

Review

The Role of Normothermic Machine Perfusion in Extended Criteria Donor Grafts: A New Direction in Liver Graft Assessment and Preservation

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Abstract: Despite improvements in short-term and long-term outcomes of liver transplant patients, the discrepancy between the number of available livers and transplant candidates continues to increase. The use of expanded criteria donors is one strategy that can be used to address donor shortages. In recent years, preservation strategies such as normothermic machine perfusion (NMP) have been explored to improve the preservation of organs and test their viability before transplantation. We reviewed the recent literature and trials assessing the use of NMP in the setting of liver transplantation. Multiple feasibility trials have demonstrated the clinical prospect of NMP and proved its numerous advantages compared to conventional static cold storage. These advantages include preservation and viability assessment of high-risk donor allografts and grafts that would have otherwise been discarded. This review aims to address the topic of liver NMP in the setting of current and future applications in the setting of extended criteria donor grafts.

Keywords: liver transplant; normothermic; machine perfusion; extended criteria donor



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1. Introduction

Organ preservation has been a fundamental part of transplantation for centuries. Since the first successful liver transplantation (LT) by Thomas Starzl in 1967, ref. [1] the short-term and long-term outcomes of transplant patients have gradually improved due to improvements in immunosuppressive therapies, donor–recipient matching, and comorbidity treatments [2,3]. Despite improved patient outcomes, there is a discrepancy between the number of available livers and transplant candidates [2,4,5]. Long waiting lists have led to a mortality risk of approximately 15% for those waiting for a LT [6]. In the US, more than 1000 patients die on the waiting list annually, and more are removed from the transplant list due to declining health [6,7]. To address the donor shortage, organs from marginal donors are being used for LT [8]. With an increase in the number of marginal organs, preservation strategies such as machine perfusion (MP) are being explored to preserve donor livers [7,9].

The most widely used method of organ donation is static cold storage (SCS), which involves flushing cold preservation solution following complete dissection. SCS is based

on the principle of reducing cellular metabolism by decreasing the temperature, therefore limiting the need for ATP [10]. However, increased vulnerability of liver endothelial cells to ischemia-reperfusion injury can be deleterious during SCS [11,12]. Anaerobic metabolism continues at a decreased rate, which leads to the depletion of ATP reserves and the accumulation of metabolic wastes [13]. These insults are exaggerated in marginal livers, which can increase the risk of initial poor function (IPF) or primary non-function (PNF) in addition to biliary complications when compared to standard criteria donors [14,15]. SCS has yielded satisfactory outcomes following LT with relative ease and low cost, but its use has been limited in high-risk or marginal organs [4]. Marginal livers are especially vulnerable to ischemia-reperfusion injury (IRI), which leads to an increased risk of death [16,17].

Marginal liver grafts, or grafts from extended criteria donors (ECD), that would have been previously discarded are becoming an increasingly essential part of the organ donor pool [18]. ECD characteristics are advanced donor age, extended duration of SCS, macrosteatosis greater than 30% or mixed steatosis of more than 60%, and organ dysfunction at procurement [18–20]. As current clinical strategies for transplantation of ECD grafts are insufficient, optimized techniques are necessary.

In recent years, the main focus of many research groups has been the development of improved organ preservation methods [21–39]. An ideal preservation method should mimic the organ's natural state within an organism as closely as possible to maintain the quality of organs as they are transported from donor to recipient [4]. Dynamic preservation methods theoretically mimic the physiological environment of the body more closely when compared to the current standard of care, SCS. SCS has yielded satisfactory outcomes following LT with relative ease and low cost, but its use has been limited in high-risk or marginal organs [4]. Normothermic machine perfusion (NMP) is a promising new modality in the organ transplantation field that can improve outcomes, particularly with marginal organs [18,40–43]. NMP also allows for the assessment of the function and viability of grafts prior to transplantation [18,42,44]. In this paper, we will review the clinical characteristics of NMP studies that have emerged in recent years.

2. Mechanism of Organ Damage during Liver Transplantation

With the utilization of ECD or marginal donors, IRI is the main underlying cause of graft dysfunction [7,8,45]. IRI is the result of cellular and histological events that occur when the blood supply is stopped and subsequently restored [5]. Restoration of blood flow is associated with exacerbation of tissue injury and inflammatory response [46,47]. There are two consecutive phases of organ injury: cold (hypothermic) ischemia and warm (normothermic) ischemia [5].

