Novel Organ Perfusion and Preservation Strategies in Transplantation – where are we going in the UK?

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Abbreviations

SCS – Static Cold Storage

NHSBT - National Health Service Blood and Transplant

KPI – Key Performance Indicator

HMP – Hypothermic Machine Perfusion

ECD – Extended Criteria Donors

DCD – Donation after Circulatory Death

OR - Odds Ratio

RR - Risk Ratio

DGF – Delayed Graft Function

PNF – Primary Non-Function

RCT – Randomised Controlled Trial

DBD – Donation after Brain Death

HTK - Histidine-Tryptophan-Ketoglutarate

UW - University of Wisconsin

COPE - Consortium for Organ Preservation in Europe

HRP – Hypothermic Regional Perfusion

uDCD - uncontrolled Donation after Circulatory Death

NRP – Normothermic Regional Perfusion

cDCD - Controlled Donation after Circulatory Death

IC – Ischemic Cholangiopathy

PV – Portal vein

HA – Hepatic artery

EAD - Early Allograft Dysfunction

HOPE – Hypothermic Oxygenated Perfusion

DHOPE – Dual Flow Hypothermic Oxygenated Perfusion

NMP – Normothermic Machine Perfusion

COR - Controlled Oxygenated Rewarming

DPP - Direct Procurement and Preservation

TA-NRP - Thoraco-Abdominal NRP

PGD - Primary Graft Dysfunction

ECMO - Extracorporeal Membrane Oxygenation

Abstract

This review article focuses on current clinical outcomes with novel perfusion strategies in organ transplantation. Broadly, these approaches can be divided into *in-situ* regional perfusion in the donor and *ex-situ* machine perfusion of individual organs. In both settings hypothermic and normothermic techniques are in clinical use. Evidence from full text articles, abstracts and data presented at scientific meetings has been considered. Animal studies have been excluded. The review focuses on kidney, liver, pancreas, heart and lungs. The level of evidence ranges from quasi-experimental work in human pancreas to multiple meta-analyses of Randomised Controlled Trials for hypothermic machine perfusion of kidneys. The data in this review was presented to experts in organ perfusion and preservation at the National Health Service Blood and Transplant Preservation and Perfusion Future Strategy Summit in London in October 2018. The outcomes of the meeting are discussed in the review after due consideration of the available evidence base.

Introduction

Rising demand for organs for transplantation has led to increased utilisation of grafts from donors that fall outside standard acceptance criteria, which are perceived as higher-risk donors. Static cold storage (SCS) may be adequate for organs from so-called 'standard-criteria' donors but is insufficient for the preservation of organs from older donors and/or those with a higher-risk of graft failure. SCS therefore doesn't facilitate the expansion of organ acceptance criteria for transplantation and likely compounds ongoing organ shortage [1]. There has been an explosion of novel strategies to perfuse, preserve, repair and resuscitate organs prior to transplantation [2]. Technological advances have supported the development of these strategies with a number of devices entering clinical practice or trials. Broadly speaking the focus of strategies has been either the donor with *in-situ* regional perfusion, or the transport and pre-implantation phase with *ex-situ* machine perfusion of isolated organs. Although no universal nomenclature is in use, in the context of deceased organ donation *ex-situ* is the preferred terminology over *ex-vivo* given that machine perfusion occurs after the organs have been removed from the body of a deceased donor [3]. A number of perfusion variables including temperature, oxygen delivery and perfusate (blood-based, blood analogues or specifically designed media) are currently being investigated [2]. The theoretical advantages of these dynamic perfusion and preservation modalities have translated into encouraging results that appear to suggest an increased graft utilisation, the ability to undertake assessment of graft viability prior to transplantation, with a potentially improved outcome for recipients [4]. However, the increasing number of devices available combined with the wide range of regimens of perfusion technology make the choice increasingly complex. Whilst

current trials focus on demonstrating the clinical benefit of individual strategies, the time has come to establish how best to tailor the techniques to specific donor types and conditions while defining their role for each organ, accounting for donor- and organ-specific risk factors.

This review undertakes a horizon scan of the clinical outcomes reported to date using various novel perfusion strategies applied in organ transplantation. The data (Figure 1, Appendix 1) was presented to experts at the National Health Service Blood and Transplant (NHSBT) Preservation and Perfusion Future Strategy Summit in London in October 2018. The outcomes of the meeting are discussed later after due consideration of the available evidence.

Kidney transplantation

The key performance indicators (KPIs) following kidney transplantation are graft utilisation, immediate vs. delayed graft function (DGF) vs. primary-non-function (PNF), graft survival, patient survival and 1-year graft function (eGFR/creatinine). The standard technique for kidney preservation is SCS in most centres.

Hypothermic Perfusion Strategies

Standard hypothermic machine perfusion (HMP) involves the kidney being connected to a perfusion device and cold acellular preservation solution is pumped continuously through the renal vasculature at temperatures ranging from 1-10°C [5]. Meta-analyses have been published comparing HMP kidney preservation with SCS in kidneys recovered from extended criteria donors (ECD) [6], donation after circulatory death

(DCD) donors [7, 8] and across all donor types [8-13]. These meta-analyses have described a significant reduction in the odds ratio (OR) or risk ratio (RR) of DGF (ranging from 0.6-0.8) following HMP but none have reported a significant reduction in PNF [6-13] (Table 1). Only one meta-analysis of ECD reported improved graft survival in kidneys following HMP compared to SCS at 1-year (OR 1.12, 1.03-1.21, p=0.005) [6]. Similarly there's a meta-analysis reporting improved graft survival at 3-years across all donor types (RR 1.06, 1.02-1.11, p=0.009) [13]. A Cochrane Review concluded that HMP is superior to SCS in both DBD and DCD kidney transplantation, even when assessing only studies that have been published in the last decade. However, because kidneys from DCD donors have an increased risk of DGF, the number needed to treat to prevent one episode of DGF is less for DCD kidneys (7.26vs.13.60 in DBD kidneys) [12].

