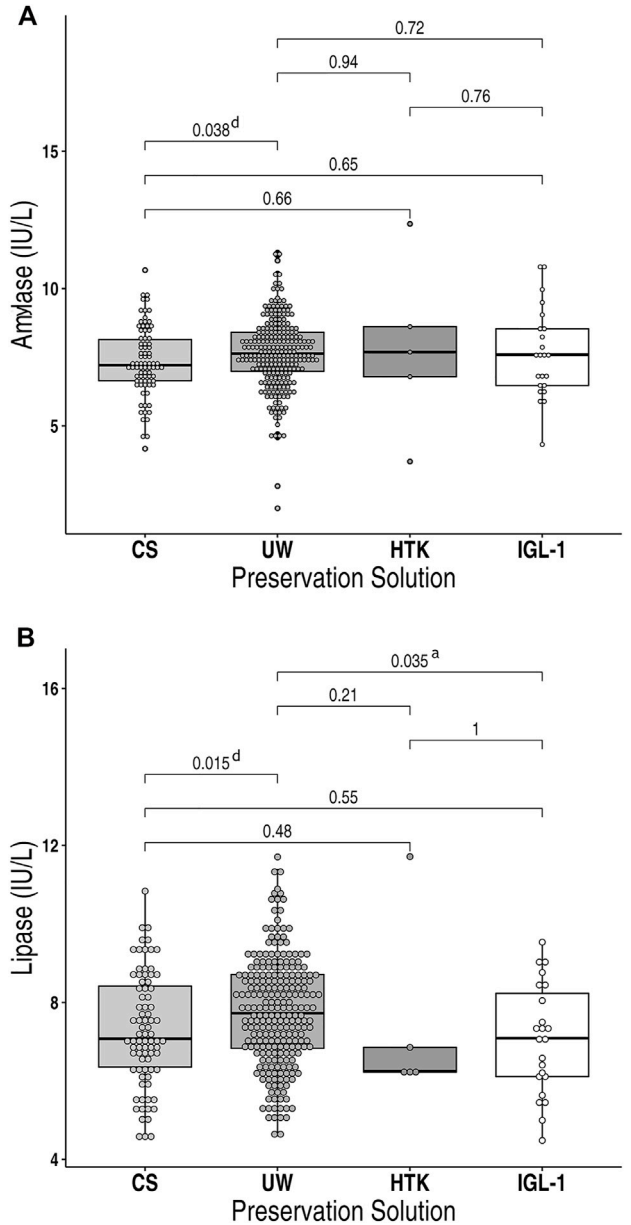



**FIGURE 1 |** Box Plot showing **(A)** post-transplant serum amylase levels and **(B)** post-transplant serum lipase levels in recipients of pancreas allografts preserved in either CS, UW, HTK or IGL-1 preservation solution. Measured values were converted into logarithmic values. Boxes represent the interquartile ranges and whiskers extend to the minimum and maximum values. The median values are shown within the boxes. UW, University of Wisconsin; CS, Celsior; HTK, Histidine-Tryptophan-Ketoglutarate; IGL-1, Institut Georges Lopez-1. [a]IGL-1 vs. UW; [d]CS vs. UW.

surgical management and technique included were the type of arterial reconstruction in the backtable, the type of vascular (arterial and venous) anastomosis and the intestinal anastomosis technique used in the recipient.

Patient and graft survival were assessed using Kaplan–Meier curves and compared with the log-rank test (LR) and Breslow. Numeric covariates were dichotomized by their median. Patient survival was calculated from the time of transplant to death or the end of follow-up. Pancreas graft survival was calculated from the time of transplant until any of the following: the need for graft removal; the return to permanent insulin therapy dependency; retransplant or death/end of follow-up with a functioning graft. $p$ values of less than 0.05 were considered statistically significant. Significant covariates were subjected to multivariate cox regression analysis.

Statistical calculations were made using SPSS for Windows software (IBM SPSS Statistics version 20.0, 1989–1995; Chicago, IL) and R statistical software (R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

# RESULTS

## Demographic Profile

A total 380 PTx's were performed in our center with the use of four different preservation solutions, which differed in terms of their chemical composition (**Table 1**). Some 267 (70.3%) patients were perfused with UW, 83 (21.8%) with CS, 7 (1.8%) with HTK, and 23 (6.1%) with IGL-1. HTK was introduced in 2013 but was associated with a high and unexpected incidence of graft pancreatitis, prompting us to cease using it and convert to IGL-1.

The four groups had similar characteristics regarding donors as shown in **Table 2**. HTK and CS groups presented older donor age as compared to IGL-1 and UW ($p < 0.05$). IGL-1 and HTK exhibited shorter CIT ($p < 0.05$), with significantly larger volumes of perfusion solution as compared to CS and UW ($p < 0.05$). The preservation solutions did not differ regarding gender, cause of death, BMI, PPASS and the levels of lipase. Nevertheless, in relation to donor amylase levels, HTK group presented lower values compared to others.

Recipient demographics showed no significant differences with respect to gender, BMI, dialysis *vintage*, type of dialysis and type of transplant (**Table 3**). By contrast, recipients in the IGL-1 group were older compared with UW group ($p = 0.003$) and had the lower proportion of patients with DM I compared to others. Thymoglobulin was the most frequently used drug as induction therapy for CS, HTK and IGL-1 groups.

## Surgical Technique

There were, by far, more SPK compared to PAK and PTA in the UW, CS and IGL-1 group (**Table 3**). Patients transplanted with HTK solution corresponded solely to SPK technique.

For the vascular reconstruction of the pancreatic graft during backtable, arterial anastomosis between the splenic artery and the superior mesenteric artery was performed in the majority of cases for all analyzed groups. Regarding enteric exocrine drainage procedures, most UW, CS and HTK-preserved grafts were transplanted intraperitoneally (duodeno-jejunostomy), except for IGL-1, for which duodeno-duodenostomy technique was used in 69.6% of cases.

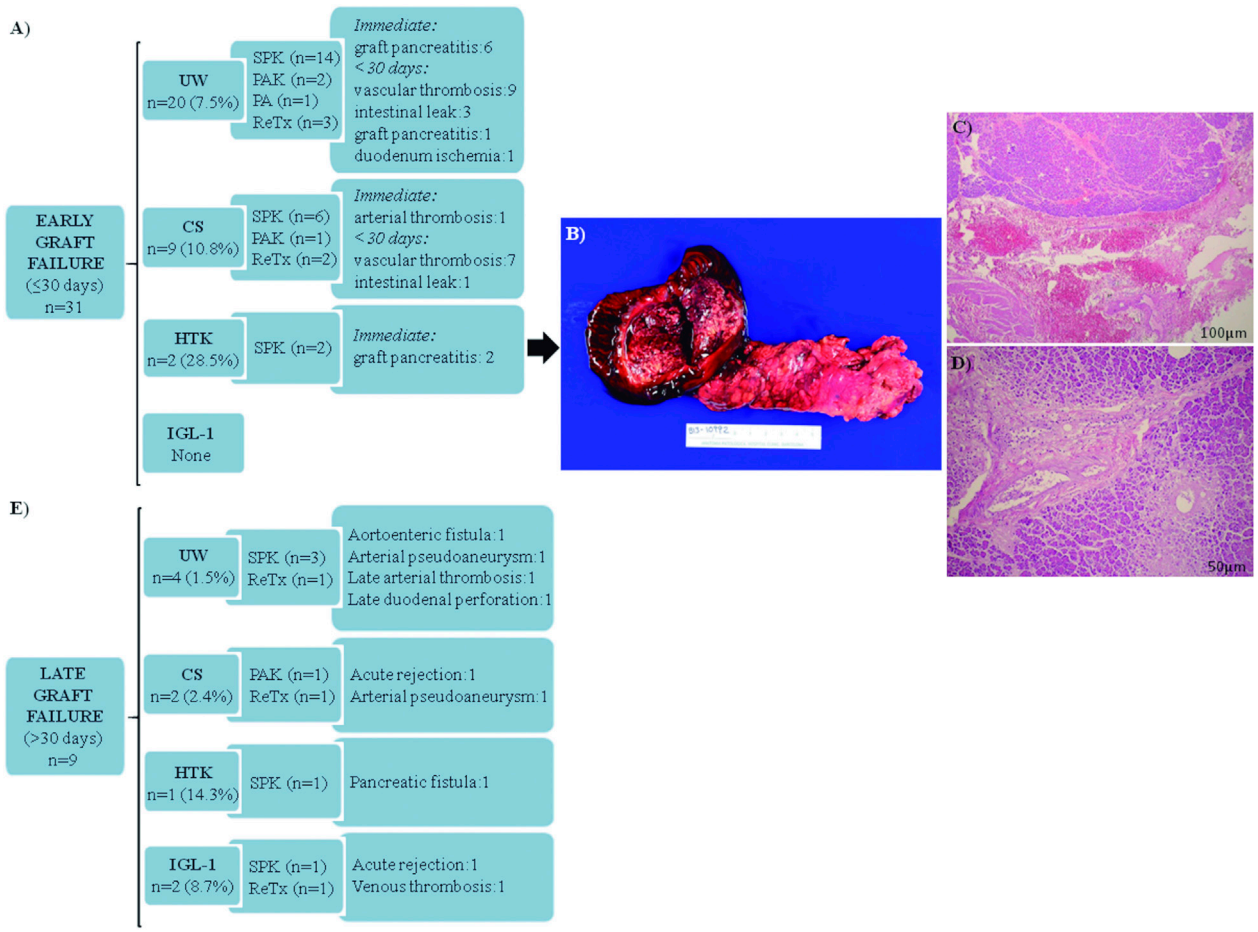



**FIGURE 2 |** Pancreas graft failure requiring early (≤30 days) and late (>30 days) transplantectomy in transplanted patients. **(A)** Main causes of transplantectomy within 30 days after pancreas transplantation. Vascular thrombosis for UW group includes: venous thrombosis ($n$ = 5); arterial thrombosis ($n$ = 3); and venous + arterial thrombosis ($n$ = 1). Vascular thrombosis for the CS group includes: venous thrombosis ($n$ = 4); venous + arterial thrombosis ($n$ = 3). **(B)** Macroscopic aspect of the graft perfused with HTK with areas of hemorrhage and necrosis in pancreatic tissue and duodenum. **(C)** Extensive hemorrhagic areas affecting the pancreatic parenchyma and peripancreatic soft tissue indicated by asterisks (H&E, scale bar 100 µm). **(D)** Pancreatic parenchyma with ischemic necrosis indicated by asterisks (H&E, scale bar 50 µm). **(E)** Main causes of transplantectomy after 30 days of pancreas transplantation. UW, University of Wisconsin; CS, Celsior; HTK, Histidine-Tryptophan-Ketoglutarate; IGL-1, Institut Georges Lopez-1; SPK, Simultaneous Pancreas-Kidney; PAK, Pancreas After Kidney; PA, Pancreas Transplant Alone; DM, Diabetes Mellitus; ReTx, Retransplant. H&E, Hematoxylin and eosin.

## Transplant Outcomes

### Early Graft Function

On the immediate postoperative days (24h–48 h), serum amylase levels were the following: UW, 198 (IQR 127–341) IU/L; CS, 148 (IQR 100–295) IU/L; HTK, 206 (IQR 62–2821.5) IU/L and IGL-1, 193 (IQR 89–375) IU/L. Statistical differences were found between UW and CS (**Figure 1A**). The serum lipase levels were as follows: UW, 212 (IQR 114–420) IU/L; CS, 135 (IQR 80.5–350) IU/L; HTK, 76 (IQR 74.5–1738) IU/L and IGL-1, 136 (IQR 66–343.66) IU/L. The highest values for lipase peak were observed in the UW group, compared with CS and IGL-1 (**Figure 1B**). Despite the fact that those patients that required immediate transplantectomy were excluded from the amylase/lipase postoperative analysis, functioning pancreatic allografts perfused and subsequently preserved in HTK solution had an elevated serum amylase and lipase peak as demonstrated by IQR-75% compared to those preserved using other solutions. Nevertheless, the differences were not statistically significant.

Interestingly, a total of 30 patients presented kidney delayed graft function (DGF): UW (7.1%); CS (7.2%), HTK (57.1%); IGL-1 (4.3%), ($p < 0.001$, HTK vs. others). Hemodialysis was required in 15 of them in the immediate postoperative period, with progressive normalization of renal function at the moment of discharge.

### Graft Transplantectomy

Early graft failure requiring transplantectomy within 30 days post-transplant occurred in 31 (8.1%) patients (**Figure 2A**), being more frequent in the case of HTK solution (28.5%). None of the IGL-1-preserved allografts required transplantectomy before 30 days. Vascular thrombosis was the main cause of early graft

**TABLE 4 |** Surgical postoperative complications.

| | Total *n* = 380 | UW *n* = 267 | CS *n* = 83 | HTK *n* = 7 | IGL-1 *n* = 23 | *P* |
|---|---|---|---|---|---|---|
| Pancreas | | | | | | |
| Abdominal hemorrhage | 32 (8.4) | 24 (8.9) | 7 (8.4) | 1 (14.3) | | 0.133[a] |
| Clavien-Dindo | | | | | | |
| I | 1 (3.1) | 1 (4.2) | | | | 0.150[b] |
| II | 3 (9.4) | 1 (4.2) | 2 (28.6) | | | 0.876[c] |
| IIIa | | | | | | |
| IIIb | 28 (87.5) | 22 (91.6) | 5 (71.4) | 1 (100) | | |
| IV | | | | | | |
| Graft pancreatitis | 14 (3.7)* | 8 (3) | 2 (2.4) | 3 (43) | 1 (4.3) | |
| Clavien-Dindo | | | | | | |
| I | 1 (7.1) | | | | 1 (100) | 0.720[a] |
| II | | | | | | 0.620[b] |
| IIIa | 5 (35.7) | 2 (25) | 2 (100) | 1 (100) | | 0.779[c] |
| IIIb | | | | | | |
| IV | | | | | | |
| Abdominal fluid collection | 10 (2.6) | 7 (2.6) | 3 (3.6) | | | |
| Clavien-Dindo | | | | | | |
| I | 2 (20) | 1 (14.3) | 1 (33.3) | | | 0.432[a] |
| II | 2 (20) | 2 (28.6) | | | | 0.355[b] |
| IIIa | 1 (10) | | 1 (33.3) | | | 0.635[c] |
| IIIb | 5 (50) | 4 (57.1) | 1 (33.3) | | | |
| IV | | | | | | |
| Intestinal complication | 25 (6.6) | 15 (5.6) | 8 (9.6) | 1 (14.3) | 1 (4.3) | |
| Clavien-Dindo | | | | | | |
| I | 3 (12) | 1 (6.7) | 2 (25) | | | 0.798[a] |
| II | 2 (8) | | 1 (12.5) | 1 (100) | | 0.421[b] |
| IIIa | | | | | | 0.197[c] |
| IIIb | 14 (56) | 10 (66.7) | 3 (37.5) | | 1 (100) | |
| IV | 6 (24) | 4 (26–7) | 2 (25) | | | |
| Vascular thrombosis** | 78 (20.5) | 57 (21.3) | 17 (20.5) | | 4 (17.4) | 0.655[a] |
| Anticoagulation protocol | 23 (29.5) | 20 (35.1) | 3 (17.6) | | | 0.742[b] |
| Conservative | 11 (14.1) | 5 (8.8) | 4 (23.5) | | 2 (50%) | 0.866[c] |
| anticoagulation | 19 (24.4) | 17 (29.8) | 2 (11.8) | | | |
| Interventional radiology | 25 (32.1) | 15 (26.3) | 8 (47.1) | | 2 (50%) | |
| Relaparotomy | | | | | | |
| Pancreas graft (no patients) | 83 (21.8) | 58 (21.7) | 21 (25.3) | 1 (14.3) | 3 (13) | 0.327[a] |
| Time after transplant (days) | 6 (2–15) | 6.5 (1.7–15) | 4 (1–12.5) | 2 | 19 (3–36) | 0.214[b] |
| — | | | | | | 0.496[c] |
| Hospital stay | 15 (11–22) | 14 (11–21) | 15 (12–24) | 30 (11–34) | 13 (11–19) | 0.475[a] |
| | | | | | | 0.257[b] |
| | | | | | | 0.384[c] |

*Categorical variables are expressed as frequencies (%) and percentages and continuous variables as median and interquartile range (IQR).*
*Comparison of the analysed variables have been made between UW, CS, and IGL-1, groups. For HTK, group only a descriptive analysis is displayed.*
[a]*IGL-1, vs. UW*
[b]*IGL-1, vs. CS*
[c]*UW, vs. CS.*
*Include hemoperitoneum, intra-abdominal/subcutaneous hematoma.*
**In 8 of the cases an immediate transplantectomy was required, not included in Clavien-Dindo classification.*
***Venous and arterial thrombosis.*
*Anticoagulation protocol (enoxaparin + aspirin).*
*Conservative Anticoagulation (systemic heparin/acenocoumarol).*
*UW, indicates University of Wisconsin; CS, Celsior; HTK, Histidine-Tryptophan-Ketoglutarate; IGL-1, Institut Georges Lopez-1.*

failure in UW and CS-preserved allografts, while graft pancreatitis was the leading cause of pancreatic failure in HTK-preserved allografts. **Figure 2B** illustrates the appearance of one of the HTK-perfused grafts, presenting an immediate severe macroscopic hemorrhagic reperfusion pancreatitis, and confirmed by histopathological data (**Figures 2C,D**).

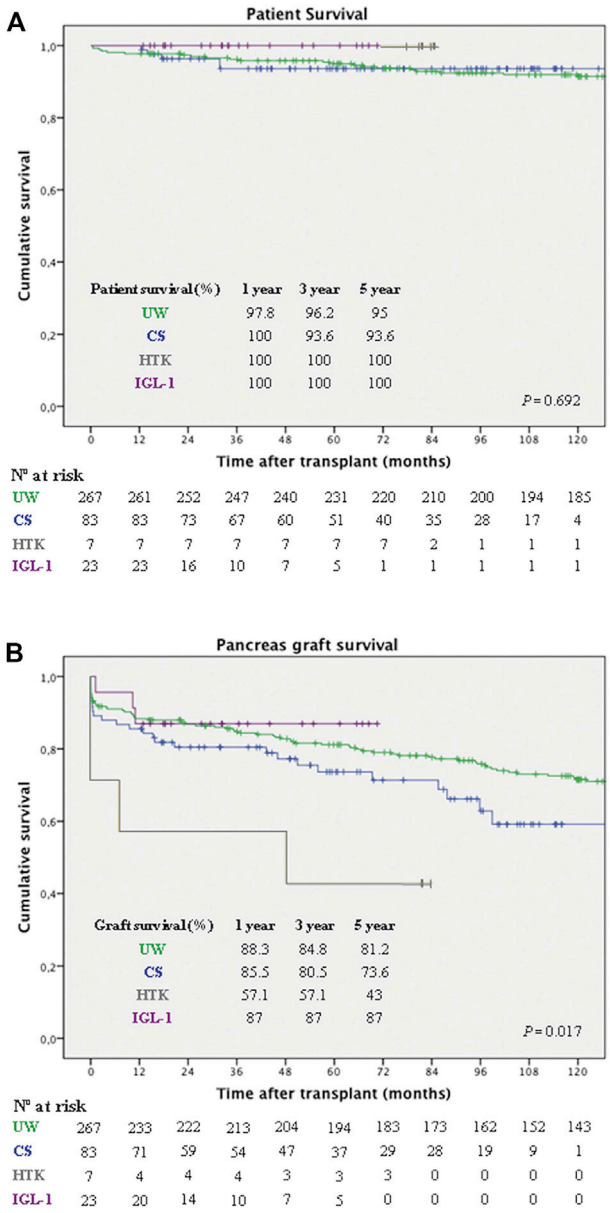



**FIGURE 3 |** Pancreas graft and patient survival in 380 consecutive pancreas transplants according to preservation solution. **(A)** Patient survival rates at 1, 3, and 5 years were: 97.8%, 96.2%, and 95%, respectively for the UW group (green line); 100%, 93.6%, and 93.6%, respectively for the CS group (blue line); 100% for the HTK group (grey line) and 100% for the IGL-1 group (purple line), with no significant differences ($p$ = 0.692, LR). **(B)** Death-censored pancreas graft survival rates at 1, 3, and 5 years were: 88.3%, 84.8%, and 81.2%, respectively for the UW group (green line); 85.5%, 80.5%, and 73.6%, respectively for the CS group (blue line); 57.1%, 57.1%, and 43%, respectively for the HTK (grey line) group; and 87%, 87%, and 87%, respectively in the IGL-1 group (purple line) ($p$ = 0.017, LR). UW, University of Wisconsin; CS, Celsior; HTK, Histidine-Tryptophan-Ketoglutarate; IGL-1, Institut Georges Lopez-1; LR, Log-rank test.

Late causes of graft failure requiring pancreas transplantectomy beyond 30-day post-transplant accounted for 2.4% of the cases (**Figure 2E**).

## Surgical Complications After Transplant

Technical failures in the total cohort amounted to 37.4% ($n$ = 142). The Clavien-Dindo grading system for the classification of surgical complications was as follow: grade I (5.3%), grade II (6%), grade IIIa (3.9%), grade IIIb (20.5%), grade IVa (1.6%).

Focusing on the most relevant postoperative events, as depicted in **Table 4**, abdominal hemorrhage was identified in 8.4% of the cases (Grade A ISGPS (3.1%) and Grade B ISGPS (96.9%)), being similar between groups, except for IGL-1 group, which had none.

In most cases, a surgical reintervention was required due to: hemoperitoneum ($n$ = 24); intra-abdominal hematoma ($n$ = 3); and subcutaneous hemorrhage ($n$ = 1). Graft pancreatitis was diagnosed in a total of 14 patients. There were numerical differences based on the preservation solution type ($p$ < 0.001), with a significantly high rate for the HTK group (43%, thymoglobulin ($n$ = 2), basiliximab ($n$ = 1)). Regarding the whole series, some 8 cases required an immediate transplantectomy because of a severe graft necro-hemorrhagic pancreatitis after reperfusion (**Figures 2A–D**). In those situations, the surgeon considered the graft not viable after checking the tightness and absence of thrombi of the vascular anastomoses. Another HTK case presented a less severe heterogeneous reperfusion with areas of intra-parenchymal hemorrhage (amylase/lipase at 24 h: 5250/3369 IU/L). In that situation, it was decided to salvage the graft, although a pancreas transplantectomy was mandatory 7 months later because of an infected persistent pancreatic fistula. The remaining 5 cases of pancreatitis, presented with a median 24 h serum values of amylase and lipase of 1017 IU/L (796.25–2007) and 776.5 IU/L (495.1–1968.7) respectively, evolved as peripancreatic fluid collection, requiring relaparotomy ($n$ = 4) at a median of 12 days (6.5–15) post-transplant and percutaneous abscess drainage ($n$ = 1). Other intra-abdominal fluid collections were diagnosed in 10 patients, without impact on graft survival. A relaparotomy was needed in half of them, performed in most cases when the patient was readmitted after discharge because of fever and abdominal pain at a median of 28 days (18–43.5) after transplant. Intestinal complications (6.6%) included post-transplant duodenal-enteric leaks and those related to small-bowel obstruction. A total of 5 patients required early transplantectomy because of: anastomotic leak ($n$ = 2); a leak of the duodenal stump site ($n$ = 2), and ischemia of the duodenum ($n$ = 1). The UW, CS and IGL-1 preservation solutions presented similar rates of vascular thrombosis (venous (77%), arterial (6.4%), both (16.6%). Note that, in a total of 25 out of 78 patients, surgery was applied as treatment. Other therapeutic options used for thrombosed pancreas grafts are also described in **Table 4**.

## Patient and Graft Survival

After a median follow-up of 118.4 months (IQR: 63.2–168.9), overall patient survival for the whole cohort at 1, 3, and 5 years was 98.4%, 96%, and 95%, respectively. Patient survival rates at 1, 3, and 5 years for the studied groups are depicted in **Figure 3A**, with no significant differences between them ($p$ = 0.692).

Overall death-censored pancreas graft survival for the whole cohort at 1, 3, and 5 years was 87.1%, 83.4%, and 79%, respectively. **Figure 3B** represents the pancreas graft survival rates at 1, 3, and 5 years for the different preservation solution groups. Overall UW, IGL-1 and CS were associated with better pancreas graft survival, compared to HTK ($p = 0.017$).

Regarding pre-procedure variables related to donor and recipient, a significantly increased risk of graft loss on univariate analysis was associated with the following: CIT (>10 h), [hazard ratio (HR) 1.51, 95% CI 1.02–2.23; $p = 0.035$], HTK as preservation solution (HR 3.48, 95% CI 1.27–9.52; $p = 0.009$), pretransplant creatinine (>5.9 mg/dl) (HR 0.66, 95% CI 0.44–0.98; $p = 0.039$), type of transplant (other than SPK) (HR 2.12, 95% CI 1.38–3.25; $p < 0.001$), recipient gender (female) (HR 1.52, 95% CI 1.03–2.23; $p = 0.031$). Other variables with no statistical significance yet presented a tendency to influence graft survival were: donor BMI >27 kg/m$^2$ ($p = 0.057$) and donor cause death other than trauma ($p = 0.06$). In a multivariate Cox regression model for graft survival, the variables associated with an increased risk for graft failure were: type of transplant (other than SPK) (HR 5.46 CI 1.63–18.28; $p = 0.005$) and recipient gender (female) (HR 1.97, 95% CI 1.00–3.86; $p = 0.04$).

## DISCUSSION

Of all solid organ transplant types, pancreas transplants are most susceptible to non-immunologic failure, with a reported graft loss rate of 5%–20% during the first year after transplantation (18-20). Because of the high vulnerability of the pancreas, an appropriate preservation solution could make a difference on graft and patient outcome. However, there is no universal consensus concerning the optimal preservation fluid in PTx (12).

Herein, we present the first retrospective single-center study comparing the effects of the four most commonly used preservation solutions in PTx, i.e. UW, CS, HTK, and IGL-1, on early pancreatic graft function as well as long-term patient and graft survival. By analyzing a large cohort of pancreas transplants in a 20–year period, this study shows that, although similar rates of graft survival were observed during the first year when comparing IGL-1, CS and UW, better results for IGL-1 were observed over the long term. Conversely, the HTK-preserved pancreas had the lowest graft survival in comparison to the other preservation solutions employed, supporting the findings of Hameed AM et al. (12) when comparing UW, HTK and CS preservation solution in a meta-analysis study.

Of note, out of the total 31 cases with early graft failure requiring transplantectomy within 30 days post-transplant, none were associated with the use of IGL-1 preservation solution. However, even though this result seems promising, they need to be interpreted cautiously because of the small sample size of IGL-1 cohort in comparison with UW or CS. When analyzing the intraoperative events, severe reperfusion pancreatitis with immediate graft removal was present in 28.5% of preserved-graft with HTK, a higher percentage when compared to other solutions. Clinical experience with HTK solution still generates controversy. It is known that its low viscosity necessitates larger solution volumes, as initially recommended by the manufacturers. However, it has been demonstrated that this factor may also be detrimental for optimal pancreas preservation, and that abdominal organs can be adequately preserved with a total volume of 5–7 L of HTK (21). In the majority of clinical studies, the HTK-flushed grafts had a higher risk of graft loss due to acute pancreatitis and thrombosis when experiencing ischemic times in excess of 12 h (22-24). In our cohort, the median of HTK-perfused solution used was 7 L. Despite the fact that HTK was used in grafts with shorter CIT, and that no changes were made in organ recovery practices, transplant techniques, or transplanting surgeons, a significant increase in the rate of pancreatitis in recipients was observed ($p < 0.001$). These findings are in contrast to a larger series published by Fridell et al. (25), who found no differences in outcomes of 308 pancreas transplants with the use of UW and HTK, suggesting that the observed differences in other studies may have been attributed to long ischemic times (19) and larger flush volumes.

A study from Ngheim et al. suggested that dual perfusion may alter pancreatic function during pancreas procurement in comparison to the aortic-only vascular perfusion (26). The authors found that the 6 pancreas retrieved by dual aortic and portal flush had higher serum amylase and lipase levels and lower levels of urine bicarbonate and pH. However, due to the lack of larger studies, both single and dual perfusion are currently considered as effective methods when procuring the pancreas for transplantation (12, 27). The impact of this factor could not be evaluated in the present series as aortic-only perfusion was not investigated. However, this method could be a source of future research to assess whether or not dual perfusion is a possible risk factor for increased graft injury resulting from venous congestion and graft edema.

Although vascular thrombosis has been shown to be a risk factor for graft loss (28-34), in this series no differences have been observed in relation to the preservation solution used. The same applies to intestinal-related morbidity.

Another important consideration when analyzing the results of our series is the quality of the pancreatic donor. Examination of the records showed no statistically significant differences regarding donor characteristics and preservation solutions used, with the exception of older pancreatic grafts in the HTK and CS groups, and longer CIT for UW and CS cases. Studied groups were also similar regarding recipient characteristics, with the exception of older patients for IGL-1 group, and longer DM *vintage* for HTK group.

No active interventions among pre-procedure factors with influence on graft survival, such as the recipient gender or type of transplant, are possible as they are unchangeable variables. Moreover, and taking into account the heterogeneous population and the long-time study period, neither the era of study (before and after 2010, as it was the midpoint of the period (2000–2019)), the type of vascular reconstruction nor the intestinal anastomosis had an impact on the early graft functioning.

In general, our findings are consistent with the scant published information in PTx using IGL-1. At the clinical level, one preliminary study suggests that IGL-1 is a safe preservation solution since it provides up to 17 h of cold ischemia. The five human pancreases preserved with IGL-1 acquired normal function immediately after reperfusion, without loss of the graft (35). Similar results were observed in a more recent study comprising a series of 47 consecutive PTx (36). Conversely, IGL-1 has been proven to be equivalent to UW or CS solutions for pancreas perfusion and cold storage before islet transplantation (37). Nevertheless, in a model of PTx in pigs, IGL-1 offered greater protection in membrane fluidity after reperfusion (38).

To the best of our knowledge, this is the only study exploring the effect of the four preservation solutions currently used for clinical PTx. We are aware that the suboptimal number of patients (mainly in the HTK group) limit the conclusions of the study, even though this factor is mitigated when evaluating the results from the point of view of "intention to treat". A low number of HTK-flushed pancreases has arisen due to an unexpected increase in the rate of immediate transplantectomy due to acute pancreatitis following reperfusion, as the latter is also an independent risk factor for impaired graft survival. This fact limited HTK's use in PTx and did not allow us to recruit an optimal number of cases for comparison with a suitable sample size. Furthermore, no hard conclusion could be obtained on the influence of induction therapy on technical failure as two out of the three cases with adverse effect were treated with thymoglobulin, which has potential broad anti-inflammatory properties that have been shown to reduce ischemia-reperfusion injury (39, 40). A long period time study carries with it inherent improvements in perioperative patient care, surgical technique and postoperative management, but the present series transplant era in question did not have statistically significant influence on the graft outcomes. Finally, the fact that surgical technique was changed in 2016 to duodenoduodenostomy does not affect immediate reperfusion injury rates, as vascular anastomoses were performed with the same technique throughout the time period in question. Despite numerous techniques to minimize exocrine pancreatic drainage complications, no universal technique has been standardized (41,42). To date, it is unclear whether duodenojejunostomy or duodenoduodenostomy provides the best long-term survival of the grafts (43). A prospective multicentre registry analysis may resolve this.

In conclusion, the fruits of this study indicate a trend towards a better graft and patient survival among IGL-1 recipients. Besides, IGL-1 composition is similar to that of the UW solution, currently considered as the "gold standard" in the reduction ischemia-reperfusion injury of the pancreas. Hence, successful PTx can be safely performed using IGL-1 solution. Further multicenter studies are still required to identify the "holy grail" of preservation solutions, especially in the current scenario of using marginal donors, including donors following circulatory death, in which the graft is exposed to a warm ischemia insult before cold storage, raising susceptibility to graft dysfunction.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Hospital Clinic of Barcelona Institutional Review Board (HCB/2020/0499). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

JF-F, EF-P, JL, JF, and JG-V conceived and designed the study. JF-F, EF-P, PV-A, GC, DP, AG-C, JB, RG-P, ML-B, RR, EE, MR, FD, CF, LF-C, JF, and JG-V contributed to the acquisition of the data or analysis and interpretation of the data. JF-F, EF-P, JL, JF, and JG-V drafted the manuscript. All authors revised the manuscript critically for essential intellectual content. All authors read and approved the final version to be published.

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## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW AND META-ANALYSIS
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# Perfusate Composition and Duration of *Ex-Vivo* Normothermic Perfusion in Kidney Transplantation: A Systematic Review

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*Ex-vivo* normothermic perfusion (EVNP) is an emerging strategy in kidney preservation that enables resuscitation and viability assessment under pseudo-physiological conditions prior to transplantation. The optimal perfusate composition and duration, however, remain undefined. A systematic literature search (Embase; Medline; Scopus; and BIOSIS Previews) was conducted. We identified 1,811 unique articles dating from January 1956 to July 2021, from which 24 studies were deemed eligible for qualitative analysis. The perfusate commonly used in clinical practice consisted of leukocyte-depleted, packed red blood cells suspended in Ringer's lactate solution with Mannitol, dexamethasone, heparin, sodium bicarbonate and a specific nutrient solution supplemented with insulin, glucose, multivitamins and vasodilators. There is increasing support in preclinical studies for non-blood cell-based perfusates, including Steen solution, synthetic haem-based oxygen carriers and acellular perfusates with supraphysiological carbogen mixtures that support adequate oxygenation whilst also enabling gradual rewarming. Extended durations of perfusion (up to 24 h) were also feasible in animal models. Direct comparison between studies was not possible due to study heterogeneity. Current evidence demonstrates safety with the aforementioned widely used protocol, however, extracellular base solutions with adequate oxygenation, supplemented with nutrient and metabolic substrates, show promise by providing a suitable environment for prolonged preservation and resuscitation.

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**Keywords: review, kidney, perfusion, normothermic, perfusate**

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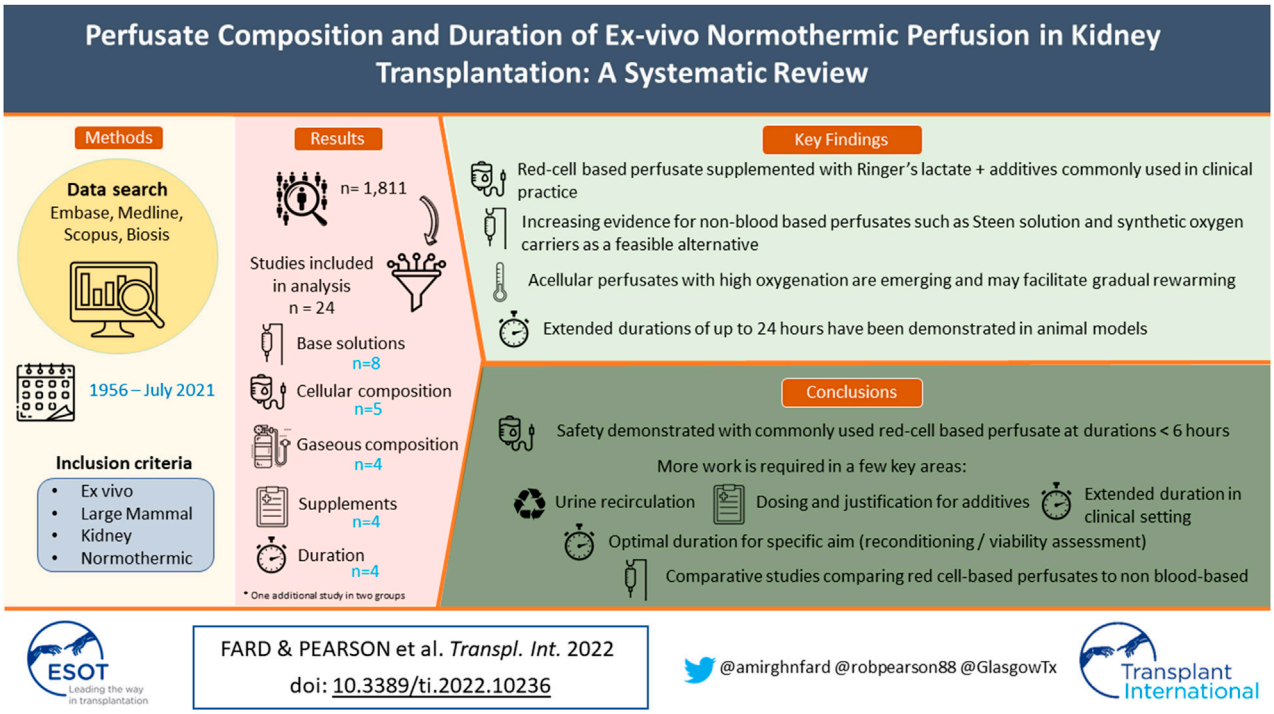
**Abbreviations:** CO, carbon monoxide; DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function; ECD, extended-criteria donor; EPO, erythropoietin; ESRD, end stage renal disease; EVNP, *ex vivo* normothermic perfusion; $H_2S$, hydrogen sulfide; HBOCs, Heam-based oxygen carriers; HMP, hypothermic machine perfusion; IRI, ischaemic reperfusion injury; RBC, red blood cells; ROS, reactive oxygen species; SCS, static cold storage; UW, University of Wisconsin.




**Graphical Abstract |**

## INTRODUCTION

Kidney transplantation is the gold standard treatment for end stage renal disease. The mainstay of organ preservation has traditionally focused on reducing metabolism by utilising hypothermic conditions with static cold storage (SCS) or, more recently, hypothermic machine perfusion (HMP) (1). The continued donor organ shortage has necessitated increased use of kidneys from donation after circulatory death (DCD) and "extended criteria" donor (ECD), (2) which are more susceptible to the effects of ischaemia reperfusion injury (IRI). IRI is multifactorial process that results in an increase in reactive oxygen species (ROS) and inflammatory mediators which stimulate vascular permeability leading to oedema and vascular endothelial damage (3–5). Furthermore, the effects of IRI are associated with higher rates of acute rejection, delayed graft function (DGF), and reduced long-term allograft survival (4). Preservation techniques to mitigate against the effects of IRI are therefore of increasing importance.

One emerging strategy is *ex-vivo* normothermic perfusion (EVNP). This involves rewarming the graft to normothermic conditions (37°C) with a perfusate that replicates the pseudo-physiological environment. Thus, facilitating the restoration of energetic substrates (e.g., ATP), metabolism and repair processes, whilst also facilitating graft viability assessment. Recently, the safety and feasibility of EVNP has been established in human clinical studies (6,7). Although unlikely to entirely counteract the process of IRI, EVNP has the potential to mitigate these deleterious effects during the period of perfusion (6).

The ideal perfusion characteristics including perfusate composition and duration remain undefined. Common clinical protocols employ a nutrient-enriched, red blood cell (RBC)-based perfusate to deliver nutrients and oxygen during 1-hour of perfusion (6,8). In addition to prolonging the duration of EVNP, variations in composition, such as synthetic and acellular preparations with varying base media, have been proposed in preclinical studies and established in liver and lung clinical protocols. However, major deviations have yet to be clinically implemented in kidneys, and limited evidence exists for the impact of different perfusion characteristics. The aim of this review was to summarise the evidence for the roles of perfusate constituents and the effects of different perfusion durations in optimising clinically relevant outcomes in the context of renal EVNP.

## MATERIALS AND METHODS

### Data Sources and Search Strategy

For this systematic review, we followed the methods proposed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, (9) and the Cochrane Handbook for Systematic Reviews of Interventions. This review was registered with PROSPERO (CRD42021231381) (10).

A limited search of the literature was conducted to identify keywords, followed by an extensive literature search on the following databases: Embase (Ovid) 1947-Present; Ovid

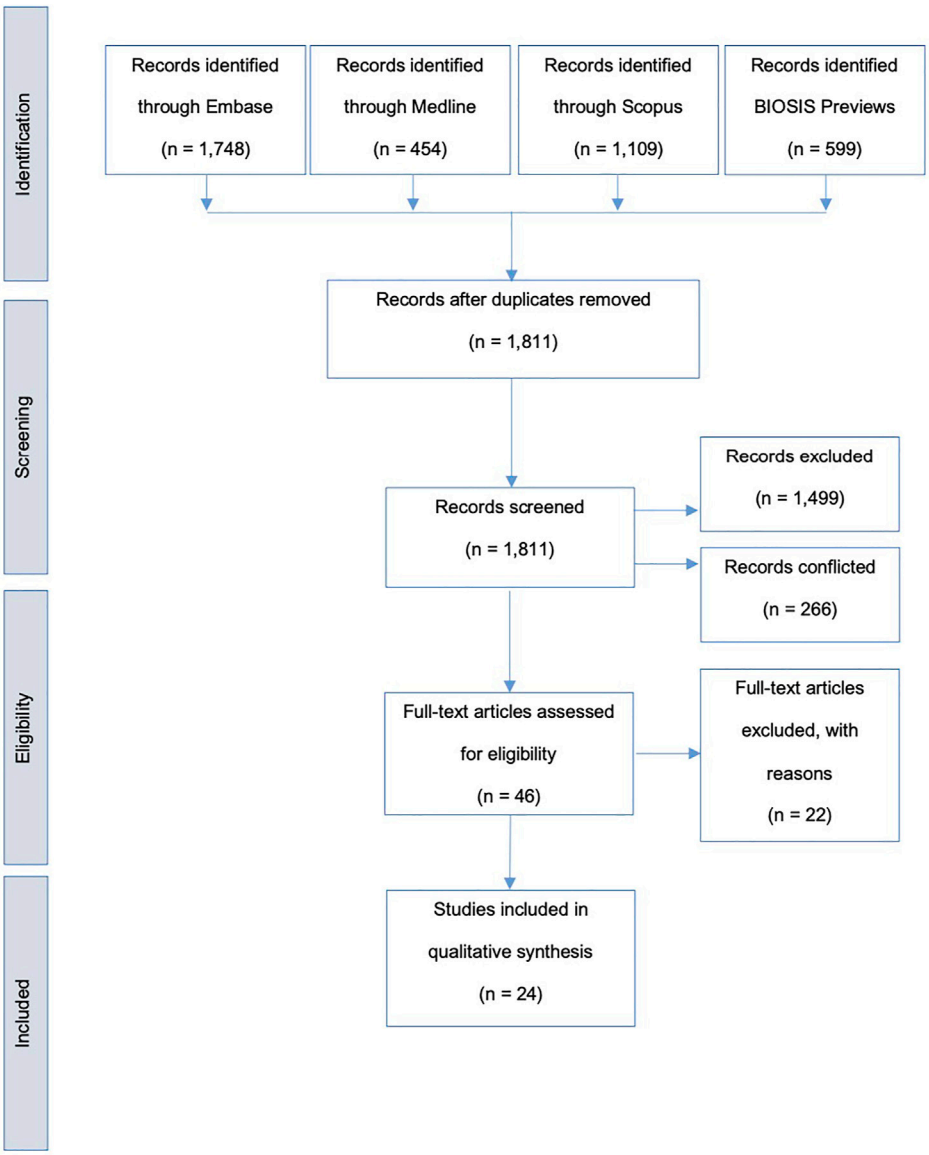



**FIGURE 1 |** Search strategy flow diagram; adapted from the preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram (9).

Medline® without Revision; Scopus; and BIOSIS Previews. The keywords used to identify relevant studies included normothermic perfusion and evnp and kidney; a comprehensive description of the search strategy can be found in **Supplementary Appendix S1**. Results were imported into Rayyan QCRI web application, where duplicate articles were removed, then two main reviewers independently and blindly screened the titles and abstracts based on predefined eligibility criteria. Thereafter, selected studies were read in full. Bibliographies of the selected articles were screened to identify landmark trials.

## Eligibility Criteria

The eligibility criteria were agreed based on the study objectives and specific research question: what are the roles of various perfusate constituents, and what are the effects of different durations of perfusion on clinically relevant outcomes in renal EVNP?

Eligible studies included preclinical and clinical, published and abstract publications from any year and any region, where English translations were available. Studies that were unpublished and those concerning *in vivo* perfusion methods, non-large mammal studies, non-kidney studies, assessment of perfusate biomarkers, and therapeutic interventions were excluded. Articles relating to sub-normothermic perfusion methods were only included where specific rationale for perfusate composition was discussed.

## Data Extraction and Analysis

The most recently dated studies were read in full first to identify up-to-date knowledge and previous related studies. Study characteristics, including name, year, design, subjects,

**TABLE 1 |** Summary characteristics of perfusate composition studies qualitatively assessed.

| Theme | Study | Design | Subject model | Objectives | Main outcome measures | Key findings |
|---|---|---|---|---|---|---|
| Whole perfusates and base solutions | Hosgood SA et al; 2011(6) | Published; clinical case report | Human patient ($n$ = 1) | First EVNP in human renal transplantation | Renal hemodynamics (renal blood flow, resistance, urine output); post-transplant serum creatinine; graft function | EVNP with plasma-free red cell-based perfusate is feasible |
| | Nicholson ML et al; 2013(7) | Published; clinical study | Human patients ($n$ = 18) | First clinical series EVNP in human renal transplantation | Graft primary nonfunction; delayed graft function (DGF)—need for dialysis; graft failure—need for nephrectomy or RRT | DGF was 5.6% in EVNP group vs. 36.2% in SCS group ($p$ = 0.014); no difference of graft or patient survival at 12 months |
| | Hosgood SA et al; 2016(8) | Published; clinical case report | Human patients ($n$ = 2) | First clinical EVNP transplantation of DCD kidneys deemed untranslatable | Graft hemodynamics; posttransplant graft function; serum creatinine | Serum creatinine at 3 months was 1.2 mg/dl and 1.62 mg/dl in the recipient of the left and right kidney—EVNP rescued kidneys previously deemed unsuitable for transplantation |
| | Hosgood SA et al; 2017(11) | Published; Protocol of clinical trial | Human patients ($n$ = 400 for recruitment) | 1-hour renal EVNP in kidneys from DCD donors versus SCS | Primary: DGF (need for dialysis in first 7-day); Secondary: renal function, hospital stay, graft & patient survival at 1 year; acute rejection; blood chemistry biomarkers | Study suspended during COVID-19 pandemic and preliminary results not yet available |
| | Horiuchi T et al; 2009(14) | Published; preclinical | Canine kidneys | Pyridoxalated hemoglobin-polyoxyethylene (Php) addition to UW solution for normothermic preservation | Oxygen consumption; histopathological assessment | Php added to UW during 12-hour normothermic preservation increased oxygen consumption, reduced damage of tubular epithelium and edematous degeneration compared to UW alone |
| | Kaths JM et al; 2015(35) | Published; preclinical | Beating-heart porcine kidneys ($n$ = 6) | EVNP using erythrocyte-based Steen solution diluted with LR perfusate | Renal hemodynamics; blood gas analysis; histopathological assessment | 10-hour DCD porcine perfusion using erythrocyte-based Steen solution diluted with ringer's lactate demonstrated stable hemodynamics, active renal metabolism and minimal renal injury |
| | Urcuyo D et al; 2017(12) | Published; preclinical | Porcine kidneys ($n$ = 15) | Whole-blood at normothermia, whole-blood with Steen solution at normothermia, and acellular Steen solution at sub-normothermia, on prolonged preservation | Primary: Hemodynamic stability and histological damage Secondary endpoints: Urine production, perfusate potassium and arterial pH | Acellular Steen solution at 21°C supported low and stable vascular resistance with adequate histological preservation during 24-hour perfusion; whole blood diluted with Steen solution at normothermia was successful but resulted in acidosis and necrosis. Whole blood alone at normothermia was unsuccessful beyond 5-hours |
| | Horn CV et al; 2021(15) | Published; preclinical | Porcine Kidneys (n = 12) | New preservation solution Custodiol-MP for *ex vivo* reconditioning of kidney grafts compared to Belzer MPS solution | Primary: renal haemodynamics Secondary: Molecular markers of renal injury and histology | No statistically significant difference in outcomes between Custodiol-MP and Belzer MPS solutions. Custodiol-MP was safe and applicable for short-term kidney perfusion |
| | Pool MBF et al; 2021(36) | Published; preclinical | Porcine Kidneys ($n$ = 20) | Comparison of four different perfusate solutions | Perfusion parameters, Urine and perfusate analysis, Markers of renal injury, Histology | All four perfusates were feasible but with differences in outcome measures. |

(Continued on following page)

**TABLE 1 |** (*Continued*) Summary characteristics of perfusate composition studies qualitatively assessed.

| Theme | Study | Design | Subject model | Objectives | Main outcome measures | Key findings |
|---|---|---|---|---|---|---|
| | | | | | | Individual influence of perfusate components remain unclear |
| Cellular Composition | Harper S et al; 2006(16) | Published; Preclinical | Porcine kidneys ($n$ = 12) | Leukocyte-depleted blood versus whole blood-based perfusates | Serum creatinine, urine output, renal blood flow, oxygen consumption, acid-base homeostasis, histological features | Leukocyte-depleted blood significantly improved post-ischemia renal function; lower serum creatinine, higher creatinine clearance and urine output ($p$ = 0.002 for all) |
| | Aburawi MM et al; 2019(17) | Published: Preclinical | Discarded human kidneys ($n$ = 14) | Hemoglobin-based oxygen carriers (HBOC) versus pack red blood cell-based perfusates | Renal artery resistance, oxygen extraction, metabolic activity, energy stores and histological features | Lactic acid levels in kidneys pRBC group was higher than HBOC group ($p$ = 0.007); other outcomes were similar |
| | Minor T et al; 2019(13) | Published: preclinical | DCD Porcine kidneys ($n$ = 12) | RBC-based perfusate versus acellular perfusate versus control during controlled rewarming | Renal hemodynamics and histological assessment | Controlled organ rewarming is superior to immediate rewarming in terms of creatinine clearance, sodium excretion, oxygen extraction, urinary protein loss and innate immune activation; inclusion of RBC added no benefit |
| | Minor T et al; 2019(18) | Published: clinical case report | Human Patient ($n$ = 1) | First controlled rewarming with an acellular Steen perfusate in human renal transplantation | Post-transplant immediate graft function; serum creatinine; urine output; patient outcomes | Postoperative course was event-free, and patient was discharged after 16 days with a serum creatinine of 143 μmol/L; Acellular controlled oxygenated rewarming was successful |
| Gaseous Composition | Adams TD et al; 2019(19) | Published; preclinical | Porcine kidneys ($n$ = 43) | Effects of reducing perfusate oxygenation on renal function and oxygen kinetics during EVNP and reperfusion | Renal function and hemodynamics; blood gas analysis; biomarkers of renal injury (NGAL) | Reducing partial pressure of oxygen significantly reduced oxygen extraction during EVNP ($p$ = 0.037) however showed no significant difference in urine output, sodium excretion, creatinine clearance or NGAL during reperfusion |
| | Maasseen H et al; 2019(21) | Published: preclinical | Porcine kidneys ($n$ = 10) | Hydrogen sulphide versus control | Renal function and hemodynamics; oxygen kinetics; histopathological assessment; metabolic activity | Hydrogen sulphide significantly reduce oxygen consumption, by 61%, ($p$ = 0.047) without directly affecting tissue ATP levels. Renal function was unchanged |
| | Bagul A et al; 2008(20) | Published; preclinical | Porcine kidneys ($n$ = 4) | Effect of carbon monoxide | Renal function and hemodynamics | Carbon monoxide improved renal blood flow ($p$ = 0.002), creatinine clearance ($p$ = 0.006), and urine output ($p$ = 0.01). Higher concentrations had negative effects |
| | Smith SF et al; 2017(22) | Published: preclinical | Porcine kidneys ($n$ = 18) | 70% argon versus 70% nitrogen versus 95% $O_2$ 5% $CO_2$ during EVNP | Renal function and hemodynamics; inflammatory mediators and histopathological assessment | Argon did not mediate any significant effects during EVNP nor reperfusion during functional parameters, inflammatory mediators or histological changes |

(Continued on following page)

**TABLE 1 |** (*Continued*) Summary characteristics of perfusate composition studies qualitatively assessed.

| Theme | Study | Design | Subject model | Objectives | Main outcome measures | Key findings |
|---|---|---|---|---|---|---|
| Supplementary Composition | Bleilevens C et al; 2019(23) | Published; preclinical | Porcine kidneys ($n$ = 10) | Vitamin C versus placebo in an *in vitro* ischemia-reperfusion porcine kidney EVNP model | Perfusate analysis (blood gas, serum chemistry, oxidative stress markers); renal hemodynamics; histological analysis | Vitamin C significantly increased antioxidant capacity and hemoglobin concentrations ($p$ = 0.02), reduced oxidative stress ($p$ = 0.002) however did not improve creatinine clearance, fractional sodium excretion or renal histology |
| | Hosgood SA et al; 2017(25) | Published; preclinical | Porcine kidneys ($n$ = 10) | Effect of a CytoSorb heme-adsorber in an isolated kidney perfusion system | Tissue and blood markers of inflammation and renal function | In the cytosorb group, interleukin-6/8, prostaglandin E2 and thromboxane were significantly lower during reperfusion ($p$ = 0.023, $p$ = 0.0001 and $p$ = 0.005 respectively) and renal blood flow was significantly higher ($p$ = 0.005); creatinine clearance was not significantly difference ($p$ = 0.109) |
| | Brasile L et al; 2003(26) | Published: preclinical | Canine kidneys ($n$ = 32) | Feasibility of cobalt protoporphyrin (CoPP) on heme-oxygenase (HO-1) expression during acellular warm perfusion | HO-1 activity; Renal hemodynamics | Induction of HO-1 during warm acellular perfusion by CoPP is feasible within clinical timeframe |
| | Yang B et al; 2011(24) | Published: preclinical | Porcine kidneys ($n$ = 6) | Impact of EPO addition to 2-hour RBC-based EVNP | Renal hemodynamics; immunohistochemistry, histopathological assessment | EPO in EVNP significantly facilitated inflammation clearance and improved and urine output |

*EVNP,* Ex-vivo *normothermic perfusion; SCS, Static cold storage; DGF, Delayed graft function; UW, University of Wisconsin solution; LR, lactate Ringer's solution; Php, Pyridoxalated hemoglobin-polyoxyethylene; DCD, Donation after circulatory death; ECD, Expanded criteria donor; HBOC, hemoglobin-based oxygen carriers; pRBC, Pack red blood cells; CoPP, Cobalt Protoporphyrin; HO-1, Heme-oxygenase 1; EPO, Erythropoietin; IRI, ischemia-reperfusion injury.*

objectives, perfusate composition, perfusion duration, main outcome measures and key findings were recorded.

## RESULTS

The search identified 3,910 articles, 2099 of which were duplicates, giving 1,811 unique articles, dating from January 1956 to July 2021. Following blinded screening by two independent reviewers, 1,499 articles were deemed ineligible, with 266 decisions conflicted. A third reviewer was used to address conflicts. Of the articles selected, 46 met the eligibility criteria. Full-text assessment reasoned a further 22 articles ineligible for qualitative analysis. Only studies utilizing human or large mammal tissue were included. **Figure 1** illustrates the search process in full.

Included studies were grouped according to common themes: Whole perfusates and base solutions ($n$ = 8); cellular composition ($n$ = 5); gaseous composition ($n$ = 4); supplementary composition ($n$ = 4); and perfusion duration ($n$ = 4), with one study applicable to both whole perfusate and base solutions, and perfusion duration. Studies comprised 5 clinical studies on human patients and 19 preclinical studies. Key findings were recorded and summarised in **Table 1** for perfusate composition and **Table 2** for perfusion duration.

Qualitative analysis found the perfusate commonly implemented in clinical renal EVNP consisted of Ringer's lactate, O-negative packed red blood cells (pRBC), Mannitol 10%, dexamethasone 8 mg, heparin, Sodium bicarbonate 8.4% as the main components, and a specific nutrient solution with insulin, multivitamins, prostacyclin 0.5 mg and glucose 5% as supplementary components, for a perfusion duration of 1-hour following SCS, pioneered by Nicholson et al. in Cambridge (7).