The purpose of cold preservation is to reduce enzyme activities with hypothermia (4 °C) [5,48,49]. The ischemia time results in limited oxygen availability due to decreases in adenylate cyclase activity and concomitant increase in vascular permeability [5,46,50]. This ultimately causes intracellular Na⁺ accumulation, edema, and swelling as aerobic respiration is inactivated [5,16]. Delivery of oxygen and substrates to graft is further reduced as Kupffer and sinusoidal cells swell, vasoconstrictors increase, and vasodilators decrease [5,51]. Altered Ca²⁺ homeostasis leads to “calcium overload”, which is associated with the activation of various cell death pathways [48,52]. Warm ischemia begins with normothermic perfusion of the organ (37 °C), which causes a release of reactive oxidative species (ROS) with consequent inflammation-mediated injury [53]. Ultimately, inflammatory Kupffer and dendritic cells cause endothelial activation, followed by neutrophil and platelet adhesion, leading to impairment of microcirculation and hepatocyte death [5]. Static cold storage has been found to exacerbate this impairment [13]. Ex-vivo normothermic machine perfusion has arisen as a potential solution to avoid cold ischemic injury altogether in marginal grafts [54].

3. Normothermic Machine Perfusion

Perfusion techniques for organ storage date back as far as the 1930s, with experiments of Nobel laureate surgeon Alexis Carrel with normothermic organ perfusion using oxygenated serum [55]. In the 1960s, the first successful liver transplant was performed, and in the early 1970s, Dr. Starzl described the potential benefits of ex vivo machine perfusion [56,57]. However, inherent financial and logistical limitations led to the discontinuation of research on machine perfusion. Additionally, the excellent results following SCS led to the further abandonment of organ perfusion research [58].

Currently, the gold standard for organ preservation is SCS. For high-quality grafts, SCS has been shown to have low rates of early allograft dysfunction, primary non-function, and biliary complications [59,60]. The use of SCS in marginal livers from ECD does not yield similar benefits and is associated with increased graft and donor complications [7]. The basic concept of NMP is to maintain organs at their physiological temperatures ex vivo while also maintaining their metabolic functions. Continuation of aerobic metabolism would reduce the incidence of IRI. Figure 1 depicts a simplified schematic of both SCS and NMP mechanisms.

NMP mimics physiologic conditions by pumping the perfusate through the portal vein and hepatic artery at different pressures while maintaining the temperature at 37 °C. The primary goal is to reduce the damage done to organs post-retrieval before implantation. Physiologic temperatures mean oxygenation is required to support metabolic demand. This mandates perfusion solutions to contain an oxygen carrier, usually red blood cells (RBC). Theoretically, since the organ is receiving oxygen, normal cellular metabolism continues.

Additionally, the use of NMP has allowed for a better assessment of organ function and viability prior to transplantation [61–63]. Assessing pre-transplant viability can become a useful tool in reducing post-LT complications. NMP has been successfully utilized for extended storage while preserving function. In 2020, Eshmunov et al. used NMP to preserve 10 discarded human livers for up to 7 days without a decline in function [64]. The ability to increase storage time without compromising graft viability can help optimize graft–recipient matching by allowing for longer transport times.

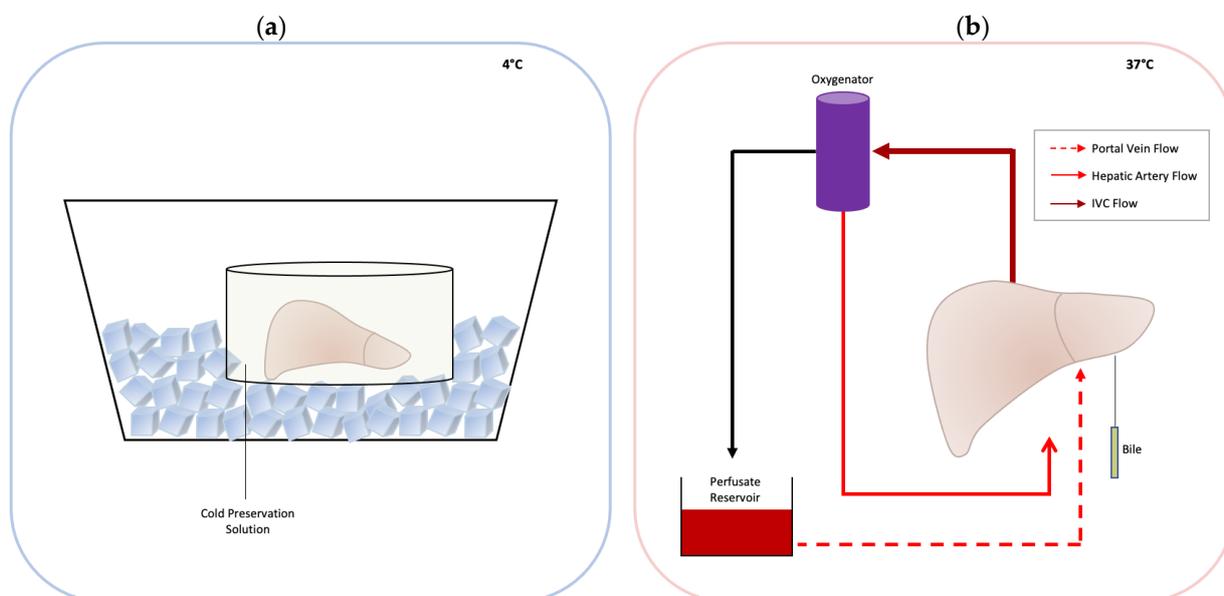


Figure 1. A simplified schematic drawing of (a) static cold storage (SCS), which is the current gold standard of preservation, and (b) the OrganOx Metra normothermic machine perfusion (NMP) device. Modified from Ravikumar et al. [65].