A randomised controlled trial (RCT) of 336 consecutive deceased donors in the Eurotransplant region that randomised in a paired design one kidney from each donor to HMP (LifePort, Organ Recovery Systems®, USA) or SCS reported a significant reduction in DGF (adjusted-OR 0.57, 0.36-0.88, p=0.01) and 1-year graft failure (adjusted-OR 0.52, 0.29-0.93, p=0.03) with HMP [5]. The reduction in DGF with HMP was confirmed in an independently powered extension of this RCT into 82 DCD donors (adjusted-OR 0.43, 0.20-0.89, p=0.025) [14], and another independent study of 91 donation after brain death (DBD) donors that were ECD (adjusted-OR 0.46, 0.21-0.99, p=0.047) [15]. The sub-analysis of ECD reported that 1-year death censored graft survival was significantly higher with HMP compared to SCS (92%vs.80%, p=0.02; adjusted hazard ratio for 1-year graft loss 0.35, 0.15–0.86, p=0.02) [15]. In the DCD population a significant reduction in DGF was observed in the HMP group

(54%vs.70%; p=0.007) but no significant difference was seen for 1-year graft survival between HMP and SCS groups (94vs.95%) [14].

In contrast to the DCD study in the Eurotransplant region, a UK RCT comparing HMP (LifePort, Organ Recovery Systems®, USA) with SCS in DCD kidneys and analysed by sequential analysis was stopped due to futility (DGF rate HMP; 58%vs.SCS; 56%) [16]. There are differences between these RCTs, most notably that in the UK trial kidneys weren't preserved with HMP from procurement and underwent an initial variable-length period of SCS. In the UK trial there was also fixed control preservation fluid in the SCS group while in the European RCT both histidine-tryptophan-ketoglutarate (HTK) (76%) and University of Wisconsin (UW) solution (22%) were used [17]. Furthermore, the DGF rate for DCD kidneys subjected to SCS was lower in the UK study than in the European DCD study (UK DGF 56%vs.70% in the European study) but DGF rates after HMP were similar in the UK (58%) and European (54%) trials [16].

In recent analysis of the NHSBT database (2007-2015), DGF rates were significantly lower in kidneys preserved with HMP compared with SCS (34%vs.42%, p<0.001; adjusted-OR 0.65, 0.53-0.80, p< 0.001) with no difference in graft survival (adjusted hazard ratio 0.88, 0.70-1.10, p=0.263) [18]. In a single-centre retrospective study from West London, pre-implantation HMP (RM3, Waters Medical Sytem, USA) following SCS (n=33) decreased DGF (24%vs.48%, p=0.04) compared to SCS alone (n=33)[19]. A further paired-kidney analysis from Germany reported a reduced rate of DGF (12%vs.21%, p=0.38; adjusted-OR 0.28, 0.07-0.94, p<0.04) with pre-implantation HMP (LifePort, Organ Recovery Systems, n=66) compared to SCS (n=43) [20].

Currently there's an ongoing UK trial replicating the EuroTransplant methods (ISRCTN 50082383). Two further RCTs of the Consortium for Organ Preservation in Europe (COPE) have completed recruitment assessing standard HMP vs. oxygenated-HMP (Kidney Assist-transport®, Organ Assist, Netherlands). One RCT has randomised kidneys from ECD to oxygentated-HMP after SCS vs. SCS alone with graft survival at 1-year as a primary endpoint (COPE-POMP, ISRCTN 63852508). The second RCT has randomised kidneys in a paired design from controlled DCD donors older than 50 years to either oxygenated-HMP (n=106) or standard HMP (n=106) with eGFR as its primary endpoint (COPE-COMPARE, ISRCTN 32967929). The results of the COPE-COMPARE study were reported at the American Transplant Congress in May 2019, showing a significant reduction in biopsy-proven acute rejection (14%vs.28%, p=0.01), reduced graft loss (3%vs.10%, p=0.021) and on sensitivity analysis a significantly higher eGFR (47.6vs.42.6 ml/min/1.73 m^2, p=0.035) at 1-year follow up for kidneys perfused with oxygenated-HMP. No statistically significant difference was seen as regards DGF and PNF (oxygenated-HMP vs. standard HMP: DGF; 38%vs.38% | PNF; 3%vs.5%) [21].

Hypothermic regional perfusion (HRP) involves isolation and perfusion of abdominal organs with continuous flow of diluted blood cooled to 4-22 °C [22]. In the largest reported series of HRP in uncontrolled DCD (uDCD) kidneys (n=320) there was a 4% rate of PNF, 61% rate of DGF and an 87% graft survival at 1-year [23]. PNF rates of 0-6%, DGF rates of 21-85% and 1-year graft survival rates of 88-97% have been reported in other single-centre studies (n=8-34) [24-27]. In a study from St Petersburg, subnomothermic regional perfusion (27–32°C) in 44 uDCD kidney donors with prolonged asystole (mean 61 minutes) led to comparable 1-year graft survival

(95%vs.96%) to DBD kidneys (n=87). A 52% rate of DGF was also observed in this study but no cases of PNF [28].

Normothermic Perfusion Strategies

Normothermic regional perfusion (NRP) is performed in a similar manner to HRP but maintaining perfusion temperature close to normothermia (35˚-37˚C) [22]. A study from France compared NRP (n=19) with SCS (n=31) in kidneys from uDCD donors. All kidneys underwent HMP (LifePort, Organ Recovery Systems®, USA) for at least 2-hours following NRP. PNF as well as patient and graft survival rates didn't differ between the groups. However, the use of NRP was associated with a significantly lower risk of DGF compared to SCS (53%vs.81%, p=0.036), which persisted in multivariate models (adjusted-OR=0.17, 0.03-0.87, p=0.034). Furthermore, the use of NRP was the only significant factor associated with a likelihood of an eGFR > 40 ml/min/1.73 m^2 at 1-year post-transplantation (adjusted-OR=3.68, 1.06-12.8, p=0.04) [29].