Preservation solutions are broadly categorised into intracellular and extracellular solutions, pertaining to whether the potassium and sodium concentrations mirror that of the intra- or extra-cellular milieu. Regarding the base solutions used for perfusate at normothermia, extracellular electrolyte compositions such as Ringer's lactate have demonstrated safety and feasibility when implemented in human clinical studies; although lacking robust data, the perfusion pressure maintained in human trials thus far ranges from 65 to 75 mmHg (6,8,11). In addition, Steen-based solutions, with or without RBCs, have been shown to support prolonged perfusion up to 24-hour of EVNP of DCD porcine kidneys (12,13). One

**TABLE 2 |** Summary characteristics of kidney perfusion duration studies qualitatively assessed.

| Study | Design | Subject model | Objectives | Duration groups | Main outcome measures | Key findings |
|---|---|---|---|---|---|---|
| Kaths JM et al; 2016(52) | Published: preclinical | SCD Porcine kidneys (*n* = 10) | Safety and feasibility of 8-hour EVNP versus SCS | (A) SCS (8 h)<br>(B) EVNP (8 h) | Perfusate injury markers (AST, LDH); Renal function (serum creatinine, 24-hour creatinine clearance); Histological assessment | Continuous EVNP is feasible and safe in good quality beating-heart donor kidney grafts |
| Kaths JM et al; 2017(28) | Published: preclinical | DCD Porcine kidneys (*n* = 20) | Brief EVNP following SCS versus prolonged, continuous EVNP in DCD porcine kidney autotransplantation | (A) 16 h SCS<br>(B) 15 h SCS + 1 h EVNP<br>(C) 8 h SCS + 8 h EVNP<br>(D) 16 h EVNP | Perfusate injury markers (AST, LDH); Renal function (serum creatinine, 24-hour creatinine clearance), Histological assessment | Prolonged EVNP significantly decreased serum creatinine, LDH, and apoptotic cells following DCD kidney transplantation compared to SCS or short EVNP after SCS. |
| Kaths JM et al; 2017(27) | Published: preclinical | DCD Porcine kidneys (*n* = 35) | Brief versus intermediate versus prolonged EVNP following 8-hours SCS in DCD porcine kidney autotransplantation | (A) 8 h SCS<br>(B) 8 h SCS + 1 h EVNP<br>(C) 8 h SCS + 8 h EVNP<br>(D) 8 h SCS + 16 h EVNP | Renal function and hemodynamics; Histological assessments 8 days post-transplantation | Intermediate and prolonged EVNP were significantly superior to brief EVNP following SCS. Brief EVNP resulted in a higher serum creatinine compared to SCS alone |
| Urcuyo D et al; 2017(12) | Published: preclinical | DCD Porcine kidneys (*n* = 15) | Whole-blood at normothermia versus whole-blood with Steen solution at normothermia, and acellular Steen solution at sub-normothermia, on prolonged preservation | (A) 24 h EVNP with whole blood<br>(B) 24 h EVNP with whole blood + Steen solution<br>(C) 24 h sub-normothermic preservation with acellular Steen solution | Primary: Hemodynamic stability and histological damage<br>Secondary endpoints: Urine production, perfusate potassium and arterial pH | Acellular Steen solution at 21°C supported low and stable vascular resistance with adequate histological preservation during 24-hour perfusion; whole blood diluted with Steen solution at normothermia was successful however resulted in acidosis and necrosis. Whole blood alone at normothermia was unsuccessful beyond 5-hour |

*SCD, Standard criteria donor; SCS, Static cold storage; EVNP,* Ex-vivo *normothermic perfusion; AST, Aspartate transaminase; LDH, Lactate dehydrogenase; DCD, Donation after cardiac death.*

**TABLE 3 |** Perfusate composition commonly used for clinical renal ex-vivo normothermic perfusion; adapted from the nicholson protocol (6, 7, 11).

| | Constituent | Volume |
|---|---|---|
| Components | Ringer's lactate solution | 300–400 ml |
| | O-negative packed red blood cells (leukocyte depleted) from blood bank | 1 Unit |
| | Mannitol 10% | 25 ml |
| | Dexamethasone 8 mg | Direct to circuit |
| | Sodium Bicarbonate 8.4% | 25 ml |
| | Heparin 1,000 iu/ml | 2 ml |
| Supplement | Nutrient solution (Nutriflex or Synthamin) | 20 ml/h infusion |
| | Sodium Bicarbonate 8.4% | 20 ml/h infusion |
| | Insulin 100 iu | 20 ml/h infusion |
| | Multivitamins (Cernevit) | 20 ml/h infusion |
| | Prostacyclin 0.5 mg | 5 ml/h infusion |
| | Glucose 5% | 5 ml/h infusion |
| | Ringer's lactate solution | Replace urine output ml for ml |

study on isolated canine kidneys showed that addition of pyridoxalated haemoglobin-polyoxyethylene (Php) to UW solution enhanced oxygen consumption and reduced oedematous damage of tubular epithelium during 12-hour normothermic preservation, however, no studies have yet translated this into clinical models (14). Custodiol-MP solution was safe and feasible for short-term perfusion of porcine kidneys, and non-inferior to clinically established Belzer MPS solution. Head-to-head comparison of four different perfusates showed feasibility in all settings during 7-hour EVNP of porcine DCD kidneys, but with substantial differences in perfusion and injury parameters (15). In this

**TABLE 4 |** Clinical Perfusate Constituent Options summary; Adapted from of Qualitative Analysis of Studies.

| | Component role | Clinical constituent options |
|---|---|---|
| Base Solution | Fluid and electrolyte balance | Ringer's Lactate<br>Steen solution |
| | Elevation of osmolality | Mannitol 10% |
| | pH Buffer | Sodium Bicarbonate 8.4% |
| | Calcium Buffer | Calcium Gluconate 10% |
| | Immune suppression | Dexamethasone 8 mg |
| | Anticoagulation | Heparin 1,000 iu/ml |
| Cells | Oxygenation | Plasma free, leukocyte-depleted packed Red Blood Cells (1 unit)<br>Synthetic Heme-based oxygen carriers<br>Acellular with no oxygen carrier |
| Gases | Oxygenation | Carbogen gas mixture (95% $O_2$, 5% $CO_2$) |
| | Hypo-metabolite | Hydrogen sulphide ($H_2S$) |
| | Vasodilation | Carbon Monoxide (CO) |
| Supplementary Component | Nutrition | Nutrient solution (Nutriflex)<br>Synthamin 17 (500 ml) |
| | pH Buffer | Sodium Bicarbonate 8.4% (25 ml) |
| | Energetic & metabolic substrates substrate | Insulin 100 iu<br>Glucose 5% |
| | Nutrition solution | Multivitamins (Cernevit) (1 vial) |
| | Vasodilation | Prostacyclin 0.5 mg<br>Verapamil 0.25 mg/h |
| | Replace fluid lost in urine output | Ringer's Lactate (ml for ml) |
| | Inflammatory suppression | Heme-oxygenase-1 (HO-1) |

instance, the influence of individual perfusate components remains unclear.

For cellular composition, leukocyte-depleted blood significantly improved post-ischaemia renal function by measure of serum creatinine and urine output ($p$ = 0.002) in porcine kidneys (16). Perfusates utilising synthetic haemoglobin-based oxygen carriers (HBOCs) were found to be non-inferior to whole blood perfusates with regard to histological injury, vascular resistance, oxygen consumption and tissue ATP, and exhibited significantly lower lactic acid levels ($p$ = 0.007) during perfusion.(17) Controlled oxygenated rewarming without any oxygen carriers resulted in successful transplantation with good immediate renal function, in a recent human clinical case study.(18).

Evidence for gaseous composition supported 95% oxygen ($O_2$), 5% carbon dioxide ($CO_2$) mixtures. Reducing oxygen levels to normoxia significantly reduced oxygen consumption during EVNP ($p$ = 0.037), however showed no difference in urine output, sodium excretion, creatinine clearance or markers of injury during reperfusion (19). The addition of carbon monoxide (CO) improved renal blood flow ($p$ = 0.002), creatinine clearance ($p$ = 0.006), and urine output ($p$ = 0.01), however higher concentrations had negative effects (20). Despite being commonly known for its toxicity, the infusion of hydrogen sulfide ($H_2S$) to the perfusate was found to induce a hypometabolic state, significantly reducing oxygen consumption by 61%, ($p$ = 0.047) without directly impacting tissue ATP levels, and renal function was unchanged (21). Argon did not mediate any significant effects during EVNP or during reperfusion (22).

Evidence for supplementary additives was limited. While vitamin C significantly increased antioxidant capacity, haemoglobin concentrations ($p$ = 0.02), and reduced oxidative stress ($p$ = 0.002); it was not shown to improve creatinine clearance, fractional sodium excretion or histological markers of renal tubular injury (23). In a porcine model EPO was found to be anti-inflammatory and anti-apoptotic, demonstrating improved urine output with the mechanism attributed to caspase-3 and IL-1β (24). Reduction of inflammatory mediators was also demonstrated to be achieved by filtration via CytoSorb haemadsorption, which significantly reduced interleukin (IL)-6/8, prostaglandin E2 and thromboxane during reperfusion ($p$ = 0.023, $p$ = 0.0001 and $p$ = 0.005 respectively), and increased renal blood flow ($p$ = 0.005) without significantly altering creatinine clearance ($p$ = 0.109).(25) In addition, induction of haem-oxygenase-1 (HO-1) was demonstrated in canine kidneys however evidence for clinical impact is yet to be elucidated (26). Commonly used protocol for clinical use and prominent variations in perfusate constituents, along with their roles, are summarized in **Tables 3**, **4**, respectively.

Continuous EVNP, with and without complete exclusion of SCS, was feasible and superior to brief EVNP (27,28). 8-hour and 16-hour durations showed significantly lower post-transplant serum creatinine compared to 1-hour EVNP ($p$ = 0.027), with no significant difference between the former (28). Acellular Steen solution at 21°C supported low and stable vascular resistance with adequate histological preservation during 24-hour perfusion, compared to whole blood alone at normothermia, which was unsuccessful beyond 5-hour (12).

# DISCUSSION

In this systematic review, the most recent evidence for roles of various EVNP perfusate constituents and durations in optimising clinically relevant outcomes of kidney transplantation were reviewed and summarised.

## Fundamentals of Perfusate Composition and Current Clinical Practice

Preservation of organs at normothermia requires a physiological milieu with adequate oxygen, nutrition, and metabolic substrates to replace depleted energy resources. Furthermore, it is necessary that the solution stabilises electrolyte balance and cell fluid content to reduce oedema and reduce free radical peroxide scavengers to diminish oxidative injury (29). Accordingly, the protocol most commonly utilised in clinical practice, (6,7) comprises a nutrient enriched, red cell-based solution, with physiological buffers and added supplementary constituents such as vitamins, insulin, glucose and vasodilators (14,30,31).

## Base Solutions

Early evidence has shown that, under normothermic conditions, colloid solutions with high-sodium, low-potassium compositions like that of extracellular fluid, such as Ringer's lactate, are superior to UW, which has a low-sodium, high-potassium composition like that of intracellular fluid, by reducing temperature-dependent oedema during IRI (31). This is consistent with evidence that clinical implementation of renal EVNP using Ringer's lactate solution is feasible (6,7,11). Further work is required to elucidate optimal mean arterial pressure (65–75 mmHg non-pulsatile is most commonly reported as target pressure), particularly in the setting of high resistance kidneys where some groups describe increasing pressure to 100 mmHg to promote perfusate flow (11).

Steen solution is alternative plasma-like solution that was initially utilised for EVNP of the lungs in the Toronto Protocol (32), and has since been developed in liver EVNP (33,34). It contains dextran and a high albumin concentration that provides oncotic force to drive water out of swollen endothelial cells, helping sustain high perfusion flow rates (12). For use with EVNP, it can remain acellular or be supplemented with RBCs. Recent studies using similar protocols in kidneys have shown that Steen solution-based perfusates can support low and stable vascular resistance during prolonged perfusion, superior to red cell-based perfusates (12). Gaining popularity is Ringer's lactate diluted with Steen solution, which has been successfully implemented in porcine kidneys for up to 10 h of EVNP, both with RBCs (35) and without (12). Further research is required to compare these different base solutions at normothermia, and to explore the potentially protective effects of Php.

Another emerging product is Custodiol-MP solution, which is reported to have antioxidant properties, specifically designed for aerobic or oxygenated machine perfusion. Compared to Belzer MPS, Custodiol-MP was deemed safe for short-term kidney perfusion, and while there were no statistically significant differences in renal hemodynamic outcomes, it remains an attractive solution which may benefit from testing in further models, as it allows flexible addition of colloids, specific to the requirements of each organ, potentially enabling wider clinical application (15).

Few studies to date have conducted head-to-head comparisons of perfusates for EVNP. A recent publication from Pool et al., however, compared four different perfusates during 7-hour EVNP of porcine kidney in a DCD model (36). While all four perfusates demonstrated feasibility, there were apparent differences between electrolyte levels, renal function parameters, and injury markers in the four groups. Perfusate 1, consisting of RBCs in Williams' Medium E-based solution, and Perfusate 2, consisting of RBCs, albumin and balanced electrolyte solution, were similar in terms of EVNP flow patterns, whereas Perfusate 3, consisting of RBCs with clinically established solution used by Hosgood et al., (7) and Perfusate 4, consisting of RBCs and a 0.9% sodium chloride-based medium (successfully used in porcine autotransplantation, (37) showed lower but more stable flow rates. This may be explained by a lack of vasodilator use in Perfusates 1 and 2. Notably, Perfusate 2 resulted in significantly lower levels of injury marker N-acetyl-β-D glucosaminidase compared to Perfusate 3 and 4, and where Perfusate 3 had the highest levels, indicating greatest tubular damage. Ultimately, this study highlighted the significant influence of different perfusate compositions on EVNP outcomes, and the importance of a harmonious protocol to enable consistent interpretation of EVNP data. The need for further comparative studies to assess these perfusate protocols is self-evident in order to further this perfusion technology.

## Cellular Composition

Most preclinical studies to date have used red cell-based perfusates; however, it is important to note that whole blood is a finite resource, particularly given that type O packed erythrocytes is most commonly used. Furthermore, the blood may contain antibodies, clotting factors, activated leukocytes and thrombocytes which potentially exacerbate IRI through generation of inflammatory mediators and activation of complement cascade (16). Accordingly, plasma-free and leukocyte-depleted perfusates have been well-established in both preclinical and clinical studies (7, 8). However, there is limited data on whether or not plasma-based perfusates, or the use leucocyte depletion filters, have a role in wider clinical use.

Nevertheless, adequate oxygenation remains a vital prerequisite, which can be delivered by several means: RBCs, synthetic HBOCs or simple diffused oxygen by carbogen gas mixtures. While RBC-based perfusates are proven, they are limited by poor availability, high cost and short-shelf life, with potentially increased risk of infection transmission and haemolysis (17). HBOCs are more accessible with reduced infection and haemolysis risks (17). Recently, preclinical studies on discarded human kidneys have demonstrated that HBOCs are non-inferior to pRBCs in terms of renal hemodynamics and histological damage (17), suggesting that HBOCs may indeed offer a logistically more convenient alternative to pRBC in EVNP of human kidneys. Further

studies, however, demonstrating improved clinical outcomes in appropriate transplant models are required.

Acellular perfusates, without any haem-based oxygen carriers, may offer a unique benefit as they better enable gradual rewarming of the organ to normothermia. At present, EVNP is performed at the receiving site after a period of SCS transport from the donor hospital. This abrupt restoration of normothermia and rise in metabolic turnover has been implicated as a secondary cause of IRI (5). This is thought to be due to disrupted cellular homeostasis at the mitochondrial level (5) and to RBCs losing their deformability in cold, leading to impaired microcirculation and tissue oxygenation, and can be mitigated by gently rewarming the organ from SCS using an acellular perfusate (13). It has been demonstrated (data presented at ATC 2019) that EVNP may be feasible without haem-based oxygen carriers for up to 6 h in discarded human kidneys (38). In this instance, the perfusate, with 95% $O_2$, 5% $CO_2$, sustained stable renal haemodynamics and restored tissue ATP levels similar to concentrations in a red cell-based perfusate. Acellular EVNP of porcine kidneys has also been shown to fully saturated venous haemoglobin when the partial pressure of oxygen was maintained above 500 mmHg (13). The same group later reinforced these findings in a first-in-man clinical case-study, in which controlled oxygenated rewarming without any oxygen carriers resulted in successful transplantation with good immediate renal function (18). Increasingly, evidence suggests that oxygen carriers may not be required to achieve adequate oxygenation during short-term renal perfusion (17,38,39).

Although beyond the scope of this review that concentrated on normothermic perfusion, there is growing evidence in favor of gradual rewarming. Comparing controlled oxygenated rewarming with continuous up-front perfusion in a porcine transplant model using steen-based solution with 95% oxygen and 5% $CO_2$, both methods effectively restored renal function after SCS to the same level, with controlled oxygenated rewarming significantly reducing tenascin C expression in tissue—a glycoprotein induced during injury—compared to SCS (40). Heat-shock proteins are well known as a defense mechanism induced by stressful stimuli such as hypoxia or hyperthemia (41,42). Minor et al. demonstrated that with gradual rewarming (or "controlled hyperthermia"), they found a 50% increase of heat-shock proteins, which correlated to improvement of tubular reabsorption of sodium and glucose upon reperfusion, and reduced loss of urinary protein compared with controls, meriting further exploration of this technique in preclinical models (43). As a result of this work, there is emerging evidence that avoiding the abrupt temperature changes may be protective against IRI.

## Gaseous Composition

Supraphysiological concentrations of oxygen, in the form of 95% $O_2$, 5% $CO_2$ gas mixtures, have been utilised in most EVNP protocols. However, excess oxygenation may exacerbate IRI through increased production of ROS (4). A porcine kidney transplant model comparing EVNP with 95%, 25% and 12% $O_2$ with 5% $CO_2$, found that while oxygen extraction was significantly reduced, reducing oxygen levels to normoxia did not significantly influence functional parameters or biomarkers of renal injury during reperfusion (19). This directly contradicts previous studies that advocate hyperoxemia (13,18). Importantly, the latter studies used acellular perfusates, signifying that higher oxygen concentrations may be necessary in the absence of oxygen carriers. In either case, theoretically neither hypoxemia nor hyperoxemia should alter renal vasomotor tone in constant $CO_2$ concentrations (44); thus, reducing oxygen tensions would not be expected to influence renal function. Further characterisation of oxidative stress in the context of EVNP may enhance this field of research.

Gases are easily absorbed into the blood, and therefore can be utilised as additives to enhance the protective effects of EVNP. In human-sized porcine kidneys, hydrogen sulfide ($H_2S$) infusion after 30 min of EVNP reduced oxygen consumption which was restored rapidly after cessation without any short-term indications of histological or biochemical damage (21). With further corroborating evidence, $H_2S$ supplementation may offer potential in reducing the extent of oxygenation required, facilitating the use of acellular perfusates or normoxic gas mixtures; further work is required, particularly, to exclude any potential long-term toxicity prior to clinical translation.

Other gases that have been utilised include carbon monoxide (CO), which has shown to significantly reduce IRI in experimental models by promoting vasodilation (20); and argon, which despite suggestion that it may potentially reduce IRI by inhibiting IL-8, did not influence renal function when administered during EVNP of porcine kidneys (22), consistent with EVNP models in porcine lungs (45). These findings may be explained by the longer durations of perfusion permitted in the experimental studies, and that benefits of argon may only be quantifiable after prolonged periods.

## Supplementary Composition

Metabolic and energetic substrates are essential for restoration of normal metabolism. Clinical perfusates have been most commonly supplemented with a nutrient solution with insulin, glucose 5%, sodium bicarbonate 8.4%, multivitamins and extracellular fluid (Ringer's lactate) to replace urine output (6,7,11). Moreover, blood-based perfusates include anticoagulants to prevent clotting within the perfusate tubing circuitry and to reduce risk of graft thrombosis, and vasodilators to reduce transient vascular constriction upon reperfusion with RBCs (46). Furthermore, liver studies have shown that maintenance of optimal microcirculatory homeostasis using vasodilators is a key factor in EVNP (34). There has been limited research, however, evaluating the impact or need for anticoagulants and vasodilators, particularly in the context of acellular perfusates.

Other supplements in the literature have aimed to further ameliorate IRI. Currently, reduction of inflammatory mediators is achieved through integration of hemadsorption technology (CytoSorb) into the EVNP circuit (25). However, such broad-spectrum hemadsorption may potentially remove important anti-inflammatory mediators. An alternative method proposed to reduce oxidative stress is the utilisation of endogenous HO-1;

a heat shock protein that catalyses degradation of haem, exerting cytoprotective effects (42). Naturally, HO-1 decreases during SCS due to reduced protein expression under hypothermia (25,26). However, one study showed that addition of cobalt protoporphyrin (CoPP) during normothermic preservation successfully induces HO-1 within clinically appropriate timeframes (26). Of note, some degree of toxicity, presented as reduced urine output and increased proteinuria, was observed at higher concentrations of CoPP, without further without increases in HO-1. Therefore, optimal HO-1 inducers and concentrations need to be explored further. Vitamin C is known to prevent apoptosis, reduce inflammation and endothelial permeability, in addition to enhancing microcirculation. However, in 6-hour animal EVNP models, no improvements in clinical parameters were observed despite a significant reduction in oxidative stress (23), consistent with negative findings of small clinical studies (47). Finally, EPO supplementation has been speculated to lessen IRI by modulation of apoptotic mediators: caspase-3, interleukin-1ß and HSP70 (24). In porcine kidneys subjected to 2-hour of haem-based EVNP, addition of EPO reduced apoptotic cells in tubular lumens and interstitial areas and facilitated renal tissue remodelling (48). While encouraging, these studies were limited by lack of clinically relevant outcome measures and did not address potential adverse effects.

Of note, no data was found on the use of antibiotics or the specific dosing of the aforementioned additives. Additionally, the administration of therapeutics such as regenerative cell therapies was deemed beyond the scope of this review.

## Duration of Perfusion

Optimising perfusion duration may be a critical step in augmenting the benefits of suitably engineered perfusates. As successfully demonstrated in clinical studies, a short period of EVNP (up to 2-hour) is acceptable following a period of SCS (6,7,11,49). However, continuous normothermic perfusion from time of retrieval may permit complete avoidance of cold ischaemic injury. Recent DCD porcine studies have verified the feasibility and safety of prolonged EVNP with near complete exclusion of SCS using whole-blood perfusates for 10-hour in livers (33,50) and acellular Steen solution for 12-hour in lungs (51). Initial evidence in kidneys showed that continuous, 8-hour EVNP is feasible and safe in good quality beating-heart donor kidney grafts, (52) and in a follow-up study on DCD porcine kidneys, the same group demonstrated that continuous 16-hour EVNP with near complete exclusion of SCS was superior to brief EVNP following SCS (28). Furthermore, sub-normothermic 24-hour preservation using acellular Steen solution has been shown to support low and stable vascular resistance whilst providing adequate histological preservation in DCD porcine kidneys (12). Notably, in this study EVNP beyond 5-hour was not feasible when whole blood alone was used, and when diluted with Steen solution, acidosis, hyperkalaemia and necrosis resulted (12). This study was limited by variable warm ischaemic times, use of inconsistent vasodilators, and lack of post-transplant reperfusion outcome measures; however, it may be of interest to further investigate the effects of different perfusates at varying durations.

Despite this emergent potential, no portable devices are yet available for continuous renal EVNP during transportation, unlike the OrganOx metra device that has shown to continuously preserve donor livers for up to 24-hour (50). Logistical burden of machine failure during transport, health-care costs, and complicated transportation procedures would also require consideration. Therefore, to evaluate outcomes of prolonged EVNP in current clinical context, brief, intermediate and prolonged EVNP following 8 h of SCS were compared in similar DCD porcine models (27). All durations maintained stable hemodynamic parameters, however posttransplant serum creatinine was significantly lower after intermediate and prolonged EVNP compared to the brief EVNP. Noticeably, serum creatinine was higher after 1-hour EVNP compared to SCS alone. This may be explained by several mechanisms: 1) 1-hour is insufficient for repair mechanisms; 2) rapid warming from hypothermia is harmful in short-term, as previously discussed; or 3) discrepancies exist due to different transplant models. Despite the higher tier evidence provided by human clinical studies (7), future studies should assess protein expression during prolonged EVNP to ascertain the specific molecular processes, whilst also exploring the feasibility of portable renal EVNP machines.

Debate remains regarding the recirculation of urine versus replacement of urine losses with colloid solution, particularly in the context of longer perfusion durations. Weissenbacher et al. demonstrated that the recirculation of urine permitted stability over a 24-hour normothermic perfusion period with urine recirculation. The control group ($n$ = 3) with fluid replacement as per urine loss were unable to be perfused beyond 4–6 h due to an inability to maintain a physiological pH (53). Subsequent work by the same group has confirmed these findings in a porcine model in which urine circulation aided the maintenance of physiological arterial pressure and acid-base homeostasis (54). Proteomic data also suggests urine recirculation may increase glucose metabolism, which may indicate an increase in metabolic activity, potentially protective against IRI (55).

## Study Strengths and Limitations

Due to the exploratory nature of this review, there lacked clear uniformity in the study designs, objectives, and outcome measures evaluated. Furthermore, high study heterogeneity precluded a meta-analysis. Moreover, a large proportion of the selected studies were experimental, yielding lower strengths of evidence and limiting our use of the recognised Cochrane bias risk assessment tool for randomised controlled trials. However, our efforts in screening a large number of databases, with wide eligibility criteria, provided some safeguard against missing relevant studies. Further identification of potentially relevant studies may have been achieved by expanding the eligibility criteria to include studies of sub-normothermic perfusion methods. The term "EVNP" was used throughout this manuscript, however, we acknowledge that the terms normothermic *ex-vivo* kidney perfusion (NEVKP), sub-normothermic kidney perfusion (SNMP), normothermic machine perfusion (NMP) are also used in the literature.

Standardisation and reproducibility of terms is an important part of collaboration with new technologies and techniques; importantly, our search strategy accounted for these additional terms.

## Overall Context and Future Direction

EVNP is a technology used for multiple reasons in the solid organ transplant field. "Optimisation" may represent different factors to different ends. For the purposes of kidney viability assessment, short-term perfusion may provide valuable information. Rapid transplantation places the kidney in a more physiological environment and may make longer perfusion undesirable. Prolonged EVNP clearly has the potential to recondition kidneys and regenerate their injured cells/tissue, not to mention the untapped potential for immunomodulation. Prolonged regeneration and immunomodulation would appear likely to require a more adaptive and physiological environment, perhaps with natural biological homeostats such as a liver in circuit, or with advanced sensors and chemical modulation beyond anything applied in the studies discussed in this review. It will perhaps be the adaptability and sensitivity of the circuit in regulating its perfusate composition, that allows the full potential of this therapy to be realised. There remains room for vast innovation and automation in this field even beyond a device such as Organox which is being taken up rapidly in the liver transplant arena.

# CONCLUSION

EVNP is an evolving technology which has the opportunity to resuscitate and evaluate kidneys prior to transplantation, and the elucidation of ideal perfusate constituents and perfusion duration remain key in the optimisation of this clinical tool. Our findings suggest that Ringer's lactate or Steen solution supplemented with nutrient and metabolic substrates provide a suitable environment for preservation at normothermia. Given logistical implications, under current protocols, blood-based perfusates may be suboptimal if synthetic HBOCs or acellular perfusates with carbogen gas mixtures are proven to support adequate oxygenation and enable gradual rewarming where continuous renal EVNP to completely bypass SCS is in development. Particularly given that longer perfusion durations (beyond 6 h) may be harmful with the use of red cell-based perfusates. However, this may relate to the limited homeostasis of established EVNP circuits and will clearly need re-evaluation as the many other biochemical parameters of kidney EVNP are optimised by improved technology. There are also emerging roles for supplementary constituents that reduce metabolism and suppress inflammation which are beyond the scope of this review. *Ex-vivo* modulatory interventions represent a brave new world of therapy in transplantation. Extensive further research is required, however, in appropriate transplant models to ascertain clinical benefits.

It is clear that co-ordinated research aims and better collaboration between the many groups involved in this emerging technology would be beneficial to progress. In conclusion, while current clinical protocols have been feasible, there is increasing evidence that there is potential to better define perfusion composition, in particular with use of non-blood-based perfusates, and prolonged duration, to optimise organo-protective benefits of EVNP.

# AUTHOR CONTRIBUTIONS

AF—screened articles, data collection, manuscript writing; RP—designed research, screened articles, manuscript writing; RL—resolved conflicts in article screening, manuscript preparation; PM—manuscript preparation and review; MC—designed research, manuscript review.

# CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10236/full#supplementary-material

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# Trends and Outcomes of Hypothermic Machine Perfusion Preservation of Kidney Allografts in Simultaneous Liver and Kidney Transplantation in the United States

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Optimal kidney graft outcomes after simultaneous liver-kidney (SLK) transplant may be threatened by the increased cold ischemia time and hemodynamic perturbations of dual organ transplantation. Hypothermic machine perfusion (MP) of kidney allografts may mitigate these effects. We analyzed U.S. trends and renal outcomes of hypothermic non-oxygenated MP vs. static cold storage (CS) of kidney grafts from 6,689 SLK transplants performed between 2005 and 2020 using the United Network for Organ Sharing database. Outcomes included delayed graft function (DGF), primary non-function (PNF), and kidney graft survival (GS). Overall, 17.2% of kidney allografts were placed on MP. Kidney cold ischemia time was longer in the MP group (median 12.8 vs. 10.0 h; $p < 0.001$). Nationally, MP utilization in SLK increased from <3% in 2005 to >25% by 2019. Center preference was the primary determinant of whether a graft underwent MP vs. CS (intraclass correlation coefficient 65.0%). MP reduced DGF (adjusted OR 0.74; $p = 0.008$), but not PNF ($p = 0.637$). Improved GS with MP was only observed with Kidney Donor Profile Index <20% (HR 0.71; $p = 0.030$). Kidney MP has increased significantly in SLK in the U.S. in a heterogeneous manner and with variable short-term benefits. Additional studies are needed to determine the ideal utilization for MP in SLK.

**Keywords: graft survival, delayed graft function, simultaneous liver-kidney transplantation, allograft preservation, allograft outcomes, primary non-function, center variability**

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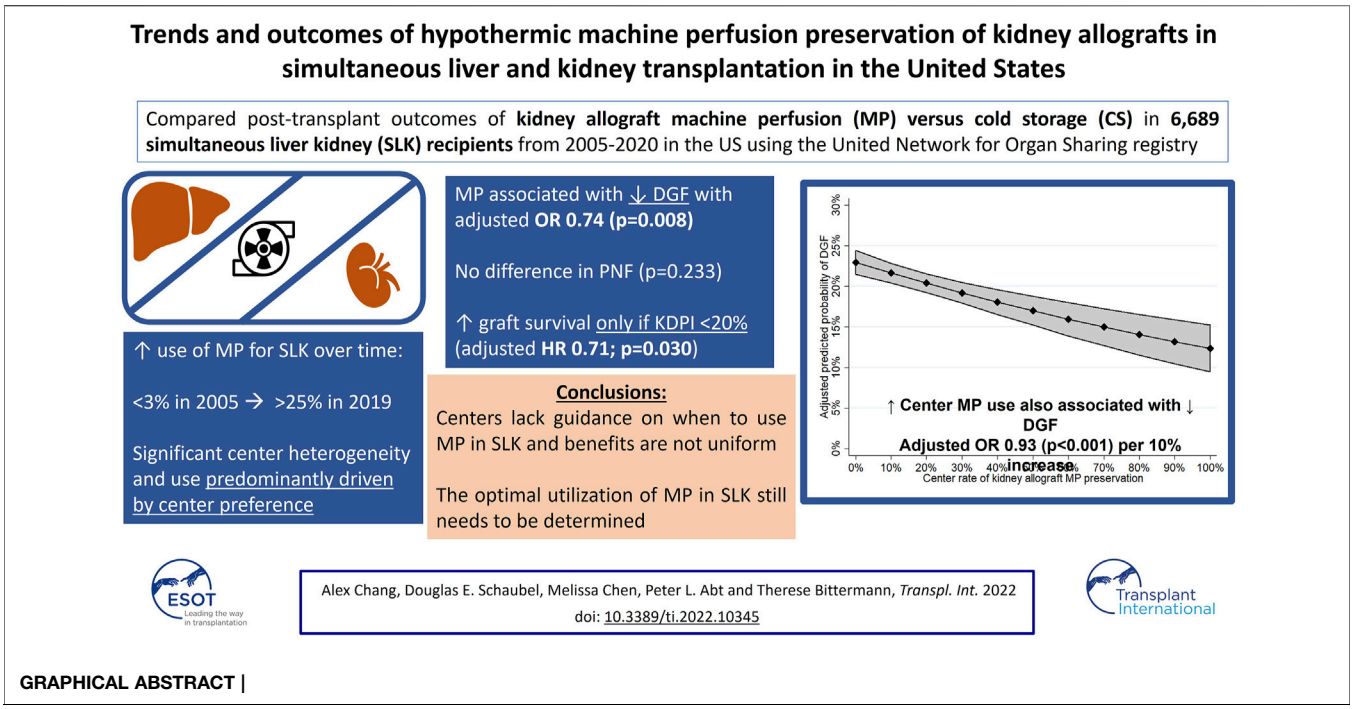
## INTRODUCTION

Outcomes after orthotopic liver transplantation (LT) are strongly associated with pre- and post-operative renal failure, and patient survival is significantly lower in recipients requiring long-term dialysis post-transplant (1). Thus, it is widely accepted that selected patients with pre-LT renal dysfunction be considered for simultaneous liver-kidney transplant (SLK) to improve their outcomes after LT (2). In the years following the introduction of the Model for End-stage Liver

**Abbreviations:** BMI, body mass index; CIT, cold ischemia time; COD, cause of death; CS, cold storage; DCD, donation after circulatory death; HCV, hepatitis C virus; HLA, human leukocyte antigen; KDPI, kidney donor profile index; LT, liver transplant; MELD, Model for End Stage Liver Disease; MP, machine perfusion; OPO, organ procurement organization; OPTN, Organ Procurement and Transplantation Network; PNF, primary non-function; SLK, simultaneous liver and kidney; UNOS, United Network for Organ Sharing.




**GRAPHICAL ABSTRACT |**

Disease (MELD) allocation system in 2002, the rate of SLKs increased dramatically (3). This partly resulted from an organ allocation system that prioritized candidates with worse renal function and from the implementation of policies that facilitated access to SLK. According to the most recently published national data, 7.1% of candidates on the LT waitlist were awaiting SLK and 8.6% of completed LTs were performed with a concurrent kidney transplant (KT) in 2018 (4). However, despite being of higher quality, kidney graft survival after SLK has been shown to be worse than after KT alone, particularly in the early post-LT period, which has been primarily attributed to the greater severity of illness of SLK recipients (5, 6).

Machine perfusion (MP) of deceased donor kidney grafts has been used as an alternative to static cold storage (CS) as a means to improve post-transplant kidney function, particularly for allografts of reduced quality (7). After the allograft is flushed free of blood, MP pumps hypo- or normothermic preservation solution through the renal vasculature in a manner that simulates natural organ perfusion, leading to clearance of toxic metabolites and reduced renovascular resistance (8). While MP has primarily been used in the setting of marginal kidney allografts for KT alone, a recent observational study by Lunsford et al. conducted at two U.S. transplant centers has suggested that MP may also improve kidney graft outcomes among SLK recipients (9).

Given these recent findings, we sought to evaluate 1) temporal and geographic changes in the use of kidney graft MP preservation in SLK and 2) evaluate the potential benefit of MP on patient and kidney graft outcomes in a national cohort.

## METHODS

This was a retrospective cohort study using the United Network for Organ Sharing (UNOS) database. All adult (≥18 years), deceased-donor simultaneous liver-kidney (SLK) transplant recipients between January 1, 2005 and December 6, 2020 were identified. Recipients of prior solid organ transplant of any kind were excluded. Status 1 (i.e., emergent LT) recipients were additionally excluded.

The primary exposure of interest was receipt of a kidney allograft preserved using MP versus CS. Given the focus of the study, all analyses were restricted to SLK recipients for whom kidney allograft preservation data was available (98.8% of the initial cohort). While detailed information regarding MP protocols used was not available (e.g., duration, flow, resistance), it should be noted that all currently approved devices by the US Food and Drug Administration are hypothermic non-oxygenated systems. Recipient characteristics obtained at the time of SLK included: age, sex, race/ethnicity, kidney disease etiology, history of diabetes, native Model for End-stage Liver Disease (MELD), cirrhosis decompensations (ascites, hepatic encephalopathy), patient location prior to SLK (home, inpatient ward, intensive care unit), severity of renal disease at SLK (on dialysis, eGFR <30 ml/min/1.73 $m^2$ not on dialysis and eGFR ≥30 ml/min/1.73 $m^2$ not on dialysis), and duration of dialysis (among those on dialysis at SLK). Donor characteristics included: age, sex, race/ethnicity, hypertension, diabetes, body mass index (BMI), terminal creatinine, hepatitis C virus (HCV) antibody status, distance from recipient hospital and cause of death (COD). Additional allograft characteristics included donation after circulatory determination of death status (DCD), cold ischemic time (CIT), whether liver allograft

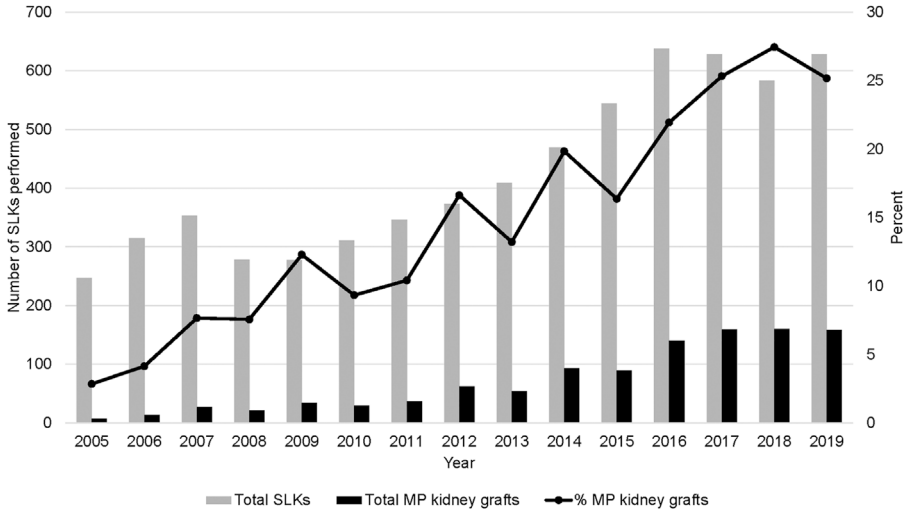



**FIGURE 1 |** Nationwide trends in MP use in SLK transplants from 2005–2019.

was split, Kidney Donor Profile Index (KDPI; categorized as <20%, 30–34%, 35–85%, and >85% (10, 11)) and share type (local, regional, national). Lastly, we also evaluated whether kidney implantation occurred on the same versus ≥1 day after the date of LT.

Recipient, donor and allograft characteristics were compared descriptively according to preservation using MP versus CS. Chi-squared tests and Kruskal-Wallis tests were used for categorical and continuous variables, respectively. Temporal, regional and center trends in MP use were also described. The geographic distribution of the 11 UNOS regions can be visualized for reference here: https://unos.org/community/regions/. In the first analysis, mixed-effects multivariable logistic regression was employed to evaluate the predictors of MP kidney allograft preservation. This model was adjusted for the aforementioned exposures as fixed effects (with the exception of KDPI to avoid collinearity, as the individual index components were already included) and transplant center as a random effect. From this model, the intraclass correlation coefficient (ICC) was obtained, which indicates the percent variability in MP perfusion across recipients that is explained by transplant center alone.

All subsequent statistical analyses evaluated receipt of MP as a predictor of recipient outcomes. Mixed-effects multivariable logistic regression was used to investigate kidney delayed graft function (DGF) and 2) kidney allograft primary non-function (PNF). Adjustment covariates included each of the aforementioned recipient and donor/allograft characteristics (except KDPI), as well as transplant era (2005–2009, 2010–2014, 2015–2020). All covariates were represented by fixed effect, with the exception of transplant center which was specified as a random effect in order to efficiently account for correlation among patients within-center. DGF was defined as receipt of dialysis within the first week after SLK (12, 13). PNF was defined as kidney graft failure ≤90 days from the date of SLK (14). Kidney graft survival was the time between transplantation and the earliest of retransplantation or death.

DGF was modeled using mixed logistic regression using all of the above-listed adjustment covariates and a random center effect. In the multivariable model investigating PNF, a parsimonious model was developed given the low number of events (a total of 124 patients experienced PNF). Stepwise forwards selection with $p$-value thresholds of <0.05 and ≥0.1 for entry and removal, respectively, was used to select covariates for the final model. Cox regression was used to model graft survival. Analogous to DGF, all of the adjustment covariates were included, with center again represented through a random effect.

After fitting each of the above-described models, we evaluated interactions with kidney allograft perfusion strategy. To evaluate the interactions with MP, we adopted the same general strategy for each of the tree outcomes. In particular, all main effects remained in the model. First, we evaluated each interaction separately one at a time. Second, any significant interactions would then be evaluated simultaneously to avoid confounding. In order to ensure clinical interpretability of our findings, we restricted attention to a pre-specified set of covariates for which interaction with MP was felt by the investigators to have biological plausibility. This set included each of the KDPI components (i.e., donor age, race/ethnicity, BMI, history of hypertension, history of diabetes, cause of death, terminal creatinine, HCV antibody status, and DCD status (15)), KDPI (categorized as <20%, 30–34%, 35–85%, and >85% (10, 11)), renal allograft CIT (continuous) and recipient renal disease severity (on dialysis, eGFR <30 ml/min/1.73 $m^2$ not on dialysis and eGFR ≥30 ml/min/1.73 $m^2$ not on dialysis). In models evaluating the interaction of MP and KDPI, the individual components of the KDPI were not included given concern for collinearity and lack of interpretability. Note that, for PNF, we excluded covariates not chosen earlier (for the main effects model) from the above list of potential interaction variables.

Next, we carried out secondary analyses. First, we evaluated unadjusted rates of each outcome according to whether kidney implantation was delayed or not among those undergoing MP preservation using descriptive statistics. Second, we replaced the

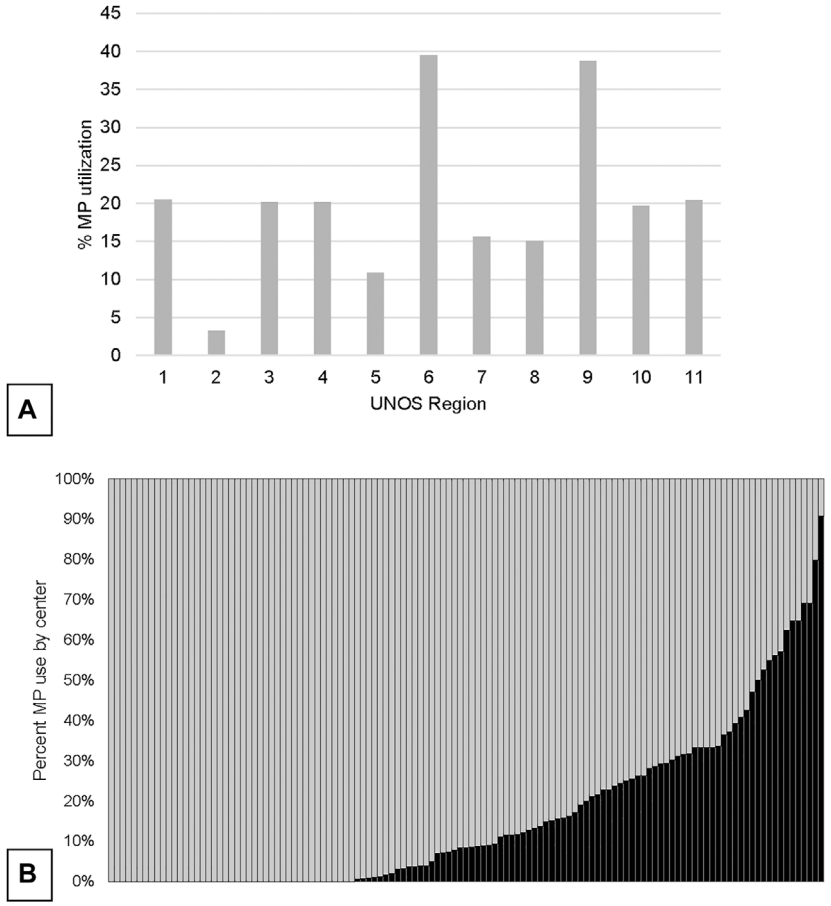



**FIGURE 2 |** Variation in overall utilization of MP in SLK by UNOS region **(A)** and by center **(B)**.

(patient-level) MP indicator with center-level percentage of patients transplanted with a perfused kidney. This equates to changing the question posed from "what is the effect of MP on patients" outcome in the primary analyses to "what is the effect of a center using more kidney MP on patients" outcome (i.e., irrespective of type of kidney perfusion strategy received). Center MP rate was evaluated as a predictor of each of the three outcomes (DGF, PNF, kidney graft survival) without adjusting for center (since doing so is inappropriate in the presence of center-level covariates). For the models evaluating DGF and kidney graft survival, the final multivariable model adjusted for all covariates. For the model evaluating PNF, the same covariate selection method described previously was used, which selected the same covariates as in the primary multivariable model.

All analyses were performed using STATA v16 (College Station, TX, United States). This study was approved by the Institutional Review Board of the University of Pennsylvania.

## RESULTS

There were 6,689 recipients of SLK between January 1, 2005 and December 6, 2020. Allograft storage type (i.e., MP vs. CS) was available in 6,610 (98.8%) recipients. Of these, 5,474 (82.8%) kidney allografts for SLK underwent static CS, while 1,136 (17.2%) received MP preservation.

Concurrent to the increase in SLK volume between 2005 and 2018, the utilization of kidney allograft MP also increased from 2.8% in 2005 to 25.2% in 2019 (**Figure 1**). There was significant geographic variability in the utilization of MP for SLK between UNOS regions and individual transplant centers (**Figure 2**). UNOS region two had the lowest utilization, with 3.3% of 668 SLKs between 2005 and 2020, while region six had the highest with 39.5% of 119 SLKs. Of the 125 centers included in the analysis, 34.4% (N = 43 centers) exclusively used CS in SLK. MP use at the remaining 82 centers ranged from 0.6% to 90.9%. There was no correlation between center MP use and center SLK volume ($p$ = 0.131), median KDPI ($p$ = 0.743) or median SLK waiting time ($p$ = 0.455).

### Donor and Recipient Characteristics According to Kidney Allograft Preservation Technique

Donors whose kidneys underwent MP were older than those undergoing CS: median 36 (IQR: 24–47) versus 34 (IQR: 26–49)

**TABLE 1 |** Donor characteristics according to kidney allograft preservation technique (N = 6,610).

| | Cold preservation | Machine perfusion | *p*-value |
|---|---|---|---|
| | N = 5,474 | N = 1,136 | |
| Sex, N (%) | | | 0.660 |
| Male | 3,388 (61.9) | 711 (62.6) | |
| Female | 2,086 (38.1) | 425 (37.4) | |
| Age (years), median (IQR) | 34 (24–47) | 36 (26–49) | <0.001 |
| Race/ethnicity, N (%) | | | 0.341 |
| White | 3,569 (62.5) | 750 (66.0) | |
| Black | 849 (15.5) | 169 (14.9) | |
| Hispanic | 836 (15.3) | 183 (16.1) | |
| Asian/other | 220 (4.0) | 34 (3.0) | |
| Hypertension, N (%) | 1,153 (21.2) | 264 (23.5) | 0.091 |
| Diabetes, N (%) | 228 (4.2) | 71 (6.3) | 0.002 |
| KDPI category, N (%) | | | 0.003 |
| <20% | 1,933 (36.5) | 356 (31.4) | |
| 20–34% | 1,022 (18.7) | 237 (20.9) | |
| 35–85% | 2,235 (40.9) | 482 (42.5) | |
| >85% | 210 (3.9) | 59 (5.2) | |
| DCD donor, N (%) | 224 (4.5) | 90 (7.9) | <0.001 |
| Kidney CIT (hours), median (IQR) | 10.0 (7.7–12.8) | 12.8 (9.4–21.7) | <0.001 |
| Liver CIT (hours), median (IQR) | 6.1 (5.0–7.7) | 6.0 (4.7–7.6) | 0.074 |
| Split liver, N (%) | 81 (1.5) | 12 (1.1) | 0.270 |
| Distance to donor (miles), median (IQR) | 59 (8–158) | 52 (8–166) | 0.492 |
| Share type, N (%) | | | 0.018 |
| Local | 4,099 (74.9) | 858 (75.5) | |
| Regional | 1,241 (22.7) | 235 (20.7) | |
| National | 134 (2.5) | 43 (3.8) | |
| Cause of death, N (%) | | | 0.002 |
| Anoxia | 1,625 (29.7) | 407 (35.8) | |
| Stroke | 1,494 (27.3) | 277 (24.4) | |
| Head trauma | 2,188 (40.0) | 420 (37.0) | |
| CNS tumor | 35 (0.64) | 5 (0.4) | |
| Other | 132 (2.4) | 27 (2.4) | |
| BMI (kg/m$^2$), median (IQR) | 25.8 (22.8–29.8) | 26.4 (23.3–30.1) | 0.001 |
| Terminal creatinine (mg/dl), median (IQR) | 0.9 (0.7–1.2) | 0.9 (0.7–1.2) | 0.267 |
| HCV antibody positive, N (%) | 399 (7.3) | 88 (7.8) | 0.599 |

years ($p < 0.001$, **Table 1**), though this difference was small. They were also more likely to have diabetes: 6.3% versus 4.2% ($p = 0.002$). There was no statistically significant difference in donor sex, race/ethnicity, terminal creatinine, or HCV antibody status between allografts preserved using MP versus CS. Kidney allografts undergoing MP were more often DCD organs (7.9% vs. 4.5%, $p < 0.001$) and had longer CIT (median 12.8 vs. 10.0 h, $p < 0.001$). Of note, there was no statistical difference in liver allograft CIT between groups ($p = 0.074$). A trend towards higher KDPI among recipients of MP preserved kidney allografts was noted ($p = 0.003$; **Table 1**).

Few recipient characteristics were associated with kidney allograft MP versus CS preservation (**Table 2**). For example, no statistically significant differences were observed with regards to age, sex, or native MELD score. While statistically significant, differences in cirrhosis decompensations such as ascites severity or hepatic encephalopathy grade were clinically less relevant, as they were very small ($p < 0.001$ and $p = 0.018$, respectively). There was no statistically significant difference in pre-LT renal disease severity between groups ($p = 0.458$). However, among recipients on dialysis pre-SLK (N = 4,590), pre-transplant dialysis duration was longer for patients receiving allografts preserved using MP: median 6.1 months versus 3.7 months ($p < 0.001$). Etiology of kidney disease was also different ($p < 0.001$) with those having hepatorenal syndrome receiving MP kidney grafts more frequently than those with cold storage (40.9% vs. 30.2%). Kidney implantation occurred ≥1 day after LT for 34.9% of patients in the MP group versus 13.9% in the CS group ($p < 0.001$).

## Predictors of Kidney Allograft MP Preservation in SLK

In adjusted analyses, several predictors of kidney allograft MP preservation were identified (**Supplementary Table S1**). These included: increasing donor age (OR 1.02 per 1 year increase, 95% CI: 1.01–1.03, $p < 0.001$), DCD status (OR 2.81, 95% CI: 1.88–4.20; $p < 0.001$), kidney allograft CIT (1.10 per 1 h increase, 95% CI: 1.08–1.11; $p < 0.001$), donor terminal creatinine (OR 1.22 per 1 mg/dl increase, 95% CI: 1.08–1.39; $p = 0.001$), and donor BMI (OR 1.02 per 1 kg/m$^2$ increase; 95% CI 1.00–1.04; $p = 0.020$). Regionally shared kidney allografts were associated with less use of MP preservation (OR 0.47 vs. local, 95% CI: 0.36–0.61; $p < 0.001$). Transplant era was strongly associated with MP use: OR 2.42 (95% CI: 1.72–3.39) for

**TABLE 2 |** Recipient characteristics at LT according to donor kidney allograft preservation technique (N = 6,610).

| | Cold storage N = 5,474 | Machine perfusion N = 1,136 | *p*-value |
|---|---|---|---|
| Sex, N (%) | | | 0.324 |
| Male | 3,482 (63.6) | 705 (62.1) | |
| Female | 1,992 (36.4) | 431 (37.9) | |
| Age (years), median (IQR) | 58 (51–63) | 58 (52–64) | 0.222 |
| Race/ethnicity | | | 0.072 |
| White | 3,386 (61.9) | 724 (63.7) | |
| Black | 807 (14.7) | 158 (13.9) | |
| Hispanic | 992 (18.1) | 196 (17.3) | |
| Asian | 211 (3.9) | 32 (2.8) | |
| Other | 78 (1.4) | 26 (2.3) | |
| Native MELD at SLK, median (IQR) | 28 (23–35) | 28 (23–35) | 0.457 |
| Ascites, N (%) | | | <0.001 |
| None | 885 (16.2) | 230 (20.3) | |
| Mild | 2,182 (40.1) | 385 (34.0) | |
| Moderate-severe | 2,381 (43.7) | 519 (45.8) | |
| Encephalopathy, N (%) | | | 0.018 |
| None | 1,697 (31.2) | 401 (35.4) | |
| Grade 1–2 | 2,991 (54.9) | 577 (50.9) | |
| Grade 3–4 | 760 (14.0) | 156 (13.8) | |
| Preop location, N (%) | | | 0.514 |
| Home | 3,142 (57.5) | 674 (59.4) | |
| Inpatient ward | 1,311 (24.0) | 262 (23.1) | |
| ICU | 1,008 (18.5) | 199 (17.5) | |
| Diabetes, N (%) | 2,356 (43.3) | 488 (43.2) | 0.905 |
| Kidney disease severity, N (%) | | | |
| eGFR ≥30 ml/min/1.73 $m^2$[a] | 677 (12.7) | 157 (14.1) | 0.458 |
| eGFR <30 ml/min/1.73 $m^2$[a] | 1,445 (27.1) | 295 (26.4) | |
| On dialysis | 3,213 (60.2) | 665 (59.5) | |
| Dialysis time[b] (months), median (IQR) | 3.7 (0.9–14.9) | 6.1 (1.5–21.5) | <0.001 |
| Etiology of kidney disease, N (%) | | | <0.001 |
| Hepatorenal syndrome | 1,655 (30.2) | 465 (40.9) | |
| Diabetes | 1,134 (20.7) | 225 (19.8) | |
| Glomerular disease | 426 (7.8) | 78 (6.9) | |
| Polycystic kidney disease | 278 (5.1) | 88 (7.8) | |
| Hypertension | 476 (8.7) | 74 (6.5) | |
| Other | 1,505 (27.5) | 206 (18.1) | |
| KT implantation ≥1 day after LT, N (%) | 760 (13.9) | 396 (34.9) | <0.001 |

[a]*Not on dialysis pre-LT.*
[b]*Among patients receiving dialysis prior to SLK (N = 4,590).*

**TABLE 3 |** Summary of findings obtained from multivariable models evaluating kidney allograft preservation type as a predictor of kidney graft outcomes after SLK.

| | Point estimate (95%CI) for kidney allograft MP compared to CS | *p*-value |
|---|---|---|
| Kidney delayed graft function | OR 0.74 (0.60–0.92) | 0.008 |
| Kidney primary non-function | OR 0.88 (0.52–1.49) | 0.637 |
| Kidney graft survival | HR 0.91 (0.78–1.06) | 0.230 |

2010–2014 and OR 6.03 (4.30–8.44) for 2015–2020 versus 2005–2009 ($p < 0.001$). The ICC for transplant center in this model was 65.0%. This indicates that nearly two-thirds of the variability in MP use across SLK recipients was explained by the transplanting center alone, while donor and recipient factors explained only a minority.

## Kidney Allograft Preservation Technique and Delayed Graft Function

DGF occurred in 256 recipients after MP and 1,311 recipients after CS (22.5% vs. 24.0%, $p = 0.293$). There was no statistical difference in DGF rates among MP allografts implanted on the same versus on a subsequent date from LT (22.0% vs. 23.6%; $p = 0.554$). Accounting for recipient and donor covariates, transplant era and transplant center, MP was significantly associated with DGF in the final multivariable model with a covariate-adjusted OR of 0.74 (95% CI: 0.60–0.92; $p = 0.008$; **Table 3**). The results of the full multivariable model are shown in **Supplementary Table S2**. There were no statistically significant interactions found between kidney allograft preservation type and any of the covariates evaluated.