4. Commercially Available Machines

There are three commercially available normothermic perfusion devices that have been used in clinical trials: OrganOx *metra*, TransMedics *Organ Care System*, and OrganAssist *Liver Assist*. Similar principles have been used in all of them. The differences are in the degree of portability, automation, recirculating perfusate pressure and pulsatility, substrate type and delivery, and perfusion through the portal vein and hepatic artery [7,13,40]. The OrganOx and Transmedics devices are fully portable and automated. OrganOx works at 37 °C, uses whole blood supplemented with plasma expander (Gelofusine), bile salts, parenteral nutrition solution, heparin, insulin, and prostacyclin through a closed perfusion, continuous, non-pulsatile portal vein, and hepatic arterial flow technique [7,54,66]. OrganAssist, on the other hand, is semi-automated with limited portability. Until now, technical and financial challenges associated with the transport of heavy, complex equipment continue to escalate the cost of this technology. Due to these challenges, some institutions now utilize limited intervention that includes the use of NMP for a liver graft only once it arrives at the recipient center [7]. Although this prevents protection against ischemic injury, it can assist in the confirmation of liver graft function prior to transplantation.

5. Outcomes of NMP versus SCS

A comprehensive search of studies regarding NMP of donor livers was performed using PubMed, Ovid, and clinicaltrials.gov. The search was conducted using the medical subject headings (MeSH) terms “machine perfusion,” “liver transplantation,” and “liver preservation” combined with the free text term “normothermic”. The included studies were limited to “human” studies. Published and ongoing clinical trials, retrospective studies, and case reports were included. Review and meta-analysis articles were not included. Studies published prior to 2016 were excluded. The cut-off date was 23 February 2022.

5.1. Donor and Recipient Characteristics

A total of 971 patients were included in 15 trials. NMP-based preservation methods were used in 362 cases, whereas SCS was used in 609 cases. The range of DCD donors in the trials ranged from 0% to 100% in both NMP and SCS groups. The reported NMP perfusion time ranged from 2 h to 24 h.

5.2. Patient and Graft Survival

In 2016, Ravikumar et al. conducted the first series of 20 liver transplants following NMP and matched those patients to 40 SCS livers [66]. They found the 30-day and 6-month graft survival to be similar in both groups (100% NMP vs. 97.5% SCS; $p = 1.00$), with one patient in the matched group dying on day 0 from a cardiovascular event. Bral et al. also demonstrated similar 30-day graft survival between NMP and SCS groups (eight of 10 NMP vs. 30 of 30 SCS, $p = 0.06$) [67]. Nasralla et al. demonstrated no significant difference in one-year graft survival with 0.950 (95% confidence interval 0.893–0.977) and 0.960 (95% confidence interval 0.897–0.985) in the NMP and SCS groups, respectively ($p = 0.695$) [68]. Jassem et al. showed no significant difference in graft (100% NMP and SCS) or recipient survival (92% NMP and 100% SCS) between the NMP and SCS groups at 1 year. One patient included in their study died from recurrent alcoholic liver disease at month 8 and was therefore excluded from graft survival calculation [69]. A clinical trial by Ghinolfi et al. in 2019 also failed to show any significant differences in graft and patient survival between NMP and SCS groups. One case of graft loss occurred in the NMP group due to hepatic artery thrombosis on day 9; however, the patient was successfully retransplanted. One patient in the CS group died on POD 31 from septic shock after readmission for intestinal occlusion [70]. Mergental et al. found no significant difference in the 90-day graft (100% NMP and 93.2% SCS; $p = 0.545$) and patient (100% NMP and SCS) survival. They additionally found no difference in the 1-year graft (86.4% NMP and SCS) and patient (100% NMP and 95.5% SCS; $p = 0.55$) survival [71]. Most recently, Hann et al. found no

significant difference in patient and graft survival at 6 months despite the NMP group having significantly more steatotic grafts and previously declined livers [72].

5.3. ICU and Hospital Stay

Median intensive therapy unit and hospital stays were similar between the two groups overall and when analyzed as DBD and DCD subsets in Ravikumar et al.'s trial [66]. Selzner et al. found no difference between the duration of intensive care unit stay and post-transplant hospital stay [73]. Nasralla et al. showed that there was no difference in median intensive care unit (ICU) stay and hospital stay (15 days NMP versus 15 days SCS; $p = 0.926$) between both groups. Comparable lengths of ICU and hospital stays between NMP and SCS groups were also reported by Jassem et al., Ghinolfi et al., and Mergental et al. [69–71]. Bral et al. reported a significantly prolonged ICU stay for NMP graft recipients. Median hospital stay in the NMP group was also significantly longer.