Three other studies have reported on kidney transplant outcomes following NRP in controlled DCD (cDCD) donors with DGF rates of 18% [30], 31% [31] and 40% [32]. Three further studies have reported on outcomes following NRP in cDCD kidney transplantation in comparison to DBD control groups [33-35]. In a study from the University of Wisconsin there was no statistically significant difference in DGF (8%vs.24%, p=0.1) in cDCD kidneys following NRP (n=24) compared to DBD kidneys (n=100) [34]. A second study from Spain showed that there was no statistically significant difference in DGF (27%vs.33%, p=0.56) or 1-year graft survival (92%vs.97%, p=0.32) in cDCD kidneys following NRP (n=37) compared to DBD

kidneys (n=36) [33]. The largest study to date reports the use of NRP followed by HMP (LifePort, Organ Recovery Systems®, USA) according to the National Protocol for kidneys from cDCD donors in France (n=92) and compares the outcomes to kidneys from DBD donors (n=5176) [35]. This study was presented at the American Transplant Congress in 2017, and reported significantly lower levels of DGF in cDCD kidneys following NRP when compared to DBD kidneys (9%vs.19%, $p<0.05$) [35]. In Italy, where declaration of circulatory death is based on absence of electrical activity and requires a minimum no-touch period of at least 20 minutes, [36] a series of 10 kidneys from cDCD donors using NRP and oxygenated-HMP reported a DGF rate of 30% and no PNF [37].

Ex-situ normothermic machine perfusion (*ex-situ* NMP) of kidneys involves perfusion with an oxygenated red cell-based plasma-free perfusate. A study of pre-implantation *ex-situ* NMP of ECD kidneys (n=18) using paediatric cardiopulmonary bypass technology (Medtronic®, UK) compared to matched control kidneys preserved with SCS using Soltran® solution (n=47) reported a significant reduction in DGF (6%vs.36%, p=0.01) with no difference in graft survival at 1-year (100%vs.98%, p=0.51) [38]. *Ex-situ* NMP is a technically challenging technique. The Cambridge group reported the assessment by *ex-situ* NMP of 10 declined DCD kidneys, five of which were transplanted, and four had initial graft function [39]. Recently, Guy's and Newcastle reported their initial experience with *ex-situ* NMP performed on 14 kidneys from 12 donors, with 12 kidneys transplanted into 10 recipients (two dual grafts). There were no cases of PNF, three patients (30%) experienced DGF and graft survival was 100% at 1-year. There were seven donors where one kidney received SCS and *ex-situ* NMP, and the other received SCS alone. Although there was a trend

towards lower DGF and PNF rates in the *ex-situ* NMP group, this didn't reach statistical significance [40]. A UK multicentre RCT (ISRCTN 15821205) of pre-implantation *ex-situ* NMP for 60 minutes (n=200) compared to SCS (n=200) in kidneys from cDCD is currently recruiting and is estimated to complete in 2020 [41].

Liver transplantation

KPIs following liver transplantation are graft utilisation, immediate vs. early allograft dysfunction (EAD) vs. PNF, hepatic artery thrombosis, biliary complications including ischemic cholangiopathy (IC), graft survival, patient survival and retransplantation. The standard technique for liver preservation is still SCS in the majority of centres.

Hypothermic Perfusion Strategies

Hypothermic liver perfusion can be accomplished either via the portal vein (PV) alone or through the PV and hepatic artery (HA) (dual-perfusion). In liver transplantation, the feasibility of end-ischemic dual-HMP using a modified bypass device (Medtronic PBS ®, USA) was demonstrated in a case-matched series of HMP preserved DBD grafts (n=20) compared with SCS (n=20) [42]. There were no cases of PNF in either group but recipients in the HMP arm demonstrated a lower peak AST (1154vs.3339 IU/ml, p=0.011), shorter length of stay (11vs.15-days, p=0.006) and lower incidence of EAD (5%vs.25%, p=0.08). A subsequent case-matched series comparing declined livers undergoing HMP (n=31) to 50 extended criteria liver grafts preserved with SCS (n=50) showed a lower incidence of biliary complications (including strictures and leaks) within 1-year (13%vs.43%, p=0.02) and reduced

hospital stay (16vs.20-days, p=0.001) without any difference in PNF (3%vs.7%, p=0.61), EAD (19%vs.30%, p=0.38) or 1-year patient survival (84%vs.80%, p=0.76) [43].

Hypothermic oxygenated perfusion (HOPE) seeks to extend HMP by oxygenating standard machine perfusion fluid (UW solution) to restore mitochondrial function with perfusion via the PV alone. In dual flow hypothermic oxygenated perfusion (D-HOPE) cold machine preservation solution is pumped via the PV and the HA and has been postulated to optimise oxygen delivery to the biliary system, although evidence that dual-perfusion is superior is lacking [44]. One matched case-series in DCD livers (25 HOPE preserved livers from Zurich vs. 50 SCS livers from Rotterdam/Birmingham) reported that patients in the HOPE arm (ECOPS®, Organ Assist, Netherlands) had significantly lower peak ALT (1239vs.2065 U/L, p=0.02), developed fewer biliary complications (20%vs.46%, p=0.04), with a reduced incidence of IC (0%vs.22%, p=0.02) and improved 1-year graft survival (90%vs.69%, p=0.04) but in the context of shorter cold ischemic times (3vs.6.5-hours, p=0.01) [45]. After 5-years of follow up graft survival was significantly better in the HOPE group compared to SCS (94%vs.78%, p=0.024) [46].

A further prospective case-control study compared DCD livers receiving D-HOPE (Liver Assist®, Organ Assist, Netherlands) (n=10) to SCS (n= 32) [44]. This study showed reduced peak ALT (966vs.1858 U/L, p=0.006), peak bilirubin (1.0vs.2.6 mg/dl, p=0.04) but no statistically significant difference in 1-year graft (100%vs.67%, p=0.052) or patient survival (100%vs.85%, p=0.21).