As a secondary analysis, center kidney allograft MP use was evaluated as an independent predictor of recipient DGF. Center practice was found to be associated with a reduction

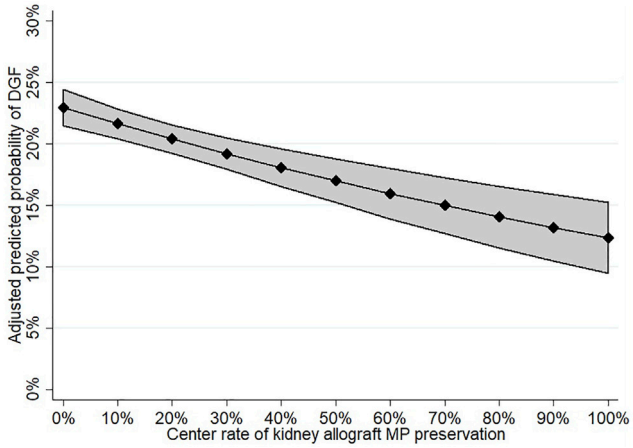



**FIGURE 3 |** Adjusted predicted probability of DGF according to increasing center MP use.

in the odds of DGF in both univariable (OR 0.94 per 10% increase in center MP use, 95% CI: 0.92–0.97; $p < 0.001$) and multivariable analyses (OR 0.93 per 10% increase in MP use, 95% CI: 0.90–0.96; $p < 0.001$; **Supplementary Table S3**). The predictive margins of DGF by increasing center kidney allograft MP are shown in **Figure 3**.

## Kidney Allograft Preservation Technique and Primary Non-function

Kidney allograft PNF occurred in 19 patients after MP and 105 patients after CS (1.9% vs. 2.1%, $p = 0.666$). There was no difference in PNF rate for MP kidneys with delayed implantation (2.0% vs. 1.9%; $p = 0.849$). MP was not associated with PNF in the final multivariable model: covariate-adjusted OR 0.88 (95% CI: 0.52–1.49; $p = 0.637$; **Supplementary Table S4**). No statistically significant interaction was found between MP use and any of the covariates studied, which included recipient renal disease severity, kidney donor KDPI, donor age, donor BMI, donor hypertension, donor cause of death or kidney allograft CIT. In secondary analyses, center MP use was not associated with kidney allograft PNF on either univariable (OR 0.94 per 10% increase in MP use, 95% CI: 0.86–1.03; $p = 0.180$) or multivariable analyses (OR 0.94, 95% CI: 0.85–1.04; $p = 0.233$; **Supplementary Table S5**).

## Kidney Allograft Preservation Technique and Kidney Allograft Survival

Kidney allograft MP was not associated with unadjusted or adjusted kidney graft survival, defined as a combined end-point of kidney graft failure or patient death: HR 0.95 (95% CI: 0.82–1.10, $p = 0.481$) and HR 0.91 (95% CI: 0.76–1.03; $p = 0.230$; **Supplementary Table S6**), respectively. Of the covariates evaluated for interaction with kidney allograft perfusion type, the following were statistically significant: donor KDPI category ($p = 0.029$) and donor cause of death ($p = 0.039$). The results of the multivariable model including the interaction of perfusion type and KDPI category are shown in **Supplementary Table S7**. In stratified models by KDPI category, MP was associated with improved graft survival in the setting of KDPI <20% (adjusted HR 0.71, 95% CI: 0.53–0.97; $p = 0.030$, but not with higher KDPI ($p = 0.677$ for KDPI 20–34%, $p = 0.339$ for 35–85% and $p = 0.071$ for >85%; **Figure 4**). Unfortunately, the interaction between perfusion type and donor cause of death was entirely driven by the "other" category, which is clinically uninterpretable and, thus, not included in the final model. Center MP use was not associated with kidney graft survival in multivariable analyses: covariate-adjusted HR 1.00 (95% CI: 0.98–1.03; $p = 0.851$; **Supplementary Table S8**). In unadjusted analyses, there was no improvement in kidney graft survival among MP allografts with delayed implantation (log-rank $p = 0.741$).

# DISCUSSION

In this analysis of national data over a 15-year period, we show that the use of MP preservation in SLK has markedly increased in the U.S. over time, accounting for 1 in 4 kidney allografts since 2017. Several studies have shown benefits of MP preservation compared to static CS in the setting of KT alone (16–18). However, its potential benefits have not been rigorously studied in SLK transplantation until now, a scenario in which 1) increased kidney allograft quality and 2) the added complexity of dual-organ transplantation may reduce the advantages of MP preservation. In this study, we find a significant reduction in kidney DGF with MP preservation and increased center MP utilization also predicted lower DGF. In contrast, we found no association between kidney allograft MP and PNF, and only benefits with respect to kidney graft survival among the highest quality kidney allografts. The present study additionally demonstrates large practice variability between transplant centers in the choice of kidney allograft preservation modality in SLK. In fact, where one undergoes SLK explained the majority of the variability in kidney allograft MP use, while the 25 other donor and recipient factors were lesser determinants. It is likely that anecdotal experience and the existing evidence-base in the KT alone population has driven the rapid expansion of MP preservation for SLKs at these centers. However, further studies are needed to more clearly delineate which SLK recipients stand most to benefit from the added resources and associated costs of kidney MP preservation.

It is well-established that SLK recipients have access to the highest quality kidney allografts (19). In several meta-analyses of clinical trials, MP preservation in KT alone has benefits with regards to short- and long-term graft outcomes. This has not only been demonstrated in marginal donor kidneys but also in standard quality organs (7, 20). This technique has also been shown to be more cost-effective over CS, irrespective of kidney graft quality (21). Yet, perhaps surprisingly, in the SLK population, we only found evidence of reduced DGF and limited improvements in long-term graft outcomes, despite accounting for other measures of inferior allograft quality in our analyses. Nevertheless, the significant reduction in DGF in this population should not be overlooked, particularly given

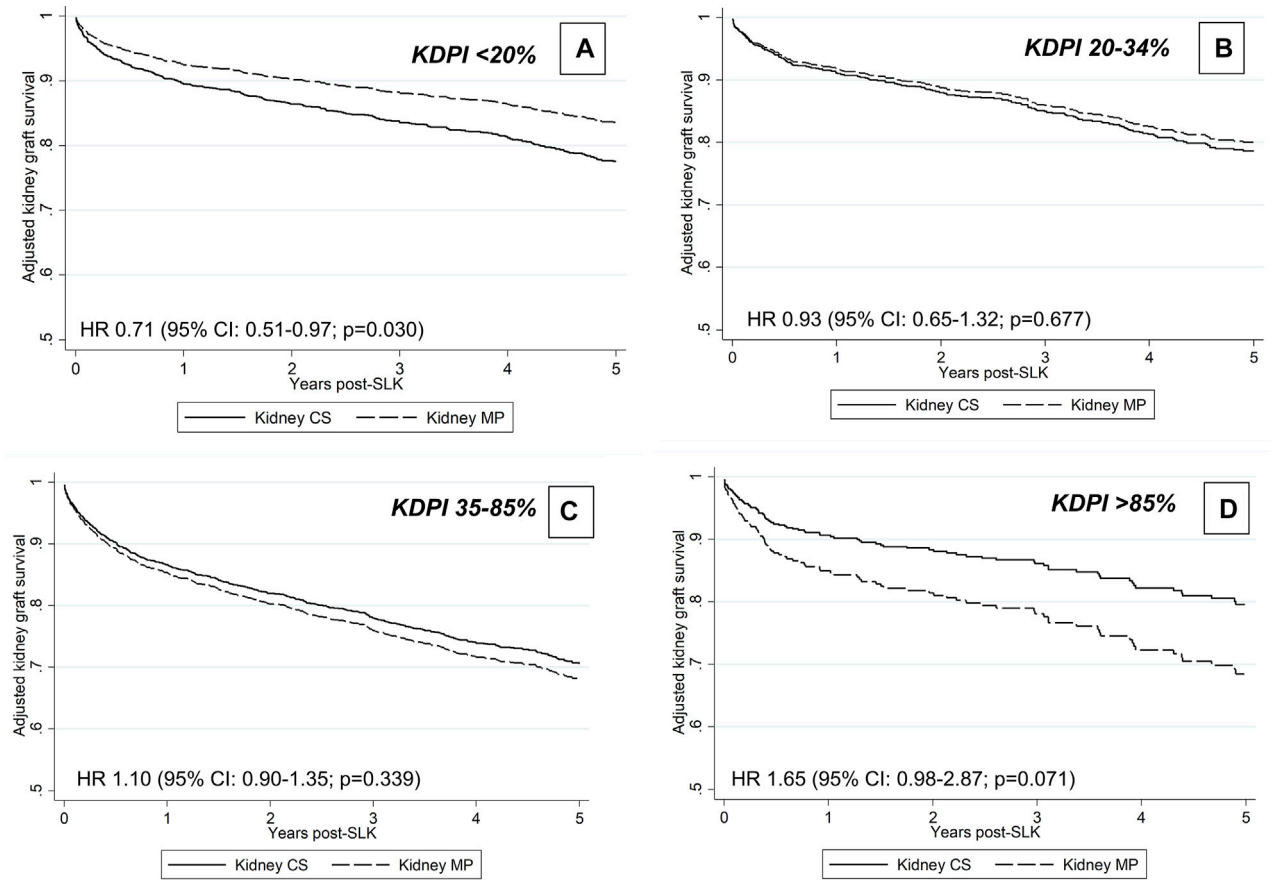



**FIGURE 4 |** Adjusted graft survival associated with MP preservation according to KDPI group.

the high quality of kidney allografts allocated to SLK recipients.

Recipient factors play important and unique roles in the development of poor kidney graft outcomes in the SLK population. These include, among others, increased liver disease severity, intra-operative challenges (e.g., volume shifts, transfusion requirements, electrolyte disturbances) and prolonged post-transplant recovery than KT alone recipients. The significant contribution of recipient factors on graft outcomes may explain why the benefits of MP were only observed in those receiving allografts with KDPI <20%, which represented 35.5% of the cohort. Delayed kidney implantation (as evidenced by the longer kidney CIT and difference in transplant dates recorded) was more frequent in the MP group. However, we did not observe any differences in unadjusted graft outcomes according to timing of kidney implantation in the MP group. Thus, the proposed benefits of MP preservation to allow for delayed KT in a more optimal recipient milieu after LT remain uncertain.

Our results using national data differ from those published by others reporting their own center-specific experiences, in which MP preservation with delayed KT implantation offered clear superior results, including resultant effects on patient survival (9, 22). These differences in findings are likely partly explained by the association between increasing center preference for MP and the associated reduction in DGF identified in this study, as centers with established MP protocols are more likely to publish on their experiences compared to those that seldom use MP. In addition, while we were able to determine type of preservation modality and duration of CIT, whether centers and organ procurement organizations (OPOs) differed with respect to the proportion of time spent on pump, time from procurement to placement on pump, other aspects of MP-related management and decision-making regarding potential delayed timing of implantation were not known. This may also explain why smaller gains were observed with kidney allograft MP preservation when this practice was evaluated nationally, and which would highlight the need for more clearly defined "best practices" regarding when and how to employ MP preservation to maximize its impact on kidney graft outcomes in SLK. Further research using more comprehensive donor data and allograft quality indicators, such as that collected from OPOs, may provide greater insights into the ideal setting to use MP preservation in SLK.

While the use of a national cohort offers advantages, there are also inherent study limitations. All commercially available MP devices for kidney allografts in the US are hypothermic non-oxygenated systems. However, more granular data regarding the duration of pumping and other MP parameters (e.g., flow, resistance) were not available and likely varied by center and OPO. This may have biased

certain results towards the null. In addition, while we were able to examine common recipient and allograft predictors of MP use, more comprehensive details on centers' decision-making and protocols are not known. Similarly, there may be differences regarding kidney allograft management that occurred at the OPO-level before the organ arrived at the transplanting center. If heterogeneity in MP protocol is indeed the explanation for the null result obtained in our study, then this speaks to the need for greater evidence-based guidance on its use and further multi-center studies are warranted that could address this evidence gap. The relationship observed between increasing center MP use and declining DGF rates may support the notion that centers with more MP experience use this technology more effectively and thus a "learning curve" for MP exists, which may further contribute to the outcomes seen.

Other limitations of registry data include diminished donor and recipient clinical detail. This could have led to unmeasured confounding and subsequent bias in our results. There were also no recipient peri- or post-operative clinical details between transplant surgeries to confirm that the longer kidney CIT and differences in KT versus LT transplant dates recorded for the MP group indeed reflected the intention to delay kidney implantation to allow for a more favorable recipient clinical status. Supporting this is the fact that indicators of kidney allograft quality and recipient factors explained only a minority of the variability in MP use across centers, and thus this decision-making infrequently takes into account key variables known to be associated with inferior kidney graft outcomes (23–25). Given the available variables, geographic trends analysis was limited to UNOS regions. This issue should be re-evaluated in the future, particularly in the context of the new liver allocation system in the U.S, which has led to greater transportation of allografts (26). Lastly, the imbalance between the MP and CS sample sizes may have led to imprecision in the point estimates and the adjustment of measured confounders in the multivariable models. In particular, given the low number of PNF events particularly among MP patients, it is likely that power was inadequate to detect a significant difference. Moreover, given the low frequency of high KDPI kidneys in this SLK cohort, a potential difference in graft survival with MP may have been missed.

A rapidly increasing use of MP for storage of kidney allografts prior to SLK transplantation has occurred in the U.S. that is predominantly driven by transplant center preference. While MP kidney allograft preservation affords a reduction in DGF, its impact on longer-term outcomes for the majority of recipients remain uncertain. There is a need to understand the cost-effectiveness and logistical implications of this increasing MP use (with or without kidney implantation delay), and more comprehensive guidance is also warranted with respect to when and how to best use this potentially valuable technology in the SLK population.

## DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://unos.org/data/.

## ETHICS STATEMENT

This study was approved by the Institutional Review Board of the University of Pennsylvania.

## AUTHOR CONTRIBUTIONS

AC: research design, data interpretation, and writing of the manuscript. DS: data interpretation, editing of the manuscript MC: research design and writing of the manuscript. PA: research design and editing of the manuscript. TB: research design, performance of the statistical analyses, data interpretation, and writing of the manuscript.

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## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10345/full#supplementary-material

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ORIGINAL RESEARCH
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# Hyperspectral Imaging as a Tool for Viability Assessment During Normothermic Machine Perfusion of Human Livers: A Proof of Concept Pilot Study

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Normothermic machine perfusion (NMP) allows for *ex vivo* viability and functional assessment prior to liver transplantation (LT). Hyperspectral imaging represents a suitable, non-invasive method to evaluate tissue morphology and organ perfusion during NMP. Liver allografts were subjected to NMP prior to LT. Serial image acquisition of oxygen saturation levels (StO2), organ hemoglobin (THI), near-infrared perfusion (NIR) and tissue water indices (TWI) through hyperspectral imaging was performed during static cold storage, at 1h, 6h, 12h and at the end of NMP. The readouts were correlated with perfusate parameters at equivalent time points. Twenty-one deceased donor livers were included in the study. Seven (33.0%) were discarded due to poor organ function during NMP. StO2 ($p < 0.001$), THI ($p < 0.001$) and NIR ($p = 0.002$) significantly augmented, from static cold storage (pre-NMP) to NMP end, while TWI dropped ($p = 0.005$) during the observational period. At 12–24h, a significantly higher hemoglobin concentration (THI) in the superficial tissue layers was seen in discarded, compared to transplanted livers ($p = 0.036$). Lactate values at 12h NMP correlated negatively with NIR perfusion index between 12 and 24h NMP and with the delta NIR perfusion index between 1 and 24h (rs = –0.883, $p = 0.008$ for both). Furthermore, NIR and TWI correlated with lactate clearance and pH. This study provides first evidence of

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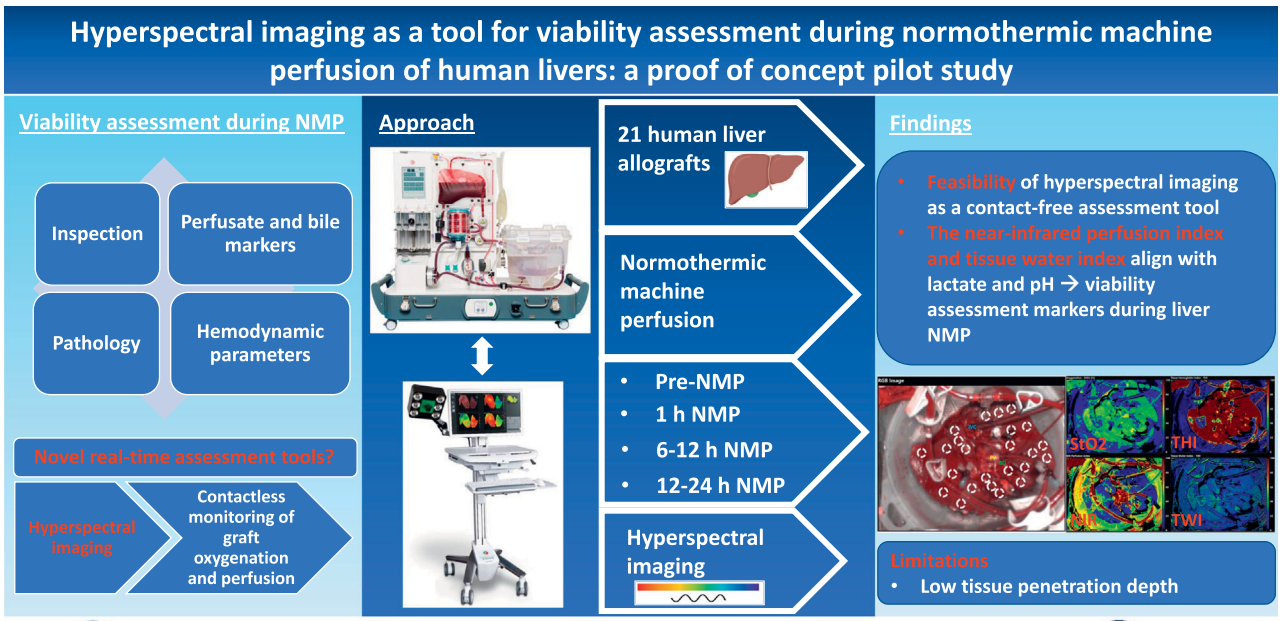
**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAR, balance of risk score; CIT, cold ischemia time; DBD, donation after brain death; DCD, donation after cardiac death; EAD, early graft dysfunction; ECD, extended criteria donors; HSI, hyperspectral imaging; HTK, histidine-tryptophan-ketoglutarate; ICU, intensive care unit; IGL-1, Institut Georges Lopez; IQR, interquartile range; IRI, ischemia reperfusion injury; ITBL, ischemic type biliary lesions; L-GrAFT, Liver Graft Assessment Following Transplantation; LT, Liver transplantation; MEAF, Model for Early Allograft Function; MELD, model for end-stage liver disease; MP, machine perfusion; NIR, near-infrared perfusion index; NMP, normothermic machine perfusion; PNF, primary non-function; RGB, red-green-blue; ROI, region of interest; StO2 (%), relative blood oxygenation index; THI, tissue hemoglobin index; TWI, tissue water index; UW, University of Wisconsin.

feasibility of hyperspectral imaging as a potentially helpful contact-free organ viability assessment tool during liver NMP.

**Keywords: transplantation, perfusion, normothermic, imaging, liver, hyperspectral, machine**




**GRAPHICAL ABSTRACT |**

## INTRODUCTION

In the light of a shortage of donor liver organs, the use of extended criteria donors (ECD) continues to rise. This poses a risk of increased rates of early allograft dysfunction (EAD), primary non-function (PNF) and biliary complications (1–10). Compared to standard criteria donor grafts, ECD livers are more susceptible towards ischemia-reperfusion injury (IRI). In the light of these developments, machine perfusion (MP) has emerged as a procedure aiming to limit IRI. Normothermic machine perfusion (NMP) is also suitable for prolongation of preservation and a comprehensive assessment of livers *ex-vivo*. While this concept is uniquely appealing, the identification of techniques and biomarkers for a meaningful determination of the quality and function of an organ remains to be established. Essentially, NMP mimics physiologic liver perfusion. During a period of up to 24 h, the liver is accessible for inspection, biopsy, perfusate and bile sampling (11). Contemporarily, viability assessment is performed by measuring biochemical parameters and synthetic function in the perfusate and bile (12–17). Further to this, innovative liver graft viability and injury markers have been applied. However, whether they are acceptable predictors of the outcomes after LT remains to be proven (2, 4, 11, 12). Novel non-invasive methods for the estimation of organ quality during NMP are necessitated. Hyperspectral imaging (HSI) represents a potentially suitable contactless tool to assess tissue morphology and organ perfusion. This technology allows a real-time quantitative evaluation of graft oxygenation and micro-perfusion, as well as organ hemoglobin and water concentration. Previous studies showed that HSI is suitable for monitoring of the oxygen saturation distribution and identifying areas with a reduced oxygen supply (18–20). This may help to detect and quantify impaired, inhomogeneous or deteriorating perfusion (18–27). We herein designed a study demonstrating the feasibility and the potential of HSI in the setting of liver NMP as a non-invasive, simple viability assessment tool.

## MATERIALS AND METHODS

### Study Design

Liver allografts accepted for transplantation were procured and subjected to NMP. The decision to apply NMP at our center was based on a previously developed concept (6). NMP was applied

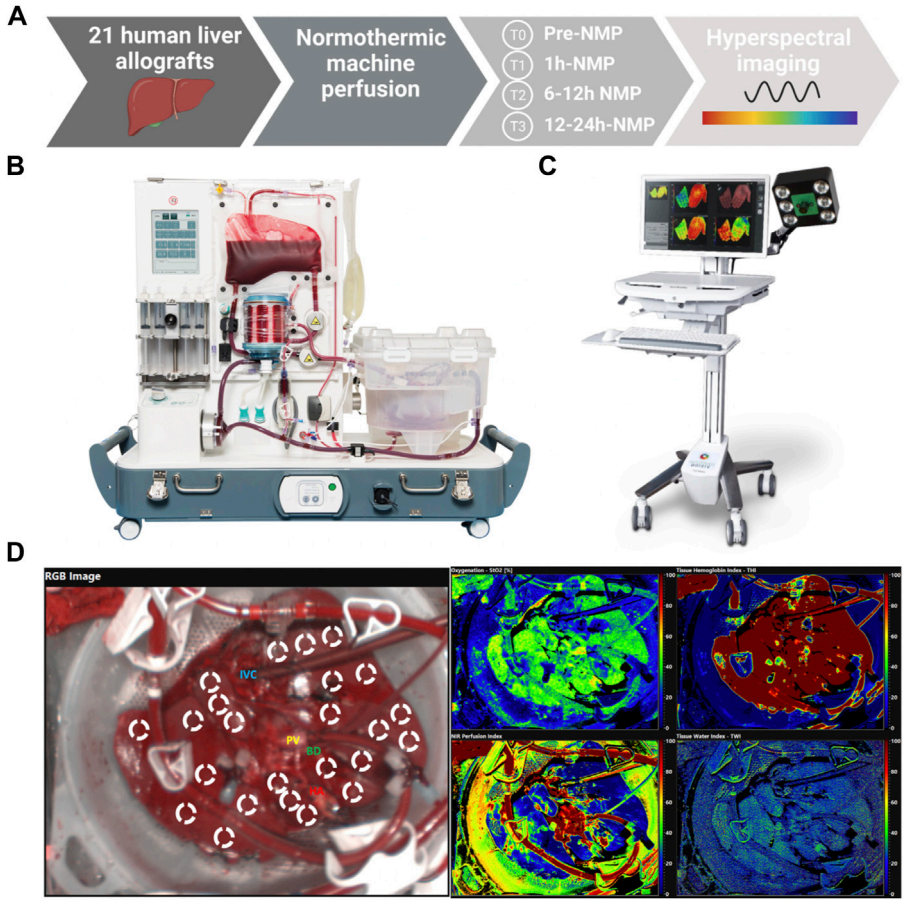



**FIGURE 1 | (A)** Overview describing the methodology and sample collection of the study; **(B)** OrganOx metra® system used for normothermic machine perfusion (14, 16, 52); **(C)** TIVITA® Tissue System used for hyperspectral imaging (21); **(D)** Images acquired during liver normothermic machine perfusion: RGB image, hyperspectral images for oxygenation (StO2), perfusion (NIR perfusion), hemoglobin (THI), and water concentration (TWI), with region of interest (ROI) markers within the parenchyma of liver allografts ICV, Inferior vena cava; PV, Portal vein; HA, Hepatic artery; BD, Bile duct; StO2, Tissue Oxygen Saturation; THI, Tissue Hemoglobin Index; NIR, Near-Infrared Perfusion Index; TWI, Tissue Water Index.

for the following indications: (I) uncertain organ quality (II) complex recipient, and (III) logistics. MP was performed using the OrganOx metra® system according to a local protocol (6), details are specified in the **Supplementary File**. Perfusion time on the OrganOx metra® system depended on the time required for assessment, decision-making and logistics. The choice to discard or transplant an organ was based on key quality parameters (6, 14): preservation of physiological pH values (7.3–7.45) without sodium bicarbonate supplementation after 2 h of NMP, a prompt decline and maintenance of lactate to physiological values (≤18 mg/dl), as well as bile production and bile pH > 7.45 are considered indicators for appropriate organ function. The decision to transplant or discard a liver graft was made after a minimum of 6 h NMP. Further to this, high aspartate aminotransferase (AST), alanine aminotransferase (ALT) (>20,000), and lactate dehydrogenase (>20,000) levels are calling for caution (1, 6). To assess the dynamics of HSI parameters during liver NMP and their correlation with perfusate parameters, serial measurements were performed before NMP (during static cold storage), at 1, 6, 12 h and at the end of NMP (**Figure 1**). HSI data points were assessed longitudinally and in reference to the established biomarkers mentioned above. Donor, recipient and NMP characteristics, transplant procedural data as well as post-operative follow-up data were collected.

## Ethics Statement

The study protocol was approved by the local institutional review board.

## Study Population

A total of 21 donor livers were enrolled in this study between December 2020 and May 2021. The majority of these livers were ECD livers. For definition of ECD, the Eurotransplant criteria were applied (28). These include liver grafts with severe macrosteatosis (>30 or >40%), prolonged cold ischemia (>12 h), DCD and high donor age (>80 years). Notably, a number of criteria that could characterize ECDs specifically for LT have been identified, but the impact of each of these remains to be defined (29). From the 21 livers studied in this trial,

**TABLE 1 |** Demographic data.

| | Total (*n* = 21) | Transplanted (*n* = 14) | Not transplanted (n = 7) | *p*-value[a] |
|---|---|---|---|---|
| **Donor data** | | | | |
| Age (y)[b] | 61 (48–70) | 66 (56–70) | 46 (43–56) | $p = 0.031$ |
| Gender | | | | $p = 0.011$ |
| • Man | 13 (61.9) | 6 (42.9) | 7 (100) | |
| • Woman | 8 (38.1) | 8 (57.1) | | |
| BMI (kg/m$^2$)[b] | 26 (24–28) | 26 (24–28) | 28 (23–31) | $p = 0.585$ |
| ICU time (d)[b] | 3 (2–7) | 4 (2–7) | 2 (2–7) | $p = 0.585$ |
| CIT (h)[b] | 6 (5–8) | 6 (5–8) | 7 (5–9) | $p = 0.856$ |
| Cause of death | | | | $p = 0.290$ |
| Cerebrovascular | 15 (71.4) | 10 (71.4) | 5 (71.4) | |
| Circulatory | 2 (9.5) | 1 (7.1) | 1 (14.3) | |
| Trauma | 1 (4.8) | | 1 (14.3) | |
| Other | 3 (14.3) | 3 (21.4) | | |
| ECD donor | 16 (76.2) | 10 (71.4) | 6 (85.7) | $p = 0.469$ |
| Donor Type | | | | $p = 1.000$ |
| DBD | 15 (71.4) | 10 (71.4) | 5 (71.4) | |
| DCD | 6 (28.6) | 4 (28.6) | 2 (28.6) | |
| DRI[b] | 2.119 (1.610–2.435) | 2.268 (1.728–2.482) | 1.760 (1.480–2.220) | $p = 0.263$ |
| Hypertension | 7 (33.3) | 4 (28.6) | 3 (42.9) | $p = 0.289$ |
| Alcohol Abuse | 4 (19) | 1 (7.1) | 3 (42.9) | $p = 0.102$ |
| Malignancy | 2 (9.5) | 1 (7.1) | 1 (14.3) | $p = 0.599$ |
| Steatosis hepatis | 11 (52.4) | 6 (42.9) | 5 (71.4) | $p = 0.279$ |
| • Mild (<40%) | 10 (47.6) | 5 (35.7) | 5 (71.4) | |
| • Moderate (40%–80%) | 1 (4.8) | 1 (7.2) | 0 (0) | |
| • Severe (>80%) | 0 (0) | 0 (0) | 0 (0) | |
| NMP indication | | | | |
| • Complex recipient | 2 (9.5) | 2 (14.3) | | $p = 0.293$ |
| • Marginal donor | 18 (85.7) | 11 (78.6) | 7 (100) | $p = 0.186$ |
| • Logistics | 8 (38.1) | 6 (42.9) | 2 (28.6) | $p = 0.525$ |
| NMP time (h)[b] | 15 (11–20) | 15 (13–20) | 12 (7–22) | $p = 0.535$ |
| Total preservation time (h)[b] | 20 (17–27) | 21 (17–27) | 19 (9–30) | $p = 0.799$ |
| **Recipient data and post-operative outcome** | | | | |
| Age (y)[b] | | 62 (58–65) | | |
| Gender | | | | |
| • Man | | 10 (71.4) | | |
| • Woman | | 4 (28.6) | | |
| BMI (kg/m$^2$)[b] | | 25.7 (21.8–28.2) | | |
| MELD[b] | | 17 (8–21) | | |
| Time on waiting list (d)[b] | | 52 (37–197) | | |
| BAR score[b] | | 7 (7–10) | | |
| BAR score ≥ 8 | | 6 (42.9) | | |
| Total hospital stay (d)[b] | | 28 (21–46) | | |
| ICU stay (d)[b] | | 6 (4–18) | | |
| Early allograft dysfunction | | 6 (42.9) | | |
| MEAF score[b] | | 5.67 (4.02–6.90) | | |
| L-Graft score[b] | | –0.73 (-1.33–0.07) | | |
| Clavien Dindo ≥3 | | 11 (78.6) | | |
| 90—days readmission rate (unplanned) | | 4 (28.6) | | |
| Biliary complications | | 9 (64.3) | | |
| • ≤ 30 d | | 6 (42.9) | | |
| • > 30 d | | 3 (21.4) | | |
| • Biliary leakage | | 4 (28.6) | | |
| • Anastomotic stricture | | 4 (28.6) | | |
| • Biliary cast syndrome | | 1 (7.1) | | |
| Arterial complication | | 2 (14.3) | | |
| Patient survival (d)[b] | | 106 (82–163) | | |
| Graft survival (d)[b] | | 106 (82–163) | | |
| Patient death | | 2 (14.3) | | |

*Values in parentheses are percentages unless indicated otherwise*

[a]*Chi-square for categorical variables and Mann-Whitney-U Test for continuous variables.*

[b]*Values are median (i.q.r.).*

*BMI, body mass index; ICU, intensive care unit; CIT, cold ischemia time; ECD, extended criteria donor; DBD, donation after brain death; DCD, donation after cardiac death; DRI, donor risk index; NMP, normothermic machine perfusion; MELD, Model for End-Stage Liver Disease; BAR, balance of risk; MEAF, model of early allograft function; L-Graft, Liver Graft Assessment Following Transplantation.*

14 were transplanted, while seven livers were discarded based on the above-mentioned performance quality criteria during NMP. An overview summarizing the most important characteristics of donors, recipients, liver allografts and MP times are displayed in **Table 1**.

## Hyperspectral Imaging of Human Liver Allografts

For the acquisition of HSI data, a contactless and non-ionizing radiation imaging system (TIVITA® Tissue System, Diaspective Vision GmbH, Am Salzhaff, Germany) was used under standardized conditions and previously reported settings (24, 30). The software (TIVITA Suite Tissue) provides a red-green-blue (RGB) image and four false color images illustrating physiologic parameters of the recorded tissue area, which quantified values of the parameters from blue (low values) to red (high values). The relative blood oxygenation in the microcirculation of superficial hepatic tissue layers (approximately 1 mm) is represented by StO2 (%), whereas the near-infrared (NIR) perfusion index (0–100) represents tissue layers in 4–6 mm penetration depth. The indices THI (0–100) and TWI (0–100) display the relative distribution of hemoglobin and water in the investigated tissue area, respectively. Serial HSI measurements were performed according to our center specific NMP protocol: before NMP, at 1h, 6h, 12 h and at the end (with a maximum of 24 h) of NMP. **Supplementary Figure S1** show the different perfusion times of the liver grafts. For the assessment protocol, circular areas, representing the ROI (10 mm diameter markers, 3 markers per liver segment), were defined within the acquired hyperspectral images (**Figure 1**). The index average was calculated from the values collected from the ROI for each image. Details regarding the application of HSI in this analysis are illustrated in the **Supplementary File**.

## Feasibility and Follow-Up

We primarily assessed the dynamic change of perfusion and oxygenation of liver tissue during NMP. Further to this, we have investigated 1) the differences in HSI dynamics between livers discarded and transplanted as well as 2) the correlations between HSI indices and perfusion parameters.

The clinical follow-up of transplanted patients included the assessment of patient survival, graft survival, Clavien Dindo post-operative complications rate, EAD, Model of Early Allograft Function (MEAF) score, Liver Graft Assessment Following Transplantation (L-Graft) score, biliary and vascular complications, 90 days readmission rate, ICU and total hospital stay.

## Statistical Analysis

To examine the Gaussian distribution, we used the D'Agostino-Pearson normality test. The data were analyzed as proportions and medians with interquartile ranges (IQR), because they were consistent with a skewed distribution. Chi-square and Fisher's exact tests for categorical variables and Mann-Whitney-U tests for continuous variables were used to compare the HSI values in the transplanted and non-transplanted groups. Ordinal variables were analyzed as continuous variables. Using the t or F distributions, Mann-Whitney-U tests were approximated for ordinal variables. The Friedman test and Sing tests were applied for paired non-parametric tests. To correlate HSI parameters and laboratory values measured in the perfusate during NMP, Spearman rank correlation tests were performed. Two-tailed $p$-values $< 0.05$ were considered significant throughout the entire analysis. Statistical analysis was performed using SPSS Statistics Version 27.0 for Macintosh (IBM Corporation, Armonk, NY, United States).

# RESULTS

## Patient Characteristics and Flow Parameters During Normothermic Machine Perfusion

During the study period, a total of 21 deceased donor livers were preserved via NMP. Night-time procedures were avoided and NMP time did not exceed 24 h. Seven (33.0%) were discarded after NMP, due to insufficient organ quality and performance. The median donor age was 61 years (48–70 years) and the median Donor Risk Index was 2.119 (1.610–2.435). Cold ischemia time (CIT) was 6 h (5–8 h) and total NMP time was 20 h (17–27 h). Six (28.6%) grafts derived from DCD donors (Maastricht category III), the remaining grafts from DBD donors. Median recipient MELD and Balance of risk (BAR) scores were 17 (8–21) and 7 (7–10), respectively. The median recipient age was 62 years (58–65 years). The median donor age of transplanted vs. discarded livers was 66 (56–70 years) vs. 46 years (43–56 years) ($p = 0.031$). All discarded liver allografts were from male donors, while 8 (57.1%) transplanted liver allografts were from female donors ($p = 0.011$).

The median ICU and total hospital stay were 6 (4–18) and 28 days (21–46), respectively. Six patients (42.9%) developed EAD, the median MEAF and L-Graft scores were 5.67 (4.02–6.90) and −0.73 (−1.33 – (−0.07)). Clavien-Dindo grade ≥3 complications occurred in 11 (78.6%) of 14 patients. Arterial complications occurred in two (14.3%) patients (one anastomotic stricture, one anastomotic aneurysm). Early (≤30 days) biliary complications were detected in six (42.9%) while late biliary complications (>30 days) in three (21.4%) patients. No patients developed non-anastomotic strictures, ischemic type biliary lesions (ITBL) or primary non-function. No patients were listed for re-transplantation. Two patients died due to multi-organ failure. The median follow-up was 106 (82–163) days. Recipient and donor demographics, as well as post-operative outcome parameters are described in **Table 1**. NMP hepatic artery and portal vein flows were >150 ml/min and >500 ml/min, for all livers during the entire course.

## Perfusion and Oxygenation of the Liver Parenchyma During Normothermic Machine Perfusion

The liver parenchyma was analyzed by HSI in cold-stored organs, at 1, 6, 12 h and at the end of NMP. The StO2, THI and NIR perfusion indices significantly increased ($p < 0.001$, $p < 0.001$ and

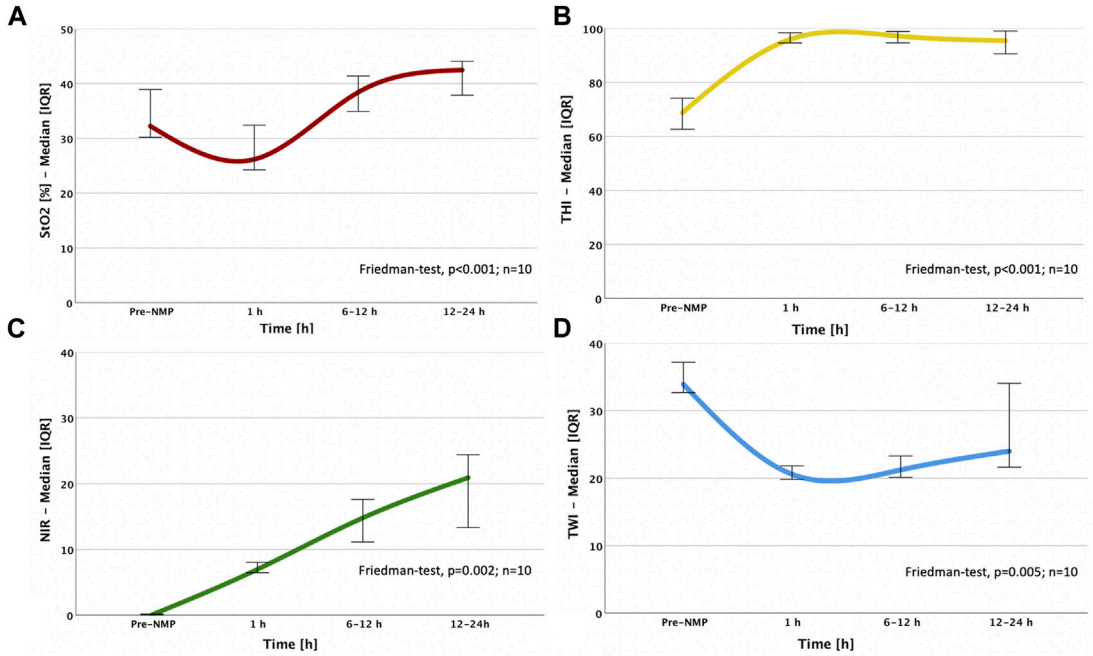



**FIGURE 2 |** Dynamic changes of HSI indices over NMP time: **(A)** StO2; **(B)** THI; **(C)** NIR; **(D)** TWI; the sample size ($n$ = 10) indicates that the Friedman test was calculated based on the ten livers perfused over 12 h and therefore, all NMP time points could be included in the statistical analysis. StO2, Tissue Oxygen Saturation; THI, Tissue Hemoglobin Index; NIR, Near-Infrared Perfusion Index; TWI, Tissue Water Index.

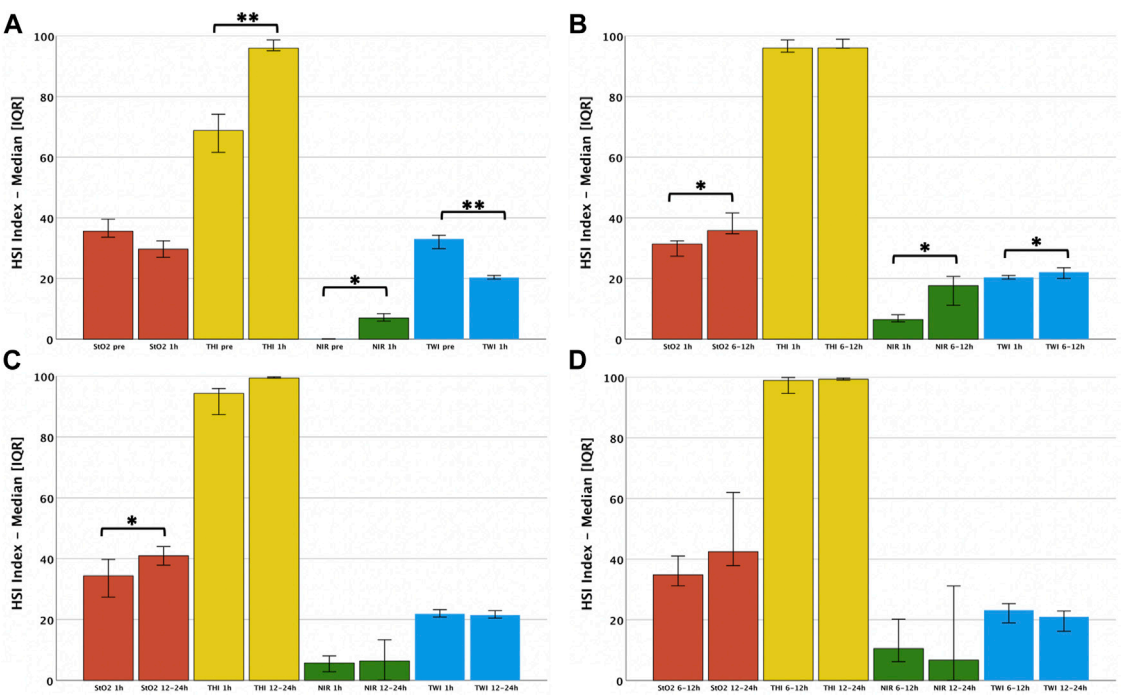



**FIGURE 3 |** Dynamics of HSI indices between single time points during NMP: **(A)** Pre-NMP to 1 h NMP; **(B)** 1 h NMP to 6–12 h NMP; **(C)** 1 h NMP to 12–24 h NMP; **(D)** 6–12 h NMP to 12–24 h NMP Sing-test: $^{*}p < 0.05$; $^{**}p < 0.01$ StO2, Tissue Oxygen Saturation; THI, Tissue Hemoglobin Index; NIR, Near-Infrared Perfusion Index; TWI, Tissue Water Index.

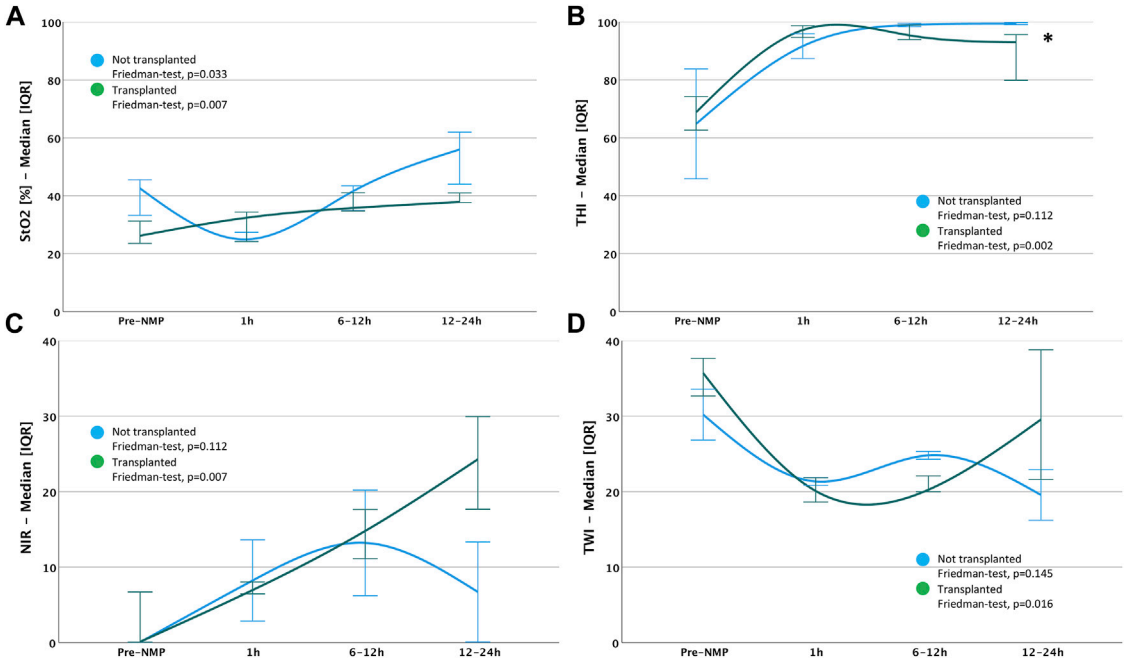



**FIGURE 4 |** Differences in dynamics of HSI indices between transplanted and not-transplanted liver grafts: **(A)** StO2; **(B)** THI; **(C)** NIR; **(D)** TWI Mann-Whitney-test: $^{*}p < 0.05$; $^{**}p < 0.01$ StO2, Tissue Oxygen Saturation; THI, Tissue Hemoglobin Index; NIR, Near-Infrared Perfusion Index; TWI, Tissue Water Index.

$p = 0.002$ respectively), while the TWI drastically decreased ($p = 0.005$) during the observational period (**Figure 2**; **Supplementary Table S1**). In the interval between static cold storage (pre-NMP) and 1 h NMP, we observed a significant augmentation of the THI and NIR (69 vs. 96, $p < 0.001$ and 0 vs. 7, $p = 0.003$, respectively), while the TWI dropped (33 vs. 20, $p < 0.001$). Contrarily, StO2 mainly remained constant. The dynamics of perfusion and oxygenation over the entire NMP period (between 1 h and 12–24 h) illustrated a significant augmentation of StO2 (31 vs. 39, $p = 0.006$), while the remaining HSI parameters remained stable. A longitudinal assessment of the tissue during NMP showed a substantial increase of the relative blood oxygenation StO2 (31 vs. 41, $p = 0.008$), the NIR perfusion index (7 vs. 17, $p = 0.008$) and the water distribution (TWI) (20 vs. 21, $p = 0.008$) during the first 6 h of NMP, while HSI values remained stable after this time (**Figure 3**; **Supplementary Table S2**).

## Discrimination of HSI Dynamics in Transplanted and Discarded Liver Grafts

Liver allografts subjected to NMP and transplantation revealed a significant escalation of the StO2, THI and NIR perfusion index ($p = 0.007$, $p = 0.002$ and $p = 0.007$, respectively) over the entire observational period, while the tissue water concentration (TWI) drastically decreased ($p = 0.016$). Livers undergoing NMP without subsequent transplantation also displayed a significant augmentation of the relative blood oxygenation (StO2%) ($p = 0.033$). However, the other HSI parameters remained mainly constant during the study period. Notably, at the end of perfusion (12–24 h), a significantly higher hemoglobin concentration (THI) in the superficial tissue layers was seen in discarded, compared to transplanted livers ($p = 0.036$). In contrast, StO2, THI, NIR perfusion index and TWI parameters did not differ during the early course of NMP (**Figure 4**; **Supplementary Tables S3, S4**). Discriminations of HSI findings between livers from DBD vs. DCD and SCD vs. ECD, as well as livers with sufficient vs. insufficient lactate clearance during the first 6 hours of NMP were performed. The **Supplementary File** displays these additional findings (**Supplementary Figures S2–S4**; **Supplementary Tables S5–S10**).

## Correlation of HSI Indices With Perfusion Parameters During Normothermic Machine Perfusion

There is currently limited evidence about the predictive value of individual perfusion parameters (12). Several biomarkers have been proposed to determine optimal clinical and metabolic liver responses during *ex vivo* NMP, including perfusate lactate clearance, or maintenance of a stable perfusate pH value (31). Lactate has traditionally been used as a marker of sepsis. Lactatemia can subsequently develop in tissue hypoxia (31). In this context, the liver is responsible for removing about 50% of circulating serum lactate, which rises in the liver in case of reduced blood flow/oxygen delivery. In line with the consideration that lactate should be interpreted as a surrogate marker of hypoxic injury and impaired hepatocyte functionality (32–34), our data displayed a negative correlation of increasing lactate values at 12 h NMP with a high NIR perfusion index between 12 and 24 h NMP and with an improved delta NIR

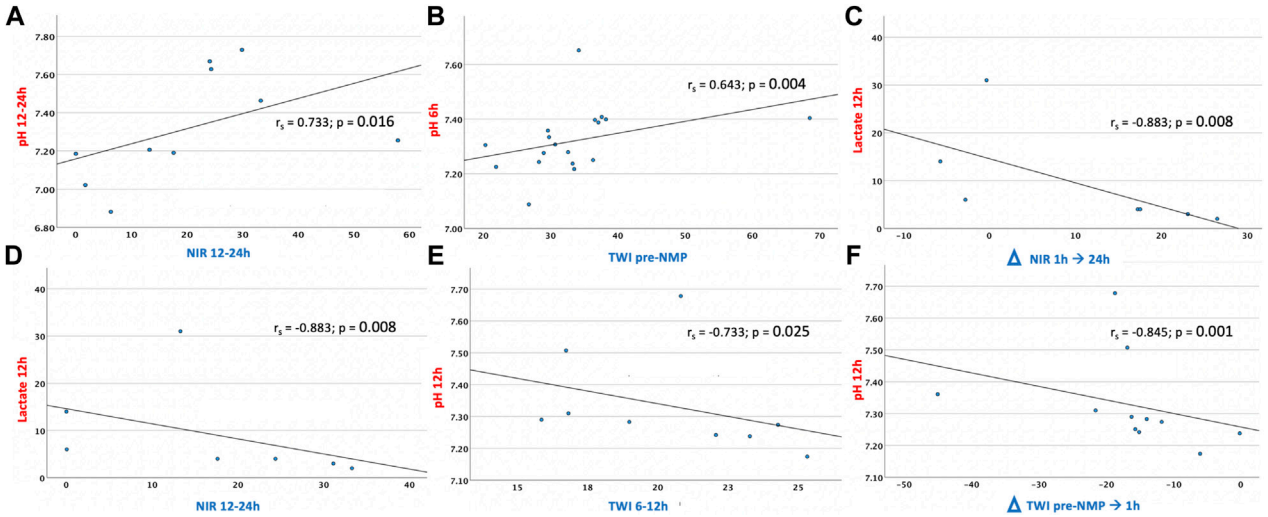



**FIGURE 5 |** Significant correlations of HSI indices and clinical applied perfusion parameters: **(A)** NIR 12–24 h NMP/pH 12–24 h NMP; **(B)** TWI pre-NMP/pH 6 h NMP; **(C)** Delta NIR 1–24 h NMP/Lactate 12 h NMP; **(D)** NIR 12–24 h NMP/Lactate 12 h NMP; **(E)** TWI 6–12 h NMP/pH 12 h NMP; **(F)** Delta TWI pre-NMP-1h NMP/pH 12 h NMP Spearman´s correlation: $r_s$, Spearman's Rank Correlation Coefficient; *$p < 0.05$; **$p < 0.01$ NIR, Near-Infrared Perfusion Index; TWI, Tissue Water Index.

perfusion index between 1 and 24 h (rs = −0.883, $p = 0.008$ for both). The perfusate pH has been introduced as a viability criterium in the context of NMP, given the association with lactic acidosis, most commonly resulting from an imbalance between oxygen delivery and oxygen demand (12). Concomitant to this assumption, our analysis revealed a positive correlation of perfusate pH with the NIR perfusion index over 12 h NMP (rs = 0.733, $p = 0.016$). The TWI concomitated a decrease in lactatemia and the rising pH. Liver oedema and the related parenchymal damage as detected with HSI during cold storage decreased during NMP. In accordance, the TWI between 6 and 12 h and between cold storage and 1 h NMP were negatively associated with the pH at 12 h (rs = −0.733, $p = 0.025$ and rs = −0.845, $p = 0.001$, respectively), while a high TWI during static cold storage correlated with a high pH at 6 h (rs = 0.643, $p = 0.004$), (**Figure 5**; **Supplementary Tables S11–S13**). These findings suggest that the NIR perfusion index and the TWI are potential markers to estimate the severity of impaired perfusion and oxygenation in livers during NMP.

## DISCUSSION

This pilot study was conducted with the intent to investigate HSI in the clinical setting of liver NMP. NMP allows to push the boundaries of organ transplantation, including the use of ECD grafts and longer preservation times (12, 13, 15, 35–37). HSI represents a user friendly imaging technology allowing for a quick and contactless, real-time viability assessment (22). *In vivo*, HSI can detect alterations at the early stages of NMP. While intra-operative haemodynamic monitoring has been limited to systemic measurements, a more organ-specific approach reflecting local oxygen delivery and microcirculatory perfusion has gained interest (38–40). In the field of hepatobiliary surgery, different imaging techniques were tested in order to evaluate liver parenchymal perfusion (41, 42). Indocyanine green fluorescence was examined as a technology aiding with intraoperative navigation, useful to detect patients at risk for developing EAD after LT (42). Moreover, this method may be utilized as tool to define boundaries of ischaemic areas by capillary flow diffusion in gastrointestinal surgery (38). Intraoperative changes in the oxygenation state of liver grafts were previously measured by near infrared spectroscopy. Mean hepatic oxygen saturation of hemoglobin in the liver was positively correlated with portal flow rate, indicating heterogeneous tissue oxygenation. This parameter was also predictive of EAD (43). Sidestream dark field imaging, a microscopic technique using polarized light to visualize erythrocytes through capillaries, was experimented as non-invasive method to visualize the microvessel architecture (38). In contrast to commonly used methods for determining the oxygenation status, HSI allows a pixel-wise analysis of chemical changes. The additional information on oxygenation status and perfusion quality, might facilitate the decision-making process in transplantation (18–20, 22, 23, 30). Currently utilized HSI parameters like StO2, NIR perfusion index and THI might be of lesser importance if measured during cold storage. However, in the context of MP, HSI may provide useful data on organ viability and performance (22). Moreover, the continuous monitoring of liver micro-perfusion, oxygenation and water content offers an early identification of functional/technical limitations during MP. For the entire observational period, we observed a significant increase in oxygen saturation, tissue hemoglobin concentration and micro-perfusion, while the organ water amount drastically diminished. Furthermore, a subgroup discrimination between transplanted and discarded liver

allografts showed an enhanced micro-perfusion in transplanted grafts, mainly after 6–12 h NMP. We observed that tissue oxygenation and micro-perfusion are specifically augmented during the first 12 h of NMP, while lesser dynamic changes were displayed in the late phase of NMP. Current liver graft evaluation is either based on scoring systems involving donor and recipient parameters, or on the invasive assessment of the parenchyma (44–51). Histopathologic examination of liver biopsies represents the current gold standard in the evaluation of liver quality in transplantation, however, several limitations such as time requirement, work-up procedure, reproducibility, intraoperative variance, inappropriate sampling, as well as the invasive nature of the retrieval represent important limitations. Further, histopathology may not always be a reliable indicator of graft quality, since this procedure only captures a snapshot of the morphological but not the functional condition (5). Other assessment technologies include perfusate/bile flow biomarkers as well as hydrodynamic parameters (11, 12). It remains to be determined, if they can be used as long-term indicators of graft outcomes (11). For livers rejected for transplantation based on particular viability criteria, no postoperative data are available and the direct comparison remains elusive (12). A decision-making process based on NMP endpoints poses the risk of incorrectly discarding organs suitable for transplantation. A definitive viability validation would require a well-powered multicenter randomized controlled trial (11). In an attempt to assess if HSI indices correspond with the perfusate biomarkers, our primary findings suggest, that NIR and TWI align with lactate and pH, considered as viability assessment markers during NMP. Based on the limited number of cases analyzed in this study, no conclusions toward an immediate clinical application can be drawn. HSI cannot replace histopathology or the viability markers currently applied. While clinical endpoints in LT trials such as EAD, MEAF and L-Graft score were applied in this study, the restricted number of transplanted patients and the selection applied through assessment during NMP did not permit the identification of discrimination towards the outcome by HSI.

The strengths of the HSI technology as applied during NMP are the immediate applicability and the comprehensive assessment of the perfusion state of an organ over the entire exposed surface (22). Integrating of a real-time imaging procedure into a clinical MP setting would require optimal acquisition distance settings and automated use under sterile conditions (22). Further to the use during NMP, utilization during donor surgery for quality assessment before cold perfusion and procurement could be of interest (30).

In addition to the small cohort analyzed in this study, the different perfusion times and the overall heterogeneity of the liver grafts represent apparent limitations. Further to these, HSI has a relatively low tissue penetration depth, which precludes the detection of injuries in deeper regions, or the potential transcutaneous measurement after transplantation. All in all, HSI during NMP appears promising and feasible and its apparent simplicity makes it attractive for clinical use, but validation in large clinical trials is needed before establishing routine application. All analyses are explorative and $p$-values $\leq 0.05$ were termed significant for descriptive reasons only.