5.4. Liver Function and Biliary Complications

Ravikumar et al. demonstrated a statistically significant difference in peak AST levels (NMP 417 vs. SCS 902 IU/L, $p = 0.034$), numerically more pronounced in the DCD cohort (422 vs. 1894 IU/L, $p = 0.283$) [66]. There was no significant difference in peak bilirubin levels on day 7 between the NMP and SCS groups. Selzner et al. found no difference in postoperative graft function between NEVLP and SCS grafts as measured by day 7 international normalized ratio and bilirubin [73]. Bral et al. showed no significant difference in peak AST levels within the first 7 days ($p = 0.52$) and post-transplant AST trends ($p = 0.24$) in NMP versus SCS preserved grafts. Additionally, there was no significant difference in bilirubin levels on day 7 ($p = 0.35$) and globally ($p = 0.17$), post-transplant alkaline phosphate levels ($p = 0.82$), coagulation parameters ($p = 0.63$), and arterial lactate levels ($p = 0.07$) between both groups [67]. Nasralla et al. found that peak AST during the first 7 days after transplant was reduced by 49.4% in the NMP group compared to SCS ($p < 0.001$), with a greater in DCD than in DBD livers ($p = 0.001$). The median bilirubin level in the first week postoperatively was lower in NMP recipients (1.23–4.28) than in the SCS group (1.52–5.00; $p = 0.029$) [68]. Jassem et al. demonstrated that peak AST within 7 days was significantly lower for patients whose procedure included NMP compared to SCS ($p < 0.01$). Additionally, the peak INR within 7 days was lower in the NMP group compared to the SCS group ($p = 0.07$). The other biochemical postoperative parameters, including alkaline phosphatase (ALP) and total bilirubin, were comparable [69]. There was no significant difference in peak AST, ALT, and bilirubin between the NMP and SCS groups, as reported by Ghinolfi et al. [70].

5.5. EAD, PNF, IRI, and Other Complications

Ravikumar et al. had no primary non-function in either group. Three patients (15%) demonstrated early graft dysfunction (EAD) in the NMP group compared to nine (23%) in the control group, but this difference was not significant. This difference was more pronounced in the DCD subset (one [25%] vs. four [50%] patients) [66]. Selzner et al. found no significant difference in instances of major complications between NMP and SCS ($p = 0.5$) and had no graft loss or patient death in either group [73]. Bral et al. reported no cases of PNF in either NMP or SCS groups. The incidence of EAD in NMP livers was 55.5% compared with 29.6% for SCS controls ($p = 0.23$). EAD was caused principally by elevated transaminases in the initial 24 h and resolved promptly without other markers of graft dysfunction. There were no cases of postreperfusion syndrome in NMP grafts [67]. Per Nasralla et al., the odds of developing EAD in the NMP were 74% lower than in the SCS ($p < 0.001$). Additionally, the proportion of patients for whom adverse events were reported was similar in the two arms, but no statistical tests were applied to these data [68]. Jassem et al. reported that NMP liver tissues showed less necrosis and apoptosis in the parenchyma and fewer neutrophil infiltration compared to SCS liver tissues [69]. Ghinolfi et al. found no significant difference in instances of PNF, EAD, or PRS between

NMP and SCS [70]. Watson et al. reported significantly lower incidences of EAD in the NRP (12% vs. 32%, $p = 0.0076$), largely as a consequence of the significantly lower peak ALT in the first week post-transplant (633 compared to 1154, $p < 0.0001$). Where NRP was used, none of the recovered livers developed cholangiopathy compared to a 27% total incidence of cholangiopathy in non-NRP livers ($p < 0.0001$). A total of 7% of the NRP DCD livers developed an anastomotic stricture compared to a 27% anastomotic stricture rate in the comparator group ($p = 0.0069$) [74]. Mergental et al. reported a significantly increased incidence of EAD ($p < 0.038$) and non-anastomotic biliary strictures ($p < 0.063$) in the study group [71].