Another alternative for oxygen delivery is persufflation, whereby oxygen is passed directly through vasculature into the organ during SCS [4]. Oxygen persufflation has been applied to a small number of marginal grafts (n=5) with 100% graft and patient survival at 2-years follow-up. This approach is currently being compared to SCS in a single-centre RCT (ISRCTN00167887) aiming to recruit 116 patients [47].

Normothermic Perfusion Strategies

Two studies from the University of Wisconsin on five and 13 cDCD liver transplants performed following NRP reported a 1-year graft survival of 86%, a 2-year graft-survival of 71% and a 14% PNF and biliary stricture rate [31, 34]. An initial UK series of 11 patients receiving cDCD liver transplantation following NRP had one reported case of PNF, an EAD rate of 36% and no incidence of IC [32]. A subsequent larger UK two-centre study of cDCD liver transplantation following NRP (n= 44) compared to SCS controls (n=185) reported a significantly lower incidence of EAD (12vs.32%, p=0.008) and anastomotic stricture rate (7%vs.27%, p<0.0001), with no cases of IC in the NRP arm (0%vs.27%, p<0.0001). A lower rate of 30-day graft loss was reported in the NRP group (2%vs.12%, p=0.06) [48]. In a single-centre study from Spain, 11 livers from cDCD donors were transplanted following NRP with a 1-year graft survival rate of 90.1% and no cases of IC [33]. In a large multi-centre report from Spain the use of NRP (n=95) compared to SCS (n=117) in cDCD liver transplantation led to decreased rates of biliary complications (8%vs.31%, p<0.001), IC (2%vs.13%, p=0.15) and graft loss (12%vs.24%, p=0.04) in patients receiving grafts following NRP [49].

A series of 20 DCD liver transplants performed in Italy with NRP reported no significant difference in 1-year patient (95%vs.94%, p=0.94) or graft survival (85%vs.91%, p=0.20) compared to DBD grafts despite the extended stand-off period of 20 minutes following donor asystole. The IC rate was 10% but no recipients underwent retransplantation due to biliary complications [50].

NRP was first utilised for uDCD donors. In a report from Spain, 34 NRP recovered grafts were compared to 538 DBD controls. Graft survival was significantly higher for the recipients of DBD versus uDCD livers (87%vs.70%, p=0.011) but patient survival wasn't significantly different (90% vs.82%, p=0.141). Biliary complications occurred in 12% of the livers transplanted from uDCD donors after NRP with an 8% rate of IC [51].

A further study from Spain comparing 20 liver transplants from NRP uDCD donors with 40 DBD liver transplants reported a non-significant difference in 1-year graft survival (80%vs.88%, p=0.77) and patient survival (86%vs.88%, p=0.77). The re-transplantation rate in the uDCD group was 15% whilst PNF was 10% with 5% IC rate [52].

The suggested standard abbreviation for *ex-situ* normothermic machine perfusion of the liver is normothermic machine perfusion (NMP) [3]. This technique mandates dual-perfusion to mimic normal liver physiology and meet metabolic demands. NMP can be instituted upon procurement at the donor centre or upon the arrival of the liver graft in the recipient centre.

A number of pilot studies initially demonstrated the feasibility of NMP in DBD, DCD and discarded livers [53-55]. A phase-1 two-centre study demonstrated feasibility, safety and demonstrated potential benefits in individual extended criteria donor livers [55]. A subgroup analysis of six of 20 livers identified more stable post-reperfusion haemodynamic parameters with a decrease in inotrope use during reperfusion [56]. As part of COPE a subsequent international multi-centre RCT of NMP (OrganOx *metra*®, OrganOx, UK) conducted in DBD and DCD livers from the time of procurement, reported that the NMP group (n=121) compared to the SCS group (n=101) had lower peak AST (485vs.974 U/L, p<0.0001) and significantly lower rates of EAD (10%vs.49%, p<0.001). This was achieved despite a lower discard rate (12%vs.24%, p=0.008) and significantly longer preservation times (714vs.465-minutes, p<0.001). There was no significant difference in IC (DBD; 7.4%vs.5.4%, p=0.68, DCD; 11.1%vs.26.3%, p=0.18), anastomotic biliary strictures (DBD; 40.7%vs.41.8%, p=0.91, DCD; 48.1%vs.57.9%, p=0.52), 1-year graft survival (95%vs.96%, p=0.71) or 1-year patient survival (95%vs.96%, p=0.67) [57].

An alternative and logistically less challenging approach is to undertake NMP pre-implantation upon the arrival of the graft in the implanting centre. A study from Birmingham described successful transplantation of five discarded livers after a period of NMP and suggested several criteria for organ viability based on perfusate lactate (<2.5mmol/L) or bile production in combination with 2 of 3 other criteria; perfusate pH>7.3, stable arterial flow of more than 150 mL and portal venous flow more than 500 mL per minute, and homogenous graft perfusion. All five recipients were reported well with normalized liver tests at a median follow-up of 7-months. These viability criteria could therefore identify extended criteria donor grafts that can

be utilized safely [53]. The initial viability criteria have since been modified by the addition of measurement of bile pH while the liver is on NMP with a pH≤7.4 associated with IC [58]. However these criteria require validation in larger trials.

In a study from Cambridge 12 declined livers were transplanted after a period of NMP (Liver Assist®, Organ Assist, Netherlands). The first six livers were perfused at high oxygen tensions and were complicated by post perfusion syndrome and vasoplegia in the recipient, complications that were not seen when the oxygen tension was lowered to physiological levels [59]. Outcomes were compared with a contemporaneous cohort of 24 other SCS livers and were found to be similar in terms of 1-year graft survival (NMP 83%vs.88% SCS), 1-year patient survival (NMP 92% vs96% SCS) and rate of IC (NMP 27%vs.29% SCS). In a subsequent study from Cambridge 22 declined or high-risk livers were transplanted after NMP with an IC rate of 18%. Whilst NMP pre-implantation was associated with an increased organ utilisation and rescue of organs that would otherwise have been discarded, there was no impact on the incidence of IC [58].