To the best of our knowledge, HSI has not yet been applied previously in the field of liver NMP. We herein proved the technical feasibility of the combination of HSI and NMP. This real-time perfusion imaging may contribute to pre-transplant viability assessment.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The study protocol was approved by the review board of the Medical University of Innsbruck.

## AUTHOR CONTRIBUTIONS

Conceptualization: MF and SS; Data curation: MF; Formal analysis: MF and LL; Investigation: MF; Methodology: MF, LL, JH, GO, and RS; Project administration: MF and SS; Resources: MF and SS; Software: MF and LL; Supervision: SS; Validation: MF and SS; Visualization: MF, LL, JH, GO, MP, BC, RO, TR, AW, MM, CM, PZ, JP, TH, DÖ, RS, and SS; Writing—original draft: MF, LL, and SS; Writing—review and editing: MF, LL, JH, GO, MP, BC, RO, TR, AW, MM, CM, PZ, JP, TH, DÖ, RS, and SS.

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## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10355/full#supplementary-material

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REVIEW
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# Sense and Sensibilities of Organ Perfusion as a Kidney and Liver Viability Assessment Platform

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Predicting organ viability before transplantation remains one of the most challenging and ambitious objectives in transplant surgery. Waitlist mortality is high while transplantable organs are discarded. Currently, around 20% of deceased donor kidneys and livers are discarded because of "poor organ quality", Decisions to discard are still mainly a subjective judgement since there are only limited reliable tools predictive of outcome available. Organ perfusion technology has been posed as a platform for pre-transplant organ viability assessment. Markers of graft injury and function as well as perfusion parameters have been investigated as possible viability markers during *ex-situ* hypothermic and normothermic perfusion. We provide an overview of the available evidence for the use of kidney and liver perfusion as a tool to predict posttransplant outcomes. Although evidence shows post-transplant outcomes can be predicted by both injury markers and perfusion parameters during hypothermic kidney perfusion, the predictive accuracy is too low to warrant clinical decision making based upon these parameters alone. In liver, further evidence on the usefulness of hypothermic perfusion as a predictive tool is needed. Normothermic perfusion, during which the organ remains fully metabolically active, seems a more promising platform for true viability assessment. Although we do not yet fully understand "on-pump" organ behaviour at normothermia, initial data in kidney and liver are promising. Besides the need for well-designed (registry) studies to advance the field, the catch-22 of selection bias in clinical studies needs addressing.

OPEN ACCESS

**Keywords: kidney transplantation, liver transplantation, hypothermic perfusion, machine perfusion, organ preservation, normothermic perfusion, organ viability assessment**

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## INTRODUCTION

One of the underlying causes of the perpetuating organ shortage is the discarding of transplantable organs based on "poor organ quality". Currently, up to 20% of kidneys and 10% of livers that are recovered in the United states are not transplanted (1). Eurotransplant data show similar figures for kidney with considerably lower utilization rates for livers donated

**Abbreviations:** DCD, donation after circulatory death; DBD, donation after brain death; DGF, delayed graft function; PNF, primary non function; GST, glutathione S-transferase; H-FABP, heart-type fatty acid binding protein; NGAL, neutrophil gelatinase-associated lipocalin; FMN, flavin mononucleotide.

after circulatory death (DCD) compared to those donated after brain death (DBD) (2). A major contributor to organ discard is the fact that organ quality and viability remain difficult to predict accurately (1). With the increasing use of DCD kidneys and livers, the need for reliable pre-transplant viability assessment has become even more important. Indeed, DCD kidneys suffer from higher rates of delayed graft function (DGF) and primary non function (PNF) leading to a significant morbidity and mortality risk for the recipient (3, 4). DGF is associated with an increased risk of acute rejection, longer in hospital stay, higher cost and lower graft survival (5, 6). Higher-risk liver grafts, especially those from DCD donors, suffer higher incidences of PNF and intrahepatic cholangiopathy ultimately leading to higher graft failure rates compared to DBD livers (7, 8).

While with static cold storage, only limited options to assess organ function and viability are available, organ perfusion preservation has been posed as a platform for organ viability assessment (9). During organ perfusion, a perfusion solution is circulated through the vasculature, driven by a pump. The perfusion solution can be cooled or heated and, often with the help of a gas-exchanger, oxygenated. During hypothermic perfusion an acellular perfusion solution is used, in normothermic conditions an oxygen carrier is needed and this are often red blood cells. In this dynamic environment, the organ can be assessed real-time by evaluating perfusion parameters and injury markers (**Figure 1**). When the organ is metabolically active, markers of organ function can also be studied. As (patho)physiology involves a complex interplay of different cells, it is likely that true prediction of organ viability will need the assessment of more than a single parameter.

This review provides an overview of the available clinical evidence on the use of organ perfusion as a platform to predict kidney and liver viability before transplantation.

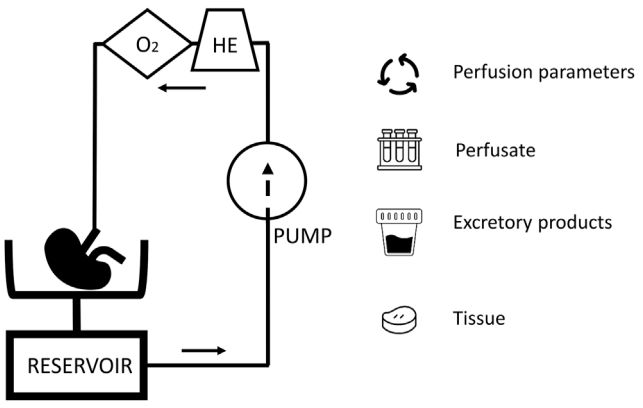


**FIGURE 1 |** A schematic overview of a kidney perfusion circuit: The presence of a heat exchanger (HE) and gas exchanger ($O_2$) depends on the perfusion mode. During perfusion, perfusion parameters, perfusate, excretory products (e.g., urine during normothermic perfusion), and tissue are available for viability assessment.

# KIDNEY

Hypothermic kidney perfusion became a clinical reality after much preclinical work in the 1960s by pioneers like F.O. Belzer (10–12). Due to refinement of preservation solutions good results with the cheaper and simpler static cold storage were obtained and kidney perfusion disappeared to the background. Nevertheless, hypothermic kidney perfusion has been reintroduced in clinical settings after it was shown to reduce the risk of DGF compared to static cold storage (13). Normothermic perfusion is being investigated in research settings with a first randomised trial underway (14).

## Pathophysiology of the Ischemic Injury

To assess kidney viability, understanding the pathophysiology of ischemia reperfusion injury is crucial. Every transplantation procedure is associated with ischemia reperfusion injury that impacts post-operative tissue injury and graft function. The biological pathways behind ischemia reperfusion injury describe functional and structural changes in the organ based on changes in cell metabolism (especially in the mitochondria). Various molecular mechanisms are active in ischemia reperfusion injury. There is the critical role of the anaerobic metabolism during ischemia, resulting in intracellular acidosis, ATP depletion, and failure of ion-exchange channels, setting the stage for reperfusion injury (15). Post-reperfusion, innate and adaptive immune responses are activated by reactive oxygen species and damage associated molecular patterns, resulting in a sterile inflammation (16–19). Ischemia reperfusion injury causes structural and functional damage to renal tubules by inducing tubular cell death which manifests as a clinical spectrum of acute kidney injury ranging from transient acute kidney injury to primary non-function (PNF). When the transient acute kidney injury is severe enough, and the patient needs dialysis in the first week after transplantation, delayed graft function (DGF) occurs. An association between DGF and acute rejection has been reported (20) and this might affect long-term graft function as persistent inflammation in scarred areas after T-cell mediated rejection has been associated with chronic scarring and fibrosis due to maladaptive injury responses (21).

This injury process leaves marks, e.g., representing epithelial cell disruption and tubular injury that might be detected as biomarkers in the perfusate (22, 23).

## Hypothermic Kidney Perfusion

In hypothermic conditions, options to assess kidney function are limited. Indeed, the metabolic rate at 4°C is limited to 10% of that at physiological temperature with a 40% lower rate of chemical reactions (24). Furthermore, in the majority of cases there is no active oxygenation during hypothermic kidney perfusion in which case aerobic metabolism is not supported (25). Focus has therefore been on identifying associations between markers of injury and post-transplant outcome.

### Perfusate Injury Markers

Injured tubular cells release proteins into the perfusate during hypothermic perfusion where they can be detected. Today, there

is good quality evidence that perfusate injury markers should not be used to assess viability of kidneys during standard hypothermic organ perfusion. In a systematic review, Guzzi et al. summarized the findings of 29 clinical studies assessing the association between PNF, DGF, and long-term graft survival and perfusate injury markers measured during hypothermic perfusion of DCD and DBD kidneys (26). Only four studies were identified as good quality prospective studies (27–30).

Glutathione S-Transferase (GST) concentrations during hypothermic perfusion have been well-studied with an independent association with DGF, however, the predictive accuracy of GST for DGF is moderate at best and no correlation with long-term outcome has been found (27–29). Similar to GST, perfusate lactate dehydrogenase independently associates with DGF and PNF but predictive accuracy is low (27, 31). While heart-type fatty acid binding protein (H-FABP) showed to be an accurate biomarker of kidney injury after transplantation in preclinical studies (32), clinical studies showed only moderate predictive power of perfusate H-FABP for DGF (27, 31). Neutrophil gelatinase-associated lipocalin (NGAL), released by renal tubular cells in response to ischemic injury, is a recognized biomarker of acute kidney injury (26, 30, 31, 33), but no reliable association of NGAL release during hypothermic perfusion with post-transplant outcomes has been found (31). Some studies assessing perfusate lipid peroxidation and perfusate interleukin-18 (a pro-inflammatory cytokine) show little promise as viability markers (28, 31). Associations between other biological parameters, like lactate, N-acetyl-D-glucosamine, Kidney injury molecule 1, and others were either not significant, not accurate, or described in single studies. Growing interest in microRNA's in multiple disease processes draws our attention for their use in viability assessment during hypothermic perfusion (34).

Whether predictive accuracy of perfusate injury markers is improved when the perfusate is actively oxygenated, is not known and subject of ongoing research (www.cope-eu.org). This is an important question as hypothermic oxygenated perfusion is already finding its way into clinical practice (e.g., the Netherlands) after it was recently shown that older DCD kidneys benefit from active oxygenation in the cold (25).

### Perfusion Parameters

Since the early days of hypothermic kidney perfusion, it has been hypothesised that kidney viability is associated with perfusion parameters. Indeed, at a stable perfusion pressure, a lower renal flow indicates a higher intrarenal resistance and reflects increased vascular injury or interstitial oedema. A correlation between perfusion parameters and DGF and PNF has been shown in retrospective studies that suffered from selection bias as kidneys were discarded based upon perfusion parameters(35–38). A large randomized controlled prospective trial, without selection bias, has shown that renal resistance at the end of hypothermic perfusion is an independent risk factor for DGF and 1-year graft survival but the predictive accuracy is low (39). These findings have been confirmed by Parikh et al. in a large prospective cohort (30).

While perfusion parameters, such as renal resistance on the pump, provide additional information on quality of the graft, they should not be used as clinical decision making tools. When the perfusate is actively oxygenated, endothelial cell integrity might be improved. This might change perfusion parameters and their predictive power which is the subject of ongoing research (www.cope-eu.org). In addition, in relating Ohm's Law to fluid flow (**Eq. 1**), it is important to remember that exact flow or resistance values will depend not only on the kidney but also on the perfusion device (pressure or flow driven) and the settings (e.g., pump pressure chosen) that are used. Perfusion parameter read-outs, and therefore any defined thresholds, are not necessarily transferable from one perfusion device to the other.

$$\Delta P/F = R \quad (1)$$

where ΔP is the driving pressure of perfusion pressure as set by the pump (in mmHg) in case of a pressure-controlled system, F is renal artery flow (ml/min), and R is the renal resistance (mmHg/mL/min).

## Normothermic Kidney Perfusion

The advantages of normothermic perfusion with regard to viability assessment relate to the use of a perfusate based on oxygenated red blood cells or oxygen carriers at physiological temperatures, meaning the graft can be fully metabolically active. In addition to assessing injury markers and perfusion parameters, normothermic perfusion would therefore allow to evaluate kidney function. Indeed, e.g., creatinine can be added to the perfusate and in this way a creatinine clearance from the perfusate over time can be calculated. In contrast to hypothermic perfusion, normothermic perfusion requires considerable technical expertise with the potential of dramatic consequences in case of technical failure as the graft would be exposed to warm ischemia.

Normothermic perfusion as mostly been developed to be used as a "resuscitation tool." This means a short period (1–2 h) of normothermic perfusion immediately before transplantation following static cold storage (40). Results of a first randomised controlled phase II trial assessing the effectiveness of normothermic perfusion as a resuscitation tool compared to static cold storage in controlled DCD kidneys are awaited (41). Meanwhile, experimental data show the feasibility, and possible benefit, of prolonged normothermic perfusion preservation starting immediately after kidney procurement (42, 43).

Initial evidence that normothermic perfusion could be used as a platform to assess viability pre-transplantation was provided by Hosgood et al. when a discarded kidney was transplanted after evaluation during a short period of normothermic perfusion (44). In a further series of kidneys, that were considered unsuitable for transplantation, a kidney quality score during normothermic perfusion was derived. This score is based on the macroscopic aspect of kidneys during perfusion, the arterial flow, and volume of urine production. Kidneys with a score ≥3 out of 5 were considered transplantable (**Table 1**) (44–46). The clinical studies leading up to development of the score suffered from selection bias because not all kidneys were transplanted. The score remains

**TABLE 1 |** Kidney quality assessment score as defined by Hosgood et al.

| Kidney quality assessment score parameter | Point |
|---|---|
| Macroscopic assessment | |
| Grade I: Excellent perfusion (global pink appearance) | 0 |
| Grade II: Moderate perfusion (patchy appearance) | 1 |
| Grade III: Poor perfusion (global mottled and purple/black appearance) | 2 |
| Renal Blood flow | |
| Threshold ≥50 ml/min/100 g | 0 |
| Threshold <50 ml/min/100 g | 1 |
| Total urine output | |
| Threshold ≥50 ml/min/100 g | 0 |
| Threshold <50 ml/min/100 g | 1 |

*Scores range from 1 to 5, 1 indicating the least injury to 5 the most severe. Reproduced from (95) with permission under Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0).*

to be validated in large series. In that light, it is important to realise that the majority of the evidence on the use of normothermic perfusion as a viability assessment platform has been obtained from kidneys that were perfused on a custom made circuit. Therefore, the threshold flow values as proposed by Hosgood et al. depend on the perfusion pressure (**Eq. 1**) (47) and are not directly transferable to settings using different perfusion pressures. Also, although the physical properties of the filter remain the same when a healthy kidney is perfused *ex situ*, the perfusate composition and perfusion pressures (pump pressures) will change oncotic and hydrostatic pressures and therefore influence filtration and ultimately "urine" production during kidney perfusion (48). Adding tubular injury markers to the kidney quality assessment score might improve its accuracy and this has been explored (49).

Importantly, Schutter et al. recently showed that early functional assessment may not reflect actual physiology. In a pig model of normothermic perfusion kidneys were mainly centrally perfused in the first 2 h of perfusion, while it took time for the outer cortex to reach its physiological dominant perfusion state (50). Before that, the functionally important renal cortex appeared severely underperfused, meaning longer perfusion times might be needed for reliable viability assessment. This point was also raised by Hosgood et al. who recently published a report on a pair of kidneys that had passed the quality assessment test but still developed PNF (51).

# LIVER

In contrast to kidney perfusion, liver perfusion has not yet reached the stage of wide-spread clinical implementation. Building on the pioneering work of Starzl and others (52–54), both hypothermic and normothermic liver perfusion are now the topic of several clinical studies investigating the value of perfusion as a preservation method but also as a platform for organ viability assessment. The need for optimized preservation and reliable viability assessment is high as an increasing number of DCD livers, at higher risk of PNF and post-transplant cholangiopathy, are offered for transplantation (7, 8). Like in the kidney, ischemia reperfusion injury in the liver causes cellular injury. Hepatocellular injury leads to a spectrum of clinical presentation, marked by increased transaminases. When severe, early allograft dysfunction occurs which is associated with increased mortality and graft loss (55–57). When irreversible, in the case of PNF, recipient mortality is high (58). While the liver regenerates, it remains difficult to assess what level of injury a liver can tolerate while still providing life sustaining function to the recipient. Furthermore, cholangiocyte injury and injury to the peribiliary plexus can lead to post-transplant cholangiopathy, a vexing complication leading to increased morbidity and reduced graft survival (59, 60). Liver perfusion offers a window of opportunity to gather additional information on both the level of injury sustained and the remaining liver function.

## Hypothermic Liver Perfusion

Like in kidney, options to assess liver function during hypothermic perfusion are likely limited because metabolic rate is severely reduced. However, in contrast to kidney, hypothermic liver perfusion is nearly always actively oxygenated and mitochondrial respiration continues (61). A short period of hypothermic oxygenated perfusion of the liver has been described to have immunomodulatory effects, preserve the endothelial cell glycocalyx and the peri-biliary vascular plexus and glands, and improve post-transplant outcomes (61–65). Recent studies have shown less post-transplant hepatocyte injury and reduced cholangiopathy rates with hypothermic oxygenated perfusion (65, 66).

### Perfusate Injury Markers

In the first clinical series of hypothermic liver perfusion, Guerrera et al. already described a correlation between perfusate and post-transplant serum transaminases (63, 67). These findings were confirmed by Patrono et al. but none of the injury markers were independently associated with outcomes (68). The detection of mitochondrial flavin mononucleotide (FMN), an integral part of mitochondrial complex I, in the perfusate might be a surrogate marker for impaired cellular energy production.

There is evidence that the release of FMN occurs independently of the other hepatocellular enzymes (69). A strong correlation of FMN with post-transplant peak transaminases and coagulation factors was found in addition to correlation of FMN with hospital stay, post-transplant complications, and graft failure within 3 months (69). FMN was also predictive of early allograft dysfunction (69). The correlation of FMN with early allograft dysfunction was also described by Patrono et al. though not found to be significant (62). Currently there is too little evidence to conclude whether injury markers measured during hypothermic oxygenated liver perfusion are helpful in predicting viability. With the completion of the first large trials, further evidence on the proper value of these markers is likely to become available in the near future (NCT01317342) (65).

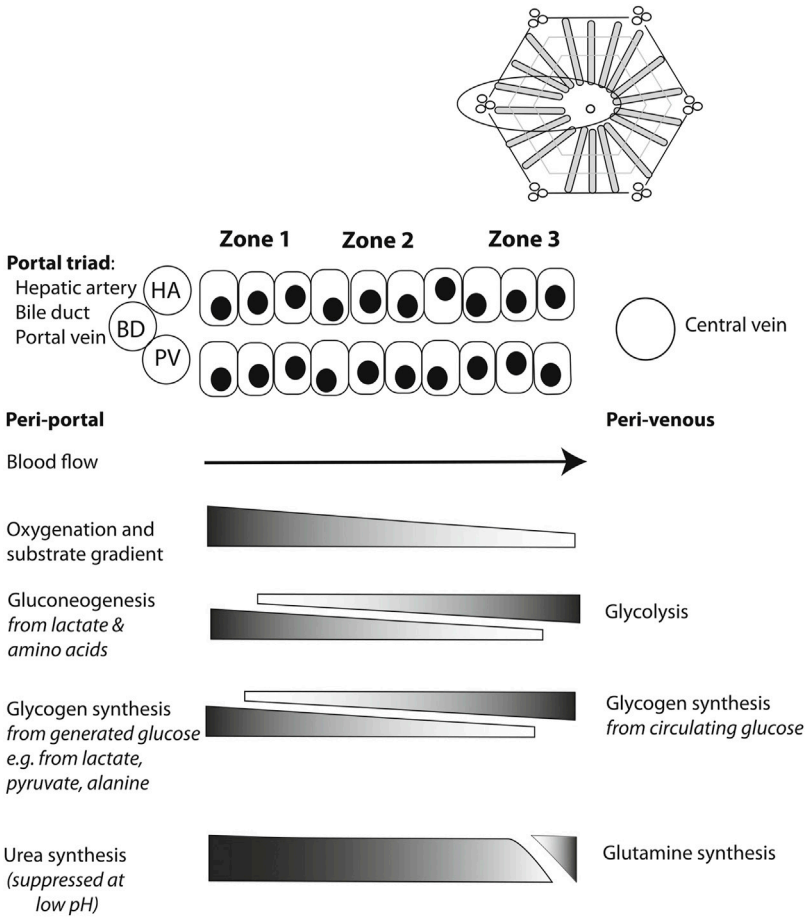


**FIGURE 2 |** Schematic overview illustrating metabolic liver zonation with reference to glucose and ammonia metabolism. Blood entering the liver lobule *in vivo* through hepatic artery (HA) and portal vein (PV) branches is rich in hormones, nutrients and oxygen. Periportal (zone 1) metabolic processes will include those requiring such conditions, while perivenous (zone 3) hepatocytes may preferentially include those metabolic processes that are less dependent on high levels of oxygen, for example, or those requiring products made in the periportal hepatocytes, such as urea. Reproduced from (84) with permission under Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0).

## Perfusion Parameters

Very little is known about the relationship between hepatic artery or portal vein flow and resistance during hypothermic oxygenated liver perfusion. Like in the kidney, an increase in flow over time and a decrease of hepatic artery resistance are observed (65, 70). Patrono et al. observed a slower decrease in hepatic artery resistance in livers that developed early allograft dysfunction but larger series need to be analysed to understand the value of perfusion parameters as predictor of post-transplant viability (70).

## Normothermic Liver Perfusion

In contrast to normothermic kidney perfusion, normothermic liver perfusion is more widely studied. In a randomised study, normothermic liver perfusion has been shown to reduce post-transplant graft injury, measured by hepatocellular enzyme release, compared to cold storage (71). Despite these findings, no differences were seen in graft or patient survival, hospital stay and bile duct complications. Remarkably, a 50% lower rate of organ discard was noticed in the perfusion arm, confirming the need for pre-transplant viability assessment to increase the number of liver transplants. It must be noted that this trial was not designed to address organ utilization and selection bias because of the non-blinded nature might have been present. Trials with organ utilization as primary outcome should randomise as early in the process as possible, ideally at the time of the organ offer or even at the time of listing the patient for transplant (72). A short period of normothermic liver perfusion to test viability has been explored by a number of groups (73–82). Encouraging results have led to the implementation of normothermic liver perfusion as a viability assessment tool in expert centres although there is considerable variability in both indications and assessment criteria (83). Because the liver is metabolically active, liver function might be assessed during normothermic perfusion. In this light it is important to remember that both hepatocytes and

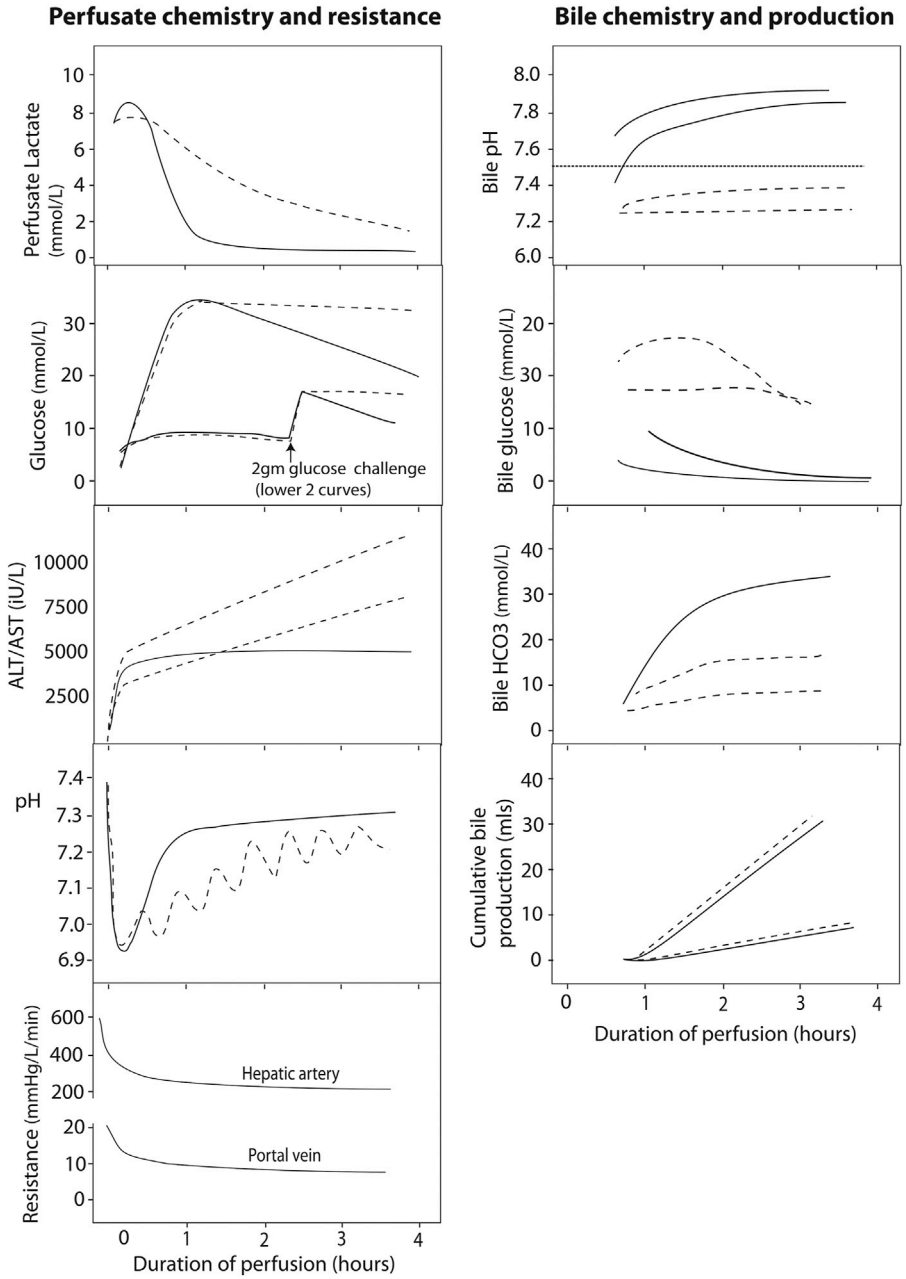


**FIGURE 3 |** Typical normothermic perfusate profiles of liver perfusion. The figure shows schematic graphs with typical biochemical and resistance profiles during normothermic liver perfusion with an interpretation regarding viability given according to current state of knowledge. Profiles of viable hepatocellular compartment livers are denoted by solid black lines, while dashed lines denote grafts where viability might be in doubt, due to a slow lactate clearance, persistently raised perfusate glucose, rising perfusate transaminase concentration or requirement for continued bicarbonate support to maintain pH. The graphs also show the different biochemical profiles of bile depending on the viability of the ducts, where viable cholangiocytes producing bile with an alkaline pH, low glucose (especially relative to the high perfusate glucose) and increasing bicarbonate levels. To date, there is no clinical evidence in support of bile production or hepatic resistance thresholds for viability. Reproduced from (84) with permission under Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0).

cholangiocytes need to be functioning for sustained graft function and survival.

## Markers of Hepatocyte Injury and Function

In assessing the hepatocyte compartment, the zonation of the hepatocytes helps when interpreting the meaning of several perfusate markers (84). As oxygen concentrations are the highest in the periportal zone, zone 1 hepatocytes are differentiated to carry out processes that require high oxygen concentrations (**Figure 2**). Near the central vein, zone 3 hepatocytes are adapted to the low oxygen concentrations that are present.

## Perfusate Transaminases

Perfusate transaminases (as opposed to postoperative systemic levels of transaminase) have been used to determine the viability of a particular graft for implantation. In viable livers, perfusate transaminases seem to plateau over time. Most livers will reach this plateau by 2 h (76, 77, 82) therefore continued transaminase increase is suggestive of ongoing injury during perfusion (**Figure 3**). It must be noted that transaminase levels may be influenced by the age of the donor, steatosis, ischemia time, among other factors (72). Perfusate transaminases should be normalized for liver weight and perfusate volume to allow comparability with other perfusion systems and different livers (72). Because aspartate aminotransferase may also rise from haemolysis on the circuit, alanine aminotransferase might be more representative of the degree of hepatocellular damage (76, 77, 85).

Perfusate transaminases seem to be correlated with post-transplant systemic levels of transaminases (77) though the usefulness of this correlation in helping predict outcome is unclear. Indeed, postoperative levels of transaminases are influenced by the perfusion itself and the large volume of perfusate (wash-out) (72). Additionally, bilirubin and INR seem to have a stronger predictive capacity for patient and graft survival compared to AST, indicating that hepatocyte injury with little involvement of the biliary tree has a more benign course (86). The usefulness of the current definition of early allograft dysfunction (using peak transaminases in the first week, total bilirubin and INR levels) (55) in case of livers transplanted after perfusion is unclear and the definition might need revisiting (86, 87).

### Perfusate Lactate

A slow clearance of lactate is associated with severe parenchymal injury where viability may be in doubt (71, 73, 77, 84, 85). Indeed, lactate metabolism occurs mainly in the periportal hepatocytes (zone 1), so a viable rim of zone 1 hepatocytes can metabolise the lactate in the relatively small volume of perfusate, even in the presence of severe parenchymal damage in zone 2 and 3 (**Figure 3**). Therefore, lactate is not recommended as a single viability marker.

### Perfusate Glucose

Glycogenolysis is an ATP-independent process that continues during cold storage, evidenced by increasing perfusate glucose levels early during normothermic perfusion (**Figure 3**). A normal level of glucose during normothermic perfusion may reflect minimal ischemia, but may point out glycogen exhaustion or extensive liver injury (77). Over time, a viable liver will re-incorporate this glucose into glycogen during perfusion (**Figure 3**) (77).

### Acid-Base Homeostasis During Perfusion

Regulation of the hepatic acid-base balance depends, among others, upon the differential metabolism of glutamine along the lobule (88). Healthy livers tend to have a better pH regulation and stabilisation (**Figure 3**). Analysing pH and the need for external regulation by bicarbonate replacement could help assessment viability of the hepatocyte compartment (76, 77).

### Coagulation Factors During Perfusion

In a preclinical study, severely injured livers have low perfusate levels of anticoagulant and coagulation factors compared to those that are minimally injured livers (89). Little information on the value of perfusate (anti)coagulation factors in human settings is available. Such proteins are detectable but no correlation between (anti)coagulation factors and severity of post-transplant injury has been shown (89, 90). Whether low factor concentrations are predictive of outcome remains to be investigated (89).

### Bile Production During Perfusion

Bile production is an important function of the hepatocyte and the volume of bile produced during normothermic perfusion has been associated with hepatocyte injury (91). However, the absence of bile production during perfusion is not necessarily a feature of a non-viable graft (71, 92).

### Markers of Cholangiocyte Injury and Function

The importance of assessing cholangiocyte viability was recently demonstrated by Mergental et al. who selected livers, thought unsuitable for transplantation on static cold storage, based on hepatocyte viability criteria. Of 31 initially discarded livers, 22 (71%) met hepatocyte viability criteria were successfully transplanted with no PNF cases. However, three out of ten (30%) DCD livers developed biliary complications requiring retransplantation (80). Indeed, while the hepatocyte is responsible for producing bile, the healthy cholangiocyte ensures an alkaline composition of bile with low glucose levels (**Figure 3**) (92, 93). Watson et al. and Matton et al. provide suggested cut off values for bile pH, glucose, and bicarbonate concentrations that need validation in large series (77, 78, 85, 94). As for kidney, clinical studies identifying these cut-off values suffer from selection bias as not all livers were transplanted, though pathological assessment of the intra-hepatic bile ducts of some of the non-transplanted livers were correlated with bile biochemistry (77).

### Perfusion Parameters

Hepatic artery and portal vein resistance decrease quickly during perfusion to reach a steady state (**Figure 3**). Little is known about the meaning of these findings though Watson et al. observed no correlation of these parameters with outcome or biochemical markers of hepatocellular injury (77).

## CONCLUSION

Organ perfusion has demonstrated it can serve as a viability assessment tool with current evidence suggesting normothermic perfusion is better suited. Indeed, although good quality evidence shows that injury markers and perfusion parameters during hypothermic kidney perfusion predict graft outcome, these markers lack the predictive accuracy needed in clinical practice. Little is known about the association of liver perfusate injury markers and perfusion parameters during hypothermic perfusion and this deserves further investigation. The recent large clinical trials, where livers were transplanted regardless of perfusate markers, provide valuable cohorts.

Normothermic perfusion, with a metabolically fully active organ, has been shown to be able to select viable grafts from

those that were thought unsuitable for transplantation. Nevertheless, to date, there are no clear, validated and accurate markers to allow routine implementation of the technique in clinical settings. Data from larger studies are needed. Ideally, selection bias should be avoided by transplanting all organs that are perfused and blinding clinical teams to the viability assessment findings. However, as these studies would involve organs of doubtful viability, and therefore a reasonable chance of post-transplant failure, this obviously poses ethical concerns exposing patients to an increased risk of complications. One way would be to accumulate cases in large international registries so that a high enough number of cases with an undesirable outcome can be analysed together.

## AUTHOR CONTRIBUTIONS

IJ conceptualized the paper. LV and IJ reviewed the literature. LV drafted the manuscript. IJ performed the critical revision.

## CONFLICT OF INTEREST

IJ has received speaker's fees from XVIVO perfusion paid to her institution.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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# Biliary Viability Assessment and Treatment Options of Biliary Injury During Normothermic Liver Perfusion—A Systematic Review

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In recent years, significant progress has been made in the field of liver machine perfusion. Many large transplant centers have implemented machine perfusion strategies in their clinical routine. Normothermic machine perfusion (NMP) is primarily used to determine the quality of extended criteria donor (ECD) organs and for logistical reasons. The vast majority of studies, which assessed the viability of perfused grafts, focused on hepatocellular injury. However, biliary complications are still a leading cause of post-transplant morbidity and the need for re-transplantation. To evaluate the extent of biliary injury during NMP, reliable criteria that consider cholangiocellular damage are needed. In this review, different approaches to assess damage to the biliary tree and the current literature on the possible effects of NMP on the biliary system and biliary injury have been summarized. Additionally, it provides an overview of novel biomarkers and therapeutic strategies that are currently being investigated. Although expectations of NMP to adequately assess biliary injury are high, scant literature is available. There are several biomarkers that can be measured in bile that have been associated with outcomes after transplantation, mainly including pH and electrolytes. However, proper validation of those and other novel markers and investigation of the pathophysiological effect of NMP on the biliary tree is still warranted.

**Keywords: normothermic machine perfusion, liver perfusion, biliary injury, biliary complication, biliary strictures, viability assessment, liver transplantation**

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## INTRODUCTION

Due to demographic change, there is a greater need for organs and the proportion of organs from older or unhealthier donors in the donor pool is growing. This leads to an aggravation of the already existing organ shortage and amplifies the need to use organs from so-called extended criteria donors (ECD). ECD include, for example, elderly donors, livers with steatosis, or donors with other comorbidities. Organs from a donation after circulatory death (DCD) donor are categorized as ECD since organs experience a harmful period of warm ischemia prior to explantation and enter the cold storage period already with an energy debt (1). A limiting factor of using ECD liver grafts is their susceptibility to postoperative complications, especially ischemic cholangiopathies (2), which are difficult to treat and are a leading cause for re-transplantation (3, 4). The pathophysiological processes involved in these ischemic type biliary lesions (ITBL) are complex and despite extensive research not completely understood. Factors that contribute to ITBL are ischemia and reperfusion,

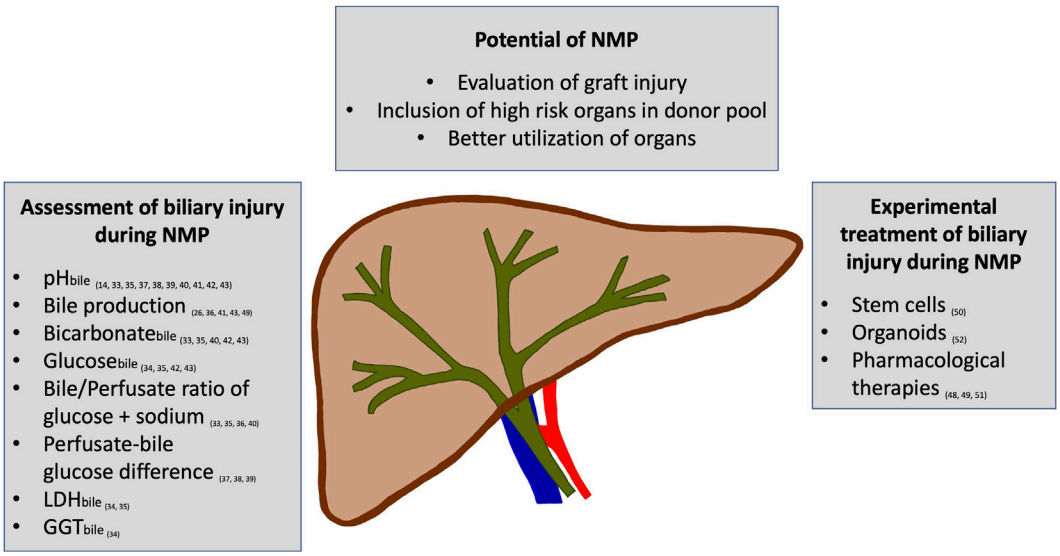


**FIGURE 1 |** An overview of the potential of normothermic machine perfusion (NMP) to assess and improve biliary injury is displayed. Several parameters have been reported to allow the assessment of biliary injury during NMP. Additionally, the first experimental studies on treating biliary injury during NMP have shown promising effects.

the associated inflammatory reaction, and the detrimental effect of non-physiologic bile composition in an already injured biliary system (4, 5). A certain degree of ischemic-reperfusion injury (IRI) is inevitable and can only be mitigated (6). Various approaches to diminish IRI in comparison to the standard preservation method of static cold storage (SCS) are currently in use. Many large transplant centers have implemented machine perfusion (e.g., hypothermic oxygenated perfusion (HOPE), normothermic regional perfusion (NRP), or normothermic machine perfusion (NMP)) to reduce organ injury. Machine perfusion aims to mitigate IRI by restoring the mitochondrial function prior to reperfusion or additionally ameliorating the injury by reperfusion of the organ in absence of immune cells (7–9). All machine perfusion strategies have shown a general benefit over SCS, however, they all have advantages and disadvantages depending on the indications they are used for. In several studies, HOPE and NRP have shown favorable effects on liver function after transplantation, including the development of ITBL (10, 11). A meta-analysis showed that HOPE was able to reduce the incidence of biliary strictures compared to SCS, while NMP was not (12). However, both HOPE and NRP are limited by their ability of organ assessment and treatment options. The implementation of NMP offers the chance for pharmacological treatment and viability assessment during perfusion (**Figure 1**) (13–16). The possibility of evaluating the biliary injury of a liver prior to transplantation or even treating it is thrilling. However, although NMP has found its place in the clinical routine, also because of its logistical benefits, literature on pathophysiological mechanisms and solid biomarkers to assess organ function are scarce. In this regard, the biliary tree is of high interest, as ITBL leads to increased morbidity and mortality of ECD organs. The benefit of HOPE or NRP is not based on assessment and there is currently no biliary-specific assessment marker that can be measured during HOPE. Therefore, the focus of this review is to summarize the available literature on the assessment and treatment options for biliary injury during NMP.

## ISCHEMIA-REPERFUSION AND BILIARY INJURY

The diverse cells of the liver are all in different ways susceptible to one or all phases of IRI. The sinusoidal endothelial cells (SECs) in the liver for example are especially susceptible to cold ischemia (17). During reperfusion, the reintroduction of oxygen leads to an expression of danger-associated molecular patterns (DAMPs) and cytokines by SECs, and an imbalance of vasodilators and vasoconstrictors results in impaired microcirculation. DAMPs can activate Kupffer cells that secrete cytokines like tumor necrosis factor α leading to platelet adherence at SECs sending them into apoptosis (18). Cholangiocytes can handle periods of anoxia quite well compared to hepatocytes. However, they produce reactive oxygen species after reoxygenation and harbor fewer antioxidants like glutathione to compensate. Thus, bile duct cells are more susceptible to injury suffered through reoxygenation (17). Extensive damage to the bile duct epithelium can be found in almost every transplanted liver. In addition, the biliary regenerative capacity has been shown to be a crucial factor for long-term outcomes (5). The peribiliary glands (PBG) and peribiliary vascular plexus (PVP) play a critical role in the viability of the biliary system. Impaired blood supply through the PVP due to injured endothelium affects the regeneration after ischemia. The biliary progenitor cells that proliferate after damage to the biliary epithelium are mainly located in PBG

**TABLE 1 |** Impact of NMP on biliary injury.

| Author | MP | Design | Aim | Major findings |
|---|---|---|---|---|
| **Preclinical animal models** | | | | |
| Op den Dries et al. (25) | Normothermic | Rat model<br>3 h preservation of 38 livers in 4 groups: non-DCD + NMP vs. non-DCD + SCS vs. DCD + NMP vs. DCD + SCS each followed by 2 h of *ex-situ* reperfusion<br>No report on systematic BD histology | Impact of MP on bile duct preservation in DCD and non-DCD rat livers | GGT + LDH in bile were lower in NMP group; bicarbonate in bile and $pH_{bile}$ higher in NMP group; ultrastructural changes most prominent in SCS-preserved DCD livers after reperfusion |
| Westerkamp et al. (26) | Hypothermic<br>Subnormothermic<br>Controlled oxygenated rewarming | Rat model<br>30 DCD livers in 4 groups<br>6 h of SCS (Ctrl) plus either 1 h of HOPE, SNP, or COR; followed by 2 h of *ex-situ* reperfusion<br>Scoring system by op den Dries et al | Impact of machine perfusion on bile duct injury comparing different perfusion temperatures | Machine perfusion groups showed lower levels of transaminases + LDH; higher mitochondrial activity; better biliary function: bile production, bicarbonate secretion, $pH_{bile}$; lower levels of biliary injury markers: $GGT_{bile}$ + $LDH_{bile}$; and less biliary epithelial injury in histological analysis |
| Boehnert et al. (27) | Normothermic acellular | Porcine model: 6 livers with 60 min WIT + 4 h SCS + 8 h NMP vs. 6 livers with 60 min WIT +12 h SCS vs. 60 min WIT + 4 h SCS; all with 12 h of whole blood reperfusion No report on systematic BD histology | Effects of NMP in porcine model of combined warm and cold ischemic injury with transplantation simulation | Reduced histologic biliary injury, reduced LDH in bile of the NMP group; higher bilirubin, phospholipids, and bile acids in bile of the NMP group |
| Liu et al. (28) | Normothermic | Porcine model<br>10 h of NMP of 5 DCD livers (60 min WIT) vs. 5 SCS livers, 24 h reperfusion with whole blood<br>Scoring systems by Hansen et al. + op den Dries et al | Impact of NMP on post-reperfusion outcomes in a transplant simulation model with DCD porcine livers | Biliary LDH and GGT higher in SCS; bicarbonate content in bile lower in SCS.<br>Ki67 absent, and von Willebrand factor higher in SCS, indicating reduced biliary regeneration and increased platelet activation in SCS liver perivascular plexus |
| **Human trials** | | | | |
| Mergental et al. (16) | Normothermic | Transplantation of 22 livers (12 DBD, 10 DCD) after NMP of 31 (17 DBD, 14 DCD) primarily discarded livers compared with control group ($n$ = 44)<br>Median follow-up 542 days (456-641)<br>No report on systematic BD histology | Feasibility of NMP as a method to push the boundaries to safe transplantation of highest risk organs | Similar graft and patient survival, higher incidence of EAD in NMP group, higher incidence of ITBL in NMP group (18% vs. 2%) but only the NMP group received routine magnetic resonance cholangiopancreatography imaging; Incidence of ITBL diagnosed by MRCP + clinical symptoms |
| **Randomized controlled trials** | | | | |
| Nasralla et al. (30) | Normothermic vs. SCS | Human RCT<br>121 NMP livers vs. 101 SCS<br>Follow-up 12 months<br>No analysis of collected BD biopsies | Effects of NMP in clinical practice compared to standard procedure (SCS) | NMP group showed<br>50% lower graft injury (transaminases, $p < 0.001$)<br>50% lower organ discard rate (11.7% vs. 24.1%, $p = 0.008$), resulting in 20% increase in transplanted livers<br>Reduction in bile duct complications statistically non-significant (11.1% in NMP DCD vs. 26.3% in SCS DCD on radiological imaging, $p = 0.18$)<br>1 case of clinically relevant ITBL in each arm |
| Markmann et al. (31) | Normothermic vs. SCS | Human RCT<br>153 NMP livers vs. 147 SCS<br>Follow-up 12 months<br>No report on systematic BD histology | Effects of NMP in clinical practice compared to standard procedure (SCS) | Significant reduction of: EAD (18% vs. 31%, $p = 0.01$); histopathologic evidence of IRI after reperfusion (6% vs. 13%, $p = 0.004$); Incidence of ischemic biliary complications after 6 months (1.3% vs. 8.5%, $p = 0.02$) and 12 months (2.6% vs. 9.9%, $p = 0.02$); Higher use of initially accepted DCD livers (51% vs. 26%, $p = 0.007$) |

*BD, bile duct; COR, controlled oxygenated rewarming; DBD, donation after brain death; DCD, donation after circulatory death; EAD, early allograft dysfunction; GGT, γ-glutamyl transferase; HOPE, hypothermic oxygenated machine perfusion; IRI, ischemic reperfusion injury; ITBL, ischemic type biliary lesions; LDH, lactate dehydrogenase; NMP, normothermic machine perfusion; RCT, randomized control trial; SCS, static cold storage; SEC, sinusoidal endothelial cells; WIT, warm ischemic time.*

deep in the bile duct walls. Whether or not the biliary system is capable to recover from IRI depends on the viability of the PBG and their blood supply through the PVP (5, 19, 20). The extra-hepatic biliary system and its connective tissue receive its blood supply only through the hepatic artery via microvascular networks. During ischemia, the endothelium is injured,

therefore promoting thrombogenesis after graft reperfusion, thus further limiting blood supply (21).

The biliary tree is a complex and delicate system, damage to one part or one cell population often results in reactive changes or excessive proliferation of another. Therefore, biliary wound healing is a complex process (21). Deep wounds in the bile duct wall and consequently activation and transformation of myofibroblasts contribute to the formation of strictures. The bile itself contains growth factors and bile salts can induce several messenger pathways that either exacerbate damage or protect cholangiocytes. Bile composition and the effects of its different constituents play an important role in the pathophysiology of various cholangiopathies (21, 22) and is a potential target for therapeutic agents (23).

Ductular reaction (DR) is a form of intrahepatic wound repair mechanism (21). DR can be triggered by cholangiocyte injury in the smallest intrahepatic ducts or any change in the intrahepatic milieu, like increased pressure in the intra-biliary tract or via a strong stimulus of liver regeneration, e.g., after partial liver resection. DR is defined as hyperplasia of reactive bile duct tissue and is common in various biliary disorders. During DR transdifferentiation of various cells from the biliary tract has been described (21, 24).

## IMPACT OF NMP ON BILIARY INJURY

A summary of studies that consider biliary injury in the context of NMP can be seen in **Table 1**.

In a rat model, op den Dries et al. compared NMP with SCS of DCD and non-DCD rat livers, followed by 2 h of reperfusion. They found increased bicarbonate and $pH_{bile}$ and reduced GGT and LDH in the bile of the NMP group (25). Westerkamp et al. used a DCD rat model ($n$ = 30) to compare hypothermic oxygenated perfusion (HOPE), sub-normothermic machine perfusion, and controlled oxygenated rewarming (COR) to SCS. All treatment groups showed an overall better outcome, lower levels of liver injury markers in perfusate, and better mitochondrial function. Furthermore, they showed higher bile production, bicarbonate secretion, and $pH_{bile}$. Biliary injury was reduced, indicated by lower GGT and LDH in bile and by histological analysis (26).

In a porcine transplantation model that compared NMP livers to livers with long and short periods of SCS, Boehnert et al. showed reduced biliary injury, reduced LDH, and higher bilirubin, phospholipids, and bile acids in bile in the NMP group (27). In 2014, Liu et al. used a porcine model to investigate the impact of NMP on the biliary system. They described higher LDH and GGT in bile in the SCS group, lower bicarbonate in bile in the SCS group, and absent Ki67 and higher von Willebrand factor in immunofluorescence in the SCS group after reperfusion of the livers with whole blood. This indicates a positive effect of NMP on biliary injury and platelet activation, biliary regeneration, and bicarbonate secretion in porcine DCD livers (28). Mergental et al. described the NMP of 31 high-risk grafts that were deemed non-transplantable by two different surgeons (16, 29). A count of 22 livers were transplanted after viability assessment based on lactate clearance, perfusate pH, and the presence of bile production. A control group was matched in order to present the results within the framework of the centers' contemporary outcomes. The control group did not receive high-risk grafts and comparisons were not powered to demonstrate any differences. Graft and patient survival were comparable, there was a higher incidence of early allograft dysfunction (EAD) (31.4% vs. 9.1%, $p$ = 0.034) and ITBL in the NMP group (18% vs. 2%, $p$ = 0.063). Only the NMP group received routine magnetic resonance cholangiopancreatography (MRCP) imaging. However, NMP was not able to prevent the development of ITBL in high-risk DCD grafts (16).

A variety of effects and benefits have been described above. These promising results all originate from non-randomized and sometimes even non-controlled trials, often with a small sample size. Recently, two larger-sized randomized controlled trials (RCT) have been published:

In 2018, Nasralla et al. published the first human RCT, comparing outcomes after NMP ($n$ = 121) vs. SCS ($n$ = 101). The NMP group showed 50% reduced graft injury measured by perfusate liver transaminases ($p \leq 0.001$). Furthermore, a 50% lower organ discard rate ($p$ = 0.008), resulting in a 20% increase of transplanted livers in the NMP group was observed. The reduction in MRCP diagnosed ITBL (11.1% in NMP-DCD and 26.3% in SCS-DCD grafts, $p$ = 0.18) was statistically non-significant (30). The second human RCT was recently published by Markmann et al., they included 300 liver transplantations (randomized after initial acceptance—NMP $n$ = 153, SCS $n$ = 147). NMP grafts showed a reduction of EAD (18% vs. 31%; $p$ = 0.01) and histopathologic evidence of IRI after reperfusion (6% and 13%; $p$ = 0.004). NMP resulted in higher utilization of DCD livers initially accepted with 51% of transplanted grafts compared to 26% in the SCS group ($p$ = 0.007). Despite the higher use of DCD organs in the NMP group, the incidence of ischemic biliary complications was reduced after 6 months (1.3% vs. 8.5%; $p$ = 0.02) and 12 months (2.6% vs. 9.9%; $p$ = 0.02). Ischemic biliary complications were defined as ITBL or bile leaks, which were confirmed either endoscopically or by magnetic resonance cholangiopancreatography. They did not mention if all patients or only symptomatic patients were examined (31).

In summary, several studies presented promising effects of NMP on LT in general, and partially regarding biliary complications. Additionally, NMP could increase the number of utilized organs. Nevertheless, our understanding of the mechanisms that influence biliary injury during NMP is incomplete. Many aspects of NMP are still vastly under-researched, such as the effect NMP has on cholangiocyte physiology. Existing preclinical studies investigated the effect either in animal models, which lack the equivalent of ITBL, or using discarded livers which represent a heterogeneous study group. Additionally, most clinical studies did not focus on mechanistic aspects and included ITBL development only as a secondary endpoint.

## HISTOLOGICAL SCORING OF BILIARY INJURY IN LIVER TRANSPLANTATION

Possible surrogate endpoints for experimental studies are histological scoring systems. Histological tissue analysis reflects

**TABLE 2 |** Biliary assessment during NMP.

| Author | Design | Aim | Biliary viability criteria | Major findings |
|---|---|---|---|---|
| | | **Non-human studies** | | |
| Linares-Cervantes et al. (35) | Porcine LT-model: transplantation after 4 h of NMP: 5 Non-DCD vs. 5 DCD30′ vs. 5 DCD70′ vs. 5 DCD120′No-PNF vs. 2 DCD120′PNF with 3-day follow-up | Investigation of biomarkers for graft function and preservation injury during NMP | Bile: LDH, pH, lactate, bicarbonate, glucose, sodium, b/p glucose + sodium ratio, lactate + urea (hepatocellular)<br>No systematic BD histology | B/p sodium ratio ≥1.1 within 4 h of NMP strongly correlated with successful transplantation |
| Kesseli et al. (34) | Primate model: NMP of 4 DCD livers with 5 min WIT vs. 4 DCD livers with 45 min WIT<br>No follow-up | Characterization of trends in POC biomarker during NMP of primate DCD livers with short and long periods of WIT | Bile: LDH, glucose + sodium; Perfusate: FMN, GGT, lactate, ALT, ALP<br>No BD biopsies collected | Perfusate GGT might be predictive of livers that are at risk of developing cholangiopathies<br>All WIT 45′ livers were nonviable and showed severe injury in the biopsies that progressed over time, GGT but not lactate discriminated between viable and nonviable livers |
| | | **Preclinical human studies** | | |
| Eshmuminov et al. (36) | 7-day NMP of 23 porcine livers with subsequent transplantation 3 h follow-up 7-day NMP of 12 human livers | Bile flow after stimulation as a viability criterion in long term NMP | B/p glucose ratio<br>No systematic BD histology | 8 human livers were viable after 7-day NMP; tazobac/methylprednisolone induce bile salt independent bile flow; UDCA is an adequate bile flow inductor; absence of bile flow despite stimulation is indicative of poor performance<br>Mean b/p glucose ratio in viable livers was <0.5 during all perfusions |
| | | **Human studies with transplanted livers** | | |
| Watson et al. (37) | NMP of 47 livers (12 DBD, 35 DCD) 22 transplanted after evaluation Median follow-up 20 months (IQR: 8.4-24.7) | | $pH_{bile}$, biliary glucose, difference in glucose and pH in perfusate and bile (<10 mmol/L suggested relevant injury), proposed glucose challenge Histology scoring by Hansen et al | Retrospect: Peak $pH_{bile}$ < 7.5 identified three livers that later developed ITBL; peak $pH_{bile}$ < 7.5 discriminated between livers with a high grade of circumferential stromal necrosis of septal bile ducts and livers without |
| De Vries (14) | DHOPE-COR-NMP 7 primarily declined DCD livers, 5 transplanted after viability testing Median follow-up 6.5 months (IQR: 5-10) | Sequential hypothermic and normothermic perfusion, 3-months graft survival after viability testing, and transplantation of marginal livers that were primarily declined | $pH_{bile}$ Bile duct biopsies were only obtained from the two discarded livers No systematic BD histology | $pH_{bile}$ > 7.45 after 150 min of perfusion used for the decision to transplant after NMP |
| Van Leeuwen et al. (38) | DHOPE-COR-NMP of 16 DCD livers, 11 transplanted Median follow-up 12 months (IQR: 8-22) | Sequential hypothermic and normothermic perfusion as a tool to resuscitate and assess marginal grafts that were initially declined | $pH_{bile}$ > 7.45 Histology scoring by Op den Dries et al | 1 ITBL Difference between bile and perfusate pH, bicarbonate, and glucose are more predictive of bile duct viability than absolute values |
| Matton et al. (33) | 6 h of NMP of 23 (18 DCD, 5 DBD) preclinical livers to identify cut-off values; 6 h NMP of 6 livers in a clinical trial to validate cut-off values, 4 transplanted after evaluation Median follow-up 8.3 months (IQR: 7.6-10.1) | Define the diagnostic accuracy of bile biochemistry for the assessment of BDI | $pH_{bile}$ > 7.48 b/p glucose ratio <0.67 bicarbonate content in bile >18 mmol/L Histology scoring adapted from op den Dries et al. (0-7) | Retrospect BDI score cut-off defined as 4.75 Biliary LDH <3689 U/l Bicarbonate in bile has highest PPV + NPV in discriminating between low and high BDI |
| Ghinolfi et al. (42) | LT of older grafts (≥70 years) randomized 10 NMP vs. 10 SCS Follow-up 6 months | Role of NMP in graft and patient survival of recipients receiving grafts from octogenarian donors | $pH_{bile}$, glucose, bicarbonate, sodium Histology scoring by op den Dries et al | NMP group showed reduced biliary injury in histological analysis; Not enough power for differences regarding graft- and patient survival between NMP and SCS |
| Cardini et al. (41) | NMP of ECD organs: 34 livers perfused; 9 livers discarded after evaluation during NMP Mean follow-up 20 months (SD: ± 5.9) | Introduce NMP into clinical practice, avoid nighttime transplantations, assessment of ECD livers | Bile production and $pH_{bile}$ were assessed, but no cut-off values were specified No BD biopsies collected | NMP feasible for clinical practice, logistic improvements compared to SCS, graft evaluation possible but not yet sufficient No cases of ITBL |

(Continued on following page)

**TABLE 2 |** (*Continued*) Biliary assessment during NMP.

| Author | Design | Aim | Biliary viability criteria | Major findings |
|---|---|---|---|---|
| Weissenbacher et al. (43) | Transplantation after viability assessment of 45 livers out of 55 NMP<br>Follow-up 3 months | Value of biomarkers that are measured repeatedly as predictors for early graft function | Bile production was a mandatory criterion for DCD livers; Biliary parameters (pH, bicarbonate, glucose, and lactate) were only assessed during 15 perfusions<br>No BD biopsies collected | Bile parameters did not correlate with the occurrence of EAD or with liver function scores<br>1 case of ITBL |
| Van Leeuwen et al. (40) | 27 bile duct biopsies + bile samples of DCD livers during NMP | Influence of donor hepatectomy time on bile duct injury in histology, bile composition, and development of ITBL | Biliary bicarbonate, pH, and b/p glucose ratio | Donor hepatectomy time 50 min as cut-off showed 17% of high BDI with ≤50 min and 64% high BDI with ≥50 min hepatectomy time |
| | Retrospective analysis of 273 DCD transplantations with ITBL development within 2 years as an endpoint | | Histology scoring | Livers with a shorter hepatectomy time and low BDI had more alkalotic bile and higher bicarbonate, b/p ratio of glucose did not differ significantly between livers with longer and shorter hepatectomy time |
| Gaurav et al. (39) | Bile samples of 100 livers (35 DCD, 65 DBD) after reperfusion, 12 cases of ITBL (5 clinically relevant) over a median follow-up period of 15 months (IQR: 11-20) | Retrospective analysis of bile samples after reperfusion | Blood-bile glucose difference, biliary sodium, $pH_{bile}$ | Blood-bile glucose difference of <6.5 mmol/L showed an 83% sensitivity and 62% specificity of predicting cholangiopathy |
| | | | Bile duct damage categorized into two groups (none to mild, moderate to severe) based on stromal necrosis | No correlation between bile chemistry and degree of bile duct damage |
| | | | ITBL was diagnosed by MRCP, in patients that showed increasing alkaline phosphatase or clinical symptoms | Sample numbers were underpowered to show subtle differences |

*ALP, alkaline phosphatase; ALT, alanine-aminotransferase; BD, bile duct; BDI, bile duct injury; b/p ratio, bile/perfusate ratio; COR, controlled oxygenated rewarming; DBD, donation after brainstem death; DCD, donation after circulatory death; DHOPE, dual hypothermic oxygenated reperfusion; ECD, extended criteria donor; FMN, flavin mononucleotide; GGT, γ-glutamyl transferase; ITBL, ischemic type biliary lesion; IQR, inter quartile range; LDH, lactate dehydrogenase; LT, liver transplantation; MRCP, Magnetic resonance cholangiopancreatography; NMP, normothermic machine perfusion; NPV, negative predictive value; PNF, primary non-function; POC, point of care; PPV, positive predictive value; WIT, warm ischemic time.*

changes on a cellular level, which however cannot always be reliably translated into clinical outcomes.