6. Graft Viability Assessment: A Beneficial Tool in Extended Criteria Donors

Compared with SCS, NMP permits graft preservation in a metabolically active state, not only reducing ischemic times but also allowing for ex situ assessment of graft metabolism [75]. If reliable predictive markers of post-transplant function can be established during NMP, then livers at higher risks of PNF can be eliminated prior to transplantation. The ability to eliminate higher-risk livers while selecting those that would have otherwise been denied based on standard criteria, the extended criteria for livers could be expanded. Different suggestions for viability criteria have been made, although clinical evaluation is still pending [44,74–78]. In 2016, Mergental et al. reported successful transplantation of five livers that were declined for transplantation by all UK centers per traditional criteria. The livers were transplanted following viability assessment (lactate clearance, bile production, perfusate pH, hepatic artery and portal vein flows, and homogeneity of graft perfusion) via NMP. The patient survival rate was 100% after 6–19 months of follow-up with no cases of primary non-function (PNF) [44]. A trial by Watson et al. in 2017 reported transplantation of 12 high-risk ECD livers assessed by NMP. The median donor risk index was 2.15 (1.47–3.14), with two grafts being allocated through an offer for research [74]. In the initial phase, post-reperfusion syndrome (PRS) was observed in five of six grafts, with one case of PNF. However, re-evaluation of the perfusion protocol led to adjustments in oxygenation and allowed for subsequent uneventful perfusion and graft evaluation [74]. Changes in lactate, glucose, and transaminase concentrations, as well as maintenance of perfusate pH, were used for viability assessment and led to a 1-year graft and patient survivals of 83% and 92%, respectively [74]. In 2018, Mergental et al. reported on the outcomes of the VITTAL clinical trial (ClinicalTrials.gov NCT02740608), which assessed declined livers using NMP. Of 31 assessed grafts, 22 met the criteria and were eventually transplanted, reaching 100% 90-day patient and graft survival [77]. A study by Nasralla et al. reported data from 120 NMP liver transplants that showed that post-reperfusion syndrome and EAD were not seen in livers that, during NMP, had low perfusate transaminase levels, low hemolysis levels, and higher levels of glucose utilization [68]. Since then, additional studies have added viability criteria has been assessed to predict post-transplant cholangiopathy: biliary bicarbonate concentration greater than 18 mmol/L, biliary pH greater than 7.48, biliary glucose greater than 16 mmol/L, a bile/perfusate glucose concentration ratio less than 0.67, and a biliary LDH less than 3.7 U/L [79]. In addition, the ability of the liver to maintain acid-base homeostasis has been demonstrated to be predictive of postoperative outcomes [80]. When comparing SCS to NMP-preserved porcine livers, the Oxford group was also able to correct the pH, while SCS livers were unable to reverse the acidosis [81,82].

7. Discussion

Utilization of livers for transplantation from organ donors is a major challenge in liver transplantation, particularly when marginal quality livers are considered [83]. Despite a rising rate of waiting list mortality in Western countries, an increasing number of ECD livers are being discarded [15,84]. Increasing demand may benefit from the successful utilization of high-risk livers, especially with the continuous increase in nonalcoholic fatty liver disease worldwide [85,86]. The increased need for liver transplantation in the Western World has prompted clinicians to implant suboptimal allografts with inferior outcomes [87,88]. In

recent years, clinical use of NMP has grown, and evidence supporting its beneficial impact on liver transplantation, particularly in the case of ECD, has increased [89,90]. A summary of published studies from livers transplanted following NMP is shown in Table 1, and a list of ongoing clinical trials is in Table 2. Multiple endpoints were examined to assess the safety and efficacy of NMP. Graft and patient survival appear comparable to SCS with the utilization of NMP despite the use of more marginal livers in certain cases. Median ICU and hospital stays were also comparable in the two groups. Bral et al. were the only group to report significantly prolonged ICU and total hospital stays between the two groups; however, they attributed these findings to patient and concurrent disease-related factors [67]. Liver function of the grafts, as determined by AST, ALT, and other biochemical markers, was comparable in the NMP and SCS groups, with multiple studies reporting decreased peak AST, INR, and bilirubin levels in the NMP groups [68,69]. Additionally, the rates of complications such as EAD in NMP groups are largely decreased or comparable to SCS livers. One study reported increased rates of EAD and non-anastomotic strictures in the study group, but they reported that these findings could be due to control patients not receiving systematic bile duct imaging and the small sample size of the study [71].

One of the main uses of NMP in clinical practice is assessing organ viability prior to utilization of higher-risk organs. The difficulty in assessing the effectiveness of NMP to SCS has been the ability to objectively evaluate the pre-transplantation viability of organs. There have been several studies examining markers of liver viability during NMP and function post-transplant. However, a universal set of parameters is yet to be established. Identification of accurate markers of viability is limited by a lack of negative controls or livers that develop PNF [91]. Nonetheless, measurement of these parameters during NMP could be used to increase the number of marginal livers suitable for transplantation. In addition to testing organ viability, NMP can help evaluate the quality of livers or how well a liver will perform following transplantation. This could be used to determine which patients might be suitable to receive specific livers.