Controlled oxygenated rewarming (COR) from 10°C to 20°C over 90-minutes has been proposed to gradually re-warm the liver and thus be less physiologically stressful. In one study of six DBD liver graft recipients compared with 106 historical DBD controls, COR was associated with lower peak AST (564vs.1204 U/L, p=0.02) [60]. Using a combined resuscitation and viability testing protocol of sequential DHOPE, COR, and NMP using a new haemoglobin-based oxygen carrier-based perfusion fluid, five of seven livers from declined DCD donors were transplanted with a 3-month graft survival of 100%. In this study use of a synthetic oxygen carrier for

end-ischemic NMP has the potential advantage of being able to perform NMP with gradual rewarming, something not possible if blood is used as a perfusate [61]. To date comparison of biomarkers and bile production in blood perfused versus cell free NMP has only been made in declined livers that have not went on to be transplanted [62, 63].

Pancreas transplantation

KPIs following pancreas transplantation are graft utilisation, graft thrombosis, graft pancreatitis, early graft failures, graft survival, and patient survival. The standard technique for pancreas preservation is SCS.

The pancreas is a low-flow organ with complex vascular anatomy that makes optimal perfusion parameters difficult to obtain [4]. As such, experimental work in terms of pancreas perfusion is still ongoing. One recent study reported successful isolation of functional islets from two of ten discarded pancreases after a period of continuous HMP (Deltastream DPII; MEDOS Medizintechnik AG, Germany) with a dual-perfusion system through the mesenteric/splenic arteries [64]. Another study from London placed discarded organs on a normothermic circuit (to mimic transplantation) after a period of HMP (RM3®, Waters Medical System, USA) and found two of three discarded organs were functional in terms of insulin production [65]. In a study from France, seven discarded human pancreases have undergone HMP for 24-hours with reducing resistive index for the first 12-hours followed by stabilization of perfusion pressures without developing oedema. Post-perfusion biopsy samples revealed normal staining for insulin, glucagon and somatostatin [66].

Pancreas preservation by oxygen persufflation in combination with SCS (n=13) has been compared to SCS alone (n=11) and reported improved β-cell function after islet cell isolation [67]. In a further study, the feasibility of *ex-situ* NMP (paediatric cardiopulmonary bypass technology, Medtronic®, UK) in declined human pancreases (n=5) using warm oxygenated packed red blood cells for 1-2 hours has been demonstrated by insulin secretion from the majority of perfused organs (n=4/5) [68]. Other than successful solid organ pancreas and islet transplantation following NRP [31-33], to date there have been no reports of pancreases transplanted into a recipient following novel preservation strategies.

Cardiac transplantation

KPIs following cardiac transplantation are graft utilisation, PNF, need for mechanical support, graft survival, and patient survival. The standard technique for cardiac preservation is SCS for DBD donors and *ex-situ* NMP for DCD donors.

Whilst *ex-situ* hypothermic heart perfusion is still under development [69], *ex-situ* NMP has been implemented clinically. PROCEED II (NCT00855712) was a multicentre RCT that compared 67 standard-criteria DBD heart transplants after *ex-situ* NMP (Organ Care System®, TransMedics, USA) with SCS (n=63) and reported similar outcomes in terms of 30-day patient/graft survival rates (94%vs.97%, p=0.45) and cardiac-related severe adverse events (13%vs.14%, p=0.90). Five hearts weren't transplanted in the *ex-situ* NMP group on the basis of lactate [70]. In a single-centre study (n=26) from Harefield, *ex-situ* NMP (Organ Care System®, TransMedics,

USA) has been reported to facilitate transplantation of hearts not initially considered suitable for transplantation or to be used for higher-risk recipients with only one reported death (3.8%) and preserved allograft function in 92% of patients [71].

DCD heart transplantation has been one of the most important developments in recent years with the potential to significantly increase the number of heart transplants undertaken and a significant reduction in waiting-list mortality [72]. The first report of successful DCD heart transplantation using *ex-situ* NMP (Organ Care System®, TransMedics, USA) was described in 2015 [73], and further case series of DCD heart transplantation have followed [74, 75]. At present, two methods for heart recovery are explored: direct procurement and preservation (DPP) which requires a rapid cooling and procurement of the heart with collection of blood from the donor with which to prime the OCS system, and thoraco-abdominal NRP (TA-NRP).

In a study from Papworth, 12 DCD heart transplants recovered with TA-NRP with *ex-situ* NMP (Organ Care System®, TransMedics, USA) were compared to 14 hearts recovered with DPP and *ex-situ* NMP (Organ Care System®, TransMedics, USA) and DBD heart transplants (n=26). There were no significant differences in the outcomes between the two approaches or in comparison to DBD heart transplantation [76]. In a recent review article the experience with DCD heart transplantation with these techniques was updated to 39 cases with a recipient survival to discharge rate of 93% [77].

Lung Transplantation

KPIs following lung transplantation are graft utilisation, primary graft function vs. primary graft dysfunction (PGD), unplanned extracorporeal membrane oxygenation support (ECMO), graft survival, and patient survival. The standard technique for lung preservation is still SCS in the vast majority of centres.