Systematic histological workup of bile ducts most frequently refers to scoring systems published by Hansen et al. (32) or op den Dries et al. (5). The scoring of Hansen et al. assesses 7 features: mucosal loss, bleeding in bile duct wall, hyaline thrombi, vascular lesions, inflammation, arteriolonecrosis, and bile duct necrosis. The authors divided each feature into grades, depending on the severity of injury. The score has been developed by analyzing 93 transplanted livers of which 18 developed ITBL. Arteriolonecrosis, bile duct necrosis, vascular lesions, and intramural bleeding correlated with the development of ITBL, but arteriolonecrosis was the only parameter that was also associated with ITBL development in logistic regression analysis (32). Op den Dries et al. analyzed 128 bile duct biopsies obtained during liver transplantation. Injury severity scores were compared between grafts that later developed ITBL (16.4%) and grafts that did not. The score is a derivative of the Hansen score and assesses biliary epithelium, mural stroma necrosis, vascular injury, thrombosis, intramural bleeding, damage to periluminal and deep PBG, and inflammation. In the original publication of op den Dries et al., injury to the deep peribiliary glands and peribiliary vascular plexus was strongly associated with the development of ITBL. Contrarily, extensive loss of bile duct epithelium was observed in nearly every liver and was not indicative of ITBL development (5).

Both scores described above however have not yet been adjusted for well-known risk factors for the development of ITBL. Furthermore, it is not known to what extent a single feature of these scores contributes to the final risk of ITBL. Matton et al. selected the three histological parameters from op den Dries et al. that were predictive of ITBL development (stroma necrosis, injury to extramural PBG, and injury to PVP) to describe the bile duct injury (BDI) score. The score ranges between 0–7 and was developed in 23 human livers subjected to NMP but not transplanted, a cut-off of 4.75 was empirically defined using the median of the histological scores. The authors investigated their results prospectively in a subsequent clinical study during NMP of 6 livers of which 4 were transplanted (33). However, the level of evidence is currently not strong enough to recommend the universal application of this score for the prediction of ITBL.

The histological scoring systems discussed above can be considered useful tools if they are interpreted with the knowledge of their insufficient validation for the prediction of clinical outcomes in mind.

Furthermore, biomarkers that were identified with histological scoring systems as a surrogate endpoint for ITBL development should only translate into clinical decision-making after proper validation to prevent possibly transplantable livers from being discarded.

The accuracy of the described histologic scoring systems in predicting ITBL should be treated with caution but they offer at least a certain degree of objectivity and enable comparison of results.

## BILIARY ASSESSMENT DURING NMP

Studies that focused on the assessment of biliary parameters during NMP are summarized in **Table 2** and classified into animal studies, preclinical studies with discarded human livers, and clinical studies with subsequent transplantation after viability assessment.

Two animal studies focused on predictive biliary markers during NMP for liver grafts that experienced different periods of warm and cold ischemia (34, 35). The bile/perfusate ratio (b/p ratio) of glucose and sodium (≤0.7 and ≥1.1, respectively) within 4 h of NMP was found to correlate with successful transplantation in a porcine model (35). In a non-human primate model (45′ warm ischemic time (WIT) vs. 5′ WIT) perfusate gamma-glutamyltransferase (GGT) levels discriminated between viable and nonviable livers with progressive injury. The authors concluded that GGT might be predictive of livers that are at risk of developing cholangiopathies (34). A long-term perfusion protocol for 7-day NMP was established by Eshmuminov et al. (36). The authors included 23 porcine livers of which 3 were transplanted. In a second phase 12 discarded human livers were evaluated and after 7 days of NMP 8 remained viable. The absence of bile flow despite stimulation with either tazobac, methylprednisolone, or UDCA was indicative of poor performance. B/p glucose ratio <0.7 was met by all porcine livers and the viable human livers. To use bile flow as a reliable viability criterion it should be complemented with bile composition parameters (36).

In preclinical human studies and clinical studies with subsequent transplantation that focused on biliary assessment and biliary complications the same markers frequently appeared in different constellations. Results were either validated prospectively with ITBL development or high grade of injury in bile duct biopsies as endpoints or in retrospective analysis. Biliary pH ($pH_{bile}$) was one of the most used biliary markers. Proposed cut-off values were >7.48 (33), >7.5 (37), and >7.45 (14, 38). Rather than assessing absolute values several groups suggested assessing the values in bile in relation to perfusate values. Matton et al. proposed several cut-off values that were all determined during NMP of 23 preclinical livers and validated in a following clinical trial with 6 livers of which 4 were transplanted after viability assessment. Upon the determined values were a b/p glucose ratio <0.67 and LDH in bile <3689 U/L. Bicarbonate in bile of 18 mmol/L discriminated between low and high BDI [positive and negative predictive value (PPV and NPV) both >80%] (33). Van Leeuwen et al. made the observation that the bile pH, glucose, and bicarbonate of a liver that later developed ITBL were similar to the perfusate levels and proposed to use the difference between perfusate and bile as markers of biliary viability (38). Gaurav et al. assessed the bile composition of recipients after reperfusion and showed that a blood-bile glucose difference <6.5 mmol/L was predictive of ITBL development (83% sensitivity) (39). Watson et al. published a study that included NMP of 47 livers that resulted in 22 transplanted grafts after viability assessment. They discovered that differences in perfusate and bile glucose levels of <10 mmol/L indicated significant injury. A $pH_{bile}$ <7.5 was identified retrospectively as a cut-off that discriminated between livers that later developed ITBL and livers that did not and in livers not transplanted the cut-off discriminated between high vs. low grade of circumferential stromal necrosis (37). Van Leeuwen et al. investigated the impact of hepatectomy time on bile composition and BDI in the histology of 27 DCD livers during NMP and validated their findings in a retrospective database analysis of 273 transplanted DCD livers with the development of ITBL within 2 years as the endpoint. Livers with longer hepatectomy time showed higher BDI, lower $pH_{bile}$ and bicarbonate in bile (40).

Several studies measured bile composition during NMP but did not use it for assessment. Furthermore, some studies did not measure bile composition consistently making results difficult to interpret (41–43).

Although several biomarkers are already used for assessment, they can only point in a certain direction but do not enable reliable decision-making at this point. Most markers were defined in livers that were not accepted for transplantation, due to a variety of reasons. The defined cut-off values of these biomarkers have been applied in clinical trials with promising results, however, it is impossible to know at this moment if livers that did not meet the criteria would have indeed shown poor performance.

## NOVEL BILIARY BIOMARKERS

Several promising experimental biomarkers assessing biliary injury have been described in the literature (**Table 3**). Currently, most of them are not established to be measured during perfusion. Novel biomarkers include microRNAs (miRNA) measured in different solutions as well as markers for tissue integrity and regeneration analyzed by immunofluorescence and immunohistochemistry. In 2013, Verhoeven et al. compared miRNA expression in graft preservation solution of 20 grafts that developed ITBL with 37 that did not. They found that the ratio of hepatocyte-derived (HD)miRNAs/cholangiocyte-derived (CD)miRNAs was higher in grafts that later developed ITBL (44). More recently Matton et al. investigated miRNA levels in perfusate and bile during NMP of 12 declined human liver grafts. The authors discovered that CDmiRNA-222 correlated with cholangiocellular injury and function reflected by LDH, bilirubin, and bicarbonate levels in bile. B/p glucose ratio correlated strongly with CDmiRNA-222 and HDmiRNA-122 in bile. Additionally, the ratio of HDmiRNA122/CDmiRNA222 at 30 min was predictive of injury of liver parenchyma after 6 h NMP (45).

In 2018, Liu et al. investigated liver function and regeneration during 24 h of NMP in 10 discarded livers. The authors described regeneration of cholangiocytes and PBG during NMP of steatotic livers indicated by increased Ki-67 staining in BD biopsies (46). In an *ex vivo* bile duct model, De Jong et al. investigated the regenerative reaction of stem cells from PBG to biliary injury.

**TABLE 3 |** Novel Biliary biomarkers.

| Author | Design | Aim | Biomarkers | Major findings |
|---|---|---|---|---|
| Verhoeven et al. (44) | Graft preservation solutions of 20 grafts that developed ITBL compared with 37 that did not | Assessment of miRNA composition and ratio at preservation is predictive of later ITBL development (defined as symptomatic and need of intervention, confirmed by cholangiopancreaticography) | CDmiRNA-30e<br>CDmiRNA-222<br>CDmiRNA-296<br>HDmiRNA-122<br>HDmiRNA-148a<br>No report on systematic BD histology, ITBL assessed in liver wedge biopsies | HDmiRNAs/CDmiRNAs significantly higher in grafts that developed ITBL |
| Matton et al. (45) | NMP (6 h) of 12 declined human liver grafts | Assessment of miRNAs in perfusate + bile of NMP liver grafts | CDmiRNA-222<br>HDmiRNA-122 and ratio<br>No report on systematic BD histology | CDmiRNA-222 in perfusate + bile correlated with cholangiocellular injury reflected by LDH in bile and cholangiocellular function reflected by bilirubin in bile |
| Liu et al. (46) | 24 h of NMP of 10 discarded livers after 4–6 h of SCS | Characterization of lipid profile and assessment of graft function in steatotic discarded livers | Bile: volume, LDH, GGT, bicarbonate<br>Ki-67<br>Scoring systems by Hansen et al. + op den Dries et al | Ki-67 staining increased in bile duct biopsies at the end of NMP indicating cholangiocyte and PBG regeneration |
| De Jong et al. (47) | *Ex vivo* model of bile duct biopsies from discarded donor livers | PBG role in recovery of bile ducts post-ischemia | HIF1-α<br>VEGF<br>Glut-1<br>Ki-67 (proliferation)<br>CK19 (cholangiocytes)<br>Sox9 (endoderm progenitor)<br>Nanog (undifferentiated Stem cells)<br>CFTR (mature cholangiocytes) | Stem cells out of PBG can proliferate and transform to mature cholangiocytes after biliary injury |
| Franchitto et al. (20) | Retrospective analysis of 62 bile duct biopsies from transplanted patients compared to 10 control ducts | Investigation of PBG phenotype, integrity of PVP, and expression of VEGF-A by PBG | VEGF-A<br>VEGF-R2<br>HIF<br>Histological scoring system by Hansen et al. and op den Dries et al | PBG in transplanted ducts contain more progenitor cells, express more VEGF-A and VEGF-R2 |

*BD, bile duct; CD, cholangiocyte derived; GGT, γ-glutamyl transferase; HD, hepatocyte derived; HIF, hypoxia inducible factor; ITBL, ischemic type biliary lesions; LDH, lactate dehydrogenase; miRNA, microRNA, NMP, normothermic machine perfusion; PBG, peribiliary glands; PVP, perivascular plexus; VEGF, vascular endothelial growth factor.*

PBG started to proliferate and transform within the first 24 h after reoxygenation which caused an increase in cholangiocytes and forming of epithelial monolayers. As a reaction to hypoxia, hypoxia-inducible factor-1α expression was increased followed by activation of metabolic and pro-angiogenic pathways characterized by expression of vascular endothelial growth factor (VEGF) and Glut-1 (47). In 2019, Franchitto et al. published a retrospective analysis of 62 bile duct biopsies from transplanted patients and compared them to 10 donor bile duct biopsies that did not experience ischemia. The authors described more progenitor cells in PBG of transplanted ducts, also VEGF-A and VEGF-R2 expression were increased (20).

## THERAPEUTIC APPROACHES DURING NMP TO IMPROVE BILIARY INJURY

An overview of therapeutic approaches targeting biliary injury can be seen in **Table 4**.

NMP offers the unique opportunity to treat livers under near physiologic conditions outside the human body. Several experimental studies have been published. In a porcine transplantation model, Goldaracena et al. described lower alkaline phosphatase and bilirubin levels during sub-normothermic machine perfusion with different anti-inflammatory agents, among others with a protective effect on endothelial cells (48). Boteon et al. described a higher volume of bile and higher $pH_{bile}$ in livers treated with lipid metabolism enhancing pharmacological agents during NMP compared to standard NMP. Furthermore, liver grafts in the treatment group showed reduced activation of immune cells and release of inflammatory cytokines (49). Additionally, oxidative stress markers, macrovesicular steatosis, and tissue triglycerides were reduced. Tian et al. discovered that administration of Heme Oxygenase-1-modified bone marrow mesenchymal stem cells (HO-1/BMMSCs) during NMP of rat livers lead to improved liver function, bile duct histology, restored epithelium, and reduced cell apoptosis (50). Haque et al. investigated the reconditioning of discarded DCD livers with tissue plasminogen activator administration during NMP. The authors described lower PVP and mural stroma injury scores after treatment with a tissue plasminogen activator (51). In 2021, Sampaziotis et al. made the exciting discovery, that cholangiocyte organoids can be used to repair damage in the biliary tree

**TABLE 4 |** Therapeutic approaches during NMP to improve biliary injury.

| Author | Design | Aim | Major findings |
|---|---|---|---|
| Goldaracena et al. (48) | Porcine transplantation model: 4 h of SNMP of 5 livers with anti-inflammatory and endothelial-protective agents vs. 4 h of NMP of 5 livers vs. 6 h of SCS<br>3-day follow-up<br>No systematic histological scoring | Improvement of NMP by applying strategies to reduce the activation of proinflammatory cascades | Serum ALP and total bilirubin levels were lower, with significantly lower bilirubin |
| Boteon et al. (49) | 6 h of NMP with vs. without a combination of drugs that enhance lipid metabolism<br>5 livers per group<br><br><br>No BD biopsies collected | Efficacy of lipid metabolism enhancement during NMP on defatting and improvement of functional recovery | Treatment group: Higher bile production and higher $pH_{bile}$ in defatted livers<br>Down-regulation of oxidative stress markers, immune cell activation, and release of inflammatory cytokines<br>Reduction in tissue triglycerides (38%) and macro-vesicular steatosis (40%) |
| Tian et al. (50) | DCD rat livers with WIT = 30min received BMMSCs, HO-1/BMMSCs, or neither during 4 h of NMP; Transplant model<br>7 days postoperative follow-up<br>No systematic histological scoring | Investigate the repair effect of HO-1/BMMSCs applied during NMP on biliary injury in a DCD rat transplantation model; Investigation of the underlying mechanisms | In the HO-1 group liver function and bile duct histology was improved; cell apoptosis was reduced; defective epithelium was restored through a large number of regenerative cells; Repair effect was inhibited through inhibition of Wnt signaling |
| Haque et al. (51) | 12 h NMP of discarded DCD livers: 3 with tPA in HA at t = 0.5 h compared with 7 controls; 2 split grafts with 1 lobe tPA and 1 lobe control | Reconditioning of discarded DCD livers with tPA during NMP | Lower PVP and mural stroma injury score (0.67 and 1.3 vs. 2.0 and 2.7) using the Hansen et al. and op den Dries et al. histological scoring systems |
| Sampaziotis et al. (52) | Cholangiocyte organoids applied in mouse model and during human NMP<br><br><br>No BD biopsies collected | Investigate the feasibility of human cholangiocyte organoids for regenerative therapy during NMP | Cholangiocytes of human organoids can adapt to cellular environment, extrahepatic bile duct derived cells were able to repair intrahepatic bile duct injury<br>Organoid-injected livers produced bile with higher pH and volume |

*ALP, alkaline phosphatase; ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; BD, bile duct; BMMSC, bone marrow mesenchymal stem cell; DCD, donation after circulatory death, GGT, γ-glutamyl transferase; HA, hepatic artery; HO-1, heme oxygenase-1; NMP, normothermic machine perfusion; PVP, perivascular plexus; SNMP, subnormothermic machine perfusion; tPA, tissue plasminogen activator; WIT, warm ischemic time.*

independent from their region of origin. Livers that were injected with organoids produced more bile with a higher $pH_{bile}$ (52).

## SUMMARY

Machine perfusion of liver grafts received tremendous attention in recent years, leading to a number of publications with promising outlooks. Ultimately, the research objective is a safe increase in the number of transplantable organs. To meet this goal, mitigation of IRI, therapeutic graft improvement, and graft assessment have emerged as the main machine perfusion-based approaches.

Most well-established viability assessment protocols mainly focus on hepatocellular criteria. However, biliary complications are one of the main challenges in liver transplantation and biliary viability criteria are lagging behind hepatocellular criteria. While the development of this field is very promising, one weakness has to be addressed. Without standardization of protocols, definitions, and sample collection heterogeneous data will be reported and results will be difficult to interpret. Especially regarding biliary complications, results from previous studies should be further validated in prospective studies, with clear primary endpoints and appropriate follow-up periods.

A variety of preclinical and clinical studies introduced different biomarkers which can be used to assess the injury and regenerative capacity of the biliary system. The majority of parameters were analyzed in small pilot studies that differ greatly in their study design. Markers of interest in preclinical studies were either determined in comparison to historical cohorts or correlated with surrogate parameters in form of histological grading systems and injury markers. The screening for ITBL and its definition varied widely in clinical studies with some performing routine cholangiopancreatography (MRCP or ERCP) alone and others in combination with clinical symptoms and/or cholestatic laboratory parameters. As for sample collection, a comprehensive description of methods helps to put results into context. In some cases, $pH_{bile}$ and bicarbonate were not reported due to contact with ambient air. Several groups suggested to cover secreted bile with mineral oil to achieve better comparability (14, 33). Nevertheless, rather than excluding results obtained with different protocols, they should be reported, and methods thoroughly described.

Currently available evidence on biliary injury from experimental and clinical studies looks very promising. First clinical machine perfusion trials reported increased graft

utilization, with comparable clinical outcomes. Just recently, the benefit of NMP regarding biliary complications was highlighted in a large randomized control trial (31), further emphasized by a higher rate of transplanted DCD grafts.

However, machine perfusion has its limits and cannot yet undo extensive damage to the organ that has already occurred (e.g., after long ischemic times, etc.). Notably, it does offer the opportunity for therapeutic interventions. Only future studies will determine if therapeutic options such as organoids, mesenchymal stem cells, and novel targeted therapeutic agents can be used to further increase organ utilization.

In conclusion, normothermic machine perfusion is a thrilling opportunity to treat the organ. Each step towards the extension of the donor pool needs to be accompanied by careful graft assessment to ensure patient safety. Every additional organ available for transplantation is a gain and with further improvement in already promising biliary viability assessment, liver transplantation in the future, without its Achilles' heel, seems within reach.

## AUTHOR CONTRIBUTIONS

JD—Drafting the work and revising it critically for important intellectual content, provide approval for publication of the content. LR—Drafting the work and revising it critically for important intellectual content, provide approval for publication of the content. GB—Revising the work critically for important intellectual content, provide approval for publication of the content. DK—Substantial contributions to the design of the work, drafting the work and revising it critically for important intellectual content, provide approval for publication of the content.

## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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# Normothermic Machine Perfusion—Improving the Supply of Transplantable Livers for High-Risk Recipients

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The effectiveness of liver transplantation to cure numerous diseases, alleviate suffering, and improve patient survival has led to an ever increasing demand. Improvements in preoperative management, surgical technique, and postoperative care have allowed increasingly complicated and high-risk patients to be safely transplanted. As a result, many patients are safely transplanted in the modern era that would have been considered untransplantable in times gone by. Despite this, more gains are possible as the science behind transplantation is increasingly understood. Normothermic machine perfusion of liver grafts builds on these gains further by increasing the safe use of grafts with suboptimal features, through objective assessment of both hepatocyte and cholangiocyte function. This technology can minimize cold ischemia, but prolong total preservation time, with particular benefits for suboptimal grafts and surgically challenging recipients. In addition to more physiological and favorable preservation conditions for grafts with risk factors for poor outcome, the extended preservation time benefits operative logistics by allowing a careful explant and complicated vascular reconstruction when presented with challenging surgical scenarios. This technology represents a significant advancement in graft preservation techniques and the transplant community must continue to incorporate this technology to ensure the benefits of liver transplant are maximized.

**Keywords: transplant, normothermic machine perfusion, liver, preservation, marginal, retransplant**

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## INTRODUCTION

Since the introduction of liver transplant as a treatment option for end stage liver disease and liver cancer in the 1960s, its role and potential benefits have expanded substantially (1, 2). In the initial phase of liver transplantation following the acceptance of the brain death concept (3), graft options were restricted to whole livers from deceased donors. In the modern day, this has expanded to include reduced size, auxiliary, domino, and split grafts from either deceased or living donors. The

**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CIT, cold ischemic time; DBD, deceased brain death; DCD, deceased circulatory death; DLI, donor liver index; DRI, donor risk index; GGT, gamma-glutamyl transferase; HBI, hypoxic brain injury; HCV, hepatitis C virus; ICU, intensive care unit; ITBL, ischemic-type biliary lesions; MELD, model of end stage liver disease; NMP, normothermic machine perfusion; PNF, primary non-function; SCS, static cold storage; UK, United Kingdom; UKELD, United Kingdom model of end stage liver disease; US, United States.

utilization of these different graft types has partly been driven by the fact that the accepted indications for liver transplant have also expanded over the last half century (4–6). Benefits from transplantation have been demonstrated in situations other than cirrhosis or primary liver cancer, including metabolic disease, colorectal liver metastases, and perihilar cholangiocarcinoma (6–8). Demand for organs continues to increase and exceeds the supply (9). Therefore, a proportion of patients on the waitlist miss their window of opportunity and are delisted. As an example, during 2018 in the United States (US), 18.6% (equating to 1471 patients) of the year's starting waitlist was removed due to either death or becoming too sick to transplant (9). Maximizing graft utility is therefore paramount, and proven strategies, such as normothermic machine perfusion (NMP), should be routinely incorporated into clinical practice for certain graft-recipient scenarios.

The assessment that a patient, with an accepted indication, can be transplanted with a good outcome is reliant on social, psychological, medical, and surgical factors. Further to this, the risk - benefit assessment must be individualized as not all perspective recipients present with the same risks, nor should they all be expected to glean the same benefits. Despite research into overall risk (10, 11), the transplantability of each patient is largely subjective. Surgically high-risk recipients are those patients with certain factors that present additional obstacles, requiring the surgical team to adapt their strategy from that of a routine transplant. These may include extensive portal vein thrombosis, previous hepatobiliary surgery, or previous liver transplantation. In these situations, altered anatomy, obliterated tissue planes, and excessive bleeding may be encountered. There may also be the requirement for complex vascular reconstruction and flow modulation techniques for both the artery and portal vein. In combination, these factors increase the surgical insult, prolong the cold ischemic time (CIT) of a traditionally preserved liver graft waiting to be implanted, and may exacerbate the ischemia-reperfusion injury process. This may result in physiological instability and poor outcomes, especially if accompanied by early graft dysfunction. Historically, graft options for surgically high-risk patients were restricted to only those of optimal quality as they could withstand the longer cold ischemic period and provide immediate graft function. NMP can minimize CIT, objectively assess graft function, and safely prolong the preservation time (12, 13). We have recently published the results of our approach for retransplant candidates, which utilizes NMP for suboptimal grafts (14). In addition to facilitating the use of "orphan" grafts for a group that were disadvantaged by the current allocation scheme, it provides logistical benefits for the completion of a difficult operation (**Figure 1**) (14). The additional challenges imparted by the COVID-19 pandemic and the consequential recipient testing protocols and strain on intensive care resources has meant that our institution has been required to increasingly apply NMP for non-patient-related reasons. Although beneficial via other means, other machine perfusion techniques such as normothermic regional perfusion and hypothermic oxygenated machine perfusion do not offer the same advantage in operative logistics (15, 16).

Selecting donors and organs that can be safely used in the transplantation process is a challenging task. Organs chosen for transplantation should pose a minimal risk of donor-derived infection or malignancy, and ideally function in both the short and longer term. Graft-recipient matching goes beyond the size and ABO blood type compatibility, it relies on clinical judgement of the donor organ and its expected graft function, with the recipient stability to withstand the physiological disturbance caused by ischemia reperfusion injury (17). The majority of western countries, including the United Kingdom (UK), rely on deceased donors as the source of liver grafts and therefore operate with a limited supply of organs. This means difficult decisions need to be made regarding allocation of grafts (18). On the other hand, numerous deceased donor liver grafts are declined for transplantation each year, undoubtedly a portion of these would pose a significant risk to the recipient and truly are unsuitable (9). However, a proportion of these grafts may be suitable for transplantation with the use of machine preservation strategies such as NMP. In this article, we will further outline the reasons for using this preservation technology for high-risk recipients, and how NMP can assist in graft assessment.

## HIGH SURGICAL RISK RECIPIENTS

A liver transplant is a challenging operation, with numerous risks due to both the technical aspects of the procedure and the recipients' physiology. Despite advances in technique, such as preservation of the recipient inferior vena cava (2, 19) and reduction in perioperative blood loss, the surgical insult remains significant. Beyond the standard recipient with either acute liver failure or chronic end stage liver disease, additional technical factors may increase the challenge and risk for the surgeon and recipient, respectively. Previous hepatectomy or liver transplant, vascularized adhesions due to portal hypertension, and displacement of anatomical structures that all result in unclear dissection planes are such examples. These factors lengthen the hepatectomy phase and prolong the CIT of a graft if preserved *via* SCS. Endovascular stents and thrombi in major vascular structures or even extending into the right portion of the heart may require extensive logistics for use of a veno-venous bypass, and involvement of cardiothoracic teams. The ability of NMP to abbreviate the cold ischemic period but prolong the graft preservation in a safe manner provides a mechanism to expand graft options for these patients, accommodate the complex logistics, and mitigate the risk of unanticipated surgical factors.

It must be emphasized that with the combination of NMP, a high risk recipient, and a suboptimal graft, the implantation time must be kept short as the graft is already warm and therefore ATP depletion will proceed at a faster rate than following SCS. Scientific evidence for this is yet to be published, however more prolonged implantation times with NMP-preserved grafts are likely unfavorable. Alternative surgical strategies can be used in complex scenarios with NMP as opposed to cold storage. Rather than striving for a short cold ischemic period with the standard preservation method and early reperfusion of the

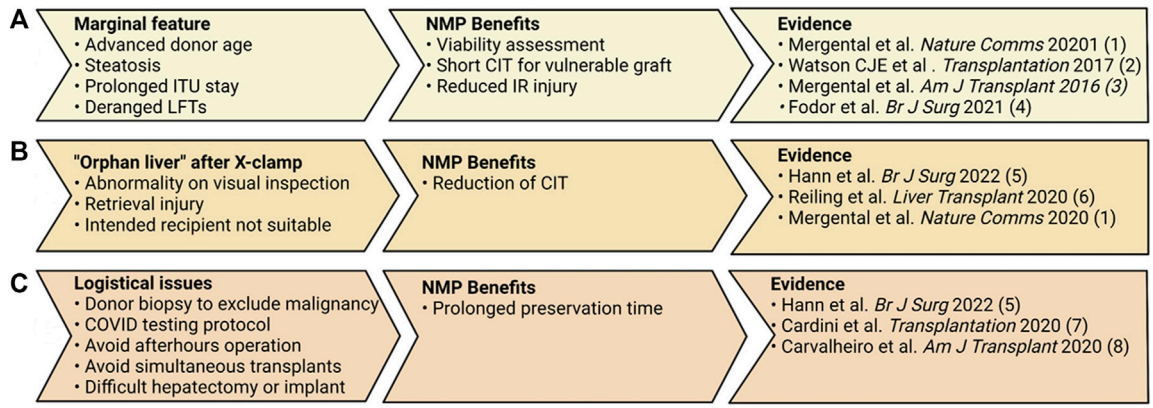


**FIGURE 1 |** Three scenarios in which normothermic machine perfusion (NMP) can applied effectively by a transplant center. **(A)** In the setting of marginal graft features, NMP can shorten the CIT these grafts are subjected to and objectively assess the grafts' function. **(B)** NMP is ideal for the situation in which an offer is received and CIT has already commenced, or one recipient is not deemed appropriate and more time is required to prepare another recipient. **(C)** Lengthening the preservation time can offer numerous benefits for the logistics around the transplant operation (12–14, 27, 30, 33, 40, 43).

graft, NMP allows time for a careful dissection to gain proper vascular control prior to the implantation phase and this is particularly useful in the setting of portal vein or late hepatic artery thrombosis when the implantation can be complicated (20). The length of this period has previously been shown to correlate with early graft function and long term outcome, with suboptimal grafts being impacted the most (21, 22). A portal cavernoma, fragile varices, and large spontaneous porto-systemic shunts may have developed following thrombosis of the portal vein. Retransplantation in the setting of late hepatic artery thrombosis is indicated when refractory or recurrent biliary sepsis occurs, often accompanied by inflamed tissues and infected bilomas. These obstacles must be overcome, and the implantation technique of the graft must ensure adequate inflow to the graft for a successful outcome (23). In the setting of late hepatic artery thrombosis, Buccholz et al. reported that aortic conduits were required in 83% of recipients (24). Regardless of whether this originates from a supraceliac or infrarenal position, the arterialization time reported in a multicenter study can be lengthy (23). The presence of a portal vein thrombus may necessitate extensive dissection in a caudad direction posterior to the pancreas to the splenic and superior mesenteric vein confluence, and a venous interposition graft if the native portal vein cannot be adequately thrombectomized. In an effort to keep the implantation and arterialization time to a minimum, we ensure the arterial and/or venous conduit is anastomosed on the proximal (recipient) side prior to disconnecting the graft from NMP and commencing the implantation.

Appropriate graft-recipient matching has been shown to be an important factor in ensuring optimal liver transplant outcomes, with marginal grafts generally preferred for recipients with lower model for end stage liver (MELD) scores (25). The rationale for this is that these recipients have a greater physiological reserve to tolerate an early period of graft dysfunction. Therefore, the suggestion that high-risk and complex recipients should be transplanted with marginal grafts contradicts the common belief that a premium quality liver graft will be required in these scenarios (26). This belief likely stems from many decades of experience with static cold storage in which the CIT was prolonged, resulting in more severe reperfusion injury and graft dysfunction as a consequence. The introduction of NMP has challenged this belief, with several successful reports of using suboptimal or "orphan" grafts for challenging cases (12, 14, 27–30). In a recent study from our center that compared a prospective group undergoing late liver retransplantation with suboptimal NMP-preserved grafts with two retrospective cold storage control groups, the outcomes were shown to be equivalent (14). Extrapolating the results from the high-risk group in this study, several of whom were undergoing a third graft, this approach can be employed for others that are complex from a surgical perspective. The utilization of NMP can therefore expand the proportion of grafts suitable for this high-risk group.

## GRAFT ASSESSMENT *via* NORMOTHERMIC MACHINE PERFUSION

Assessment of graft viability *via ex situ* machine perfusion remains an imperfect science, with many unanswered questions (31). Many different institutions have reported the criteria they apply to determine if a graft is viable, and these are being constantly altered to reflect the growing experience (12, 13, 28, 32–34). These have been extensively reviewed by other authors (35–37). The different markers reportedly used for graft assessment during NMP can be considered as surrogate indicators of graft injury and hepatocellular or cholangiocyte function (**Figure 2**). Graft injury sustained prior to NMP and poor hepatocellular function will manifest as severe graft dysfunction in the early post-operative period (38). Cholangiocyte injury sustained during the preservation process

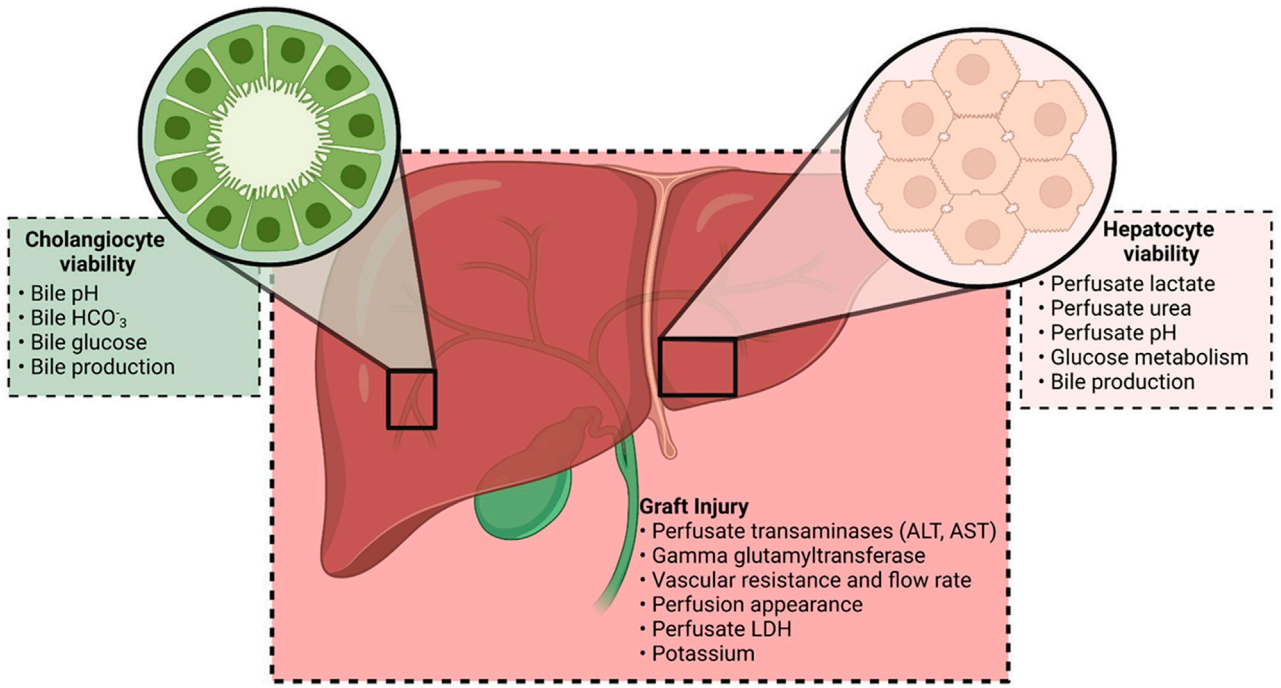


**FIGURE 2 |** Markers currently being used by transplant centers to assess viability on the NMP circuit. Markers can be considered as indicating graft injury and cholangiocyte or hepatocyte viability. The combination of these markers is used in the viability criteria reported by different institutions.

may be detectable through altered bile composition, and manifest as ischemic type biliary lesions (ITBLs). Although there is growing evidence that NMP can reduce the incidence of ITBLs, the risk of this complication is undoubtedly higher in DCD grafts (13, 39, 40).

Lactate clearance is the one viability marker that is consistent across all reported liver NMP viability criteria. Prior to connection of the liver to the NMP circuit which contains preserved human red blood cells, the lactate is usually high (10–20 mmol/L) due to the high concentrations of this substance within preserved bags of human erythrocytes (41). Following connection of a liver with functional hepatocytes, lactate is converted to pyruvate by lactate dehydrogenase in the hepatocyte cytosol (42). This lactate uptake and metabolism has been reported to occur predominantly in zone 1 hepatocytes which receive the best perfusion and oxygenation, therefore it has been proposed that a failure to clear lactate indicates an extensive and panlobular injury (35). Despite this, adequate lactate clearance and post operative graft function have been demonstrated in the setting of extensive hepatocyte necrosis (29). The normal pattern of lactate clearance on NMP is a rapid early phase, that slowly tapers off and reaches a plateau for the remaining period of perfusion (33, 43). This early change in lactate concentration is clinically useful as it gives an early indication of the likelihood the graft is transplantable as other indicators such as bile production and glucose metabolism generally become evident later. It has previously been demonstrated that some transplantable grafts do not follow the above "normal" pattern (28). Instead, they may demonstrate a slower but gradual decline or a slight increase in lactate concentration following the initial rapid decline (28). In these situations, we recommend continuing the duration of the perfusion to determine if the ability to clear lactate and maintain it at ≤2.5 mmol/L is possible. In our experience with using a closed circuit device (Organox®, Oxford, United Kingdom), we have noted that a rise in lactate (after previously rapid clearance) is often the result of bleeding from the liver hilum and this should be addressed. It may occur due to a larger proportion of blood returning directly to the reservoir bag, and therefore bypassing the metabolism and clearance by the hepatocyte. We apply the previously reported viability criteria regardless of the recipient, and also have reported successful outcomes recently with further expansion of the time cut-off for DBD grafts (13, 28).

## EXPANDING THE POOL OF TRANSPLANTABLE GRAFTS

Optimizing the safe utilization of deceased donor livers should be an ambition of all transplant programs. Despite recent improvements, the organ discard rate for retrieved organs from deceased donors in the US and UK is 8.4% and 18.2%, respectively (9, 44). Previous work has revealed concern regarding organ quality or donor history which accounts for the majority of transplant center declines (45, 46). Additional reasons for organ decline that emerge at the time of retrieval

include the macroscopic appearance, a prolonged donor warm ischemic time in the setting of DCD donation, and unexpected laparotomy findings suggestive of malignancy. Despite sub-optimal donor features, many centers have reported acceptable results using livers "that nobody wants", otherwise known as "orphan" livers (13, 27, 45, 46). This is a real-world demonstration that there is capacity to increase the "standard" acceptance criteria of liver grafts and NMP is a safe way to continue expanding these boundaries.

## Donor Medical History

The only absolute contraindications to transplanting a deceased donor's liver based on their medical history is an established diagnosis of cirrhosis, primary central nervous system lymphoma, hematological or metastatic malignancy, active JC viral infection, or a transmissible spongiform encephalopathy (47, 48). The more common reasons in the current era for graft decline include donor age, alcohol history, abnormal liver function test results, and peri-mortem events (49, 50). Separating out the proportion of these grafts that can be safely transplanted is a challenging task, and we find NMP technology beneficial.

### Donor Age

The physiological effects of age have less of an impact on the liver compared with the kidney and heart. However, both structural and functional differences exist in livers of older donors. The metabolic function of the hepatocyte has been shown to be decreased in the elderly. Peterson et al. demonstrated that the phosphorylation and oxidative capacity of mitochondria within the hepatocytes of the elderly (61–84 years) was reduced by 40% compared with young controls (18–39 years) (51). This was also accompanied by an increase in the triglyceride content of hepatocytes, which is further increased with an insulin-resistant state (51, 52). These deficiencies make the graft of an older donor less tolerant of the ischemic periods inherent in the transplant process. Structural changes that have been described in the livers of elderly people include increased fibrosis, possibly as the result of enhanced Th2 cytokine expression from macrophages (53).

The threshold for considering a donor to be of advanced age varies considerably, ranging from ≥40 to ≥80 years of age (54–56). Recent evidence demonstrates that elderly donors of DCD (>70 years) and DBD (>80 years) grafts can be used safely with 5 years graft survival rates of 74% and 77%, respectively, and therefore advanced age should not be a contraindication (54, 57). Grafts from older donors should be viewed as more susceptible to cold preservation injury for the aforementioned reason, therefore every effort should be made to minimize the CIT and NMP is an ideal strategy. Through the application of NMP on receipt of the graft at the recipient center, even recipients that will likely require a prolonged hepatectomy can be transplanted with a graft from an elderly donor with a short CIT.

### Alcohol History

Alcohol-induced liver damage progresses in severity from hepatic steatosis to alcoholic steatohepatitis and then cirrhosis (58). Approximately 90% of individuals who consume excessive quantities of alcohol for at least 2 weeks, will develop macrovesicular hepatic steatosis (58). However, this will resolve following even a short period of abstinence but approximately one third of individuals with hepatic steatosis from alcohol will progress to steatohepatitis (58). Both the transaminases and gamma-glutamyl transferase (GGT) are relevant as they may give an indication of acute hepatocyte injury and steatosis, respectively (59).

The literature describing the transplant outcomes of donors with an excessive alcohol history is limited. Mangus et al. reported similar short and medium-term outcomes in groups of recipients that received a graft from either a donor with or without a history of excessive alcohol consumption (60). The graft recipients peak alanine aminotransferase (ALT) was higher in the excessive alcohol group, however the incidence of graft loss within the first 90 days was similar (6% vs. 7%, $p = 0.75$) (60). These authors analyzed the histological evidence provided from the post reperfusion biopsies of the excessive alcohol consumption group, and only 8% had fibrosis (any severity) and 9% had steatosis (>20%), respectively (60). However these authors defined excessive alcohol consumption as ≥2 more alcoholic drinks per day on a chronic basis ("at least several years") (60). This may mean that these findings underestimate the risk in donors who may consume in excess of 10 alcoholic drinks per day, which is not uncommon in the United Kingdom. At our institution, donors with a history of excessive alcohol consumption are given full consideration if there is no established diagnosis of alcoholic liver disease. Visual inspection of the graft occurs at the time of retrieval and if concerns regarding significant steatosis or steatohepatitis exist, a graft biopsy is performed and our experienced liver histopathologists review the frozen sections before proceeding to transplantation. If the donor (and retrieval procedure) are occurring at a distant hospital, utilizing NMP provides additional time for histological and functional assessment.

### Liver Function Tests

The commonly described "liver function tests", actually provide minimal insight into the liver's synthetic function, if at all. Despite this, even minimal elevations of liver enzymes are considered as criteria for defining marginal donors as per Eurotransplant criteria (61, 62). Alanine aminotransferase (ALT) is found predominantly in the cytoplasm of liver and kidney cells, whereas AST is found in both the cytoplasm and mitochondria of all cells (63). Therefore sources of elevation outside the liver, such as skeletal muscle damage and hemolysis, should be considered when considering a potential donor's transaminases (63). In addition, factors such as hemodynamic instability, trauma, and sepsis may result in deranged liver function results through various mechanisms (50, 59, 64). Mangus et al. reported comparable outcomes in patients transplanted with grafts from donors with elevated peak ALT levels >1000 IU/L and 500–1000 IU/L when compared to those with peak ALT <500 IU/L. In the group with elevated peak ALT

levels (≥500 IU/L), anoxia was the cause of death in a significantly higher proportion of patients compared to those with peak ALT <500 IU/L. Therefore, the authors concluded that an acute anoxic event likely accounted for the liver function derangement (50). Interestingly, the extent of necrosis on graft biopsy did not positively correlate with the peak ALT elevation (50). Adding to the argument that these type of donors are an acceptable way to expand the pool, data from the Scientific Registry of Transplant Recipients (US) demonstrate the discard rate being approximately 70% for a donor's high transaminases (>1000 IU/L) as opposed to 22% for low transaminases (<1000 IU/L) (65).

The viability assessment provided by NMP is the ideal preservation platform for these grafts in the three aforementioned clinical scenarios that lead to organ discard, and has previously been shown to be effective even when the transaminase elevation is accompanied by significant hepatocyte necrosis (29). Our institution gives full consideration to the utilization of livers from donors with deranged transaminases, and places a greater importance on the trend rather than the peak level. The half life of AST and ALT in the human circulation differs, with it being approximately 17 (±5) h for AST and 47 (±10) h for ALT (63). Therefore following any transient insult to the liver during the terminal illness, it should be expected that the peak AST will be higher, occur earlier, and more rapidly decline than ALT, and therefore a decline in donor AST will be the first sign of a resolving insult. Donor bilirubin is undoubtedly an important biochemical test in the context of donor evaluation and is more useful in identifying donors that likely have a poorly functioning liver than transaminases. In support of this belief, it was the only biochemical variable identified as having a significant relationship to transplant outcome in the construction of a UK donor liver quality model by Collet et al. (66). In the absence of an extrahepatic cause of donor hyperbilirubinemia, our upper limit of acceptance for donor bilirubin is 2 x the upper limit of normal (40 μmol/L, 2.4 mg/dl). In the series of 14 donors with transaminases >1000 IU/L reported by Martins et al., only one had a peak bilirubin above this value. Other causes of jaundice in the donor such as hemolysis or Gilbert syndrome (UDP glucoronosyltransferase-1A1 deficiency) should be considered, and useful additional tests such as lactate dehydrogenase and conjugated and unconjugated bilirubin should be requested.

### Peri-Mortem Events

In the context of deceased organ donation, consideration of the events and pathological process that resulted in brain death (or the consideration for DCD donation) is required. Consensus guidelines are available for the scenario of donor malignancy or infection and are beyond the scope of this review (47, 48). In a seminal publication by Feng et al. using US registry data, which reported the donor risk index (DRI), death from trauma or hypoxic brain injury (HBI) was associated with better outcomes than cerebrovascular accident or other causes (55). However, this finding was not replicated with UK data in derivation of the donor liver index (DLI) (66). Despite these statistical models taking into account several donor and graft features, no significant difference exists between those that satisfied our institution's viability criteria and those that did not (**Figure 3**).

A donor ICU length of stay >7 days is considered an extended criteria donor by Eurotransplant, whereas previous consensus meetings have stipulated an ICU length of stay <5 days is ideal for DCD donors (59, 62). It seems that a consensus on what comprises a prolonged donor ICU stay is yet to be reached (67). A longer ICU stay does appear to be associated with increased donor infections, however a positive culture in the donor does not appear to influence the recipient's outcome (67, 68). In a study by Misar et al., which compared the outcomes of pediatric recipients who received a graft from a donor following a short (<5 days) or long (≥5 days) ICU stay, there was no difference in overall graft or patient survival (67). In our practice, we agree with the notion put forward by Strasberg et al. in which it is not the length of ICU stay per se that is deleterious, rather the events that transpire during this period (69). These could include hypotensive episodes, sepsis, exposure to hepatotoxic medications, surgical procedures, and lack of adequate nutritional support.

A blunt traumatic injury may result in serious intracranial injuries and brain death as a result. There may also be an associated traumatic liver injury, which is most commonly lacerations or contusions of the liver parenchyma (70). These traumatically injured livers may still be transplantable and certain strategies, including a back table graft reduction, have been reported with success (70). A concern with utilizing NMP in this scenario is that uncontrollable bleeding may occur on the perfusion circuit due to the high concentration of heparin and lack of coagulation factors. In general, we have seldom had problems with bleeding on the NMP machine from traumatic injuries sustained prior to or at the time of organ retrieval. Application of gauze swabs and hemostatic products have been used to control minor bleeding from capsular damage.

## Graft Steatosis

A frequent reason for a graft to be assessed as suboptimal and discarded is the presence of steatosis (71). Although the cause of hepatic steatosis is likely multifactorial, there is a strong association with body mass index (BMI) (72). This presents a significant issue for the field of liver transplantation as the western population is becoming more obese. Therefore, transplant clinicians must aim to better understand the implications and limitations of steatotic livers and further develop effective medical and surgical strategies to facilitate their safe utilization.

Multiple studies have demonstrated that even severe microsteatosis does not have a deleterious effect on transplant outcome (73–75). It is the extent of macrovesicular steatosis which is the main concern of the transplant surgeon, as an association with primary non-function and reduced graft survival has been demonstrated in the past (76, 77). Despite

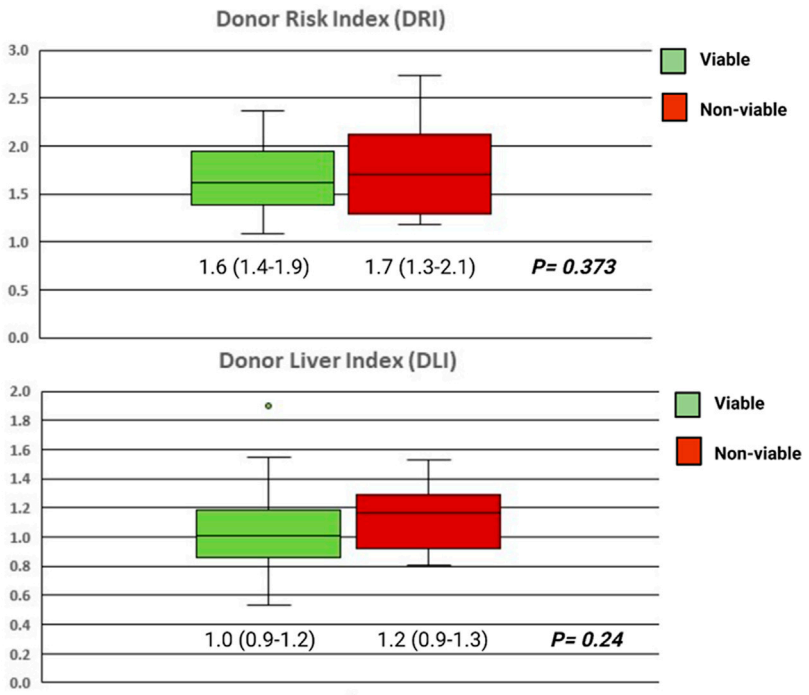


**FIGURE 3 |** Box plots showing the DRI and DLI of the grafts that were assessed as viable and transplanted following NMP (green, *n* = 95), and those that were assessed as non-viable (red, *n* = 12). Groups compared with the Mann-Whitney U test and independent samples T-test for the DRI and DLI, respectively.

graft biopsy and fresh frozen histological assessment being considered the gold standard (71), the method (visual inspection or histology) used to assess graft steatosis is variable. As an example, the UK organ donation service does not have pathology services available after-hours and therefore retrieval surgeons rely on visual inspection and palpation. Furthermore, the implanting surgeon is limited to photographic assessment alone. A limitation of visual assessment and palpation of graft texture is the inability to reliably distinguish microvesicular from macrovesicular change. In a publication by Yersiz et al. that compared surgeons' visual assessment to pathologists' microscopic assessment, the positive predictive value of a surgeons' assessment of >30% macrovesicular steatosis as per histology was only 52% (78). It must also be noted that histological assessment also has its own limitations including interpretation differences between pathologists (79).

The extent of macrosteatosis that represents a graft that is totally unusable remains unclear (80). Studies have frequently failed to define the type of steatosis, making it difficult to draw conclusions draw given the aforementioned differing implications (79, 80). Nevertheless, it is well known by liver transplant clinicians that recipients of livers with moderate to severe steatosis have a more turbulent early post-operative period. In general, more than 30% of macrosteatosis is viewed as increasing the risk of clinically relevant early graft dysfunction. Safely utilizing steatotic livers will become increasingly important due to the obesity epidemic. As described by Jackson et al., drawing comparisons between outcomes of steatotic and non-steatotic livers is most often meaningless as the patient (and surgeon) do not get to decide simultaneous offers of each (81). Therefore the risk-benefit decision is really between survival with a steatotic transplanted liver, and no transplant at all. In a large US study that used national registry data, waitlisted recipients for whom a steatotic liver (≥30% macrosteatosis) offer was made but declined, 22.8% died on the waitlist and 17.6% were delisted. Overall, accepting a steatotic donor liver offer reduced the risk of mortality by 62% in comparison to declining it (81). Furthermore, the reduction in mortality seen with receiving a steatotic liver was greatest in the subgroup with the highest MELD score (81). Further demonstrating that steatotic livers offer a survival benefit in even the sickest of patients.

NMP has previously demonstrated benefits in the setting of steatotic livers, and probably represents one of the most frequent indications for its use (12, 13, 82). The experience with NMP preservation and transplantation of steatotic livers appears to be accruing, and the viability assessment provided by this modality clearly has a role in distinguishing those that will function adequately in the early post operative period. Our center's experience is that one of the main reasons for a graft to be "orphaned" is steatosis, and only severely (>60% macrovesicular) steatotic livers fail the viability assessment. Minimizing the CIT of these grafts is paramount for a good outcome and NMP can facilitate this.

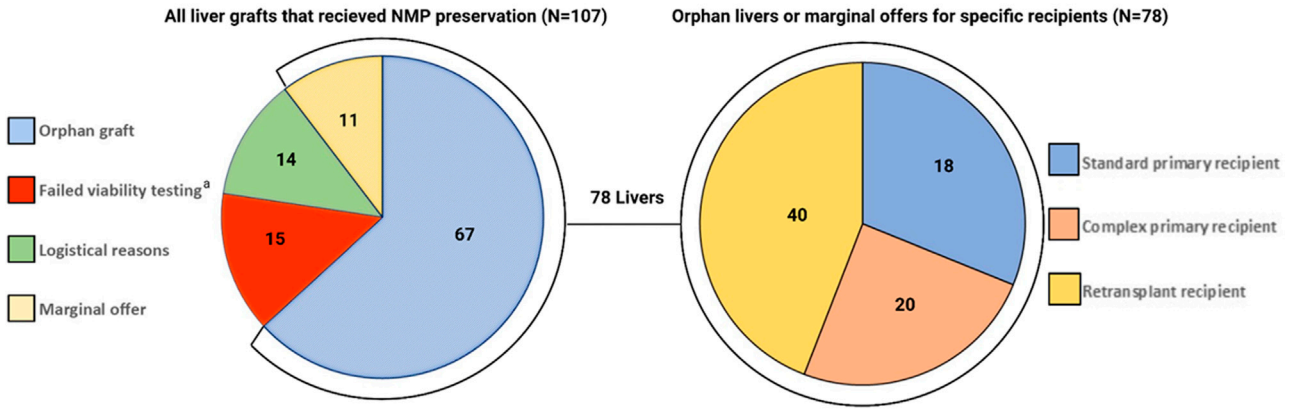


**FIGURE 4 |** Pie charts demonstrating all grafts that underwent normothermic machine perfusion (NMP) at QEHB between October 2018 and April 2022 (left). The high-risk recipient-specific offers with marginal features ($n$ = 11) or "orphan" grafts ($n$ = 67) comprised 78/92 (85%) of the grafts transplanted. The right pie chart demonstrates that the majority of the livers went to either retransplant (40/78, 51%) or complex primary transplant recipients (18/78, 23%). Complex primary transplant recipients were those recipients with previous major hepatobiliary surgery or Yerdel grade ≥III portal vein thrombosis. [a]Two grafts not transplanted due to recipient reasons and one due to equipment failure.