Another primary use for NMP is improvement in transplant logistics, therefore extending preservation time. When using SCS, generally, preservation times over 10 h are not well tolerated [91,92]. This limits the distance organs can travel and the urgency of which they must be transplanted. These limitations with complex conditions require prolonged surgical preparation, which further prolongs CIS. Patients with long travel times to hospitals may be denied the opportunity to receive high-risk organs. Finally, a deciding factor in whether a recipient unit can accept an organ depends on its ability to immediately proceed with the procedure upon receiving the organ. This ability can be impeded if another transplant is already underway [91]. In these situations, extended preservation time could be beneficial. Many publications have demonstrated successful extension of preservation times with the use of NMP [44,66,74,77,93,94]. The studies included in this paper had preservation times on NMP varying from 2 h to 24 h. In a randomized trial, the median preservation time for the 121 transplanted livers in the NMP group was almost 12 h, with no evidence of a detrimental impact on the graft [68]. Another study showed histological and biochemical evidence of successful preservation of discarded human livers for up to 7 days [64].

Interestingly, a recent study by Javanbakht et al. evaluated the cost-utility of NMP with OrganOx *metra* compared to SCS [95]. Using a de novo decision analytic model based on current treatment pathways, it was found that NMP was more costly and more effective than SCS [95]. However, the higher cost of NMP was attributed to the extra available transplantable grafts and, therefore, an increase in post-transplantation costs. Ultimately, the use of OrganOx *metra* was shown to have a 99% probability of being cost-effective at the GBP 20,000 threshold and led to the utilization of 54 additional livers with improved outcomes [95]. Another study by Webb et al. showed that NMP was cost-effective in comparison to SCS and resulted in greater incremental quality-adjusted life years (QALYs) gains over 5 years [96].

The clinical benefits of NMP, particularly with marginal livers, have been well demonstrated. However, multiple challenges remain to be addressed with regard to this technology. The use of NMP has been reported to prolong the organ retrieval process by 2 h due to the increased time required for back-bench preparation in addition to cannulation and connection to the device [91]. Furthermore, the use of NMP was originally intended for the entirety of the preservation period. Special logistical considerations must be made when using NMP as intended, including having trained personnel at the donor and recipient hospitals. Using NMP during the transportation of livers requires suitable transportation with sufficient space, power, and personnel. These issues highlight the marked logistical, financial, and legal complexities that arise with the use of this technology, which is not an issue with SCS [97]. Additionally, not all of the commercially available machines are portable.

The ideal perfusion solution, temperature, rewarming time, and perfusion protocols remain unknown. In contrast to cold preservation, NMP requires an oxygen carrier/blood and is more complex to monitor [81]. Additionally, there have been reports of graft loss for various reasons, including user error or device error in clinical trials [67,68]. It remains debatable who should decide what organs should be pumped and how organs are allocated in case the primary center declines the organ [81].

There are also limitations to be considered when conducting clinical trials. Clinically relevant primary endpoints require large sample sizes, thus increasing trial costs and requiring time. Additionally, there are no agreed-upon biomarkers that accurately predict clinical outcomes.

Table 1. Published manuscripts of transplanted NMP livers.

Author (Year)	Experimental Groups	Donor Type (DCD/DBD)	Perfusion Device	Perfusate Characteristics	Perfusate	MP Time	Endpoints	Outcomes
Ravikumar, et al. (2016) [66]	NMP (<i>n</i> = 20) vs. SCS (<i>n</i> = 40)	NMP (4/16) SCS (8/32)	OrganOx <i>metra</i>	HA: 60–75 mmHg PV: not recorded HA \approx 0.2 L/min PV \approx 0.8 L/min	3 units of cross-matched PRBC + 1 unit of Gelofusine® (B Braun)	9.3 h (3.5–18.5 h)	Primary: graft survival at 30 days Secondary: AST/ALT at 7 days and 6 months	Median peak aspartate aminotransferase in the first 7 days was significantly lower in the NMP group. Thirty-day graft survival was similar between NMP and SCS.
Selzner, et al. (2016) [73]	NMP (<i>n</i> = 10) vs. SCS (<i>n</i> = 30)	NMP (2/8) SCS (8/24)	OrganOx <i>metra</i>	Pressure: Not described HA: 0.3 L/min (0.2–0.4) PV: 1.25 L/min (1.2–1.3)	3 units PRBC + Steen solution	8 h (5.7–9.7 h)	Lactate, bile production, ALT/AST, ICU stay, hospital stay, complications	No significant difference in graft function, hospital stay, or complications
Mergental, et al. (2016) [44]	NMP (<i>n</i> = 5)	NMP (3/2)	Liver Assist and OrganOx	Not described	3 units of the donor liver-specific blood group, Rhesus-negative, packed red blood cells, supplemented with 1000 mL human albumin solution 5%, 30 mL sodium bicarbonate 8.4%, and 10 mL calcium gluconate 10%	332 min (318–564 min)	Hospital stay, 6-mon survival	Median in-hospital stay was 10 (range 6–14) days. All recipients were well, with normalized LFTs at median follow-up of 7 (range 6–19) months
Watson, et al. (2017) [74]	NMP (<i>n</i> = 12) (normoxic vs. hyperoxic)	NMP (9/3)	Liver Assist	PV: 660–1130 mL/min HA: 208–390 mL/min Oxygen: 621–671 mmHg or 153–187 mmHg	leucocyte-depleted washed red cells, succinylated gelatin, or Steen solution (cases 6 to 8 only)	284 (122–530 min)	Post-reperfusion syndrome, vasoplegia, PNF, oxygen tension	Significantly decreased peak ALT in normoxic group at post-transplant day 7, significantly decreased post-reperfusion syndrome and vasoplegia in normoxic group
Bral, et al. (2017) [67]	NMP (<i>n</i> = 10) vs. SCS (<i>n</i> = 30)	NMP (4/6) SCS (8/22)	OrganOx <i>metra</i>	Pressure: Not described Flow: Not described	Gelofusine® (B Braun) + 3-unit type “O” PRBC	11.5 h (3.3–22.5 h)	Primary: graft survival at 30 days Secondary: patient survival at 30 days, peak ALT/AST at 7 days, EAD at 7 days, liver biochem on days 1–7, 10 & 30, major complications defined by Clavien-Dindo score \geq 3, patient and graft survival at 6 mo, biliary complications at 6 mo	No difference in graft survival at 30 days, prolonged hospital stays in NMP group, no difference in any other secondary endpoints
Nasralla, et al. (2018) [68]	NMP (<i>n</i> = 121) vs SCS (<i>n</i> = 101)	NMP (34/87) SCS (21/80)	OrganOx <i>metra</i>	HA \approx 0.28 L/min PV \approx 1.1 L/min	Gelofusine® (B Braun) + 3-unit donor-matched PRBC	9.13 h (1.42–24 h)	Primary: peak AST at 7 days Secondary: organ discard rate, post-reperfusion syndrome, PNF, EAD, graft function, hospital stay, need for renal replacement therapy, cholangiopathy on MRCP at 6 months, graft and patient survival at 1 year	Significant reduction of peak AST, odds of early allograft dysfunction, and median bilirubin during first 7 days post-transplant in NMP vs. SCS. No significant difference in hospital stay, need for RRT in the first week, or 1-year survival