In lung transplantation there are two main systems that have been used for *ex-situ* NMP. The XVIVO Perfusion System (XVIVO Perfusion, Gothenburg, Sweden) is a static system utilising either acellular or red-cell supplemented perfusion while the Organ Care System (Organ Care System®, TransMedics, USA) is a portable system that uses red cell supplemented perfusion [78]. The Steen group (Lund, Sweden) described the first successful lung transplantation after *ex-situ* NMP with six out of nine donor lungs initially rejected for transplantation. The six recipients survived the first 3-months and four of the six were alive at 1-year [79]. After modification of Steen's initial *ex-situ* NMP protocol, a matched-controlled study from Toronto of 20 high-risk lungs preserved with *ex-situ* NMP (XVIVO Perfusion System) compared to conventional-risk SCS lungs (n=116) reported no statistically significant difference in the primary endpoint of PGD 72-hours post transplantation (15%vs.30%, p=0.11); or secondary endpoints of 30-day mortality, bronchial complications, duration of mechanical ventilation, intensive care unit length of stay or hospital length of stay [80].

In a follow up study, the *ex-situ* NMP group (n=50) was compared to a SCS group (n=235) and the incidence of PGD grade-3 at 72-hours was lower (2%vs.9%, p= 0.14)

with similar 30-day mortality (4%vs.3.5%, p=1.0), and 1-year survival (87%vs.86%, p=1.0) [81]. In a further study from Toronto, patients transplanted with DCD lungs after *ex-situ* NMP (n=28) were compared to patients transplanted with DCD lungs after SCS (n=27) and had similar patient survival (86%vs.92%, p=0.68) but shorter hospital stay (median 18vs.23-days, p=0.047) and a shorter length of mechanical ventilation (2vs.3-days, p = 0.059) [82]. In a retrospective study from Harefield, lungs initially deemed unusable for transplantation (n=13) underwent *ex-situ* NMP (adapting the Toronto protocol) and 46% (n=6) were transplanted with 100% survival at 3-months [83]. The reported conversion rate from *ex-situ* NMP to transplantation is lower in the Harefield experience at 46% compared to the Swedish (67%) and Toronto (87%) experience [83]. In a combined analysis of UK, Sweden and Toronto experience, only two deaths within 90-days were reported in over 65 *ex-situ* NMP lung transplants [84].

In a further study from France, *ex-situ* NMP (XVIVO Perfusion System) was performed in lungs initially considered unsuitable for transplantation (n=32) and compared to SCS controls (n=81) with similar rates of PGD after 72-hours (9.5%vs.8.5%, p=1.0), 30-day mortality (3.3%vs.3.7%, p=0.69) and 1-year survival (93%vs.91%, p=0.8) [85]. In a single-centre RCT from Vienna standard-criteria lungs were randomised to *ex-situ* NMP and SCS (XVIVO Perfusion System, n=39) or SCS (n=41) with no significant difference in PGD (6%vs.20%, p=0.10), need for post-operative prolonged ECMO (6%vs.12%, p=0.44), 30-day survival (97%vs.100%, p=0.46) or intubation time, intensive care stay and hospital stay. There was also loss of some standard-criteria donor lungs due to technical issues during perfusion making exposure of all donor lungs to *ex-situ* NMP unattractive [86]. In the largest multicenter

RCT (INSPIRE, NCT01630434) of *ex-situ* NMP (n=151) (Organ Care System) compared to SCS (n=169), a composite end-point of a 30-day patient survival (96%vs.100%) and the incidence of PGD grade-3 within 72-hours (18%vs.30%, p=0·015) was not statistically significantly different between the groups (70%vs.79%, p=0.068). Patient survival at 1-year post-transplant was also similar (89%vs.88%) [87].

DEVELOP-UK was a multicenter (n=5) observational study that assessed *ex-situ* NMP (Vivoline LS1, Vivoline Medical AB, Sweden) in extended criteria lungs (53 assessed and 18 transplanted) in comparison to standard donor lungs (n=184). The study was terminated early due to higher rate of very early PGD grade-3 (44%vs.18%) and a need for unplanned ECMO (39%vs.3%) at increased cost (approximately £35,000) in the *ex-situ* NMP group. Survival at 30-days was similar (94%vs.97%) but by 12-months of follow-up the hazard ratio for mortality in the *ex-situ* NMP arm relative to the standard arm was 1.96, 0.83 to 4.67 [84]. The use of *ex-situ* NMP in ECD lung transplantation is still under evaluation in the EXPAND I (NCT01963780) and II trials (NCT03343535), with preliminary data suggesting good lung utilisation [78]. Other series of *ex-situ* NMP lung transplantation not discussed but published in the literature are summarised in Table 2 [88-102]. Overall clinical outcomes of *ex-situ* NMP treated lungs appear equivalent to SCS despite the use of *ex-situ* NMP for lungs not initially considered suitable for transplantation [90, 91, 96, 100, 102].

Discussion

This review has undertaken a horizon scan across the available literature on novel strategies for the perfusion and preservation of the solid organs currently used in

clinical transplantation. The review has identified a range of evidence levels for different techniques spanning from quasi-experimental work in pancreas up to multiple meta-analyses of RCTs in the case of HMP of kidneys (Figure 2).

In kidney transplantation, HMP is well established and over time has accumulated evidence to support a reduction in the rate of DGF compared to SCS [6-13]. However, HMP is not universally adopted due to conflicting results from major RCTs [5, 14, 16], and a lack of evidence to support reduced rates of PNF or improved longer-term graft survival [12]. Another potential reason is that it hasn't been evaluated how to best introduce a national system providing devices for HMP. In a post–hoc subgroup analysis of the RCT performed in the Eurotransplant region it has been reported that a significant effect of HMP on reducing the incidence of DGF was most significant in kidneys transplanted with cold ischemic times <10 hours, and was statistically non-significant at longer cold ischemic times. This analysis suggests that HMP cannot compensate for cold ischemia, and is still beneficial with short cold ischemic times [103].