## Prolonged Cold Ischemic Time

Reducing the cellular temperature to approximately 4°C slows cellular metabolism and therefore slows ATP consumption to approximately 10% of activity at normal body temperature (83). Nevertheless, ATP consumption continues and advanced donor age, BMI, and poor nutritional status may result in lower baseline ATP levels and therefore the organs tolerate the ischemic period poorly. The hypothermic conditions result in direct injury to cellular structures, such as the cytoskeleton and various organelles (84). Therefore the energy-conserving effect of cold preservation is partly offset by it non-physiological nature. An ischemia-reperfusion injury is the consequence of this preservation period and this impacts early graft function, and is the main reason for primary non-function (38). An improved understanding of the effect a cold ischemic period has on outcomes may have lead to the shortening of the cold ischemic period that is evident in registry data, and represents one of the main drivers for machine preservation technology (9).

Feng et al. demonstrated that in reference to a graft with an 8 h CIT, every additional hour resulted in a 1% decrease in 1 year graft survival (55). This suggests that the beneficial effect of minimizing CIT demonstrates a continuous pattern, rather than one with a clear threshold effect. Although NMP applied in a back-to-base model cannot alter the CIT associated with the donor procedure, it can abrogate the CIT that is associated with a lengthy recipient preparation period or hepatectomy. In our institution, we aim to keep the CIT at less than 6 h when applying NMP to suboptimal livers. This is achievable in the majority of instances, even if the graft is accepted very late in the retrieval process, as long as adequate personnel are available to prepare both the machine and the graft for perfusion. The timespan from arrival of the graft at our center until the commencement of NMP is approximately 2 h and this should be considered in the estimates of CIT.

# NORMOTHERMIC MACHINE PERFUSION FOR HIGH-RISK GRAFT-RECIPIENT COMBINATIONS

In this section we discuss how we tie up the previously discussed issues together—the novel technology of NMP and the utility of this in the context of high-risk recipients who are significantly disadvantaged due to the scarcity of good-quality organs and timely transplantation of these candidates with marginal grafts. At our institution, Queen Elizabeth Hospital Birmingham, we have incorporated NMP technology into our service on a selected basis for the high-risk graft-recipient combination. The overall benefit of NMP technology over and above cold storage for standard graft-recipient combinations remains debatable. Although NMP is known to mitigate ischemia reperfusion injury and somewhat mitigate short-term beneficial outcomes, it is our belief that NMP should be utilized with a greater aim. Therefore, we utilize NMP in a manner that will allow high-risk recipients to benefit through improved graft access. The results of this approach have been previously published and presented at international conferences (14, 20). To update the current status of our program, between October 2018 and March 2022, we have perfused 107 liver grafts and 92 of these have proceeded to transplantation (**Figure 4**). Delivering a much

**TABLE 1 |** Donor, graft and recipient characteristics.

| **Donor** | **SCS group (N = 56)** | **NMP group (N = 40)** | ***P*** |
|---|---|---|---|
| Donor age, (IQR) | 52 (44–69) | 50 (42–56) | 0.67 |
| Female | 25 (45%) | 27 (67%) | 0.03 |
| Donor BMI (IQR) | 24.9 (22.5–28.3) | 24.1 (21.4–27.7) | |
| Days in ICU (IQR) | 2 (2–4) | 3 (2–5) | 0.22 |
| DRI (IQR) | 1.55 (1.40–1.73) | 1.57 (1.38–1.69) | 0.92 |
| DLI (IQR) | 1.05 (0.92–1.21) | 0.99 (0.86–1.13) | 0.16 |
| Inotrope requirement | 48 (86%) | 36 (90%) | 0.53 |
| Smoker | | | |
| History of alcohol excess | 11 (20%) | 15 (38%) | 0.07 |
| Donor cardiac arrest | 24 (44%) | 13 (32%) | 0.27 |
| Downtime minutes (IQR) | 30 (8–48) | 38 (28–52) | 0.142 |
| Liver biochemistry | | | |
| Peak ALT, IU/L (IQR) | 53 (21–99) | 109 (40–669) | <0.01 |
| Peak bilirubin, mg/dL (IQR) | 9 (7–16) | 13 (8–20) | 0.03 |
| Donor ALT ≥1000 IU/L | 0 (0%) | 9 (23%) | <0.01 |
| **Graft** | **SCS group (N = 56)** | **NMP group (N = 40)** | ***P*** |
| Declined by at least 1 other center[a] | 14 (26%) | 31 (78%) | <0.01 |
| Steatosis | | | <0.01 |
| None | 40 (73%) | 21 (53%) | |
| Mild | 13 (24%) | 7 (18%) | |
| Moderate | 2 (4%) | 12 (30%) | |
| Cold ischemic time, min (IQR) | 482 (409–596) | 372 (325–425) | <0.01 |
| Perfusion time, min (IQR) | — | 759 (488–953) | N/A |
| Total preservation[b], min (IQR) | 482 (409–596) | 1107 (746–1330) | <0.01 |
| **Recipient** | **SCS group (N = 56)** | **NMP group (N = 40)** | ***P*** |
| Age (IQR) | 43 (29–56) | 36 (24–50) | 0.05 |
| UKELD | 58 (55–63) | 58 (53–61) | *0.73* |
| MELD | 19 (14–25) | 21 (13–26) | *0.82* |
| Number of previous grafts | | | 0.06 |
| One (first retransplant) | 49 (87%) | 29 (72%) | |
| Two (second retransplant | 7 (13%) | 9 (21%) | |
| Three (third retransplant) | 0 (0%) | 2 (7%) | |
| Indication | | | *0.40* |
| Hepatic artery thrombosis | 17 (30%) | 14 (35%) | |
| Chronic rejection | 5 (9%) | 8 (20%) | |
| Biliary complications | 18 (32%) | 9 (22%) | |
| Disease recurrence | 13 (23%) | 6 (15%) | |
| Waitlist duration (days) | 72 (26–151) | 235 (60–423) | *<0.01* |
| Follow up (Median, months) | 40 (25–56) | 21 (11–29) | <0.01 |

*Categorical variables compared with Chi-square test. Independent sample T-test used to compare continues variables that were normally distributed. Mann -Whitney U test used to compare normally distributed continuous variables. AL, alanine aminotransferase; BMI, body mass index; ICU, intensive care unit; DRI, donor risk index; DLI, donor liver index; UKELD, United Kingdom model for end stage liver disease; MELD, Model for end stage liver disease; SCS, Static cold storage; NMP, Normothermic machine Perfusion.*

[a]*Reason related to donor or graft quality.*

[b]*Total preservation time comprised cold ischemic time and perfusion time.*

needed organ transplantation service during the COVID-19 pandemic has provided additional challenges, however NMP has proven useful to overcome COVID testing requirements and hospital logistics regarding bed access. NMP provided a prolonged preservation time that would not have been possible, even in the standard graft-recipient combinations (85).

The ability to accept and safely transplant marginal and 'orphan' livers into high-risk recipients has been made possible with NMP due to a number of reasons. Firstly, the NMP viability assessment of a graft provides additional confidence that the graft will function adequately in the early post operative period. This is supported by the fact that PNF has not occurred in any of the 92 recipients of a liver preserved with NMP. Secondly, the ability to inspect and connect the liver to NMP expediently on arrival at our center allows the CIT to be kept to an acceptable minimum. This means that grafts that are declined at other transplant centers, in other parts of the UK, can still be reperfused within an adequate time period. The additional insult of a prolonged CIT on a graft with marginal attributes should not be underestimated, as undoubtedly there is an interaction between these variables as described previously. Finally, with the current national organ retrieval system in the

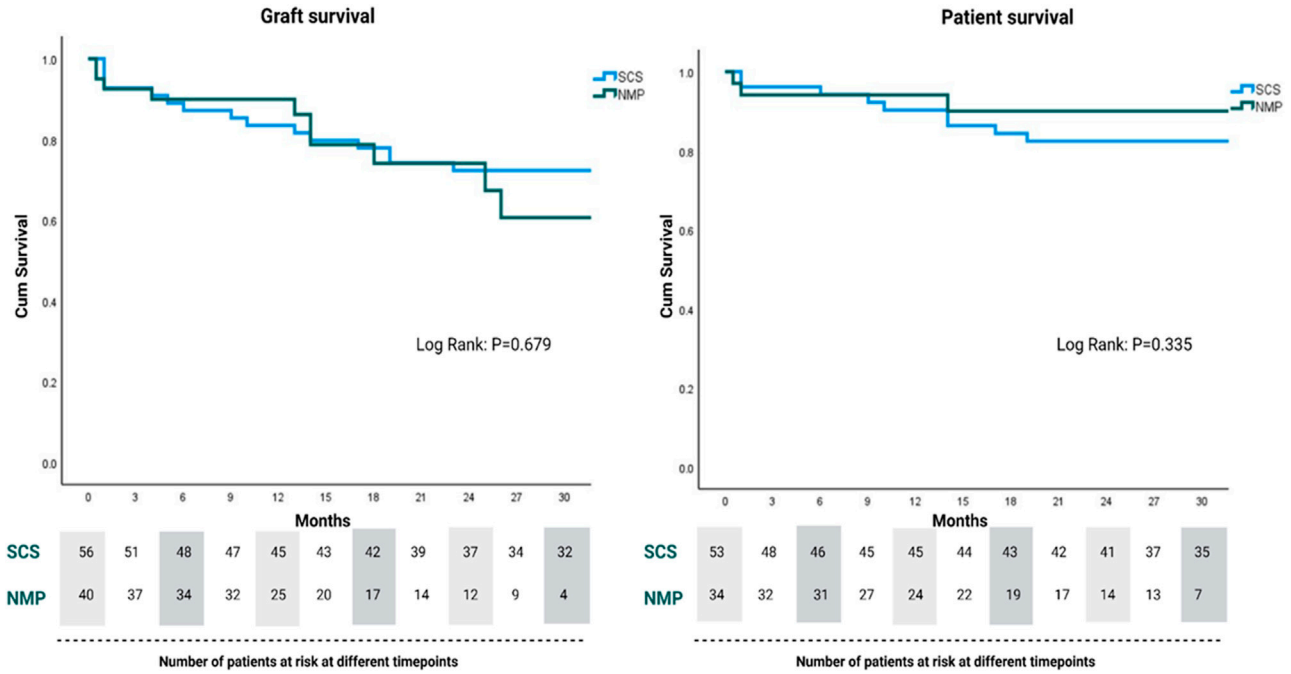


**FIGURE 5 |** Graft and patient survival for normothermic machine perfusion (NMP) and static cold storage (SCS)-preserved grafts for retransplant recipients.

UK being relatively inflexible about donor retrieval timing, it allows for a difficult operation to be performed during daylight hours with adequate and appropriate staffing.

In regards to graft physiology following NMP, we have experienced a lower than expected incidence of reperfusion syndrome and clinically significant early allograft dysfunction than would be expected given the graft and recipient characteristics (86). In the cohort of NMP-preserved grafts depicted in **Figure 4**, 40 out of the 78 marginal grafts were transplanted into patients undergoing retransplantation. The outcomes of the initial 26 patients in this cohort have been published previously (14). Since completing enrolment in this previous study and the reassuring results, we have performed an additional 14 retransplants using NMP-preserved grafts and all of these have reached at least 3 months follow-up. Therefore we have experience with a unique cohort of 40 patients undergoing retransplantation with NMP-preserved marginal liver grafts. The donor and graft characteristics of this group, in comparison to a retrospective cold storage control cohort transplanted at our institution over the last 5 years, are demonstrated in **Table 1**. Similar to our previously reported findings, the graft and patient survival did not differ between groups (**Figure 5**) despite the NMP group having significantly more steatotic grafts (moderate steatosis; 30% vs. 4%, P=<0.01), donors with peak ALT >1000 IU/L, and grafts declined by at least one other transplant center (78% vs. 26%, *P*=<0.01). Furthermore, in this expanded cohort, the peak alanine transaminase in the initial post operative 7 days was significantly lower (521 IU vs. 796 IU, $p = 0.02$) in the NMP group, and the EAD rate was not significantly different (47% vs. 37%). The rate of early acute rejection was higher (50% vs. 27%, $p = 0.02$) in the NMP cohort and has been discussed in a previous publication. We acknowledge that the incidence EAD rate is higher than previous reports, however this did not translate to early graft loss. Furthermore, a large majority were "biochemical" EAD due to raised bilirubin only (>177 mmol/L) in the presence of severe immune-mediated rejection, without any clinically relevant organ failure.

## CONCLUSION

The transplantability of a liver graft has long been a subjective assessment. It is understandable that surgeons may therefore err on the side of caution, in an effort to do no harm. This is of course particularly important when the predicted surgical risks are already high. The ability of NMP technology to extend liver graft preservation time can allow more complex recipients to be transplanted in a controlled and safe manner. Furthermore, it can safely facilitate the usage of liver grafts for these recipients that would otherwise not have been considered. Evidence supporting the use of NMP for marginal organs and high-risk recipients is starting to emerge with promising data in the retransplantation setting. The problem inherent with researching this topic is the lack of appropriate controls for comparison. Retrospective control cohorts, or even propensity matching, have their limitations as under different preservation conditions these grafts would not have been transplanted previously (87). The benefits of NMP in the setting of a suboptimal graft and high-risk

recipient justify the additional resources required for this technology. Similar to the improved understanding of cold preservation techniques throughout the second half of the 20th century, the application of NMP continues to be refined through research at many centers around the world. As experience with machine perfusion technology grows, this will hopefully translate into improved transplant outcomes.

## DATA SHARING AGREEMENT

Data collected for this study, including individual participant data and the data dictionary, will be made available to others at publication. The data will be in an anonymized form to protect participants' privacy. The authorship agrees to provide access to all additional study documents.

## AUTHOR CONTRIBUTIONS

AH wrote and edited the first draft of the manuscript. AN, GC, IP, DS, and YO reviewed and edited the manuscript. MP and HH conceptualized, reviewed, and edited the manuscript.

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## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# From Haphazard to a Sustainable Normothermic Regional Perfusion Service: A Blueprint for the Introduction of Novel Perfusion Technologies

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Normothermic Regional Perfusion (NRP) has shown encouraging clinical results. However, translation from an experimental to routine procedure poses several challenges. Herein we describe a model that led to the implementation of NRP into standard clinical practice in our centre following an iterative process of refinement incorporating training, staffing and operative techniques. Using this approach we achieved a four-fold increase in trained surgical staff and a 6-fold increase in competent senior organ preservation practitioners in 12 months, covering 93% of the retrieval calls. We now routinely provide NRP throughout the UK and attended 186 NRP retrievals from which 225 kidneys, 26 pancreases and 61 livers have been transplanted, including 5 that were initially declined by all UK transplant centres. The 61 DCD(NRP) liver transplants undertaken exhibited no primary non-function or ischaemic cholangiopathy with up to 8 years of follow-up. This approach also enabled successful implementation of ex situ normothermic liver perfusion which together with NRP contributed 37.5% of liver transplant activity in 2021. Perfusion technologies (*in situ* and *ex situ*) are now supported by a team of Advanced Perfusion and Organ Preservation Specialists. The introduction of novel perfusion technologies into routine clinical practice presents significant challenges but can be greatly facilitated by developing a specific role of Advanced Perfusion and Organ Preservation Specialist supported by a robust education, training and recruitment programme.

**Keywords: donation after circulatory death, education, normothermic machine perfusion, training model, normothermic regional perfusion, training, simulation**



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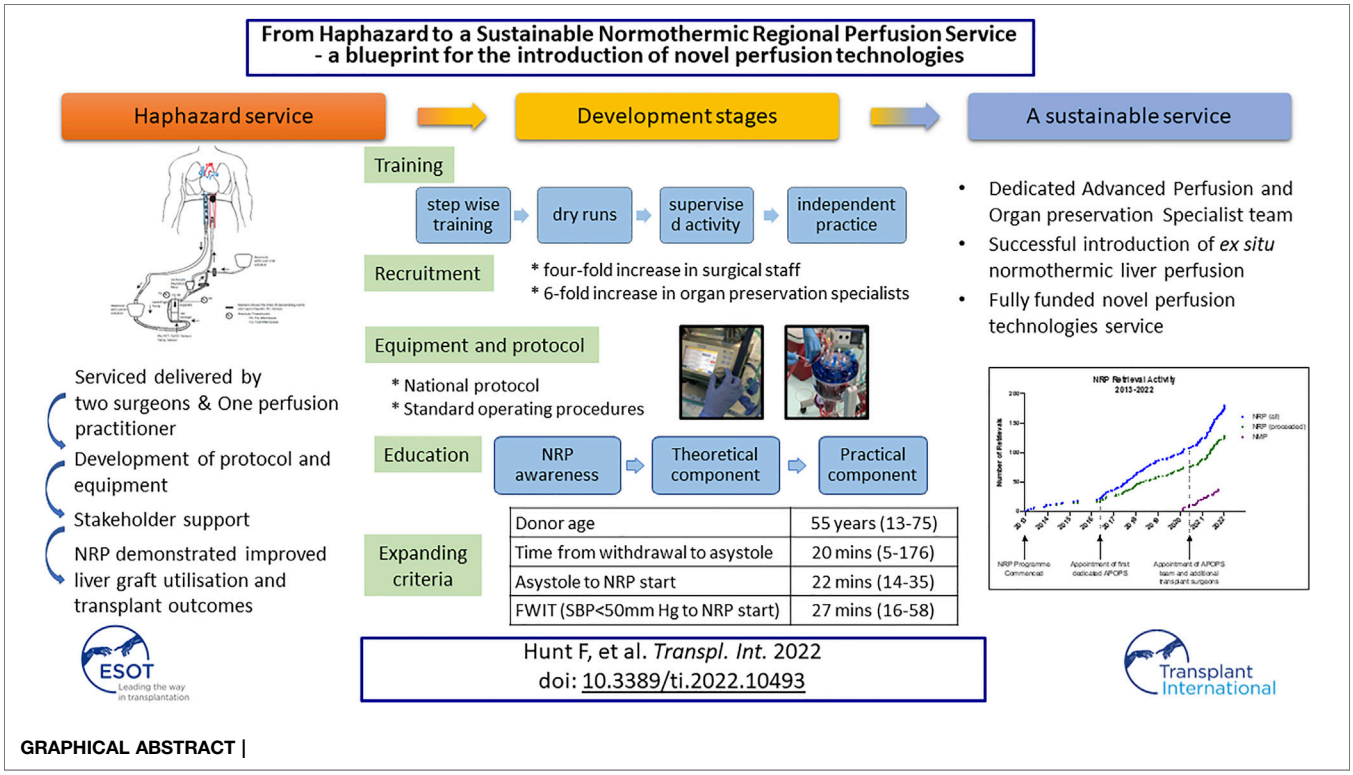


**GRAPHICAL ABSTRACT |**

## INTRODUCTION

Donation after circulatory death is now accepted in many countries across the globe. The confines of widely varying regulatory arrangements lead to significant variation in clinical practice with regards to the duration of the "no touch" period after asystole (from 2 min in some areas of the US though to 20 min in Italy) and the use of ante-mortem interventions such as heparinisation and femoral vessel cannulation (1).

In the United Kingdom, donation after circulatory death (DCD) currently accounts for 43% of all deceased organ donors (2). DCD donation has had a considerable impact on kidney transplantation in the UK with utilisation rates comparable with DBD donation and very good clinical outcomes (1). In contrast, only a minority of DCD livers are utilised, and, as a result, DCD liver transplantation contributed to only 16.2% of all deceased donor transplants in the UK in 2020-21 (3). Despite a selective approach, including minimisation of cold ischaemic time and careful recipient selection, the burden of additional complications specific to DCD liver transplantation is significant. Specifically, higher rates of early allograft dysfunction in the short term and multi-focal biliary stricturing (ischaemic cholangiopathy) in the long term lead to considerable morbidity and result in a retransplantation rate of nearly 25% in the recent UK experience (4). Similarly, the rates of DCD pancreas utilisation are low, with increased complication rates (5) but good long-term clinical outcomes (6).

In recent years, several approaches have been adopted to mitigate the complications of DCD organ transplantation. These range in ambition and complexity from optimisation of the retrieval process (7), through the use of novel perfusion and preservation technology, in the form of *ex situ* machine perfusion [hypothermic (8, 9), oxygenated hypothermic (10, 11), or normothermic (12)], or by re-establishing an oxygenated blood supply to donor organs *in situ* after donor asystole with Normothermic Regional Perfusion (NRP) (4, 13) or a combination of these strategies (14). *Ex situ* technologies have shown a variable degree of benefit in DCD transplantation in terms of either prolonged preservation, a reduction in the rate of complications, immunomodulation, and improved graft outcomes. In contrast, the use of *in situ* NRP offers several advantages over these approaches, including a beneficial impact for all abdominal organs with a single intervention (15).

Novel perfusion and preservation technologies are an exciting innovation that so far has been driven by enthusiasts who have designed the technology, refined the protocols, and generated the initial outcome data. However, translation into routine clinical use is more complex and requires adequate and continuous evaluation (16), an appropriate skill mix in the team, training, and education as well as institutional support for innovation and adequate funding. The absence of such an environment has often been cited as a reason for the lack of adoption (17). Furthermore, the individual technology complexity and the magnitude of change required to the existing practices play important roles when considering their adoption. There is no doubt that the use of NRP is at the higher end of technological complexity, as it requires supplementation of skills as well as additional equipment to travel to the donor centre with a modified Extracorporeal Membrane Oxygenation (ECMO) device.

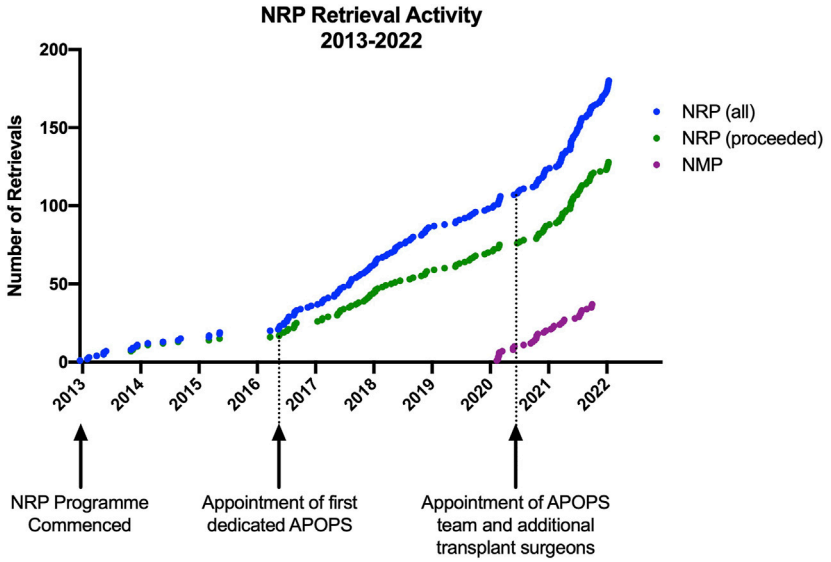


**FIGURE 1 |** Edinburgh normothermic regional perfusion and *ex situ* normothermic machine perfusion activity and key points in the evolution of the programme.

For this reason, logistical feasibility has been cited as a barrier to NRP implementation into clinical practice by many transplant teams.

Herein, we describe the Edinburgh Transplant Centre's experience in implementing NRP from a haphazard use to a routine and sustainable clinical service. Whilst the focus here is on NRP specifically, the approach described can act as a framework for the introduction of other novel technologies more widely.

## NORMOTHERMIC REGIONAL PERFUSION—THE EXPLORATORY STAGE

NRP was first described in Spain in the context of unexpected cardiac arrest, with potential donors commenced on Extra-Corporeal Membrane Oxygenation (ECMO) *via* femoral vessel cannulation whilst CPR was ongoing (18). In the context of unexpected cardiac arrest, NRP presents effectively the only viable solution for proceeding to successful solid organ donation ["uncontrolled" or Maastricht Category II DCD donation (19)] and transplantation. This approach was subsequently expanded to include all DCD donors and has since been considered in several European countries.

The cumulative insults of the initial warm ischaemic time followed by ongoing ischaemia during static cold storage affects all DCD organs to various degrees. The liver is particularly sensitive, as reflected in higher rates of early allograft dysfunction and late ischaemic biliary stricturing (ischaemic cholangiopathy) compared to livers transplanted from DBD donors. Accordingly, in the context of Category III DCD, the use of NRP was driven primarily by a need to improve liver graft utilisation and outcomes.

To assess the role of NRP with regard to the liver outcomes, the procedure was first introduced in the UK in 2011-2012 as part of a research study (Cambridge), to support the development of a pilot of uncontrolled DCD donation (Edinburgh) (20) and as a service development in Birmingham. The initial combined report from the three centres demonstrated a reduction in early allograft liver dysfunction, a complete absence of ischaemic cholangiopathy, and hinted at an improved function for the renal transplants undertaken following NRP (21). Based on these encouraging data, NRP provision continued as part of a formal service evaluation project in two centres (Edinburgh and Cambridge) overseen by NHS Blood and Transplant (NHSBT) to enable further data acquisition whilst allowing for refinement of the surgical procedure and technology as well as considering how the NRP service could be configured and integrated into the established UK national retrieval service (22) to build a robust business case for funding.

## NORMOTHERMIC REGIONAL PERFUSION—THE DEVELOPMENT STAGE

The development phase had several key targets, including promotion and wider acceptance, refinement of the protocol, technique and equipment, and defining the standard operating procedure, and it involved a formal four-step process for implementation: recruitment, education and training, implementation, and review for evaluation.

### Stakeholders Support

Successful implementation of NRP involves many stakeholders including transplant co-ordinators, specialist nurses in organ donation and donor hospital staff, patients, and transplant surgeons for all organs at a national level, given the current allocation protocols in place in the United Kingdom. The plans and proposed approach were discussed extensively and support was gained from stakeholders groups at the local and regional levels to facilitate logistics, deployment, and operating room planning in the referring hospitals. At the national level,


Donor location:
78 Local
83 Regional
19 National (16 by air)

**FIGURE 2 |** Distribution of NRP retrievals by location and footprint of standard NRP retrieval equipment. (Map by Datawrapper©) (locally defined as Edinburgh; Regional defined as Scotland; National defined as the rest of the United Kingdom).

discussions with NHS Blood and Transplant and organ-specific advisory groups ensured governance support and organ acceptance. A wider consultation about the ethical implications of NRP took place and several technical modifications were put in place to ensure that the use of NRP does not invalidate the circulatory determination of death and does not lead to accidental brain perfusion (23), thus addressing issues that may preclude its adoption in other countries (24).

## Recruitment and Definition of Roles in the Team

One of the key points in ensuring sustainability was the definition of the roles in the team. Our standard retrieval team comprises a lead surgeon and an assistant surgeon, a scrub practitioner, and a theatre practitioner for cold perfusion. During the exploratory stage, it became clear that the additional tasks needing completion during NRP combined with the management of the pump will require additional manpower. To that extent, a new role of Advanced Perfusion and Organ Preservation Specialist (APOPS) with the knowledge and skills to manage all aspects of machine perfusion and the NRP process was created. APOPS travel to the donor centre with the standard retrieval team, manage the pump during perfusion and coordinate the NRP process (blood sample processing, data recording, and allocation of roles prior to the start of the procedure to ensure a rapid setup in theatre). The team of five routinely travels within the standard logistical footprint of a regular retrieval team in a single vehicle or aircraft anywhere in the United Kingdom.

The development of the APOPS role also had an instrumental impact on the expansion of the NRP programme within our service (**Figure 1**) by coordinating logistics, setting up training and education, and overseeing the governance of the equipment.

Concomitantly with the development of the APOPS team, additional surgeons were recruited to undertake NRP (six currently) to ensure robust service delivery and a sustainable rota, which allowed us to increase the reach of NRP within and outwith our allocated retrieval area (**Figure 2**).

## Equipment and Surgical Procedure

The initial equipment included a Medtronic pump, a Maquet heat exchanger, and a complex bespoke circuit that had two bypass loops to mitigate against potential problems such as clots in the reservoir or the leukocyte filter. Biochemical and blood gas analyses were undertaken in the local hospitals. However, this limited portability and presented logistic challenges with regional and national donors and lengthen the response time to biochemical interpretation and decision making. As a result, the equipment was minimised to allow portability and ease of use and currently consists of a Maquet Cardiohelp® device, a heat exchanger, and a simplified circuit (Maquet®– BE-MECC 50312 Edinburgh NRP pack) (**Figure 3**). Point of care devices are used for biochemical (Abbott Picolo®) and blood gas analysis (I-Stat®) to provide real-time decisions and make the team fully self-sufficient.

In terms of surgical approach, initially, minimal changes were made to a standard DCD procurement to enable adoption, with aortic (24Fr DLP® Single Stage Cannula, Medtronic Inc, Minneapolis, MN, United States) and inferior vena cava cannulation (29/37Fr Edwards Trim-Flex™ Dual Stage Venous Cannula, Edwards Lifesciences LLC, Irvine, CA, United States), and descended aorta occlusion using an intra-aortic balloon (Coda® LP Balloon Catheter, Cook Medical Inc, Bloomington, IN, United States) as previously described (21). Recently we have adapted the surgical cannulation to the donor situation using femoral cannulation if there is a hostile abdomen (i.e., extensive previous surgery) or if there is concomitant cardio-thoracic

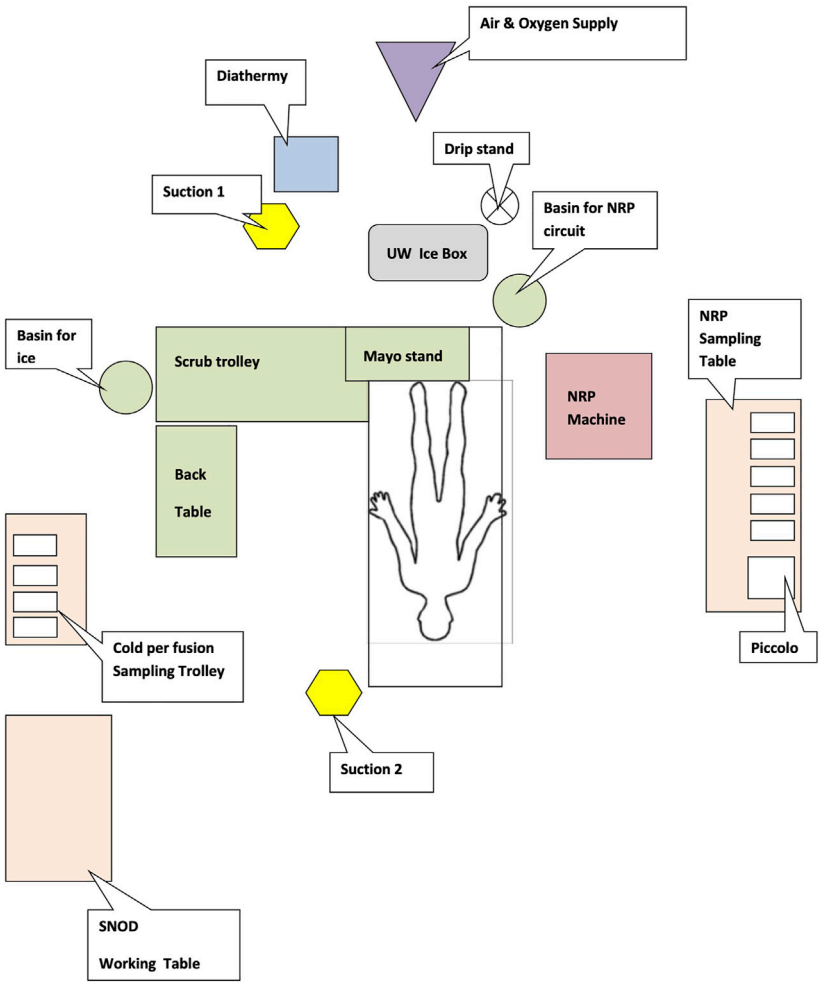


**FIGURE 3 |** NRP theatre setup (floor map).

retrieval. If cardiothoracic organs are to be retrieved, the intention to undertake NRP is discussed with the cardiothoracic team as early as possible and the skill mix of the on-call retrieval teams is reviewed, with the provision of additional support if needed. To prevent inadvertent brain perfusion (which is unlikely in abdominal NRP as long as complete occlusion of the descending aorta is achieved) we added a safeguard that involves the insertion of a large cannula (9Fr DLP® Aortic Root Cannula, Medtronic Inc, Minneapolis, MN, United States) in the ascending aorta that is left open to atmosphere before A-NRP is commenced (23).

## Protocol and Standard Operating Procedures

Over the last 9 years, a comprehensive NRP protocol and standard operating procedures have been developed and refined to incorporate all aspects of the process, including pre-departure equipment checklists, quick reference cards for equipment set-up, and troubleshooting potential problems during perfusion. An overview of the process is outlined below, and the entire operating procedures and relevant material are included in **Supplementary Appendix SA**.

Upon arrival at the donor hospital the APOPS set up the theatre as illustrated in **Figure 3** (often repositioning the operating table to facilitate access to air and oxygen supplies for the NRP machine) before setting up the NRP machine and point of care blood gas and biochemistry analysers (iStat® and Piccolo®) whilst the surgeons take a handover from the Specialist Nurse in Organ Donation (SNOD) and check through relevant donor documentation.

The entire team (retrieval team, SNOD, local theatre staff, and intensive care doctor) then join together for a detailed briefing to include all aspects of the process, timings, communication with recipient transplant teams, and what to expect for those who have not been previously involved in an NRP retrieval (e.g., awareness of small bowel peristalsis). The APOPS will then set and prime the disposable NRP circuit

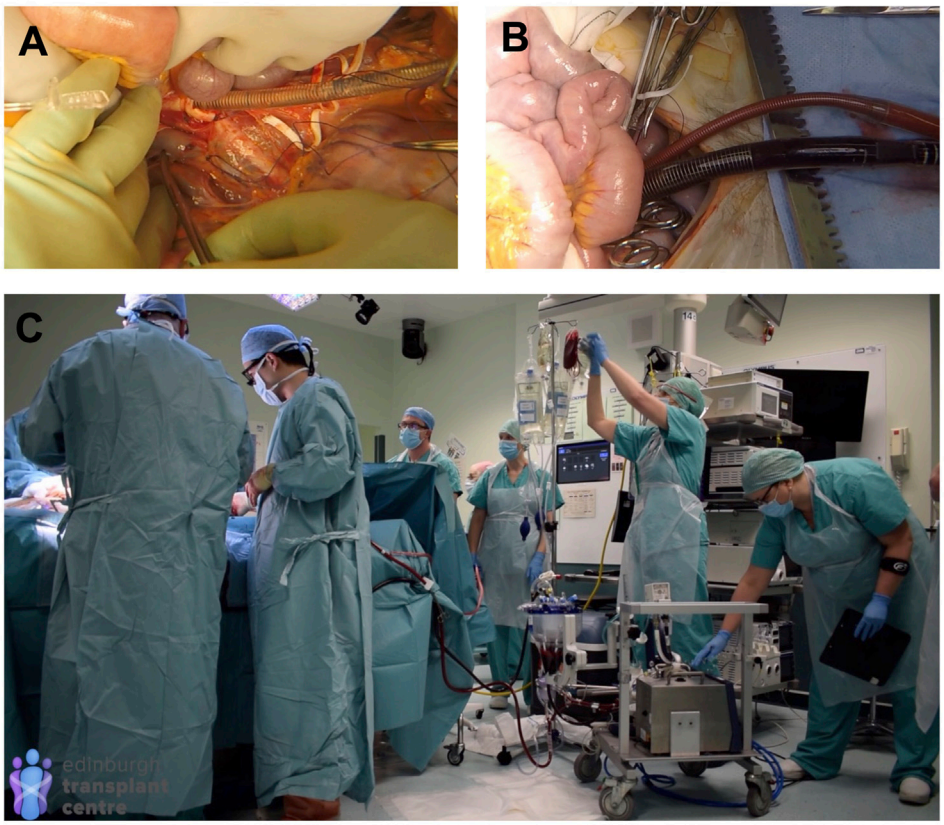


**FIGURE 4 |** NRP Technique (see also full protocol and operative video demonstration in **Supplementary Appendix**). **(A)** cannulation of the abdominal aorta and IVC; **(B)** NRP circulation established; **(C)** theatre arrangement shortly after commencing NRP circulation (all operative images obtained with donor family permission).

whilst the surgeons prepare all antibiotics and drugs required for addition to the perfusate solution. The entire set-up can be completed within 30 min, minimising any additional time required before withdrawal of life-sustaining treatment from the donor. After asystole and the legally mandated "stand off" period, the donor is transferred into the operating theatre. Rapid laparotomy and a limited Cattel-Braasch manoeuvre are undertaken to allow access to the abdominal aorta and vena cava, which are then cannulated with 26F and 29F cannulas respectively and connected to the NRP circuit (**Figure 4**). A median sternotomy is carried out, the descending thoracic aorta is cross-clamped and a cannula is placed in the aorta arch and left open to air to confirm the complete absence of any circulation to the brain. At this point (usually 7–10 min after knife to skin), tubing clamps are released and NRP circulation commenced. Any bleeding points in the abdomen or chest are secured whilst continuously communicating with the APOPS managing the NRP pump until a stable circulation is achieved. After 5–10 min of stable circulation, a T-tube is placed in the bile duct (to measure bile production) and no/minimal further dissection is undertaken during the 2 h of NRP circulation to minimise haemodynamic disturbance and allow time to focus on dynamic functional organ assessment. Biochemistry and blood gas samples are taken every 30 min for the 2-hour duration of the NRP after which retrieval proceeds in a similar manner to DBD donation (21).

## Education and Training

Mastering all aspects of NRP DCD organ retrieval, pump management, and organ assessment required training to ensure repeatability and routine. An education programme was designed involving three key components: an NRP awareness session followed by theoretical and practical components (**Table 1**). Training sessions were delivered initially on a daily basis focusing on the practical aspects of NRP and evolved as the programme expanded with regular group/team sessions, weekly drop-in sessions, tailored one to one meetings depending on staff availability and level of skill and knowledge and fully simulated retrievals using bespoke mannequins developed in-house. The NRP awareness sessions were instrumental in creating wider support and were open to all stakeholders in the donation, retrieval, and transplant process. These sessions covered the need for NRP, the known benefits for transplanted organs, and the practicalities of how NRP is undertaken (**Table 1**).

Theory covered the fundamentals of physiology and basic scientific principles underpinning the effects of organ ischaemia during the retrieval process and how this can be mitigated with NRP. Practical sessions were designed to teach the technical skills

**TABLE 1 |** Normothermic regional perfusion training programme structure and content (APOPS = Advanced Perfusion and Organ Preservation Specialist; SNOD = Specialist Nurse in Organ Donation; ICU = Intensive Care Unit).

| Training session | Content | Attendees | Session approach |
|---|---|---|---|
| NRP Awareness | • What is the need?<br>• What is NRP?<br>• Why NRP?<br>• Benefits of NRP?<br>• Outcomes<br>• Simulation of NRP Retrieval<br>Theatre set-up<br>Practical demonstration | • Surgeons<br>• APOPS<br>• Theatre team<br>Scrub<br>Cold preservation<br>• SNODs<br>• Recipient Coordinators<br>• Blood bank staff<br>• Donor hospital team<br>ICU team<br>Theatre staff | • Drop-in<br>• Team session<br>• Seminars |
| Theoretical Component | • Anatomy<br>• Physiology<br>What happens in cells during DBD<br>What happens in cells during DCD<br>Impact of DCD on organ function<br>What happens in cells when using NRP<br>• Equipment configuration and circuit dynamics | • Surgeons<br>• APOPS<br>• Theatre team<br>Scrub<br>Cold preservation<br>• SNODs | • One:one<br>• Team session<br>• Interactive case-based discussion |
| Practical Component | • Pre–retrieval setup<br>• Composition of Priming solution<br>• Surgical Protocol/Cannulation<br>• Pump/ Circuit Training<br>• Troubleshooting<br>• Blood Sampling/Blood Analysers<br>• Interpretation of Blood Results<br>• Communication<br>• Paperwork/documentation<br>• Simulation | • Surgeons<br>• APOPS<br>• Theatre team<br>Scrub<br>Cold preservation<br>• SNODs | • One:one<br>• Team session<br>• Video debrief<br>• Case-based discussion<br>• Focus tutorials |

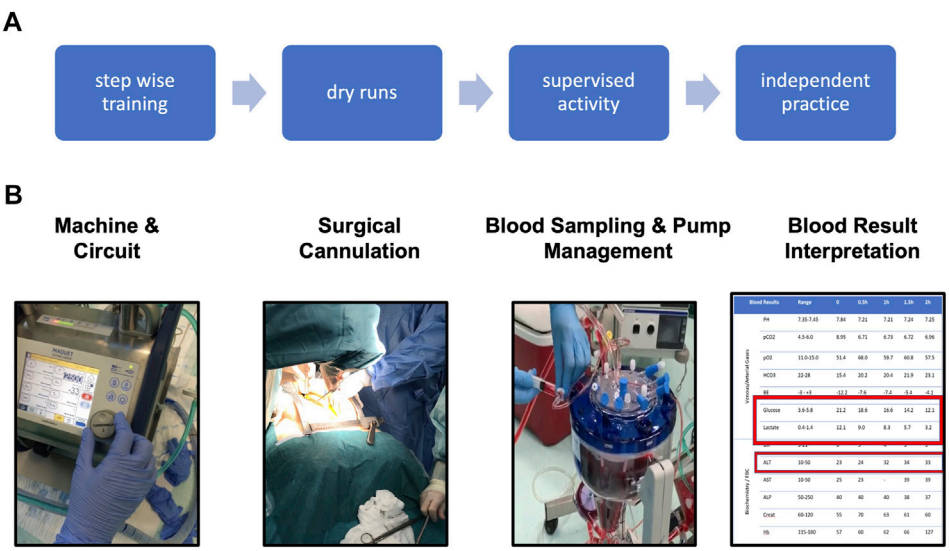


**FIGURE 5 |** NRP training. **(A)** overview of training strategy. **(B)** Fundamentals of NRP knowledge and skills required for independent practice.

of all aspects required to establish and maintain NRP (from machine setup, through surgical technique, to blood result interpretation) and were delivered in tasks specific workstations. Dry-runs, which included troubleshooting scenarios, were considered essential for all core retrieval team members prior to actual donor attendance (**Figure 5**).

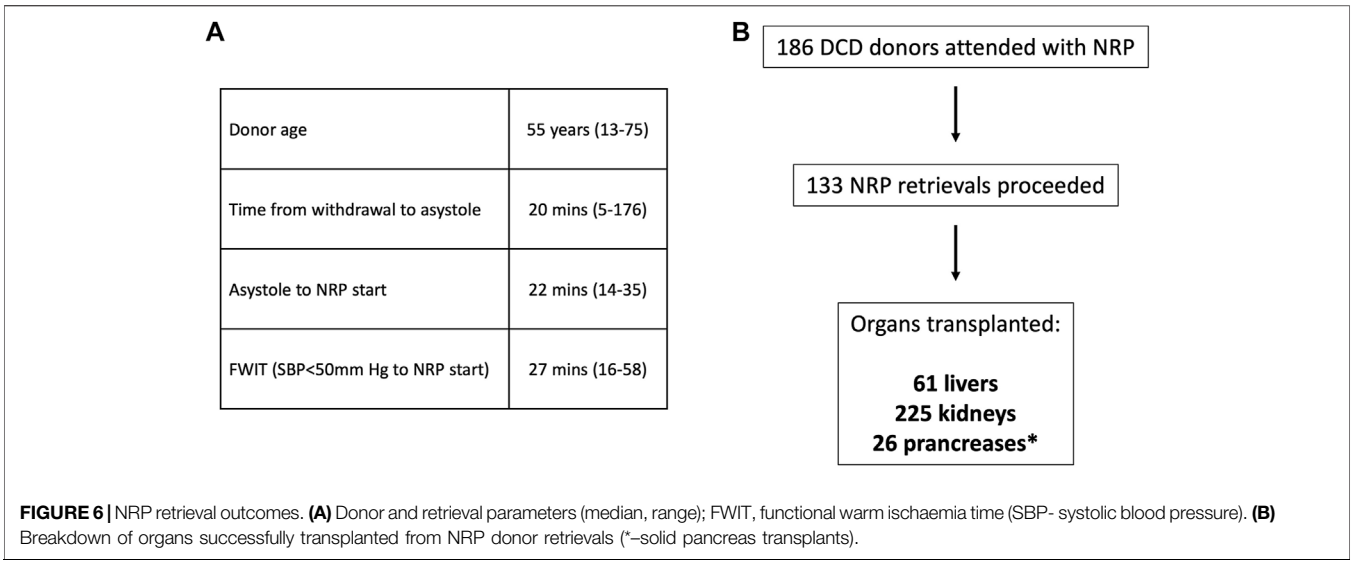


**FIGURE 6 |** NRP retrieval outcomes. **(A)** Donor and retrieval parameters (median, range); FWIT, functional warm ischaemia time (SBP- systolic blood pressure). **(B)** Breakdown of organs successfully transplanted from NRP donor retrievals (*–solid pancreas transplants).

Training was delivered by the APOPS with support from clinicians and technical experts from the companies providing the NRP equipment.

A comprehensive competency framework (**Supplementary Appendix SA**) was designed to guide trainees safely through the detailed aspects of the process; this acted as a record of achievement of knowledge, skills, and competencies, ensuring accountability. A training pack (**Supplementary Appendix SA**) was also developed to support the training sessions and as a practical guide directing the learner through the entire NRP retrieval process in a logical way.

Regular team debriefings include high-definition intraoperative videos, which provide invaluable material for teaching, particularly after technically challenging donors, and focused tutorials on historical donor cases complementing the accumulating experience on organ retrievals runs.

Whilst the road to achieving independence in NRP practice varies greatly and depends on several factors including previous experience with standard DCD retrievals, following this training programme most of our senior trainees became comfortable running NRP after five mentored cases.

## RESULTS AND CURRENT STATUS

Over the last 9 years, our team has attended 186 DCD donors with NRP. A total of 133 donors progressed to donation, resulting in 61 liver transplants, 225 kidney transplants, and 26 pancreas transplants (**Figure 6**). Changes in the team structure outlined above have allowed for a considerable increase in capacity, reaching 93% NRP cover for all DCD donors attended by our team anywhere in the United Kingdom. The functional warm ischaemic time has been extended to 1 h and the time from withdrawal to asystole to 3 h (**Figure 6**) with no detrimental effects. All livers transplanted (52 locally and 9 in other centres) remain free of ischaemic biliary complications (4). A total of five livers that were declined by all UK centres based on donor history prior to retrieval were rescued and transplanted following assessment during NRP; this led to a change in acceptance policy and we only decline livers after functional assessment during NRP. The criteria for accepting a DCD NRP liver for transplantation have evolved with time and currently are based on a composite index of liver function tests (ALT less than 500–600 iu/L), lactate (downward trend), glucose (evidence of consumption), pH (normalisation), as detailed in the attached national protocol (**Supplementary Appendix SA**). Livers where ALT >1000 iu/L or lactate is increasing or not falling are usually discarded whilst for those in-between *ex situ* NMP is used for additional functional assessment.

Using NRP transferable skills and employing the strategy outlined above, we successfully implemented *ex situ* Normothermic Machine Perfusion using the OrganOx *Metra*® in clinical practice in 2020 and undertook 41 liver perfusions and transplanted 27 grafts. The donor and recipient demographics and preservation times for the NMP programme are illustrated in **Table 2**. All grafts are monitored and managed on the device by the APOPS. Our current perfusion and preservation strategy is detailed in **Figure 7**. Essentially, all DCD donors that we attend undergo NRP. Additional use of *ex situ* NMP is indicated if further functional assessment is required or if there are logistics or complex recipient issues. If a DCD liver is retrieved without NRP by a different team, these organs will undergo *ex situ* NMP at base for functional assessment prior to transplantation. The use of *ex situ* NMP for DBD livers is driven by logistics or if a functional assessment is needed for marginal grafts.

The use of these two technologies had a significant impact on the liver transplant activity mitigating a considerable reduction in liver transplant activity in the wake of the introduction of the new UK National Liver Offering System for DBD grafts (25) and the COVID-19 pandemic and represented 37.5% of activity in 2021 (**Figure 8**).

### Funding a Novel Perfusion Technologies Programme

The development of the NRP programme required financial support from several sources at the different stages of

**TABLE 2 |** Demographic data, indications, and preservation times for *ex situ* normothermic machine perfusion.

| | All Perfusions (*n* = 41) | Livers Transplanted (*n* = 27) | Livers Not Transplanted (*n* = 14) |
|---|---|---|---|
| Donor demographics | | | |
| Gender M:F | 20:21 | 12:15 | 8:6 |
| Age median (range) | 52 (15–77) | 51 (15–71) | 58.5 (21–77) |
| BMI median (range) | 26.5 (19.7–37.0) | 26.1 (20.0–36.3) | 27.8 (19.7–37.0) |
| Cause of death | | | |
| Hypoxic Brain | 15 | 10 | 5 |
| Intracerebral haemorrhage | 21 | 13 | 8 |
| Intracerebral thrombosis | 2 | 2 | 0 |
| Meningitis | 1 | 1 | 0 |
| Trauma | 2 | 1 | 1 |
| Donor type | | | |
| DBD | 32 | 20 | 12 |
| DCD | 1 | 0 | 1 |
| DCD/NRP | 8 | 7 | 1 |
| Indication for *ex situ* NMP | | | |
| Further Assessment | 10 | 5 | 5 |
| Logistics | 26 | 20 | 6 |
| Complex Recipient | 5 | 2 | 3 |
| Preservation time (min) | | | |
| CIT (1st) | 453 | 470 | 415 |
| Normothermic machine preservation time | 508 | 621 | 297 |
| CIT (2nd) | | 22 | — |
| Total preservation time | | 1113 | 712 |
| Recipient demographics | | | |
| Gender M:F | | 22:5 | |
| Age median (range) | | 58 (24–71) | |
| BMI median (range) | | 27 (20–44) | |
| UKELD median (range) | | 53.5 (45–74) | |
| Indication | | | |
| ALD | | 8 | |
| HCC* | | 9 | |
| HCV | | 1 | |
| NAFLD | | 6 | |
| PBC | | 2 | |
| PSC | | 4 | |
| Cryptogenic cirrhosis | | 1 | |

*BMI, body mass index; DBD, donation after brain death; DCD, donation after circulatory death; NRP, normothermic regional perfusion; CIT(1st), Cold ischaemic time from* in situ *cold perfusion to liver perfusion on device; CIT (2nd), cold ischaemic time from liver disconnected from device to reperfusion in recipient; UKELD, UK model for end stage liver disease; HCV-hepatitis C; ALD, alcoholic liver disease; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; PBC, primary biliary cirrhosis; PSC, primary Sclerosing cholangitis; *–HCC cases as primary indication or in association with other liver disease.*

development (**Figure 9**). Initial setup (equipment, consumables) was funded as part of a clinical pilot for uncontrolled donation (20) during which the feasibility of donation from A&E was explored. This allowed the parallel development of the use of NRP in controlled DCD donation which generated the initial clinical data (21) that helped secure a more significant financial support from NHS Blood and Transplant as part of a wider Novel Technologies service evaluation. Although this funding covered the consumables for the procedures, it was insufficient to support the development of the team infrastructure and therefore additional (initially temporary) funding was secured from local funds, leading to the appointment of the first APOPS in 2016. The expanded programme demonstrated good clinical outcomes (4) that supported a successful funding application for additional APOPS from the local hospital to support the conceptual change in service delivery by a dedicated and self-sufficient team. At the same time, a cost-effectiveness study was undertaken based on clinical data generated by the UK programmes (26) which demonstrated that the incremental cost of using NRP to recover DCD livers (£4,500/procedure) is economically justified, leading to a cost saving of £1.17 million for every 100 NRP DCD cases. These data mirror the Spanish experience where cost utility is one of the drivers for the increased use of NRP. The new Donation and Transplantation Plan for Scotland: 2021 to 2026 (27), identified the use of novel technologies as a key strategy to increase organ utilisation and supported the roll-out of machine perfusion technology as a medium-term goal. The results from the NRP and the *ex situ*

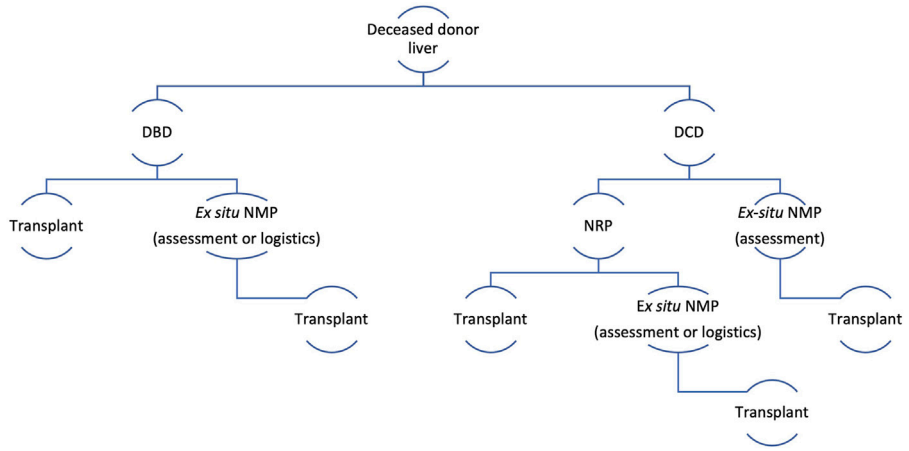


**FIGURE 7 |** Perfusion and preservation strategy for deceased donor liver transplantation. *Ex situ* NMP, *Ex situ* Normothermic machine perfusion (back at base model); DBD, donation after brain death; DCD, donation after circulatory death.

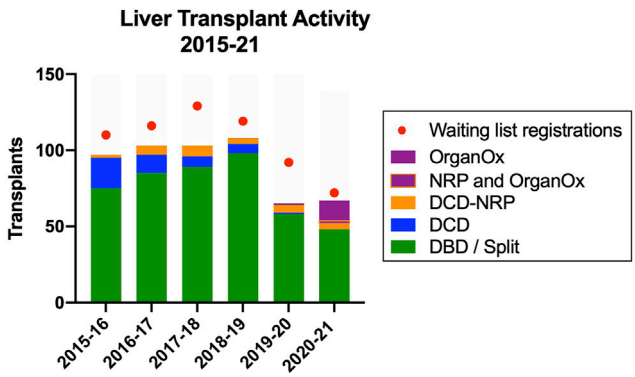


**FIGURE 8 |** Impact of NRP and *ex situ* NMP on liver transplant activity in the Edinburgh Transplant Centre.

NMP feasibility work led to full funding of the Organ Perfusion and Preservation Service as of 1 April 2022.

## DISCUSSION

DCD donation has seen a rapid expansion and in many countries provides a significant contribution to the donor pool (28). Whilst very good DCD renal organ utilisation and transplant outcomes have been achieved, the situation is different for other solid organs where lower utilisation rates, increased complication rates, and inferior long-term survival rates have been reported (29). Despite several refinements in the donation process and more stringent selection criteria, it is really the advent of novel *in situ* and *ex situ* perfusion technologies that holds the greatest promise for a leap improvement in utilisation and outcomes. While the place of individual technologies in the transplant process and the benefit each provides is yet to be fully defined, they all share the common challenge of translation from experimental to routine clinical procedure.

The novel perfusion and preservation strategies are inherently technologically complex and the magnitude of change to routine practice required has often been seen as a barrier to implementation and risk for non-adoption. NRP sits at the higher end of complexity as it requires not only supplementation of skills but also a significant change in logistics. Furthermore, despite excellent reported results (13, 15, 21), the technique has not been subjected to randomised controlled trials. When introduced in our centre, NRP was still in a developmental stage, requiring further refinement of the technical details and equipment. As such, the service evolved from selected donors attended by a small group of enthusiasts when available, through to a detailed evaluation of safety, clinical and non-clinical outcomes, protocols, standard operating procedures, and reporting mechanisms, leading to the current arrangement of NRP for all DCD donors attended by the team (regardless of donor history).

Failure to adopt new clinical techniques (even with unequivocally proven benefits) is unfortunately common (30), and therefore the implementation strategy requires just as much attention as the technique itself. This is not usually the result of technology failure but the resistance of the organisation to embrace change, which appears to be independent of size, academic status, resources, innovation history, or support from senior management (31). To minimise the risk of failure and non-adoption, we progressed through an iterative process, with several key elements such as recruitment, education, and training that had a transformative impact on service delivery and outcomes. This was supported by an awareness campaign to reach all stakeholders in the donation, retrieval, and transplant process.