Table 1. Cont.

Author (Year)	Experimental Groups	Donor Type (DCD/DBD)	Perfusion Device	Perfusate Characteristics	Perfusate	MP Time	Endpoints	Outcomes
Ceresa, et al. (2019) [94]	NMP (<i>n</i> = 31)	NMP (8/23)	OrganOx <i>metra</i>	HA: 0.44 L/min (0.29–0.59) PV: 1.08 L/min (0.96–1.2) HA: 67 mmHg (64–70)	Gelofusine® (B Braun) + unspecified blood products	8.4 h (4.3–12.5 h)	Primary: 30-day graft survival Secondary: AST/ALT, EAD, MEAF, PNF, PRS, RRT, hospital stay, adverse events, graft histology, adverse events, biliary complications and survival at 1 year	94% 30-day graft survival. 13% developed EAD. PRS was observed in 10% of livers. Median duration of initial critical care stay was 3 days (1–20 days), and median hospital stay was 13 days (7–31 days). 23% developed complications of grade 3b severity or above. 6% developed biliary complications. 12-month overall graft survival rate (including death with a functioning graft) was 84%
Jassem, et al. (2019) [69]	NMP (<i>n</i> = 12) vs. SCS (<i>n</i> = 27)	DBD only	OrganOx <i>metra</i>	HA: 60–75 mmHg PV: not recorded HA ≈ 0.2 L/min PV ≈ 0.8 L/min	3 units of cross-matched PRBC + 1 unit of Gelofusine® (B Braun)	9.3 h (3.5–18.5 h)	Peak AST, INR, ALP, bilirubin. AST, INR, ALP, bilirubin at 7 days. ICU stay length, rejection, graft survival at one year	Peak AST and INR within 7 days were significantly lower in the NMP group compared with the CS group. Alkaline phosphatase (ALP) and total bilirubin together with post-transplant clinical parameters such as the days of ITU stay, the rates of acute rejection and one-year graft and recipient survival, were comparable between the two groups.
Ghinolfi, et al. (2019) [70]	NMP (<i>n</i> = 10) vs. SCS (<i>n</i> = 10) All patients older than 70 yo	DBD only	LiverAssist	HA: 0.205–0.420 L/min PV: 1.1–1.7 L/min	Gelofusine® (B Braun) + ABO-compatible RBC concentrate	4.2 h (3.25–4.7 h)	Primary: graft and patient survival at 6 months Secondary: AST/ALT at 7 days, 6 mo biliary complications histology	No significant difference in graft and patient survival, lower lactate in NMP, decreased mitochondrial volume density at steatosis, and increased volume density of autophagic vacuoles in NMP
Watson, et al. (2019) [98]	NMP (<i>n</i> = 43) vs. non-NMP (<i>n</i> = 187)	DCD only	Medtronic, Cardiohelp or the Extra-Corporeal Organ Procurement System (ECOPS) or the Donor Assist	Abdominal flow = 2.5–3 L/min Thoracoabdominal flow = 4–6 L/min	Hartmann's solution (Baxter Healthcare Ltd., Thetford, UK) and Gelofusine® (BBraun)	2 h	Early allograft dysfunction, 30-day graft loss, freedom from ischemic cholangiopathy and anastomotic strictures	NRP was associated with a reduction in early allograft dysfunction, 30-day graft loss, freedom from ischemic cholangiopathy, and fewer anastomotic strictures

Table 1. Cont.