The risk of DGF is greater in DCD kidney transplantation [12], and the lowest reported rates of DGF (<10%) following transplantation of kidneys from DCD donors are from the French Protocol that uses a combination of NRP and HMP [35]. However, it is unclear whether the low rate of DGF reported with this protocol relates to strict donor and recipient selection criteria, NRP alone, or the combination of NRP with HMP [35]. The results of RCTs assessing *ex-situ* NMP (ISRCTN 15821205) [41], and oxygenated-HMP (COPE-POMP, ISRCTN 63852508) are still awaited. There is currently no data to compare HMP and *ex-situ* NMP. The potential of *ex-situ* NMP to extend

preservation times also remains untested. Although DGF prolongs hospital stay, increases costs, may mask rejection and prompts graft biopsies, it is not an accurate marker for kidney graft outcome, particularly in the context of DCD kidney transplantation [104]. Therefore, use of DGF as an endpoint of many of the studies published is a limitation.

A further limitation is the lack of long-term follow up for graft survival after HMP. In the recent Cochrane review[12], only two trials were identified that assessed long-term graft survival up to 3-years [105, 106], and one trial assessed 10-year graft survival [107]. In the RCT from the Eurotransplant region, 3-year graft survival was better for kidneys in the HMP group compared to SCS (91%vs.87%; adjusted hazard ratio for graft failure, 0.60; p=0.04) [105]. In a RCT involving 282 recipients of DCD kidneys, the 3-year graft survival rate in the HMP group was significantly higher than that in the SCS group (93%vs.82%, p=0.036) [106]. In the study with 10-year follow up, the kidneys that received HMP (n=37) compared to SCS (n=37) had a statistically non-significant improvement in 10-year graft survival (68%vs.43%, p=0.08) [107].

In liver transplantation SCS remains the practice against which emerging strategies have been compared. A RCT has demonstrated that compared to SCS, NMP can increase liver utilisation and reduce EAD despite increased preservation time [57]. Case series suggest that liver NMP can be applied pre-implantation with comparable results with application from procurement. Whilst there is an increased liver utilization in the pre-implantation application of NMP, there is no reduction in the incidence of IC, indicating that perhaps the intervention takes place too late. However, it should be appreciated that the multicentre RCT of liver NMP trial was not powered to examine

IC, which was identified on magnetic resonance cholangiopancreatography scan performed 6-months after transplantation [57].

In the UK, NRP programmes were introduced in two centers in 2011 and 2012 with the aim of improving clinical outcomes in DCD liver transplantation [30, 32]. Initially NRP was performed in the context of an approved clinical research study that required donor families to consent for NRP treatment of the donor, and for recipients to consent to receiving a NRP treated organ. Following this a service evaluation of the technique has been performed by NHSBT and the safety of NRP has been confirmed. In the service evaluation phase of the introduction of NRP, recipients have consented to receive an organ from a DCD donor, irrespective of whether the donor procedure has involved NRP or not [48]. In large case-controlled studies of DCD liver transplantation, NRP has been reported to limit the incidence of IC and lead to a significant increase in organ utilisation [48, 49]. However, one potential advantage of NMP is the opportunity to perform viability testing on livers prior to implantation. Following on from case-controlled studies, RCTs of HOPE (NCT01317342, NCT03124641) and DHOPE (NCT02584283) are awaited [44, 45, 108, 109].

It's possible that the discussed techniques may be best used in concert, in an individualised manner based on the specific requirements of the donor/recipient combination or the set-up available in the transplant unit accepting the organ. Currently, NRP is followed by a period of SCS [32], but it could be combined with NMP as a transport strategy or as a pre-implantation strategy to allow further assessment of grafts that are slow to recover during the NRP. Although techniques like oxygen persufflation[47] and COR[60] are being tested, clinical implementation may

be challenging and restricted by current perfusate developments. Therefore, to date the most developed pre-implantation strategies to consider are HMP [45] and NMP [57]. NMP, which has RCT level evidence to support its use, has the potential advantage of facilitating longer preservation times [57]. It can also be used to assess higher-risk grafts [53, 59]. In contrast, viability assessment during HMP is challenging as there is decreased metabolic activity in the liver [110] . However, unlike HMP, any technical problems in NMP could lead to graft loss if not recognised promptly, as the default position of SCS does not apply. Previous studies have reported device-related technical complications such as twisting of the PV or hypoperfusion of the HA but overall these appear rare [57, 111]. In steatotic liver grafts, which are expected to increase in the donor pool due to the obesity pandemic, it is anticipated that novel perfusion strategies will be key to increasing acceptance and improving outcomes after liver transplantation [112].

Novel perfusion strategies are having an impact on the transplantation of cardiothoracic organs. With the assistance of novel perfusion strategies, DCD heart transplantation has become established and results are comparable to DBD heart transplantation [77]. *Ex-situ* NMP of lungs has accumulated evidence to support improved short and long-term clinical outcomes as well as increasing organ utilisation. However, the main issue in interpretation of the clinical studies is that all use different definitions of ECD lungs. The definitions differ in terms of donor age, smoking history and use of lungs from DCD donors, which are included in the standard arm of some studies but in the *ex-situ* NMP arm of other studies making direct comparison of results challenging. It has been suggested that a RCT or a registry analysis may be required to compare static *ex-situ* NMP (XVIVO Perfusion

System) to portable *ex-situ* NMP (Organ Care System) to help decide the optimal approach [78]. Such studies could guide decision making around integration of *ex-situ* NMP into recovery of lungs, or whether organ-reconditioning hubs or specific centres with their own *ex-situ* NMP technology are required.

From a clinical perspective we are at cross roads in terms of selecting the optimal strategy from the vast array of options currently at our disposal. It's essential that any clinical studies report similar data to enable post-hoc comparisons and to assist the design of future trials. There's also an imperative need for a universally agreed terminology in this field [3]. Whilst the results of studies are encouraging, we must caution against over interpretation of benefit and careful consideration of current data prior to wide-scale implementation without additional evidence.