Several key steps to the successful implementation of novel technology have been described (31) with the use of "full team dry-runs" as a fundamental step in the training process which enable the team to work together in standard as well as troubleshooting scenarios. The training process evolved from covering theoretical aspects and practical skills to including

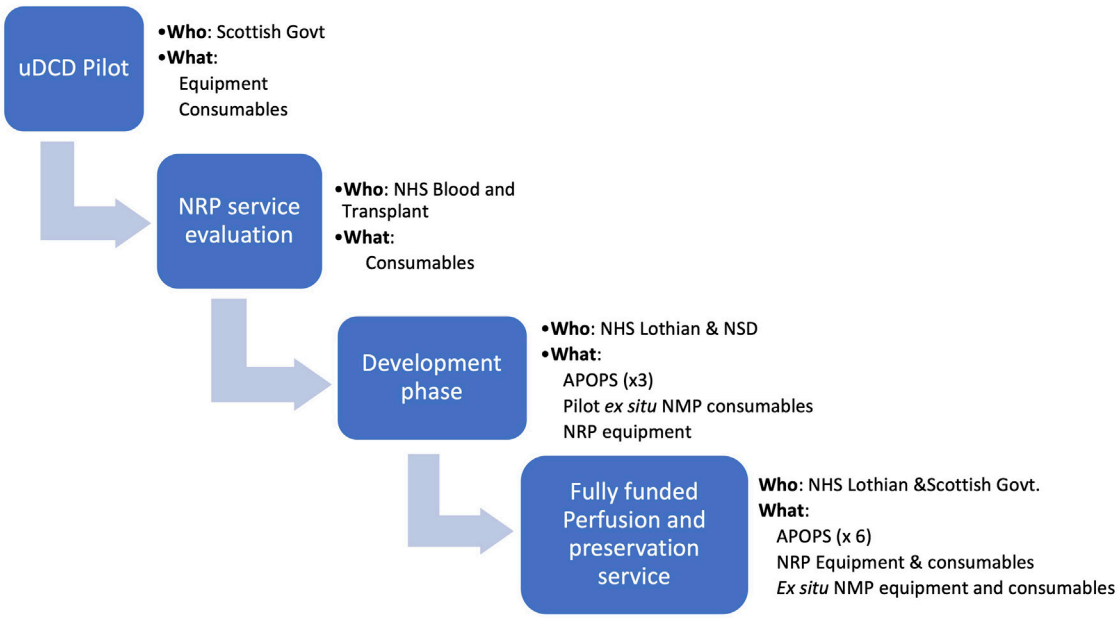


**FIGURE 9 |** Funding strategy from pilot project to a fully funded service. APOPS, advanced perfusion and organ preservation specialists; NMP, normothermic machine perfusion.

dry runs and full simulations that provided the opportunity for a technical run but also highlighted the necessity for interdependence and communication between all team members, fostering trust and confidence prior to actual donor attendance. This was supported by the development of relevant educational material, competency frameworks, and a training pack to support the training session which helped participants to implement similar programmes in their home institutions. This structured approach allowed us to support training teams that implemented the Dutch and Swedish NRP programmes.

Leadership is critical in driving change and enabling the successful introduction of any innovation. Whilst the input of senior clinicians and support from the organisation was critical in the initial stages when we demonstrated the safety and feasibility of NRP, it was the creation of a dedicated advanced perfusion and organ preservation specialist role that led to a significant change in the delivery of the service. This role focused exclusively on the delivery of perfusion technologies and took ownership of the NRP process and logistics, provided leadership and training, and facilitated the implementation of the changes in the pathways. The expansion of the NRP service enabled by these appointments was followed by the seamless implementation of the *ex situ* normothermic liver perfusion programme, with the two programmes contributing 38% of the current liver transplant activity in our centre.

Such developments could not have been possible without funding. Whilst the initial stages were supported through projects or local finances, the full development of the programme required a cost-effectiveness analysis. This was timely as it coincided with a new donation and transplantation strategy that identified the novel perfusion technology as a key priority. The clinical results of the NRP and the *ex situ* NMP programmes and the impact on organ utilisation and clinical activity lead to the establishment of a fully funded organ perfusion and preservation service.

At a systems scale, NRP and all *ex situ* perfusion technologies will operate in a complex and dynamic socio-technical environment with a sophisticated web of structural, socio-political interdependencies and continuous technological evolution (17). Therefore, it is important to define the framework for these technological developments. As the evidence for a beneficial impact gathers pace, the implementation will be dependent on the willingness to adopt and integrate these innovations into practice. The approach described herein demonstrates that this is achievable and provides adaptability and organisational resilience to continued change as illustrated by the successful implementation of two different perfusion technologies. This can only be made possible by support from a healthcare organisation with the capacity to innovate and fund these disruptive changes into routine clinical practice as was the case for us (17).

In summary, NRP is a highly-disruptive technology with a transformative impact on DCD transplantation. Herein we advocate a stepwise approach to training and service delivery, coupled with a well-defined framework, face-to-face leadership, and teamwork that has proven to be very effective in our centre. Whilst some details may require local adaptation, the blueprint described should accelerate wider and sustainable adoption of all organ perfusion and preservation technologies in the future.

## DATA AVAILABILITY STATEMENT

De-identified data for the purpose of meta-analyses, statistical analysis plans and data dictionary are available on request which should be sent to the corresponding author (gabriel.oniscu@ed.ac.uk). Data requestors will need to sign a data access agreement.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10493/full#supplementary-material

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REVIEW
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# Hospital-Based Health Technology Assessment of Machine Perfusion Systems for Human Liver Transplantation

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Based on published data, we have carried out a hospital-based health technology assessment of machine perfusion in adult liver transplantation using cold storage as a comparator, and within the perspective of a national health system-based hospital practice and disease-related group reimbursement policy. A systematic literature review on machine perfusion for adult liver transplantation was conducted exploring the Pubmed, CINAHL, Scopus, Embase, and Cochrane databases. The literature was analyzed with the intent to provide information on 6 dimensions and 19 items of the hospital-based health technology assessment framework derived from previous studies. Out of 705 references, 47 (6.7%) were retained for current analysis. Use of machine perfusion was associated with advantages over cold storage, i.e., a 10%–50% reduced risk for early allograft dysfunction, 7%–15% less ischemia reperfusion injury; 7%–50% fewer ischemic biliary complications, comparable or improved 1-year graft and patient survival, and up to a 50% lower graft discard rate. Hospital stay was not longer, and technical failures were anecdotal. Information on costs of machine perfusion is limited, but this technology is projected to increase hospital costs while cost-effectiveness analysis requires data over the transplant patient lifetime. No hospital-based health technology assessment study on machine perfusion in liver transplantation was previously conducted. From the hospital perspective, there is evidence of the clinical advantages of this novel technology, but strategies to counterbalance the increased costs of liver transplantation are urgently needed. Further studies should focus on the ethical, social, and organizational issues related to machine perfusion.

**Keywords: liver transplantation, machine perfusion, health technology, health technology assessment, hospital, patients**

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**Abbreviations:** CS, cold storage; DBD, donation after brain death; DCD, donation after circulatory death; DRG, disease-related group; EAD, early allograft dysfunction; ECD, extended criteria donors; HB-HTA, hospital-based health technology assessment; HT, health technology; HTA, health technology assessment; IBC, ischemic biliary complications; IRI, ischemia/reperfusion injury; LT, liver transplantation; NHS, national health system; SCS, static cold storage; SD, standard deviation.

## INTRODUCTION

Health technology assessment (HTA) is a research-based, practice-oriented assessment of available knowledge on both the direct and intended consequences of health technologies (HT), and on their indirect and unintended consequences, in the short and long term (1). The consequences include clinical benefits (i.e., efficacy, effectiveness) and the economic and organizational impact (efficiency), as well as the social, ethical, and legal implications associated with the HT being assessed (1) (**Table 1**).

Science-based information is of special importance for hospitals as they are the entry point for new technologies. Over the last decade, the practice of liver transplantation (LT) has witnessed the introduction of *ex-vivo* machine perfusion (MP) systems for both donation after brain (DBD) and circulatory death (DCD) (2). This emerging technology has the potential to improve the outcome of LT, especially when extended criteria donors (ECD) are used (2). However, post-marketing HTA of MP is still inadequate as per the standards of national HTA agencies, and to the best of our knowledge no assessment of MP from an HTA agency has ever been performed.

Hospital-based HTA (HB-HTA) includes the processes and methods used to produce HTA reports with special focus on hospital practice (3) (**Table 2**). The overarching principle of HB-HTA is to provide hospital decision-makers with relevant, comprehensive, objective, and reliable information on the effects and implications of introducing a new HT into the hospital, and the information provided by HB-HTA is analyzed considering the specific context of the hospital where the HT is to be introduced (**Table 2**). In order to be able to support decision-making in hospitals, HTA should also focus on local infrastructure, prevailing treatment options, patient populations, learning curves, and competing priorities (3).

Given the paucity of HTA reports on novel HT implemented in LT in general, and on MP in particular, the current paper presents the result of an evidence-based HB-HTA of MP devices for human LT with reference to the European hospital practice and a disease-related group (DRG) reimbursement policy.

## MATERIALS AND METHODS

In January 2022, we carried out a systematic literature review on MP for adult LT. The literature search explored the Pubmed, CINAHL, Scopus, Embase, and Cochrane databases using a combination of the following MeSH entries with no time limit: #liver transplant (ation), #liver graft, #machine perfusion, #hypothermic machine perfusion, #normothermic machine perfusion, #subnormothermic machine perfusion, #*ex-vivo* machine perfusion, #*ex-situ* machine perfusion, #safety, #complication(s), #risks, #cost(s), #utility, #effectiveness, #efficacy, #outcome(s), #results, #resource(s), #training, #acceptability, #quality of life, #access, #equity, #usability, #population(s), #health technology, #health technology assessment, #hospital(s), and #hospital-based health technology assessment.

The resulting list of references was checked by both investigators, and only papers published in English on clinical application of MP were included. Non-original research works, such as letters to editors, personal points of view, commentaries, and state-of-the-art papers were excluded. Reviews and meta-analyses were considered for data relevant to the current research strategy. The abstracts of all retrieved references were analyzed by the investigators for consistency with the scope of the current research, and if considered relevant the corresponding full papers were included. The articles' references lists were scanned for evidence of papers not reported in the above databases. In the event of duplicates or manuscripts from the same institution, only the most recent or comprehensive reports were retained. Qualitative assessment of published manuscripts was according to the Currency, Relevance, Authority, Accuracy, Purpose (CRAAP) methodology described elsewhere (5).

Two different clinical settings were included, i.e., DBD and DCD LT using static cold storage (SCS) as the comparator. For both clinical scenarios, the literature was analyzed with the intent to provide information on any of the 6 dimensions and 19 items of the HB-HTA framework as derived by previous works (**Table 2**) (3, 4). The hospital perspective was that of a national health system (NHS)-based health payer, this being the system in place in Italy and most EU-27 countries, and the corresponding reimbursement policy was that of a DRG-based system. Data on the commercially available MP devices were pooled, since superiority of any HT was beyond the scope of the present analysis. As for any HTA report, the literature review was completed with recommendations and identification of unexplored and underexplored areas and/or items to be investigated in future research. Due to its noninterventional design, no approval by the local ethics committee was necessary as per current Italian regulations.

## RESULTS

Out of 705 references initially retrieved through the databases, 312 (44.2%) papers were excluded being experimental works both in the pre-clinical and clinical setting, 254 (36.0%) were non-original works (letters, expert opinions, state-of-the-art articles, or position papers), 70 (9.9%) were not consistent with the research scope (i.e., combined organ transplantation, pediatric populations, mixed animal and human studies, etc.. . .), 12 (1.7%) focused mainly on perfusion solutions, 9 (1.3%) were duplicates, and 1 (0.1%) was a survey. Finally, 47 (6.6%) references were retained for current analysis (6–52) (**Figure 1**). The selected references were published between 2010 and 2022, and all were available as full-length papers.

No previous reference on HTA of MP in human LT was retrieved. The majority of published evidence focused on efficacy/effectiveness of MP in the setting of ECD DBD (6, 8, 10–16, 21–26, 28–38, 40, 41,45, 46, 48, 49, 51) and of type-2 and 3 DCD (7, 8, 13, 16–24, 26, 27, 29–31, 33, 35–48, 50), with most series including both donor populations (**Table 3**). Information was frequently provided on incidence of biliary complications, which were considered as a surrogate of MP efficacy in most clinical trials together with markers of acute liver injury (9, 17, 30, 31, 33, 34–37, 38, 40, 41). Universal consensus was shared on use of MP in the setting of DCD, especially for type-2 donors, while identification of ECD DBD categories in need of MP was more controversial and yet not entirely agreed upon (10, 16, 28, 32). No consensus on recipient populations to be treated with

**TABLE 1 |** The scope, aims, and perspectives of HB-HTA.

| Domains | Definition |
|---|---|
| Scope | • Provide hospital decision-makers with information on the effects and implications of introducing a new HT into the hospital |
| Pre-requisites | • Information on HT has to be relevant, comprehensive, objective, and reliable<br>• It has to be specific to the context of the hospital where the HT of interest is to be introduced |
| Aims | • Take better-informed decisions supporting effective health practices<br>• Facilitate more efficient investment decisions<br>• Allow hospitals to save money by reducing unnecessary use or avoiding inappropriate investments<br>• Facilitate best clinical practices<br>• Improve patient safety<br>• Engage key opinion leaders in decision-making processes<br>• Inform stakeholders on the rationale of managerial decisions and resource investments |
| Perspectives[a] | • Hospital managers<br>• Policy makers<br>• Healthcare payers<br>• Key opinion leaders<br>• Hospital healthcare staff<br>• Patients and their families<br>• Community<br>• Stakeholders<br>• Scientists, researchers<br>• Industry |

*Note. HT, health technology; HB-HTA, hospital-based HTA; HTA, health technology assessment.*
*[a]In HB-HTA reports, the pre-eminent perspective is that of hospital managers. However, due to the multidisciplinary character of any HTA process, all of the indicated perspectives are to be considered.*

**TABLE 2 |** The dimensions of HB-HTA investigated in the current paper (derived from refs 3 and 4).

| Dimension | Item |
|---|---|
| Clinical | • Safety/risk<br>• Efficacy/effectiveness<br>• Mortality/survival rates<br>• Population to be treated (donors, recipients)<br>• Incidence/prevalence of illness |
| Economic(al) | • Costs<br>• Cost-effectiveness, cost utility, cost opportunity<br>• Resource(s) |
| Ethical | • Patient acceptance/comfort<br>• Access to novel HT<br>• Equity<br>• Potential patient harm |
| Social | • Patient quality of life<br>• Pain/discomfort<br>• Time in hospital/patient burden |
| Organizational | • Training<br>• Equipment availability/location<br>• Resource constraints |
| Human factors | • Acceptance/acceptability<br>• Usability/ease of use |

*Note. HT, health technology; HB-HTA, hospital-based HTA; HTA, health technology assessment.*

MP has so far been reported in the international literature, and this choice is usually based on local center allocation policies and regional/national donation rates. Limited information was published on costs and cost-effectiveness/cost utility of MP (13, 21) with original studies originating from Canada (13) and the United Kingdom (21) only. No reference was retrieved on human factors or organizational issues related to the use of MP. Similarly, no information was available on quality of life, access to MP, equity in using MP across diverse patients' categories, or potential patient harm connected with this novel HT (i.e., competitive models).

From a quantitative point of view (**Table 3**), use of MP was associated with definite advantages over SCS. In comparative studies, in light of similar rates of transplant-related (i.e., artery thrombosis) and unrelated (i.e., bleeding) complications (15, 24, 30, 32, 35, 37), MP allowed for prolonged total graft preservation time (15, 35), a 10%–50% reduced risk for EAD (7, 17, 24, 30, 36, 37, 41, 47), 7%–15% less IRI (7, 17, 28, 32, 39, 42), 7–50% fewer ischemic biliary complications (IBC) (7, 15, 17, 24, 30, 31, 36, 37, 47), comparable (22, 24) or improved 1-year graft (30, 47) and patient survival (30, 35), and up to a 50% lower discard rate (31, 35, 43). Hospital stay was not longer for MP patients (15, 24, 30), and technical failures were anecdotal (36). Costs of MP have limitedly been investigated in two studies only (13, 21). One Canadian paper reported a minimum cost per MP run of 18,593.02 $Can, and hypothesized potential cost savings by decreasing night-time salary premiums, complications, and length of hospital stay (13). A study from the United Kingdom focused on costs and cost utility of OrganOx metra™ only, demonstrating higher per-patient costs versus SCS (46,711 versus 37,370£) in light of an anticipated increase in quality of life years (QALY) (10.27 versus 9.09) gained by this novel HT versus SCS (21).

**Table 4** illustrates the recommendations derived from the current HB-HTA report.

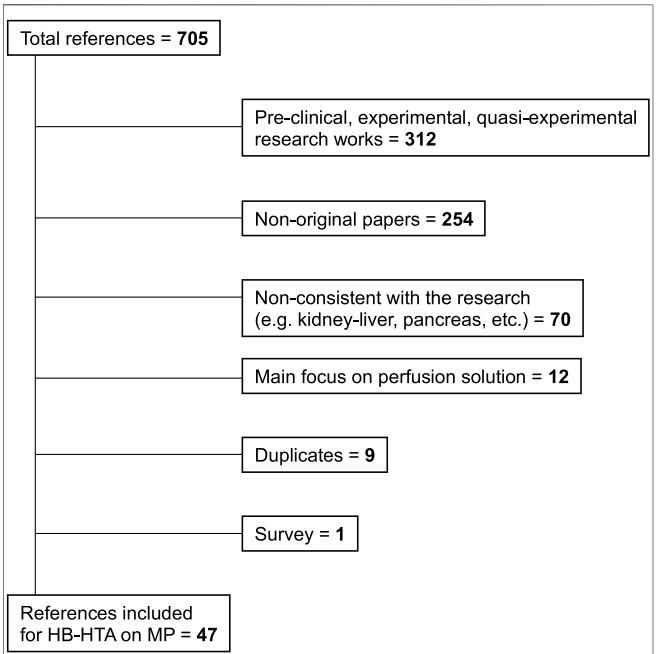


**FIGURE 1 |** The literature search algorithm. HB-HTA, hospital-based health algorithm; MP, machine perfusion.

## DISCUSSION

Modern healthcare systems are under pressure and facing challenges that govern their sustainability. One of these challenges is the expansion in technical developments that are fueling innovative and attractive HT to provide answers for unmet medical needs. Innovation is highly rewarding, since it contributes to improved population health status, prolonged life expectancy, and better quality of life. On the other hand, healthcare managers are more accurate in their decisions concerning public expenditure due to the global economic shrinkage. In this scenario, HTA and HB-HTA reports are even more crucial to guide decisions on innovative HT.

MP technology is a recently introduced and expensive intervention whose benefits are under evaluation. With most evidence focusing on patients' outcomes, limited information is available on the impact of this novel technology on hospitals, healthcare systems, and communities. The paucity of information compliant with standard HTA reports is due to the incredible velocity of research on MP, introduction of this technology at a higher pace than anticipated, and also on the lack of consideration on the part of scientists and clinicians. To the best of our knowledge, this is the first HTA report on MP in LT to be published in the international literature.

Our study confirms that use of MP for LT is safe and associated with frequently improved graft and patient survival for recipients of DCD and ECD DBD transplants, with an associated reduced risk for EAD and ischemic biliary complications. MP seems necessary for implementation of a DCD LT program with special reference to type-2 DCD grafts, due to its striking superiority versus SCS in this setting. But MP also seems to expand use and rescue of ECD grafts, although its implementation in this scenario is frequently driven by regional/national yearly donation rates, proportion of utilized marginal liver grafts, disposition of the waiting list, and single center allocation policies. Future studies should focus on identification of the ideal recipient populations to be treated and on long-term post-transplant survival.

MP is not economically neutral and is projected to increase costs of LT in the hospital setting. Additionally, the evolving scenario of technology advancements is anticipated to increase costs of future MP devices and of those ancillary technologies (i.e., MP-facilitated graft reconditioning) that are currently being explored worldwide. Based on its impact on graft and patient survival, MP-facilitated LT is anticipated to be cost-effective compared to non-transplant best care practices for liver disease patients, but cost-effective and cost-utility analyses require implementation of appropriately powered studies on long-term transplant recipients.

In the economic evaluation of healthcare technologies, costs are usually calculated by multiplying the quantities of resources used per patient by the unit costs of the resources, but economic evaluations for MP technology require alternative approaches to the standard patient-specific modeling by considering all of the following: 1) procedures that generated transplantation versus those that did not generate suitable grafts (i.e., per-run cost); 2) transplantation of liver grafts that would not be otherwise used, and 3) the impact of expanded graft utilization on patients, hospitals, and populations. Especially for NHS-based transplant programs, generating more transplants from use of ECD grafts may increase the economic burden for hospitals (as per the increased number of pre-transplant investigations, surgeries, perioperative care, and post-transplant medical treatment), but these costs should be balanced against those associated with non-transplant care while waiting for a standard quality graft and those derived from loss of transplant-related survival benefit.

To this regard, the choice of the most appropriate costing models and resource-use items is crucial for future analyses, and will require broad consensus across the healthcare professionals involved in LT programs. The decision on which types of cost to include depends on several key factors, including the perspective to be adopted (e.g., hospital managers versus patients versus payors), the form of economic evaluation (e.g., cost-effectiveness versus cost utility versus cost opportunity), the quantitative importance of the type of cost along the entire transplant continuum (i.e., what is the economic burden of MP technology as compared to that related to chronic immunosuppression?), whether the cost can be attributed to the intervention (i.e., can we anticipate reduced cost for treatment of post-transplant ischemic cholangiopathy), and the time horizon of the economic evaluation (perioperative versus early-term versus long-term versus life-long). Collection of detailed data on resource use for all patients may not be necessary, but can be limited to key cost-generating events (normothermic regional perfusion, MP technology, re-transplantation, etc.) where there is economic variation between standard patients and those treated with the novel

**TABLE 3 |** Quantitative results of HB-HTA of MP versus SCS (information is presented for items where qualitative information was available).

| Dimension | Available information |
|---|---|
| Clinical | • No increased complication rate (15, 24, 30, 32, 35, 37)<br>• Prolonged total graft preservation time (15, 35)<br>• 10–50% reduced risk for EAD (7, 17, 24, 30, 36, 37, 41, 47)<br>• 7–15% less IRI (7, 17, 28, 32, 39, 42)<br>• 7–50% fewer IBC (7, 15, 17, 24, 30, 31, 36, 37, 47)<br>• Comparable (22, 24) or improved 1-year graft (30, 47) and patient survival (30, 35)<br>• Up to a 50% lower discard rate (31, 35, 43) |
| Economic(al) | • Increased costs [per-run cost of 18,593.02 $Can (13); per-patient increase of 9,341£ (20)]<br>• Theoretically improved cost-effectiveness and cost utility (21)<br>• Increased use of economic resources (13, 21) |
| Ethical | • Anecdotal single reports of MP-related adverse events (37) |
| Social | • No difference in length of hospital stay (15, 24, 30) |

*Note. EAD, early allograft dysfunction; HB-HTA, hospital-based health technology assessment; HT, health technology; HTA, health technology assessment; IBC, ischemic biliary complications; IRI, ischemia reperfusion injury; MP, machine perfusion; SCS, static cold storage.*

**TABLE 4 |** Key considerations on introduction of MP in the hospital setting based on HB-HTA.

| Dimension | Information |
|---|---|
| Clinical | Available<br>• Current MP technology is safe and associated with equal-to- superior graft and patient short-term survival versus SCS<br>• Main advantages of MP are a reduced risk for IRI, EAD, and IBC, and a reduced graft discard rate<br>• MP facilitates implementation of a DCD LT program, especially for type-2 DCD grafts<br>Needed<br>• Better identification of ECD DBD grafts to treat with MP<br>• Better identification of recipient populations to be treated with MP<br>• Long-term data in transplant populations exposed to MP |
| Economic(al) | Available<br>• MP is not economically neutral<br>• MP is projected to increase costs of LT in the hospital setting<br>• HT advancements are projected to increase MP-related costs in the near future (i.e., graft reconditioning)<br>Needed<br>• Cost-effective and cost-utility analyses on long-term recipients of MP-facilitated LT<br>• Best strategies to neutralize increased costs of MP (i.e., introduction of *ad hoc* DRG, reimbursement of marginal gains achieved from increased proportion of transplants, etc.) |
| Ethical | Available<br>• Limited information is currently available and consists of reports of numerically low MP-related adverse events<br>Needed<br>• Patient acceptance has to be investigated<br>• Strategies to allow for equitable access to MP across LT centers should be identified<br>• Potential patient harm from non-implementation of MP-facilitated transplantation should be investigated with simulation models (i.e., competitive risk analysis) |
| Social | Available<br>• None<br>Needed<br>• Patient quality of life has to be investigated in the setting of MP-facilitated LT<br>• Time in hospital/patient burden should be the focus of future studies |
| Organizational | Available<br>• None<br>Needed<br>• Future studies should focus on staff training and learning curves, equipment availability with regard to comparative analysis of the different commercially available devices, and on the impact of resource constraints (staff and/or financial) on implementation of an MP-facilitated LT program |
| Human factors | Available<br>• None<br>Needed<br>• As technology evolves, acceptance/acceptability of novel devices and information on usability/ease of use has to be provided |

*Note. DCD, donation after circulatory death; DRG, disease-related group; EAD. Early allograft dysfunction; ECD, extended criteria donors; HB-HTA, hospital-based HTA; HT, health technology; HTA, health technology assessment; IBC, ischemic biliary complications; LT, liver transplantation; MP, machine perfusion; SCS, static cold storage.*

technology. As clinicians, we are challenged to think through all these methodological issues related to MP technology and build empirical evidence in our future practice.

From the hospital perspective, strategies to neutralize the costs of MP are urgently needed, such as introduction of specific DRG categories, reimbursement of marginal gains retrieved from the increased proportion of transplants, or from out-of-pocket co-pays. Additional avenues for future research should also focus on patient acceptance, on strategies to offer equitable access to MP across different LT centers, and on potential patient harm from non-implementation of MP-facilitated transplantation.

Finally, we advocate future research on staff training and learning curves, on equipment availability with regard to comparative analysis of the different commercially available devices, and on impact of resource constraints (staff and/or financial) on implementation of an MP-facilitated LT program. As technology evolves, acceptance of novel devices and information on usability and ease-of-use from healthcare professionals is also highly needed.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## CONFLICT OF INTEREST

PDS and DG have conducted studies on machine perfusion systems with financial and logistic support from Avionord Srl and OrganOx Ltd. DG has received speaker's honoraria from Aferetica Srl.

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# Normothermic Regional Perfusion and Hypothermic Oxygenated Machine Perfusion for Livers Donated After Controlled Circulatory Death With Prolonged Warm Ischemia Time: A Matched Comparison With Livers From Brain-Dead Donors

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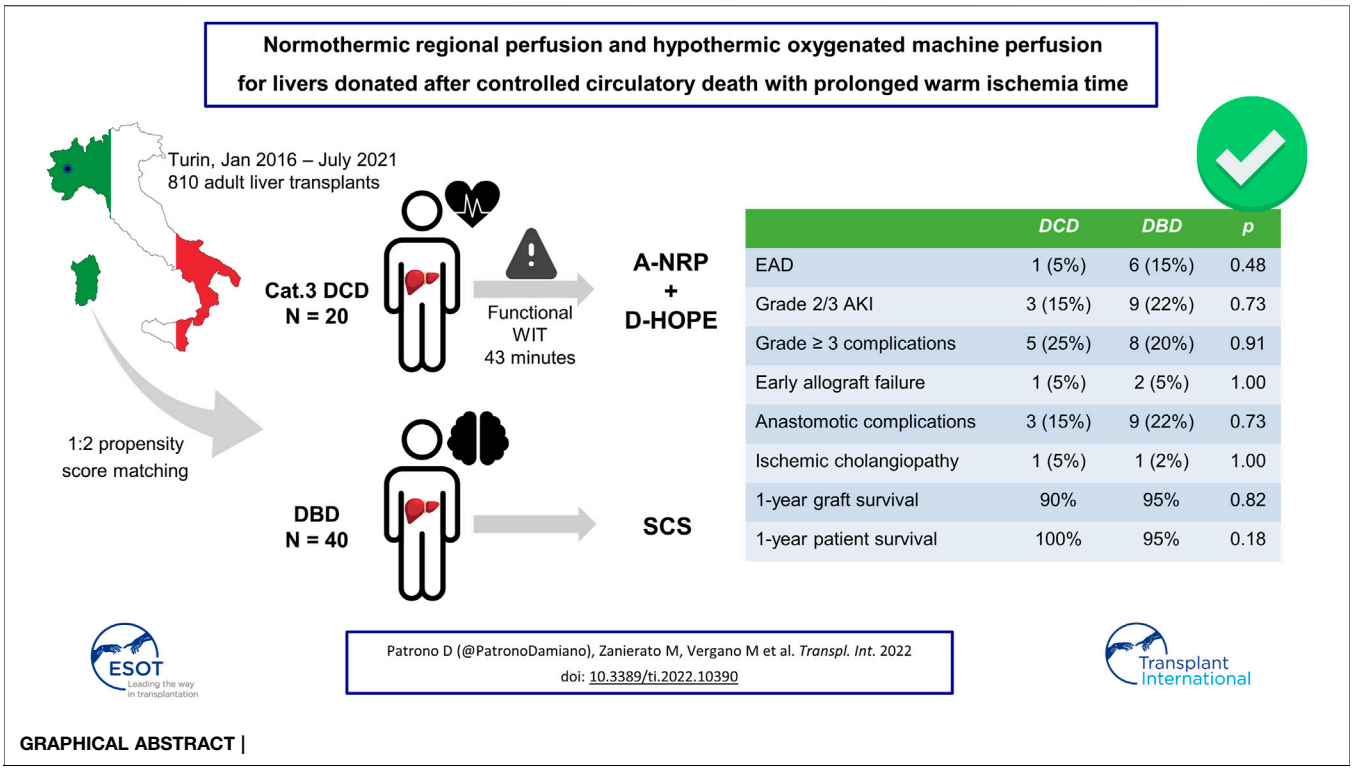
Prolonged warm ischemia time (WIT) has a negative prognostic value in liver transplantation (LT) using grafts procured after circulatory death (DCD). To assess the value of abdominal normothermic regional perfusion (A-NRP) associated with dual hypothermic oxygenated machine perfusion (D-HOPE) in controlled DCD LT, prospectively collected data on LTs performed between January 2016 and July 2021 were analyzed. Outcome of controlled DCD LTs performed using A-NRP + D-HOPE ($n$ = 20) were compared to those performed with grafts procured after brain death (DBD) ($n$ = 40), selected using propensity-score matching. DCD utilization rate was 59.5%. In the DCD group, median functional WIT, A-NRP and D-HOPE time was 43, 246, and 205 min, respectively. Early outcomes of DCD grafts recipients were comparable to those of matched DBD LTs. In DCD and DBD group, incidence of anastomotic biliary complications and ischemic cholangiopathy was 15% versus 22% ($p$ = 0.73) and 5% versus 2% ($p$ = 1), respectively. One-year patient and graft survival was 100% versus 95% ($p$ = 0.18) and 90% versus 95% ($p$ = 0.82). In conclusion, the association of A-NRP + D-HOPE in DCD LT with prolonged WIT allows achieving comparable outcomes to DBD LT.

**Keywords: donation after circulatory death, abdominal normothermic regional perfusion, hypothermic oxygenated machine perfusion, warm ischemia time, ischemic cholangiopathy, liver transplantation outcome**




**GRAPHICAL ABSTRACT |**

## INTRODUCTION

In liver transplantation (LT) using grafts from donors whose death has been determined by circulatory criteria (DCD), warm ischemia time (WIT) has a major impact on the outcome. Prolonged WIT has consistently been associated with an increased risk of primary non-function, ischemic cholangiopathy (IC) and inferior graft survival (1–5). In contrast with most countries with active DCD transplant programs, Italian law requires a 20-min period of absent cardiac electrical activity for death declaration (6), which significantly increases the risks associated with the use of these grafts and has slowed down implementation of DCD LT in Italy (7).

However, mainly prompted by the favourable Spanish experience with the use of abdominal normothermic regional perfusion (A-NRP) to recover DCD liver grafts from Maastricht category 2 donors (8), DCD LT was introduced in Italy in 2015 (9, 10). Given the unique characteristics of the Italian setting, use of A-NRP has been established as mandatory, while subsequent ex-situ machine perfusion (MP) has been encouraged and adopted by most centres.

A growing body of literature supports the benefits of A-NRP for livers procured from Maastricht category 3 (controlled) DCD donors (11–17). In the same setting, use of end-ischemic (dual)-hypothermic oxygenated machine perfusion (HOPE/D-HOPE) has been consistently associated with better liver graft function and lower incidence of IC as compared to static cold storage (SCS) (18–21). However, these studies reported shorter WIT compared to what can possibly be achieved in Italy.

In the Italian setting, previous studies have shown that the association of A-NRP with ex-situ machine perfusion for controlled DCD liver grafts allows achieving good LT outcomes (22–24), which appear to be comparable to those of DCD livers preserved by ultra-rapid recovery and preserved by SCS (25). However, a formal comparison with LT using livers from donors after neurologic determination of death (DBD) accounting for potential confounders and demonstrating comparable outcomes is still lacking.

Thus, the aim of the study was to report our results with the use of A-NRP + D-HOPE for controlled DCD liver grafts with prolonged WIT. To assess the effectiveness of this approach, outcomes of DCD grafts recipients were compared to those of a matched cohort of DBD LTs, selected using propensity score matching.

## MATERIALS AND METHODS

Prospectively collected data on adult (≥18-year-old) patients who underwent LT at our centre from January 2016 to July 2021 were retrospectively analyzed. Collected data included donor and recipient baseline characteristics, operational details, and prognostic scores (26, 27). The UK-DCD risk score (4), a prognostic score for DCD LT based on 4 donor and 3 recipient variables, was used to grade the risk profile associated with each case. Recipients of a combined transplant, retransplant, partial graft or suffering from on-table death were excluded. To limit confounding, recipients of a DBD graft treated with any type of machine perfusion were also excluded, as well as recipients of Maastricht category 2 DCD grafts and of Maastricht category 3

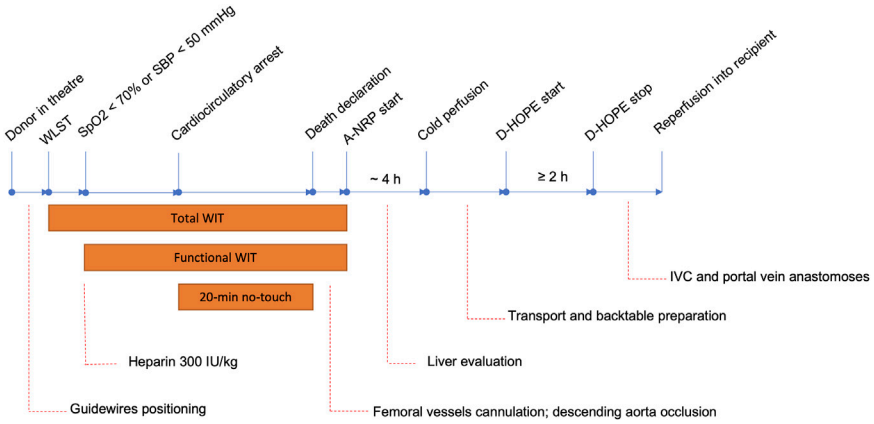



**FIGURE 1 |** DCD procurement protocol. Abbreviations: WLST, withdrawal of life-sustaining treatment; SBP, systemic blood pressure; A-NRP, abdominal normothermic regional perfusion; D-HOPE, dual hypothermic oxygenated machine perfusion; WIT, warm ischemia time; IVC, inferior vena cava.

DCD grafts treated with a machine perfusion modality other than D-HOPE. To control selection bias, two comparable cohorts of DBD and controlled DCD LTs were selected using 1:2 propensity score matching. Minimal patient follow-up was 6 months. The study was conducted according to the principles of the Istanbul and Helsinki declarations and was approved by the ethics committee of our Institution (protocol 506/2021).

Our procurement and machine perfusion protocols are depicted in **Figure 1**. Briefly, withdrawal of life-sustaining treatment (WLST) took place in the operating theatre, after guidewires for subsequent femoral vessels cannulation had been placed under ultrasound guidance (pre-mortem cannulation is not allowed in Italy). At the onset of functional warm ischemia (peripheral $O_2$ saturation ≤70% or systolic blood pressure ≤50 mmHg, whichever occurred first) 300 IU/kg heparin was administered. After 20-min electrical asystole, death was declared, femoral vessels were cannulated and descending aorta was occluded by an endovascular balloon or a surgical clamp, depending on theatre logistic, after which A-NRP was started. During A-NRP, pump flow was maintained ≥1.7 L/min/$m^2$ and temperature at 35–36°C (28). Target perfusion pressure was 55–70 mmHg, which was sustained using low dose vasopressin or norepinephrine when necessary, in addition to flow settings and fluid replacement. The circuit sweep gas levels ($FiO_2$ and air flow) were adjusted to maintain $PaCO_2$ between 35 and 45 mmHg, $SaO_2$ about 96–98%, and SvO2 > 60%. If needed, packed red blood cells were transfused to maintain haematocrit ≥20%. Heparin boluses were administered based on activated clotting time values. Blood samples were obtained prior to A-NRP start, at 30 min and then hourly to adjust A-NRP parameters (gas flow, blood flow, $FiO_2$, pump speed) and to assess liver injury and function. Target A-NRP duration was 4 h and it was never less than 2 h or more than 6 h. During A-NRP, liver viability assessment was based on a modified version of the criteria proposed by De Carlis et al. (29), including pump flow >1.7 lt/min/$m^2$, transaminase level <1,000 IU/L, downward lactate trend, absence of significant (≥15%) macrovesicular steatosis or Ishak >1 fibrosis, good liver and abdominal viscera perfusion, and evidence of bile production. A liver biopsy was systematically obtained to rule out significant necrosis or macrovesicular steatosis. At the end of A-NRP, the liver graft was cold flushed with Celsior (IGL, Lissieu, France) solution through the arterial cannula and trough a portal vein tributary. Liver was prepared on the backtable immediately upon arrival at our transplant centre and subsequently underwent a minimum of 2 h of D-HOPE using the LiverAssist device (XVivo, Groningen, Netherlands), primed with 3 lt of Belzer MP solution (BridgeToLife, Northbrook, IL). Temperature, portal vein and hepatic artery pressure were set at 8–10°C, 3–5 mmHg and 25 mmHg, respectively. D-HOPE was not used with the purpose of viability assessment and all grafts treated by D-HOPE were subsequently transplanted. At the end of recipient hepatectomy, the liver was disconnected from the device and brought to the operating table for implantation.

In DBD group, the liver was flushed with Celsior and preserved by static cold storage until implantation into the recipient. In both groups, the liver was flushed with chilled 5% albumin solution before implantation.

As a rule, liver transplant was performed by the piggyback technique with portal reperfusion first. Following hepatic artery anastomosis, an end-to-end biliary anastomosis was performed using a 2.5 mm T-tube. In all patients graft histology was assessed on time-0 biopsies, which were systematically obtained at the end of transplant operation. Standard immunosuppression included basiliximab, tacrolimus, steroids and mycophenolate mofetil, and was not modified according to treatment group.

Early outcome endpoints included rate of post-reperfusion syndrome (30, 31), transaminase peak, early allograft dysfunction (32), rate and severity of acute kidney injury (AKI) (33), requirement for renal replacement therapy, hospital and intensive care unit (ICU) stay, postoperative complications (34, 35), and the rate of early graft failure (EAF), defined as death of relisting for LT withing 90 days from transplant.

Post-reperfusion syndrome was defined as a drop in mean arterial pression ≥30% from the baseline for at least 1 min and within 5 min from reperfusion (30), whereas severe post-reperfusion syndrome was defined as the onset of severe hemodynamic instability, persistent hypotension, cardiac arrest

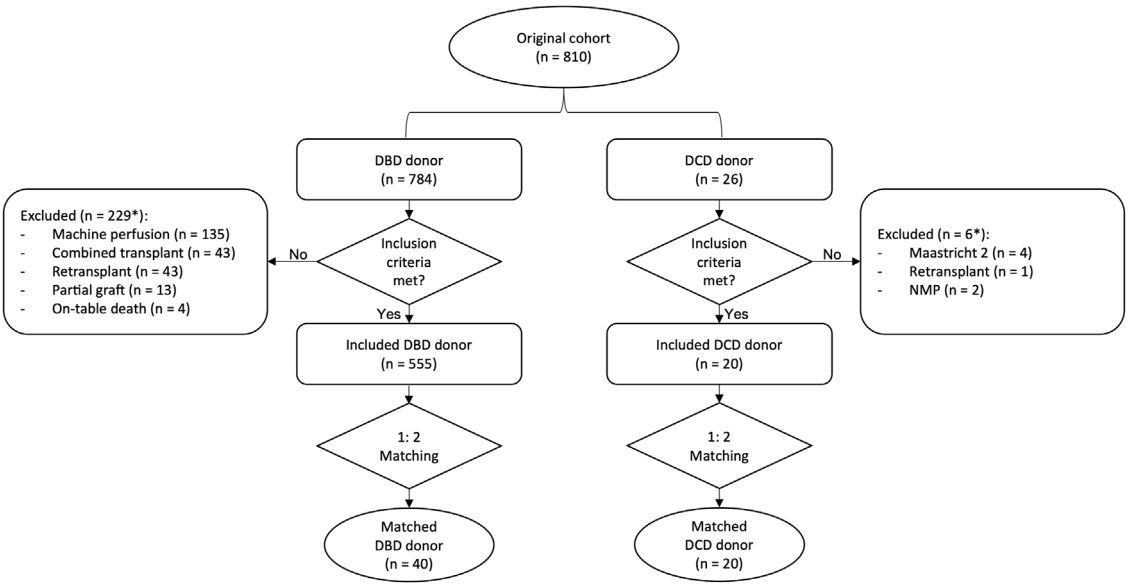



**FIGURE 2 |** Patient selection flowchart. *some patients met more than one exclusion criterium. NMP, normothermic machine perfusion.

or hemodynamically significant arrhythmias (31). EAD and AKI were defined according to Olthoff et al. (32) and KDIGO guidelines (33). Postoperative complications were graded according to Clavien-Dindo classification (34), which was also used to calculate comprehensive complication index (CCI) (35).

Biliary complications (36) were diagnosed based on the 3-month cholangiogram obtained before removing the T-tube, or by magnetic resonance cholangiopancreatography (MRCP), which was performed if clinically indicated. Recipients of a DCD graft underwent systematic 6-month and 12-month MRCP.

Variables are presented as number (percentage) of median (interquartile range), as appropriate, and compared using Fisher's, Chi-square and Mann-Whitney tests. To control selection bias, 1:2 propensity score matching without replacement and using the nearest method was used to select two patient cohorts with comparable characteristics. Variables included in the model were recipient age, body mass index (BMI) and model for end-stage liver disease (MELD) score, hepatocellular carcinoma (HCC) as an indication for LT, donor age and BMI, percentage of macrovesicular steatosis and presence of macrovesicular steatosis ≥15%. Standardized mean differences were used to assess balance obtained by propensity score matching. Patient and graft survival was analyzed using Kaplan-Meier curves. Statistical analysis was performed using R: a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

During study period, 810 adult LTs were performed, of which 26 using organs proceeding from a DCD donor (cat. 3, $n = 22$; cat. 2, $n = 4$). A total of 37 category 3 DCD donors were signalled in our region during study period, of which 22 were transplanted by our centre. As per Italian regulations, livers from regional DCD donors were allocated locally to our centre, which is the only liver transplant centre in our region, and referred elsewhere only upon refusal by our unit. Four livers were refused based on donor characteristics and the organs were reallocated to other centres. Three of these grafts were successfully transplanted, whereas one was discarded during A-NRP due to elevated transaminases and lack of lactate clearance. Of the remaining 11 livers, 6 were discarded by our and all other Italian centres based on donor features, whereas of 5 offers initially accepted by our centre, 2 were subsequently discarded due to excessive functional WIT, and 3 during A-NRP. The reason to discard the liver during A-NRP was mainly elevated transaminases, which was associated to persistently elevated lactate levels in one case and evidence of gallbladder and bile duct necrosis in another. No liver was discarded based on histological findings. Overall utilization rate of livers from category 3 DCD donors was 25/37 (67.6%), whereas it was 22/37 (59.5%) if we consider only those transplanted at our centre.

Based on exclusion criteria, 229 and 6 patients were excluded from DBD and DCD group, respectively (**Figure 2**). In the DCD group, besides 4 recipients of livers from category 2 DCD donors, 2 further cases, including one retransplant, were excluded due to the use of normothermic machine perfusion instead of D-HOPE. Thus, 555 DBD and 20 DCD liver transplants were included for analysis. Finally, outcomes of the 20 DCD LTs were compared to those of 40 recipients of a DBD graft, selected by 1:2 propensity score matching.

Baseline patient and donor characteristics and operational details are summarized in **Table 1**. In the DCD group, median donor age and BMI were 60.1 (55.1, 61.5) and 25.0 [23.0, 26.1], and only one liver had 15% macrovesicular steatosis, reflecting our policy of avoiding overlap of additional donor risk factors in this high-risk cohort, characterized by a functional WIT of 43 (35, 46) min. A-NRP and D-HOPE times were 246 (221, 269) and 205

**TABLE 1 |** Baseline covariates balance.

| | Whole cohort | | | | Matched cohort | | |
|---|---|---|---|---|---|---|---|
| | **DBD** | **DCD** | **p** | **SMD** | **DBD** | **DCD** | **SMD** |
| *n* | 555 | 20 | | | 40 | 20 | |
| Rec. age | 57.5 [52.4, 62.1] | 60.7 [57.4, 66.7] | 0.02 | 0.64 | 60.6 [56.2, 65.6] | 60.7 [57.4, 66.7] | 0.04 |
| Gender (male) | 404 (73) | 16 (80) | 0.65 | 0.17 | 30 (75) | 16 (80) | 0.12 |
| Rec. BMI | 25.0 [22.7, 27.7] | 25.3 [22.6, 27.3] | 0.90 | 0.05 | 25.2 [22.5, 27.8] | 25.3 [22.6, 27.3] | 0.01 |
| Indication | | | 0.28 | 0.65 | | | 0.76 |
| Viral hepatitis | 276 (50) | 9 (45) | | | 27 (68) | 9 (45) | |
| Alcoholic cirrhosis | 98 (18) | 6 (30) | | | 7 (18) | 6 (30) | |
| Cholestatic disease | 39 (7) | 2 (10) | | | 0 (0) | 2 (10) | |
| NASH | 17 (3) | 2 (10) | | | 1 (2) | 2 (10) | |
| Autoimmune | 16 (3) | 0 (0) | | | 0 (0) | 0 (0) | |
| Acute liver failure | 3 (1) | 0 (0) | | | 0 (0) | 0 (0) | |
| Other | 106 (19) | 1 (5) | | | 5 (12) | 1 (5) | |
| MELD | 13.0 [9.0, 18.0] | 10.5 [8.8, 14.5] | 0.17 | 0.21 | 11.5 [8.0, 17.2] | 10.5 [8.8, 14.5] | 0.10 |
| Creatinine (mg/dl) | 0.8 [0.7, 1.1] | 0.8 [0.7, 1.0] | 0.95 | 0.02 | 0.9 [0.7, 1.2] | 0.8 [0.7, 1.0] | 0.20 |
| Dialysis pre-LT | 11 (2) | 0 (0) | 1.00 | 0.20 | 1 (2) | 0 (0) | 0.23 |
| Prev. abdo. surgery | 206 (37) | 10 (50) | 0.35 | 0.26 | 21 (52) | 10 (50) | 0.05 |
| Life support | 17 (3) | 1 (5) | 1.00 | 0.10 | 1 (2) | 1 (5) | 0.13 |
| Ascites | 211 (38) | 7 (35) | 0.96 | 0.06 | 14 (35) | 7 (35) | <0.01 |
| Encephalopathy | 114 (21) | 2 (10) | 0.38 | 0.30 | 7 (18) | 2 (10) | 0.22 |
| HCC | 296 (53) | 16 (80) | 0.03 | 0.59 | 33 (82) | 16 (80) | 0.06 |
| Donor age | 65.4 [52.4, 74.4] | 60.1 [55.1, 61.5] | 0.13 | 0.30 | 63.1 [44.8, 71.7] | 60.1 [55.1, 61.5] | 0.04 |
| Donor BMI | 25.3 [22.9, 27.7] | 25.0 [23.0, 26.1] | 0.57 | 0.17 | 25.3 [23.3, 27.6] | 25.0 [23.0, 26.1] | 0.14 |
| Macrosteatosis (%) | 1.0 [0.0, 5.0] | 0.0 [0.0, 1.2] | 0.05 | 0.35 | 0.0 [0.0, 3.5] | 0.0 [0.0, 1.2] | 0.02 |
| Macrosteatosis ≥15% | 64 (12) | 1 (5) | 0.57 | 0.24 | 2 (5) | 1 (5) | <0.01 |
| Microsteatosis (%) | 10.0 [1.0, 25.0] | 5.0 [0.0, 10.0] | 0.04 | 0.53 | 10.0 [4.5, 20.0] | 5.0 [0.0, 10.0] | 0.36 |
| D-MELD | 800 [573, 1117] | 542 [488, 1014] | 0.05 | 0.33 | 699 [533, 977] | 542 [488, 1014] | 0.12 |
| BAR | 5.0 [3.0, 19.0] | 5.0 [3.0, 8.0] | 0.99 | 0.18 | 5.0 [3.0, 17.0] | 5.0 [3.0, 8.0] | 0.09 |
| WIT (min) | | 43 [40, 48] | | | | 43 [40, 48] | |
| Functional WIT (min) | | 43 [35, 46] | | | | 43 [35, 46] | |
| A-NRP time (min) | | 246 [221, 269] | | | | 246 [221, 269] | |
| CIT (min) | 431 [379, 482] | 261 [229, 295] | <0.01 | 2.06 | 418 [375, 510] | 261 [229, 295] | 1.86 |
| D-HOPE time (min) | | 205 [146, 277] | | | | 205 [146, 277] | |
| Total pres. time (min) | 431 [379, 482] | 492 [426, 531] | 0.01 | 0.65 | 418 [375, 510] | 492 [426, 531] | 0.58 |
| Portal rep. time (min) | 23.0 [21.0, 27.0] | 22.0 [20.5, 26.2] | 0.47 | 0.19 | 23.0 [21.0, 26.2] | 22.0 [20.5, 26.2] | 0.01 |
| Total rep. time (min) | 38.0 [24.0, 50.2] | 48.5 [42.0, 59.5] | 0.01 | 0.51 | 41.0 [24.0, 55.2] | 48.5 [42.0, 59.5] | 0.41 |
| PRBC units (n) | 3.0 [0.0, 8.0] | 2.5 [0.0, 7.2] | 0.70 | 0.04 | 5.0 [0.8, 9.2] | 2.5 [0.0, 7.2] | 0.01 |
| Graft weight (gr) | 1490 [1290, 1720] | 1455 [1222, 1610] | 0.39 | 0.19 | 1475 [1295, 1692] | 1455 [1222, 1610] | 0.09 |

*Abbreviations: SMD, standardized mean difference; BMI, body mass index; NASH, non-alcoholic steatohepatitis; MELD, model for end-stage liver disease; prev, previous; HCC, hepatocellular carcinoma; D-MELD, donor age * MELD score; BAR, balance of risk score; WIT, warm ischemia time; A-NRP, abdominal normothermic regional perfusion; CIT, cold ischemia time; D-HOPE, dual hypothermic oxygenated machine perfusion; pres, preservation; rep, reperfusion; PRBC, packed red blood cells.*

(146, 277) min, respectively. DCD livers were preferentially allocated to low-MELD (10.5 [8.8, 14.5]) patients, with HCC being the indication for LT in 80% of cases. However, with increasing experience, livers from elderly donors were also accepted and procured organs were more frequently allocated to higher-MELD recipients (**Figure 3**). Despite donor and recipient selection, median UK-DCD risk score (4) was 13 (11, 14) with 17 cases being classified as "futile" and 3 as "high-risk".

Patient cohorts selected by propensity score matching showed good comparability, as reflected by a standardized mean difference ≤0.10 for all major confounders, including recipient age, BMI and MELD score, HCC as the indication for LT, donor age, graft macrovesicular steatosis, balance of risk (BAR) score and portal reperfusion time (**Table 1**).

Outcomes in the unmatched and matched cohort are reported in **Table 2**. Overall, early outcomes in the DCD group were comparable to those observed in the DBD group.

In the DCD and DBD group, EAD and grade 2/3 AKI rates were 5% versus 15% and 15% versus 22%, respectively, with no patient requiring renal replacement therapy after LT. Five (25%) and 8 (20%) recipients of a DCD or DBD liver, respectively, developed grade ≥3 complications and median comprehensive complication index was 16.5 (0.0, 33.9) versus 21.8 (8.7, 35.4). Intensive care unit and hospital length of stay was 4 (2, 5) versus 4 (2, 6) and 10 (8, 19.5) versus 12 (9, 19) days, respectively. Two grafts were lost in the DCD group, which were the first and the second in our series. The first graft loss resulted from a hepatic artery injury that occurred during an attempt at performing hepaticojejunostomy for a late biliary fistula 97 days after LT. The vascular injury resulted from the severe inflammatory reaction caused by the biloma involving the porta hepatis and was deemed not amenable to repair. The second graft loss was caused by hepatic artery thrombosis occurring on postoperative day 2. Despite the graft was showing good function, large necrotic areas were apparent at computed tomography scan, so a decision was made

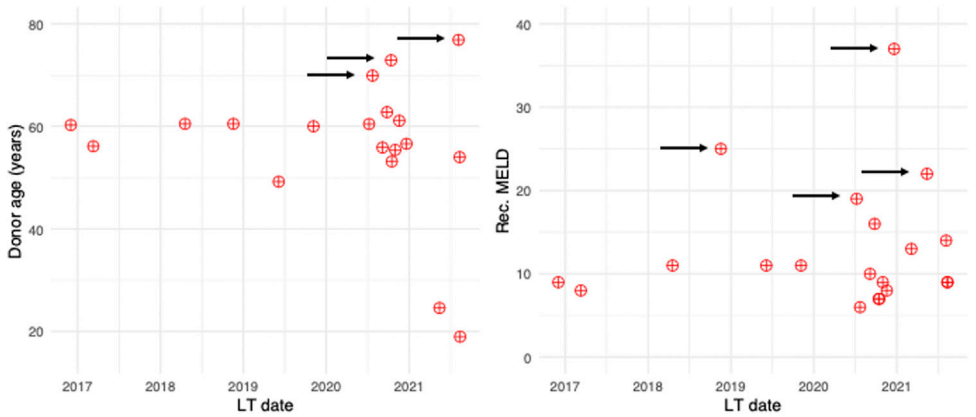



**FIGURE 3 |** Scatter plot depicting donor age and recipient MELD as a function of study period. During study period, donors of increasing age were considered, and DCD grafts were more frequently allocated to higher-MELD recipients (arrows).

**TABLE 2 |** Outcome.

| | Whole cohort | | | Matched cohort | | |
|---|---|---|---|---|---|---|
| | **DBD** | **DCD** | **p** | **DBD** | **DCD** | **p** |
| *n* | 555 | 20 | | 40 | 20 | |
| Severe PRS | 77 (14) | 3 (15) | 1.00 | 4 (10) | 3 (15) | 0.89 |
| End-LT lactate (mmol/l) | 2.0 [1.4, 2.8] | 1.6 [1.0, 2.4] | 0.13 | 2.0 [1.4, 2.9] | 1.6 [1.0, 2.4] | 0.26 |
| AST peak (IU/L) | 1111 [692, 1752] | 761 [589, 1345] | 0.13 | 937 [663, 1438] | 761 [589, 1345] | 0.63 |
| ALT peak (IU/L) | 702 [448, 1126] | 461 [385, 608] | 0.01 | 632 [360, 835] | 461 [385, 608] | 0.18 |
| EAD | 157 (28) | 1 (5) | 0.04 | 6 (15) | 1 (5) | 0.48 |
| AKI stage | | | 0.53 | | | 0.27 |
| 0 | 178 (32) | 8 (40) | | 10 (25) | 8 (40) | |
| 1 | 226 (41) | 9 (45) | | 21 (52) | 9 (45) | |
| 2 | 107 (19) | 3 (15) | | 4 (10) | 3 (15) | |
| 3 | 44 (8) | 0 (0) | | 5 (12) | 0 (0) | |
| Grade 2/3 AKI | 151 (27) | 3 (15) | 0.34 | 9 (22) | 3 (15) | 0.73 |
| Renal replacement therapy | 13 (2) | 0 (0) | 1.00 | 0 (0) | 0 (0) | NA |
| Early rejection | 46 (8) | 1 (5) | 0.91 | 3 (8) | 1 (5) | 1.00 |
| Grade ≥3 complications | 126 (23) | 5 (25) | 1.00 | 8 (20) | 5 (25) | 0.91 |
| ICU stay (days) | 3.0 [2.0, 5.0] | 4.0 [2.0, 5.0] | 0.92 | 4.0 [2.0, 6.0] | 4.0 [2.0, 5.0] | 0.55 |
| Hospital stay (days) | 12.0 [9.0, 17.0] | 10.0 [8.0, 19.5] | 0.59 | 12.0 [9.0, 19.0] | 10.0 [8.0, 19.5] | 0.35 |
| Hospital CCI | 22.6 [12.0, 33.7] | 16.5 [0.0, 33.9] | 0.10 | 21.8 [8.7, 35.4] | 16.5 [0.0, 33.9] | 0.26 |
| Early allograft failure | 28 (5) | 1 (5) | 1.00 | 2 (5) | 1 (5) | 1.00 |
| Biliary complications | | | | | | |
| Anastomotic | 85 (15) | 3 (15) | 1.00 | 9 (22) | 3 (15) | 0.73 |
| Fistula | 10 (2) | 1 (5) | 0.85 | 2 (5) | 1 (5) | 1.00 |
| Stricture | 75 (14) | 2 (10) | 0.91 | 7 (18) | 2 (10) | 0.70 |
| IC | 28 (5) | 1 (5) | 1.00 | 1 (2) | 1 (5) | 1.00 |
| Treatment | | | 0.06 | | | 0.15 |
| Operational | 69 (71) | 1 (33) | | 7 (78) | 1 (33) | |
| Surgery | 24 (25) | 1 (33) | | 2 (22) | 1 (33) | |
| Retransplant | 4 (4) | 1 (33) | | 0 (0) | 1 (33) | |
| N° of treatments | 2.0 [1.0, 3.0] | 3.0 [2.5, 4.5] | 0.33 | 2.0 [2.0, 3.0] | 3.0 [2.5, 4.5] | 0.43 |
| Determining graft loss | 5 (1) | 1 (5) | 0.51 | 0 (0) | 1 (5) | 0.72 |
| Determining patient death | 1 (0) | 0 (0) | 1.00 | 0 (0) | 0 (0) | NA |

*Abbreviations: PRS, post-reperfusion syndrome; LT, liver transplant; EAD, early allograft dysfunction; AKI, acute kidney injury; ICU, intensive care unit; CCI, comprehensive complication index; IC, ischemic cholangiopathy.*

to relist the recipient for urgent retransplantation. Both patients were successfully retransplanted.

The rate of anastomotic biliary complications and ischemic cholangiopathy was comparable between groups (**Table 2**). In particular, only one case of IC was observed in the DCD group. This patient had a percutaneous biliary drain inserted before undergoing hepaticojejunostomy for a tight anastomotic stricture. Cholangiogram showed an isolated posterior duct

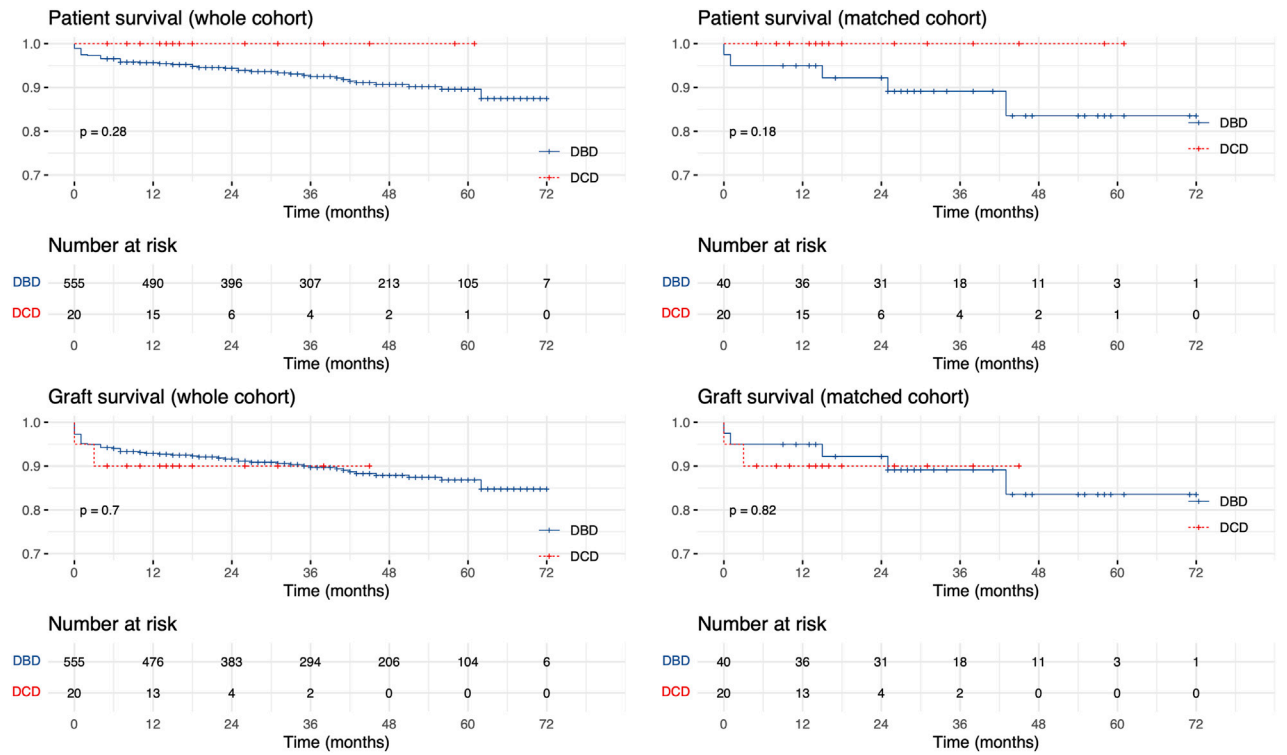



**FIGURE 4 |** Kaplan-Meier patient and graft survival curves in the unmatched and matched cohorts.

stricture, likely representing an incidental finding. Patient was treated with a single balloon dilatation and has neither clinical nor radiological evidence of recurrence 8 months after the procedure.

Median follow-up was 40 (21, 56) and 15.5 (12, 27) months in the DBD and DCD group, respectively. Graft and patient survival was comparable between groups (**Figure 4**). In the matched cohort, 1-year patient survival in the DCD and DBD group was 100% (confidence interval [CI] = 100%, 100%) and 95% (CI = 88.5%, 100%), respectively, whereas 1-year graft survival was 90% (CI = 77.8%, 100%) and 95% (CI = 88.5%, 100%).

## DISCUSSION

This study shows that a combination of A-NRP followed by D-HOPE is effective in preserving grafts from controlled DCD donors with prolonged WIT and allows obtaining comparable outcomes to DBD LT. These results appear to be even more remarkable if some peculiarities of the Italian setting are considered. Besides the 20-min no-touch time, which is unique among countries with active DCD programs (6), pre-mortem cannulation is not allowed in Italy, which further prolongs WIT due to the time necessary to cannulate femoral vessels and occlude the descending aorta (**Figure 1**). Furthermore, as the required 20 min of flat EKG recording are preceded by a variable time of pulseless electric activity, procured organs are exposed to a no-flow time that is frequently much longer than the 20-min no-touch time. If these livers were procured by ultra-rapid recovery and preserved by static cold storage, a poor outcome would be expected (1–4). In contrast, reconditioning and preservation by A-NRP + D-HOPE appears to allow obtaining good results, which are not different from those observed after DBD LT. It is worth noting that, despite initial concerns and logistic obstacles, our ~60% utilization rate compares favourably with that observed in other realities (37, 38).

Overall, our results confirm the benefits of both A-NRP and D-HOPE in controlled DCD LT. As compared to ultra-rapid recovery followed by static cold storage, use of A-NRP has been associated with better graft function, lower rate of overall biliary complications and ischemic cholangiopathy, and improved graft survival (11–13, 15–17, 39). A recent large Spanish study has shown that use of A-NRP alone in DCD LT allows achieving comparable outcome to DBD LT (13). Additionally, use of A-NRP appears to positively impact on utilization rate and post-transplant function of other abdominal organs, especially kidneys (40, 41). On the other hand, DCD LT is the setting in which the advantages of end-ischemic D-HOPE have been more convincingly demonstrated (18, 19, 21, 42–44), with a recent randomized controlled trial showing that use of D-HOPE in this context is associated with a significant reduction of symptomatic non-anastomotic biliary stricture incidence from 18% to 6% (19). However, these data come from countries where local regulations allow usually limiting WIT to 10–15 min, which is much shorter than what is currently observed in Italy. Therefore, Italian centres have frequently considered to combine these two approaches. In

Italy, successful use of controlled DCD donors by combining A-NRP and D-HOPE or normothermic machine perfusion has already been reported (9, 10, 22–24, 29), with a recent study by De Carlis et al.(25) showing that, despite longer WIT, outcome of liver grafts procured by this approach is comparable to those of DCD liver grafts procured by ultra-rapid recovery and SCS. To our knowledge, the present study is the first suggesting that the outcome of controlled DCD LT performed by combining A-NRP and D-HOPE, despite a functional WIT almost invariably exceeding 40 min, is not inferior to that of matched DBD LT.

Undoubtedly, these favourable results also issue from accurate donor selection and liver function assessment during A-NRP. In our experience, four (12.9%) initially accepted grafts were discarded based on parameters obtained during A-NRP. Different criteria for liver viability assessment during A-NRP have been proposed in different countries (8, 16, 17, 45, 46). Given the expected long WIT, we chose to adopt a modified version of the rather unrestrictive criteria proposed by De Carlis et al.(29). These criteria were not modified during study period and are still currently adopted at our centre. The good outcome observed in our series seems to confirm their validity. However, these data must be considered preliminary and future larger studies should investigate whether these criteria could be safely expanded further.

As LT outcomes are also influenced by recipient condition (26, 27), it is likely that recipient selection also played a role in achieving the good results observed in this series. This is the reason why, in order to allow a meaningful comparison, recipient characteristics were accounted for in the matching process. However, although initially DCD livers were preferentially allocated to low-MELD patients undergoing LT for HCC, the good results observed during the initial phases of this study fostered an increased confidence with DCD grafts utilization, which led to consider donor of progressively increasing age and to allocate DCD grafts also to patients with severe hepatic disease (**Figure 3**), without observing any detrimental effect on outcomes. This was also associated with an increasing number of DCD LTs per year (**Figure 3**). Overall, these findings are in keeping with the good outcome achieved and reflect how utilization of DCD liver grafts has become standard practice.

Limitations of our study include retrospective single-centre design and limited numerosity. Given the exploratory nature of this analysis, formal sample size calculation was not made. Also, as the majority of DCD LTs were performed in 2020–2021, follow-up was shorter in DCD group. Although 6-months minimal follow-up should have allowed capturing the majority of biliary complications, late-onset complications could have been missed. We are aware that an updated definition of functional WIT has been recently introduced (47). However, all cases included in this study were antecedent to its introduction and a retrospective recalculation of functional WIT was not possible. Finally, as all grafts included in this study were treated with D-HOPE, we could not evaluate the additional value of D-HOPE after A-NRP. It could be argued that use of machine perfusion could be omitted in selected cases, whereas additional viability assessment by normothermic machine perfusion could be indicated in others (48). In our experience, use of D-HOPE has been systematic for grafts meeting all viability criteria during A-NRP, which are those included in this series. So far, use of normothermic machine perfusion has been limited to cases characterized by doubtful evaluation during A-NRP (24), or in which logistics constraints imposed prolonging preservation time. Well designed and appropriately powered randomized studies are needed to define when and by which modality machine perfusion after A-NRP is indicated in DCD LT.

In conclusion, despite apparently prohibitive WIT, outcome of LT using livers from controlled DCD donors treated by a combination of A-NRP and D-HOPE is comparable to that of DBD LT, suggesting that a wider implementation of this approach could contribute improving the results of DCD LT and expand donor pool. Larger studies are required to confirm these findings, refine our evaluation process, and establish when and by which modality machine perfusion is indicated in this setting.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico Interaziendale A.O.U. Città della Salute e della Scienza di Torino–A.O. Ordine Mauriziano–A.S.L. Città di Torino. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

DP designed the study, collected and analysed data, and drafted the paper; MZ, MV, and RP contributed to data collection, data analysis and paper drafting; CM and ED collected data and critically revised the paper; GR, SC, SM, and DC contributed to data collection and critically revised the manuscript; SL, RB, and RR critically revised the manuscript.

## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Assessment of Mitochondrial Function and Oxygen Consumption Measured During *Ex Vivo* Normothermic Machine Perfusion of Injured Pig Kidneys Helps to Monitor Organ Viability

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Donor kidney assessment may improve organ utilisation. Normothermic Machine Perfusion (NMP) has the potential to facilitate this advance. The mechanism of action is not yet determined and we aimed to assess mitochondrial function during NMP. Anaesthetised pigs ($n$ = 6) had one kidney clamped for 60 min. The healthy contralateral kidney was removed and underwent NMP for 8 h (healthy control (HC), $n$ = 6). Following 60 min warm ischaemia the injured kidney underwent HMP for 24 h, followed by NMP for 8 h ($n$ = 6). Mitochondria were extracted from fresh tissue for analysis. Injured kidneys were analysed as two separate groups (IMa, $n$ = 3 and IMb, $n$ = 3). Renal resistance was higher (0.39ï, ± 0.29 vs. 1.65ï, ± 0.85; $p$ = 0.01) and flow was lower (55ï, ± 28 vs. 7ï, ± 4; $p$ = 0.03) during HMP in IMb than IMa. NMP blood flow was higher in IMa versus IMb (2-way ANOVA; $p < 0.001$) After 60 min NMP, $O_2$ consumption was significantly lower in IMb versus IMa ($p \leq 0.002$). State-3 respiration was significantly different between the groups (37ï, ± 19 vs. 24ï, ± 14 vs. 10ï, ± 8; nmol$O_2$/min/mg; $p$ = 0.049). Lactate levels were significantly lower in IMa versus IMb ($p$ = 0.028). Mitochondrial respiration levels during NMP may be suggestive of kidney viability. Oxygen consumption, renal blood flow and lactate can differentiate severity of kidney injury during NMP.

**Keywords: kidney, mitochondria, normothermic machine perfusion, ischemia/reperfusion injury, preservation**

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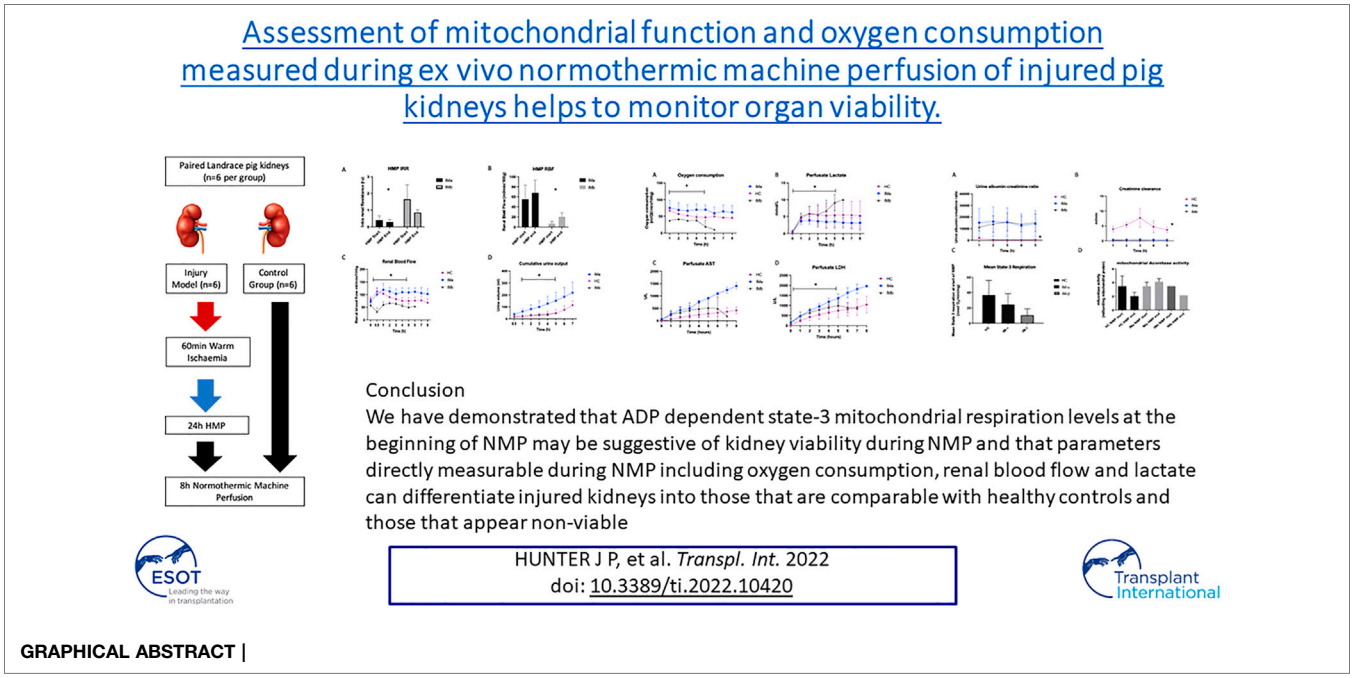


**GRAPHICAL ABSTRACT |**

## INTRODUCTION

The population of organ donors has shifted in recent decades from younger healthy patients with isolated cerebral trauma to older and higher risk patients with more co-morbidities. The result is that clinicians accepting organs for transplant have increasing uncertainty about organ quality. The ability to accurately predict the quality of an organ by a safe and reproducible assessment prior to transplant is desirable for both patients and clinicians. As a result of the drive to utilise more organs there has been a steady increase in the use of donation after circulatory death (DCD) kidneys [1], which sustain warm ischaemic injury. Following transplantation this injury is amplified and results in ischaemia-reperfusion injury (IRI) which is a complex cascade involving immune mediation, mitochondrial dysfunction, complement activation and oxidative damage to cells and tissues [2]. Attempts to minimise IRI by improving organ preservation methods may improve outcomes following transplant.

Kidney normothermic machine perfusion (NMP) is a preservation method that is based on pumping an oxygenated red blood cell-based perfusion solution *via* the renal artery at physiological temperature. NMP reanimates the organ and allows the measurement of perfusion parameters such as renal blood flow and renal resistance, metabolic parameters such as pH and acid-base balance and measures of function such as creatinine clearance. NMP also offers the opportunity to assess organ viability and in future may provide enough information for clinicians to base decisions on whether to accept an organ for transplant [3]. Cerebral injury followed by organ preservation with standard cold preservation causes hypoxia with a significant stunning of mitochondria and cellular energy metabolism whilst adequate function after transplantation of the kidney depends on immediate cellular ATP production. Short periods of NMP can replenish ATP and may improve early outcomes after transplant [4]. Recently published work showed that prolonged NMP of discarded human kidneys was feasible and there was some evidence of histological improvement at the end of perfusion [5]. However, the mechanisms of action of NMP are not clear and to target interventions or improve means of assessment a better understanding is necessary.

The impact of IRI on mitochondrial function is significant and has direct and indirect consequences. Unrecoverable mitochondrial injury leads to mitophagy and loss of mitochondria which has a direct impact on cell energetics and may lead to apoptosis/necrosis [6]. Indirect consequences of mitochondrial injury result from the production of free radicals which cause damage to DNA, proteins, cell organelles and lipids [7]. To date, assessment of mitochondrial function during hypothermic oxygenated machine perfusion (HOPE) and NMP has been performed in rat and clinical models of liver transplant, but not in kidneys [8]. Given the pivotal role of mitochondria as the cellular engine we hypothesised in this study that NMP would aid mitochondrial recovery in a pig model of kidney injury.

## MATERIALS AND METHODS

### Animals and Materials

Animal welfare and experimental procedures were in adherence with Home Office codes of practice and the Animals (Scientific

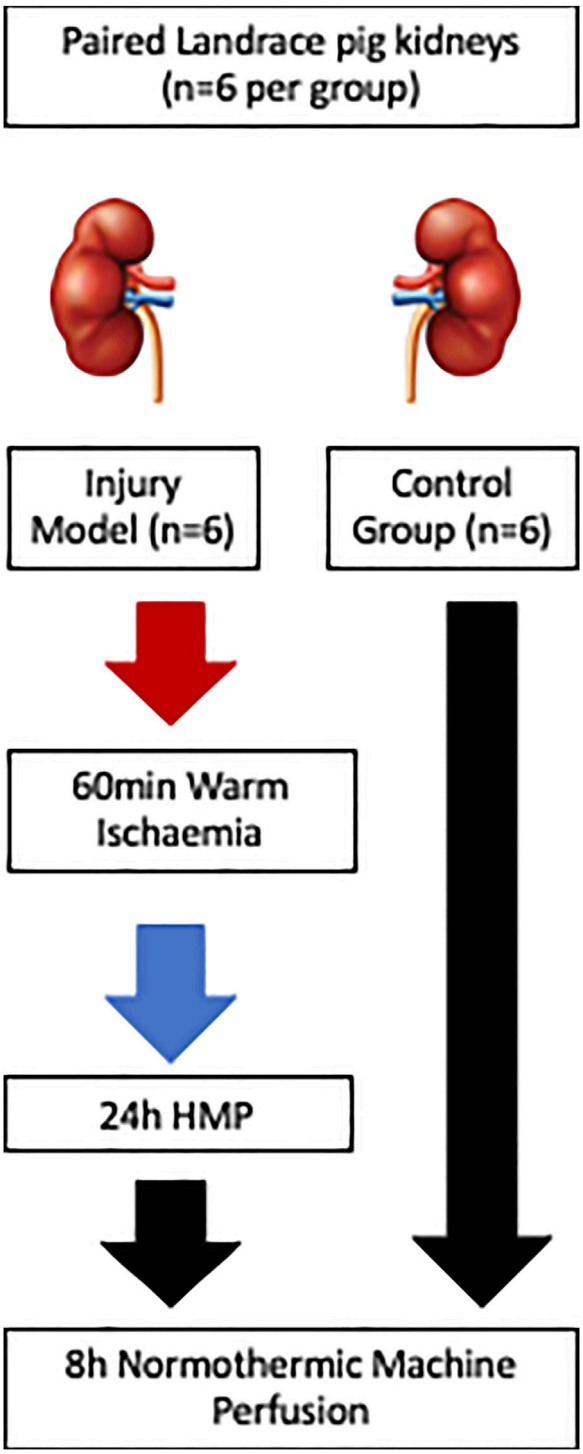


**FIGURE 1 |** Schematic diagram of the study methods illustrating the differing injury sustained and preservation methods between the injury groups ($n$ = 6) and the control group ($n$ = 6).

Procedures) Act 1986. Study design was ratified and local ethical approval was obtained through standard University of Oxford procedures. Landrace pigs (50–60 kg) were used.

## Sample Size Calculation

Sample size was based on Mitochondrial oxygen consumption, ATP levels and Complex I activity and calculated from previous rat kidney studies. The calculations yielded groups sizes of 3, 7, and 8 respectively and following discussion with our local animal ethics board, $n$ = 6 was selected to balance the principle of Reduction with statistical power. Full power calculations are detailed in **Supplementary Appendix SA2**.

## Study Design

Paired kidneys from pigs ($n$ = 6) were allocated to either uninjured control or injury group. Healthy controls (HC) were retrieved, static cold stored (3 h) during transfer back to the laboratory and perfusate preparation, and placed on normothermic machine perfusion (NMP) for 8 h. The injury model group kidneys (IM) were subjected to 60 min warm ischaemia followed by 24 h hypothermic machine perfusion (HMP) and then 8 h NMP, **Figure 1**. The combination of warm and cold ischaemia was used to simulate the injury sustained in DCD donation. The degree of injury was selected following pilot pig auto-transplants performed by collaborators in the MePEP consortium, a collaboration between University of Aarhus, Erasmus University Rotterdam, University Medical Centre Groningen and University of Oxford funded by the Lundbeck Foundation. The experiments demonstrated that 75 min warm ischaemia followed by 16 h cold ischaemia (CI) and auto-transplant caused significant acute kidney injury but no mortality [9]. For logistical reasons 16 h CI was not practical so to account for the increase in CI to 24 h, we reduced the WI from 75 to 60 min.

## Anaesthesia

Animals were sedated and anaesthetized following standard protocols for the study centre. Anaesthesia was maintained with isoflurane in oxygen *via* positive-pressure ventilation. Remifentanil 2 μg/ml (GlaxoSmithKline) was given as a continuous intravenous infusion for analgesia, and maintenance fluids were delivered at 2 ml per kg per h (Hartmann's solution, Aqupharm; Animalcare, York, United Kingdom) throughout surgery. Oxygen saturation, heart rate, respiratory rate, expired carbon dioxide level, body temperature, electrocardiography and blood metabolic parameters were monitored throughout surgery. 25,000 units heparin was given intravenously prior to clamping of the renal vessels.

## Surgical Technique

The pig was placed supine and the abdomen opened through a midline incision. The kidney allocated to the injury group was dissected and the renal artery and vein were exposed and cross-clamped for 60 min. During the period of warm renal ischaemia, the contralateral kidney, allocated to healthy control, was dissected free from surrounding attachments. 10 min prior to the end of 60 min WI the healthy kidney was removed, flushed with 250 ml Soltran and placed on static cold storage (SCS). At the end of 60 min WI the kidney was removed, flushed with 250 ml Soltran and placed on HMP.

## Hypothermic Machine Perfusion

The renal artery was cannulated and the kidney was connected to the Kidney Assist-transport device (Kidney Assist transport, XVIVO. Groningen, Netherlands). The circuit was filled with 500 ml of cold Belzer MPS® UW solution (Bridge to Life Ltd., United Kingdom). Perfusion was commenced at an arterial pressure of 25 mmHg and renal blood flow, renal resistance and temperature were monitored throughout perfusion.

## Normothermic Machine Perfusion

The Kidney Assist device (Kidney Assist, XVIVO, Groningen, Netherlands) was used for NMP. Kidney Assist consumables were used however the circuit was modified to minimise the length of tubing and to accommodate a custom organ chamber. The device was primed with perfusate containing albumin and an autologous red blood cell (RBC) suspension added to produce a haematocrit of 25%. Following cannulation of the renal artery the kidney was connected and perfusion was commenced at a pressure of 70 mmHg. Urine volume was replaced with Ringer's lactate diluted with sterile water to half strength to maintain electrolyte homeostasis and avoid hypernatraemia.

## Perfusate

Autologous red blood cells (RBC) were prepared from whole blood. Blood was filtered using a leukocyte filter (CompoFlex, Fresenius Kabi, Bad Homburg vor der Hohe, Germany) and then centrifuged at 3,000 x g for 20 min at room temperature. The RBCs were washed with 1x phosphate-buffered saline (PBS) and removed by centrifugation at 3,000 x g for 20 min. The washed, isolated RBCs were used for perfusion and added to obtain a haematocrit of 25%. The perfusion solution contained: 250 ml 5% human serum albumin (Alburex, CSL Behring UK Limited, West Sussex, RH16 1AH, UK); 6 ml glucose (B. Braun); 3 ml calcium gluconate (B. Braun); 10 mg mannitol (Sigma Aldrich) and 1000 µmol/L creatinine (Sigma Aldrich). The pH was buffered with 5 ml 8.4% sodium bicarbonate to a physiological pH of 7.2–7.45. The perfusate was supplemented with 1200 mg Co-amoxiclav. A Verapamil bolus of 0.25 mg was added at the beginning of NMP and then 0.25 mg/h was added. Prior to commencing NMP, perfusate was analysed using ABL90 FLEX blood gas analyser to ensure values were physiological. Any alterations to normalise electrolyte and acid-base balance were made prior to connecting the kidney and commencing perfusion.

## Sampling

Perfusion characteristics including renal blood flow, intrarenal resistance and urine production were monitored. Arterial perfusate, venous perfusate and urine samples were taken hourly and blood gas analysis was carried out hourly on the ABL90 FLEX blood gas analyser (Radiometer, Copenhagen, Denmark). Blood and urine samples were centrifuged at 12,000 x g for 12 min at 4°C and the supernatant stored at -80°C for further analysis. Punch and tru-cut needle biopsies were taken prior to NMP after 1 h NMP and at the end of perfusion. Early analysis of mitochondrial function in the injured kidney group suggested evidence of recovery and so additional biopsies were taken at 2, 4 and 6 h in the final 3 kidneys. Biopsies were either prepared immediately to extract mitochondria, stored in formalin or snap-frozen in liquid nitrogen and stored at −80°C.

## Wet/Dry Ratio

Punch biopsies (5 × 8mm) were collected, weighed and dehydrated for 24 h in an incubator set at 60°C. They were then re-weighed and the wet/dry (W/D) weight ratio was calculated by dividing the wet by the dry weight. This ratio determines the proportion of accumulated fluid during the perfusion and the greater the ratio, the greater the volume of fluid accumulated.

## Mitochondrial Assessment

### Mitochondrial Isolation and Respiration

Mitochondria were isolated from the biopsies collected during perfusion and their function was assessed by $O_2$ consumption in a Clarke-type electrode, as previously described [10]. Briefly, biopsies were placed on a Petri dish on wet ice in 1:10 (weight: volume) ice-cold mitochondria isolation buffer (IBc, Tris-MOPS 10 mM, EGTA/Tris 1mM, Sucrose 0.2M, pH 7.4). Biopsies were minced with a scalpel and subsequently homogenised in a pre-cooled glass-teflon homogeniser. Homogenates were centrifuged at 600 x g for 10 min at 4°C. The supernatants were transferred to pre-cooled clean tubes and further centrifuged at 7,000 x g for 10 min at 4°C. The supernatant was then discarded and the pellet containing the mitochondria was gently suspended by pipetting. 25 uL of the mitochondrial suspension were then added to an oxygen electrode (Oxygraph, Hansatech) chamber, equilibrated at 30°C with 1 ml of EBc buffer (KCl 0.125M, Tris/MOPS 10 mM, EGTA/Tris 10 mM, $P_i$ 1 mM). Oxygen concentrations (nmol/ml) were recorded. Using Hamilton micro syringes, succinate (final concentration in chamber 5 mM) was added to the chamber and state 2 respiration $O_2$ consumption was recorded. After 5 min, ADP was injected into the chamber (final concentration 150 µM) and the state 3, ADP-dependent mitochondrial respiration was recorded. Mitochondrial $O_2$ consumption following the addition of ADP was calculated and normalised to mitochondrial protein concentration in each sample. Mitochondrial protein concentration was determined by BCA assay (Thermo Scientific) according to manufacturer's instructions.

## Mitochondrial Aconitase Activity Assay

Aconitases are a family of iron-sulfur enzymes that catalyse the conversion of citrate to isocitrate. Aconitases are reversibly inactivated by reactive oxygen species, and aconitase activity in the mitochondrial compartment is thus indicative of mitochondrial oxidative stress. Aconitase activity in the mitochondria isolated from the kidney biopsies collected before and at the end of NMP was assessed by a colorimetric aconitase activity assay (Sigma Aldrich), per manufacturer's instructions. Mitochondrial aconitase activity in these samples was normalised to the mitochondrial protein concentration.

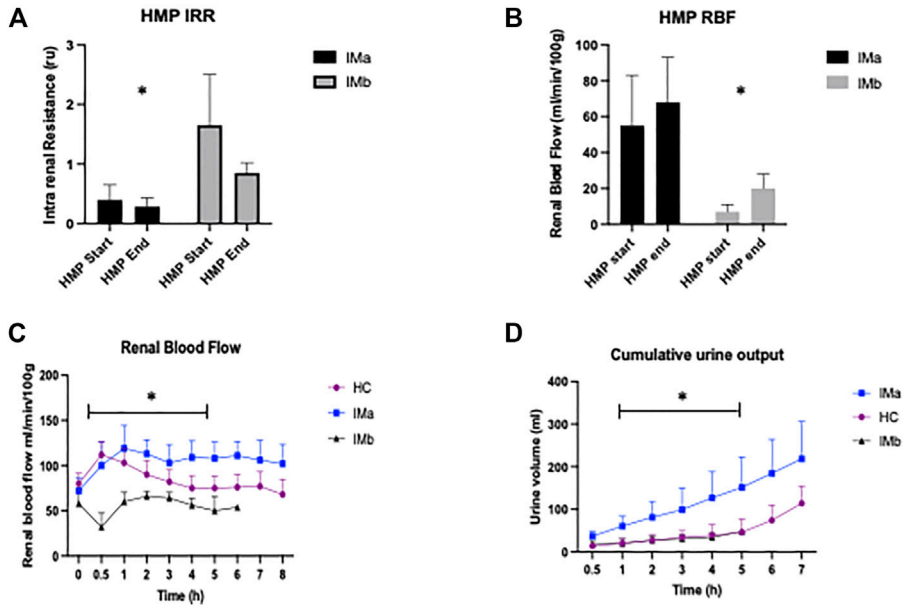


**FIGURE 2 | (A)** Histogram showing Intrarenal resistance (IRR) and **(B)** Renal blood flow (RBF) during Hypothermic Machine Perfusion (HMP) of Injury model kidney groups IMa and IMb (1-way ANOVA with = $p < 0.05$ *). **(C)** Line graph showing renal blood flow and **(D)** Cumulative Urine Output during Normothermic Machine Perfusion of Healthy and Injury model kidney groups IMa and IMb (2-way ANOVA analysed over 5 h NMP with = $p < 0.05$*).

## Histology

Samples were preserved in formalin, processed, sliced and stained using Haematoxylin and Eosin. Tissue injury was assessed using four categories; tubular dilatation, tubular sloughing, cytoplasmic vacuolation and apoptosis. For each category, a severity of injury was ascribed from 0–3; 0 = normal, 1 = mild, 2 = moderate and 3 = severe. The sum of the scores were calculated for each kidney biopsy at three time points (T0 = prior to perfusion, T1 = after 1 h NMP and Tend = end of NMP). Depending upon the sum of the scores, kidneys were then allocated to one of three groups of injury for each time point: A = 0–2, B = 3-4 and C =>5. Slides were assessed by a renal pathologist who was blinded to the grouping (HC or IM).

## Statistical Analysis

Data are presented as mean (s.d.) or as median (range). Normality was assessed using the Shapiro-Wilk test, and non-parametric data were analysed with the Mann–Whitney *U* test. Continuous variables were analysed using Area under the curve (AUC) and One or two-way analysis of variance (ANOVA) and multiple groups were compared using one or two-way analysis of variance (ANOVA) with Tukey's multiple comparisons. Categorical data were compared using Chi-squared test. Statistical analysis was performed using InStat and Prism 8 and 9 software (GraphPad Software, San Diego, CA, United States). $p < 0.050$ was considered statistically significant.

# RESULTS

One kidney allocated to the injury group was hydronephrotic and anatomically abnormal and was excluded. To replace this excluded kidney and ensure $n = 6$ in each group an additional experiment was performed. Three kidneys in the injury model (IM) group had perfusion stopped prior to 8 h due to macroscopic appearance (globally poor perfusion) and according to the criteria for perfusion termination in **Supplementary Appendix SA1**. Kidneys in the IM group were either able to perfuse successfully for 8 h ($n = 3$) or were terminated early ($n = 3$). As a result, we have analysed the IM kidneys in two groups, those that perfused successfully for 8 h (IMa) and those that were terminated early (IMb).

## Weight and Wet/Dry Ratio

There was no significant change in weight during NMP in any of the groups, although HC and IMa kidneys had a mean increase in weight of 20% and 10%, respectively and IMb kidneys had a mean reduction in weight of 12% (data missing for final IMb kidney, therefore $n = 2$ so unable to perform any statistics).

## Hypothermic Machine Perfusion

Intrarenal resistance decreased in both IM groups and there was a significantly lower mean intrarenal resistance at the start of HMP (T0) between IMa and IMb (2-way ANOVA: 0.39 ± 0.29 vs. 1.65 ± 0.85; $p = 0.01$) but not at the end of HMP (2-way ANOVA: 0.25 ± 0.14 vs. 0.51 ± 0.17; $p = 0.76$), **Figure 2**. Renal blood flow increased throughout HMP in both groups and was significantly higher at the start (2-way ANOVA: 55 ± 28 vs. 7 ± 4; $p = 0.03$) and end (2-way ANOVA: 68 ± 25 vs. 20 ± 8; $p = 0.03$) of HMP in the IMa compared to IMb group, **Figure 2**.

## Normothermic Machine Perfusion

### Renal Blood Flow

There was a significant difference in RBF between the three groups (2-way ANOVA: $p$=<0.001). RBF was significantly

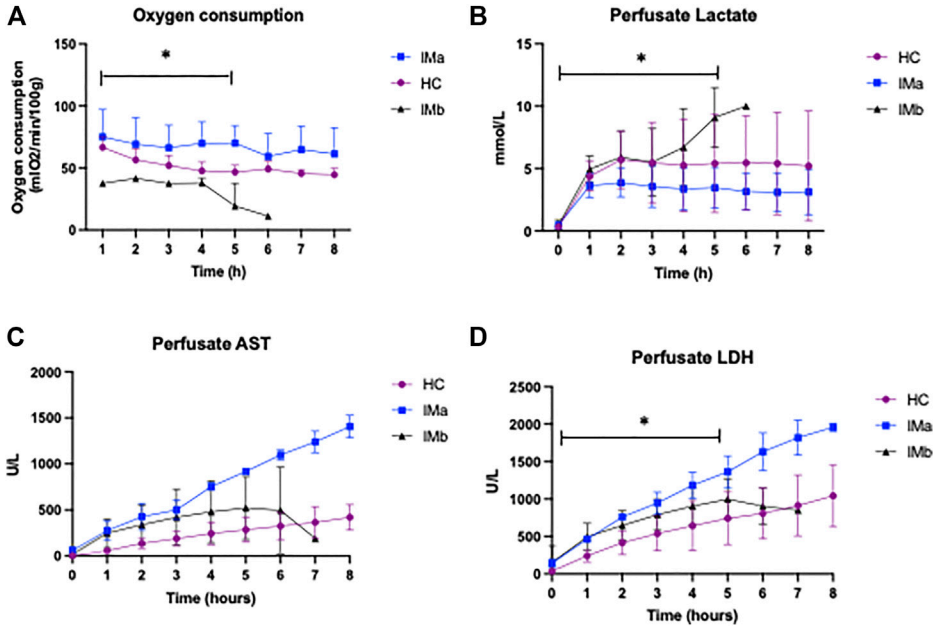


**FIGURE 3 | (A)** Line graph showing oxygen consumption **(B)** Perfusate lactate levels **(C)** Perfusate AST and **(D)** Perfusate LDH levels during Normothermic Machine Perfusion of Healthy and Injury model kidney groups IMa and IMb (2-way ANOVA analysed over 5 h NMP with = $p < 0.05$ *). AST, aspartate transaminase, LDH, lactate dehydrogenase (* = $p < 0.05$).

higher in HC compared with IMb (2-way ANOVA with Tukey's multiple comparisons; $p = 0.029$) and IMa compared with IMb (2-way ANOVA with Tukey's multiple comparisons; $p < 0.001$) and interestingly RBF was higher in IMa compared with HC (2-way ANOVA with Tukey's multiple comparisons; $p = 0.002$), **Figure 2**. The groups, including Tukey's multiple comparisons were compared over 5 h NMP, which was the duration of perfusion with all 12 kidneys included.

## Cumulative Urine Production

There was a significant difference in cumulative urine production between the three groups (2-way ANOVA: $p$=<0.0029). Urine production was significantly higher in HC and IMa when compared with IMb (2-way ANOVA with Tukey's multiple comparisons; $p = 0.005$ and $p = 0.004$, respectively). There was no difference in urine output between HC and IMa (2-way ANOVA with Tukey's multiple comparisons; $p = 0.8$). The groups including Tukey's multiple comparisons were compared over 5 h NMP, which was the duration of perfusion with all 12 kidneys included, **Figure 2**.

## Oxygen Consumption

There was a significant difference in $O_2$ consumption between the three groups over 5 h NMP (2-way ANOVA $p$ = <0.0001). $O_2$ consumption was significantly higher in IMa compared with IMb and HC (2-way ANOVA with Tukey's multiple comparisons $p$ = 0.002 and $p = 0.04$, respectively) and HC compared with IMb (2-way ANOVA with Tukey's multiple comparisons over 5 h NMP, $p = 0.04$), **Figure 3**. Two of the three ischaemic kidneys that were terminated early, at hours 5 and 6 showed an inability to consume oxygen with $PvO_2$ increasing from 9 to 76 kPa and 16–50 kPa over the final hour and with oxygen consumptions of 1 and 11 kPa/ml/min/g respectively. The median $PvO_2$ and oxygen consumption of the 3 injured kidneys that completed 8 h NMP was 8.6 kPa and 57 kPa/ml/min/g, respectively.

## Lactate

There was a significant difference in perfusate lactate levels between HC, IMa and IMb groups respectively over the first 5 h NMP, which included all 12 kidneys (2-way ANOVA, $p$ = <0.031). Perfusate lactate levels were significantly lower in IMa compared with IMb but not HC versus IMa or IMb (2-way ANOVA with Tukey's multiple comparisons over 5 h NMP, $p$ = 0.028), **Figure 3**.

## Renal Function

Creatinine clearance was significantly higher in the healthy control group compared to both IMa and IMb groups (Two-way ANOVA, $p \leq 0.0001$) although there was no difference in CrCl over time (Two-way ANOVA with Tukey's multiple comparisons over 5 h NMP, $p = 0.88$). Urine albumin: creatinine ratio was significantly lower in the healthy control group compared to both IMa and IMb groups (2-way ANOVA, $p \leq 0.0001$) but with no difference over time (2-way ANOVA with Tukey's multiple comparisons over 5 h NMP, $p = 0.99$), **Figure 4**.

## Markers of Injury

Perfusate LDH levels were significantly different between groups over 5 h NMP (AUC and One-way ANOVA: 2070 ± 952 vs. 4106 ± 319 vs. 3168 ± 700IU/L; $p \leq 0.02$) with a significant

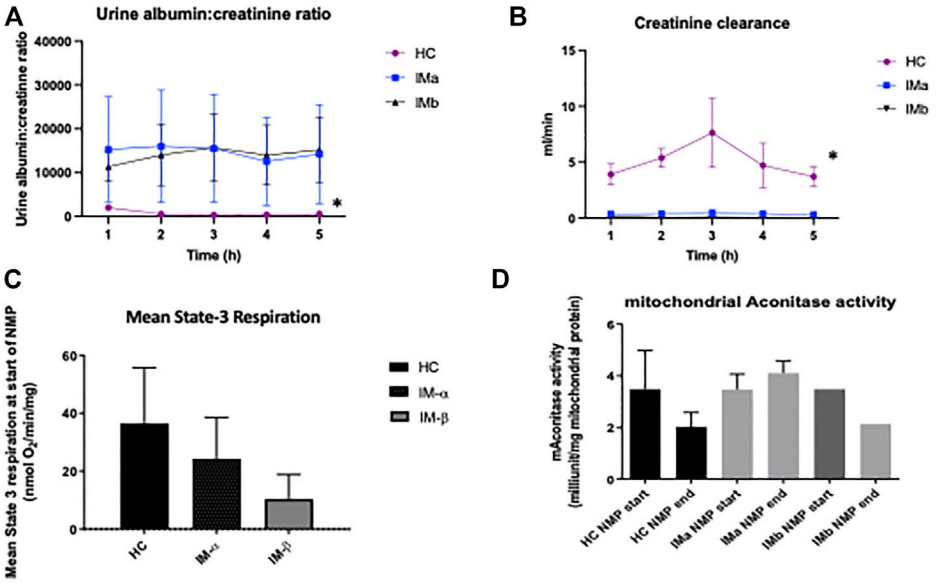


**FIGURE 4 | (A)** Line graph showing urine albumin:creatinine ratio and **(B)** creatinine clearance during Normothermic Machine Perfusion of Healthy and Injury model kidney groups IMa and IMb (2-way ANOVA analysed over 5 h NMP with = $p < 0.05$ *). **(C)** Graph showing mean State-3 respiration and **(D)** mitochondrial aconitase activity during Normothermic Machine Perfusion of Healthy and Injury model kidney groups IMa and IMb (* = $p < 0.05$).

difference between HC and IMa but not HC and IMb over 5 h NMP (AUC and One-way ANOVA with multiple comparisons ($p < 0.05$). Perfusate AST was no different over 5 h NMP (AUC and One-way ANOVA: 1089 ± 985 vs. 2447 ± 271 vs. 1757 ± 1189IU/L; $p = 0.19$), respectively, **Figure 3**.

## Mitochondrial Assessment

ADP-dependent (State-3) respiration is defined as ADP-dependent oxygen consumption, and reflects the mitochondrial respiration coupled with ATP production. This was measured "real-time" in mitochondria isolated from biopsies taken immediately prior to normothermic machine perfusion. A significant difference was seen between the 3 groups at the beginning of NMP (Kruskal-Wallis: 37 ± 19 vs. 24 ± 14 vs. 10 ± 8; nmol$O_2$/min/mg; $p = 0.049$). Comparison between the groups showed a higher level of state-3 respiration in the HC group compared to IMb (Mann-Whitney, $p = 0.0024$) but not IMa (Mann-Whitney, $p = 0.38$). There was no difference in mean mitochondrial respiration throughout perfusion between the 3 groups (One-way ANOVA: 33 ± 23 vs. 29 ± 13 vs. 14 ± 7 nmol$O_2$/min/mg; $p = 0.38$). Mitochondrial aconitase activity was measured as a marker of mitochondrial oxidative stress and there was no difference at the beginning and end of NMP in either healthy kidneys or injured kidneys and no difference between the two groups (One-way ANOVA $p = 0.506$), **Figure 4**.

## Histology

There was a significant difference in injury severity (ISS) score between the healthy control kidneys and both ischaemic model groups at the beginning of preservation (T0) (Chi-Squared: $p = 0.01$), however at the end of preservation (T8) there was no difference between HC and IM groups (Chi-squared $p = 0.76$). There was a significant worsening of injury severity score in HC from T0 to T8 (Chi-squared $p = 0.007$), but no difference in ISS in the IM kidneys from T0 to T8 (Chi-squared $p = 0.99$).

# DISCUSSION

This study set out to assess whether mitochondrial respiratory capacity, analysed during normothermic machine perfusion (NMP), had the potential as an indicator of kidney viability. ADP-dependent state-3 respiration assessed from freshly isolated mitochondria at the end of the preservation period was lower in ischaemic kidneys that performed poorly during NMP (IMb), compared to healthy controls. This difference was significant despite the small numbers ($n = 3$) present in the two injury model groups. The original study design included paired kidneys to compare a "gold standard" healthy control with kidney injury that was at the extent of what would be considered viable. 60 min warm ischaemia with 24 h cold ischaemia was selected, as the combination of WI and CI was clinically relevant, and the durations were based on previous work that showed 75 min WI plus 16 h CI followed by auto-transplant led to significant but recoverable acute kidney injury [11]. During the project it became clear that in some of the kidneys the injury sustained was unrecoverable, as they were significantly deteriorating and appeared to be non-viable during NMP. We used pre-established criteria to decide upon perfusion termination (**Supplementary Appendix SA1**) and of the 6 injury group kidneys 3 were terminated early. This resulted in the injury group being split into two groups, those that completed 8 h of NMP successfully (IMa, $n = 3$) and those that were terminated early (IMb, $n = 3$). The authors recognise the limitation that the

small group size has on the strength of the findings. With hindsight, it would have been beneficial to have continued the perfusion of all kidneys to the common endpoint of 8 h. However, the clear differences between the 2 sub-groups are discussed in detail below and to minimise the effect of the small group sizes we have mainly analysed the data over 5 h NMP which includes all 12 experimental kidneys.

The first interesting finding was the difference in perfusion parameters between the two injury groups during HMP. Kidneys from the injury model group that completed 8 h NMP successfully (IMa) had lower renal resistance at the beginning of HMP and higher renal blood flow at the beginning and end of HMP compared with kidneys in the injury model group that were terminated on NMP early (IMb). Despite these differences, the renal resistance significantly reduced in the IMb group throughout HMP and was no different to the IMa value at the end of HMP. This suggests that for IRR, the values obtained at the beginning of HMP may be more predictive of viability than the end values or the change during perfusion. Clinical studies have shown that renal resistance, both during HMP and at the end of perfusion may be predictive of long term graft survival [12, 13]. In a sub-group analysis of the European MP-trial a cut off of >0.4 mmHg/ml/min at the end of perfusion was suggested, which was based on HMP of human kidneys on the Lifeport Kidney Transporter [13]. Our study used pig kidneys on the Kidney Assist Transporter device and the IRR values at the end of perfusion were 0.25 (IMa group) and 0.51 (IMb group) which correlates with the >0.4 mmHg/ml/min, despite these clear methodological differences. Systematic review and meta-analysis data demonstrate that HMP of deceased donor kidneys reduces DGF but the effect on graft survival is less clear and there is no effect on PNF [14, 15]. As a predictor of function, there is not enough evidence that IRR during HMP can be used in isolation. However, it may be valuable in selecting those kidneys that are marginal and would benefit from additional testing such as NMP and/or biopsy.

ADP-dependent mitochondrial respiration was lower in the IMb group immediately prior to NMP compared with the HC and IMa groups. Despite the small numbers and variation at different time points throughout NMP, mitochondrial respiration remained stable across the groups, with similar values at the beginning and end of NMP. This suggests that 8 h NMP supports mitochondrial respiration but does not aid mitochondrial recovery. It is unclear whether this is due to the kidneys having sustained unrecoverable injury or whether the pseudo-physiological state during NMP does not provide the environment or substrates required. From the perspective of potential translation into organ assessment in the clinical field, it was interesting to observe that mitochondrial respiration showed the same pattern as calculated oxygen consumption. Calculated oxygen consumption can be assessed in real-time and requires only venous and arterial blood gas analysis. This is in contrast to isolating mitochondria and analysing fresh samples for respiratory function, which is very resource intensive and impractical in the clinical context. An alternative possibility would be to assess respiratory capacity directly in permeabilized tissue biopsies, avoiding the need for mitochondrial isolation, but unlike net oxygen consumption, allowing a thorough measurement of respiratory capacity [16]. In addition, the level of mitochondrial respiration at the beginning of NMP may have the potential to be predictive of organ viability. This study has not conclusively demonstrated a link between oxygen consumption/mitochondrial respiration and organ viability during NMP but it is clear that organs with poor perfusion parameters during NMP were those with low $O_2$ consumption and low ADP-dependent state-3 respiration at the end of cold preservation.

Mitochondrial aconitase activity was measured as a marker of oxidative damage, as oxidative stress reversibly inactivates the enzyme. No significant differences were found between the groups, although interestingly the levels in HC kidneys dropped from the beginning to the end of perfusion. This suggests that NMP itself may cause oxidative damage, consistent with a reperfusion event after a period of sustained ischaemia [17] despite the lack of kidney injury. Histological data also show that NMP has an effect on healthy tissue, with an increase in the injury severity score (ISS). The ISS at the beginning of NMP showed, as expected, almost no injury, but after 8 h there was evidence of tubular injury and significant cytoplasmic vacuolation (**Figure 5**). However, the change in ISS in the IM groups during perfusion was not significant which makes histology difficult to interpret in the context of viability. A further interesting observation was that although the macroscopic appearance of the IMb kidneys was globally discoloured and ischaemic, the histology scores were no different from IMa and no different at the end of perfusion compared with the beginning. There was no cortical necrosis and tubular injury was mostly moderate, not severe. Other groups have shown that tubular injury and inflammation were lower in the 8 h NMP group 30 mins after auto-transplant compared to controls however the findings were marginal and did not persist at 3 days after transplant [18].

Healthy kidneys were deliberately selected as a control to determine the impact of NMP as a preservation technique on uninjured organs and to see what readings NMP of a "normal" kidney produced. Kidneys were perfused for 8 h and had stable blood flow and oxygen consumption throughout. Lactate levels remained stable, although cellular injury markers, AST and LDH did rise throughout perfusion. This pattern was also observed by Kaths et al in their DCD auto-transplant model [19]. Healthy kidneys in this study retained glomerular and tubular function, demonstrated by the ability to clear creatinine and the absence of proteinuria, which remained stable throughout the 8 h NMP. This was in contrast to kidneys in both injury groups which, despite the IMa group producing a good urine volume, did not clear creatinine and had gross proteinuria. This suggests that tubular and glomerular function during NMP may not be a helpful marker of viability in injured kidneys and could be considered the clinical equivalent of delayed graft function. Other groups have effectively used CrCl and fractional excretion of sodium during NMP to demonstrate function and compare kidneys although the kidney injury was less severe than in our model [20–22].

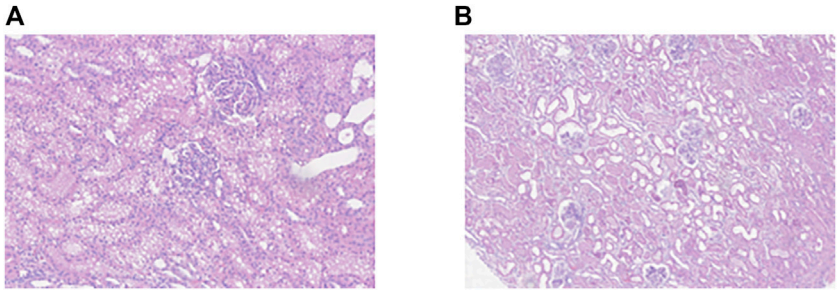


**FIGURE 5 | (A)** Representative histological image of a healthy kidney section at 20x magnification after 8 h NMP showing significant cytoplasmic vacuolation. **(B)** histological image of an injury model kidney after 8 h NMP showing tubular dilatation.

The striking feature of the IMa group that perfused successfully for 8 h was that absolute values of renal blood flow, oxygen consumption and urine output were higher than the healthy control group. In order to sustain ATP production, oxygen consumption increases which results in an increase in renal blood flow [23–25]. This results in an increased glomerular filtration rate, and hence increased urine output, as seen in the IMa group. Therefore, the tubular load of electrolytes destined for active reabsorption rises and the increased oxygen delivery is matched by increased demand. However, the inability of the kidney to compensate for increases in oxygen consumption renders it particularly sensitive to alterations in oxygen metabolism that result in decreased kidney oxygen tension ($pO_2$) [26]. We did not directly measure tissue oxygen tension in this study but it is certainly worth further investigation and may be the most accurate way to assess this complex equilibrium during NMP and help decide on optimal $pO_2$ value and oxygen delivery.

Although the objective of the study was the assessment of NMP as a preservation technique, the main limitation was the absence of a transplant end point, which offers clinically relevant functional data. In the context of animal ethics and refinement, we minimised the number of animals required, the cost and the severity of the model, without a significant compromise to the outcome [27]. A pig model was selected as the heterogeneity of discarded human kidneys makes mechanistic work challenging. The pig has genetic, anatomical, and immunological similarities to the human and is recognised as an excellent translational model [28–30]. The authors acknowledge that the concept of organ viability during NMP is difficult to define and for the kidneys that were considered non-viable (IMb group) and terminated on NMP early, they all scored 5 (data not shown) using the published viability score [3], which is considered un-transplantable. This score was designed for human kidneys undergoing 1 h of NMP and may not be directly translatable but is widely used in experimental work. We developed our own criteria for termination of pig kidney NMP on the basis of previous experience (**Supplementary Appendix SA1**). In addition, long term outcomes after DCD transplant are equivalent to DBD, despite the warm ischaemic injury the kidneys sustain. Other factors, such as chronic rejection, fibrosis and drug toxicity impact on graft survival and longevity, which are unlikely to be linked to biomarkers during NMP [31].

In conclusion, we have shown that this pig kidney model with 60 min warm ischaemia and 24 h cold ischaemia offers an extreme of injury that appears to be at the interface between viable and non-viable. We have shown the impact of NMP on uninjured, healthy kidneys as a benchmark for future work. We have demonstrated that ADP-dependent state-3 mitochondrial respiration levels at the beginning of NMP may be suggestive of kidney viability during NMP and that parameters directly measurable during NMP including oxygen consumption, renal blood flow and lactate can differentiate injured kidneys into those that are comparable with healthy controls and those that appear non-viable.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The animal study was reviewed and approved by the University of Oxford.

## AUTHOR CONTRIBUTIONS

JH and LF: participated in research design, writing of the paper and/or article approval, performance of the research, and data analysis and/or statistics. KR: participated in the performance of the research and data analysis and/or statistics. FD, AO, and LK: participated in the performance of the research. AW: participated in the performance of the research and writing of the paper and/or article approval. JM: participated in the performance of the research, contributed new reagents or analytic tools, and participated in the writing of the paper and/or article approval. KG: contributed new reagents or analytic tools and participated in data analysis and/or statistics. RP: participated in research design and writing of the paper and/or article approval.

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## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10420/full#supplementary-material

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