With these goals in mind for the UK, NHSBT convened a Preservation and Perfusion Future Strategy Summit in London in October 2018. The findings of this review were considered as the basis for discussion. After considering the available clinical evidence and reports from Industry representatives, the delegates representing abdominal/cardiothoracic organ transplantation units were asked to discuss the clinical use of the various novel technologies/methods alone or in combination to enhance organ utilisation. Discussions in the cardiothoracic group centered on the development of organ reconditioning hubs for high-risk organs as a strategy that allows a rapid development of expertise leading to capabilities for novel organ therapy prior to transplantation. The abdominal transplant group acknowledged the challenges with DCD donation in the UK and identified this group as the main target for future RCTs (Figure 3). Future trials should move away from the traditional

comparison versus SCS and allow for a more innovative modeling of single or combined use of various perfusion approaches in order to define their benefit for specific donor-recipient combinations. Future trials should also involve collaborations that ensure appropriate sample sizes are achieved to detect statistically significant differences between groups. This will avoid over-interpretation of results or under-appreciation of potentially important clinical differences, which was a risk when analysing the numerous small studies identified by this review.

A final consideration is the logistical advantages of the available technology to increase the capacity solid organ transplantation by allowing extended periods of preservation and reduced time pressures. This would reduce the stress of organ recovery and could facilitate the performance of complex implant procedures during daytime hours. Taking the time pressure off the movement of transplantable organs is another potential benefit of transportable devices as it could lead to a minimisation of urgently arranged and high cost flights to transport organs.

Conclusion

A clinical evidence base is rapidly emerging for novel perfusion and preservation strategies in organ transplantation. In the UK, improving clinical outcomes following DCD transplantation of abdominal organs is a high priority area, and should be the main focus for future RCTs modeling single and combined use of different perfusion and preservation strategies. In cardiothoracic transplantation the development of organ reconditioning hubs for high-risk organs will allow for rapid development of expertise and enable clinical application of available techniques.

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Author	Year	Donors	Kidneys	DGF	PNF	1-year graft loss^ 1-year graft survival*	1-year patient death^ 1-year patient survival*	3-year patient death^ 3-year patient survival*
Lam[10]	2013	All	1353	**RR 0.83 (0.72-0.96)**	RR 0.78 (0.36-1.68)	RR 0.87 (0.64-1.19)^	RR 0.91 (0.60-1.37)^	-
O'Callaghan[9]	2013	All	2203	**RR 0.81 (0.71-0.92)**	RR 1.15 (0.46-2.90)	-	-	-
Deng[7]	2013	DCD	351	**OR 0.56 (0.36-0.86)**	OR 1.30 (0.49-3.44)	OR 0.64 (0.28-1.46)^	OR 0.37 (0.09-1.64)^	-
Bathini[8]	2013	DCD	3316	**OR 0.64 (0.43-0.95)**	-	OR 0.74 (0.48-1.13)^	-	-
Jiao[6]	2013	ECD	11090	**OR 0.59 (0.54-0.66)**	OR 0.54 (0.21-1.40)	**OR 1.12 (1.03-1.21)***	OR 0.98 (0.94-1.02)*	-
Martinez Arcos[11]	2018	All	1764	**RR 0.79 (0.71-0.88)**	RR 0.92 (0.73-1.16)	-	-	-
Peng[13]	2018	All	2048	**RR 0.78 (0.69-0.87)**	RR 1.08 (0.71-1.65)	RR 1.03 (1.00-1.07)*	-	**RR 1.06 (1.02-1.11)***
Tingle[12]	2019	All	2266	**RR 0.77 (0.67-0.90)**	RR 0.88 (0.58-1.33)	-	RR 0.99 (0.95-1.03)^	-

Table 1 | Results of meta-analyses of transplantation of kidneys following HMP compared to SCS

Additional abbreviations: ECD – extended criteria donors

Author	Year	No. *ex situ* NMP patients or lungs*	*Ex situ* NMP not transplanted	No. SCS patients or lungs*	Machine	Outcome differences
Lindstedt[88]	2011	6	-	15	Medtronic	None
Valenza[89]	2012	2	-	4	Self-made	None
Aigner[90]	2012	13	4	0	Self-made	-
Wallinder[91]	2014	11	2	47	Vivoline	***Ex situ* NMP group had longer median time to extubation (12 vs. 6 h, p=0.05) and median ICU stay 152 vs. 48 h, p=0.01)**
Henriksen[92]	2014	8	1	36	Vivoline	-
Boffini[93]	2014	11	3	28	Self-made	None
Fildes[94]	2015	9	0	46	-	None
Zeriouh[95]	2016	14	7	308	OCS	***Ex situ* NMP had significantly better postoperative FEV1 at 3 (69 vs. 93, p < 0.001) and 6 (77 vs. 94, p=0.006) months**
Wallinder[96]	2016	64*	13*	290*	Vivoline	None
Valenza[97]	2016	13*	3*	42*	Self-made	None
Luc[98]	2017	7	0	4	OCS	***Ex situ* NMP had lower grade of primary graft dysfunction at 72 h (0.4 +/- 0.5 vs. 2.1 +/- 0.7, p = 0.003)**
Koch[99]	2018	11	2	41	XVIVO	None
Zhang[100]	2019	11	2	140	XVIVO & Lung Assist	None

Schiavon[101]	2019	16	1	47	OCS	-
Nilsson[102]	2019	122*	22*	529*	Vivoline LS1	***Ex situ* NMP group had longer median time to extubation (18 vs. 7 h, p=0.002) and median ICU length of stay (4 vs. 3 days, p=0.002)**

Table 2 | Results of other studies of transplantation of lungs following *Ex situ* NMP compared to SCS

Additional abbreviations: OCS - Organ Care System

Figure 1| Search results

Figure 2 | Summary of evidence level for each the various different novel perfusion strategies applied in kidney, liver, pancreas, heart and lung transplantation. Additional abbreviation: OPAL – Oxygen Persufflation as adjunct in Liver Preservation.

Figure 3 | Potential RCT of novel perfusion strategies for controlled DCD donor liver and kidney transplantation