Author (Year)	Experimental Groups	Donor Type (DCD/DBD)	Perfusion Device	Perfusate Characteristics	Perfusate	MP Time	Endpoints	Outcomes
Mergental, et al. (2020) [71]	NMP (<i>n</i> = 22) vs. SCS (<i>n</i> = 44)	NMP (10/12)	OrganOx <i>metra</i>	Not described	Not described	4–24 h	Primary: (A) feasibility of NMP in discarded organ recovery and (B) achievement of successful transplantation. Secondary: LFTs, 90-day graft survival, hospital stay, vascular complications, biliary strictures with MRCP at 6 months	Patient and graft survival is similar at 12 months. Higher rate of EAD in study group. Higher incidence on non-anastomotic biliary strictures was higher in study group. No differences in other parameters.
Chen et al. (2021) [99]	NMP (<i>n</i> = 2)	NMP (1/1)	Not described	Not described	Gelofusine® + cross-matched leukocyte depleted RBC, 5% NaHCO ₃ , heparin, 10% Ca gluconate, 25% MgSO ₄ , methylprednisone, compound AA injection, Impenem cilastatin, metronidazole	7 h	Evaluate efficacy and safety of transplanting ECD directly under NMP without recooling	Continuous NMP without recooling is safe for LT with ECD livers
Seidita, et al. (2022) [100]	NMP (<i>n</i> = 17)	NMP (3/14)	Not described	Not described	Perfusion solution based on heparinized human plasma and red blood cells	195–330 min	Kaplan–Meier survival estimates at 30, 90, 180, and 1 year after transplant, estimated 3-year survival	Overall survival rates did not differ from those of patients transplanted with non-perfused grafts from an ECD
Hann, et al. (2022) [72]	NMP (<i>n</i> = 26) vs. Historical CS (1) (<i>n</i> = 31) Contemporaneous CS (2) (<i>n</i> = 25)	DBD only	OrganOx <i>metra</i>	Not described	Gelofusine® + 3-unit O-negative red blood cells	Minimum of 4 h	Primary: graft and patient survival at 6 months	No difference at 6 months despite NMP group having significantly more steatotic grafts and previously declined grafts

Table 2. Ongoing registered clinical trials involving NMP.

Study Title	Study Type	Number Enrolled	Outcomes	Start Date	Device	Identifier	Group
OCS Liver PROTECT Trial: Preserving and Assessing Donor Livers for Transplantation	Randomized	300	Primary: incidence of EAD at 7 days, incidence of serious adverse events at 30 days	February 2016	OCS™ Liver System (TransMedics, Andover, MA, USA)	NCT02522871	TransMedics, Andover, MA, USA
Efficacy Evaluation of Normothermic Perfusion Machine Preservation in Liver Transplant Using Very Old Donors (CEFEMA)	Randomized pilot	30	Primary: 6-month graft survival rate Secondary: IRI through biopsy at 1 day and AST at 7 days, IRI through ischemic type biliary lesions	October 2016	Liver Assist (Organ Assist, Groningen, The Netherlands)	NCT02940600	UO Chirurgia Epatica e del Trapianto di Fegato Pisa, Italy
Normothermic Liver preservation Trial	Phase 2	50	Primary: Graft survival rate at 30 days Secondary: patient survival rate at 30 days. EAD at 7 days, peak blood AST and lactate at 7 days, perfusate ALT, bilirubin and lactate levels	February 2017	OrganOx <i>metra</i> (OrganOx Ltd., Oxford, UK)	NCT03089840	University of Alberta Edmonton, Alberta, Canada

Table 2. Cont.

Study Title	Study Type	Number Enrolled	Outcomes	Start Date	Device	Identifier	Group
Post Static Cold Storage Normothermic Machine Liver Perfusion	Phase 2	30	Primary: patient and graft survival at 30 days Secondary: peak AST at 7 days, EAD at 7 days, PNF at 10 days, adverse events, transplantation and organ discard rates, biliary investigation or intervention at 6 months, patient and graft survival at 6, 12 months	May 2017	Not specified	NCT03176433	University of Oxford
Efficacy of Ex-situ Normothermic Perfusion Versus Cold Storage in the Transplant With Steatotic Liver Graft. (ORGANOXLAFE)	RCT	50	Primary: peak AST & ALT at 1, 3, 5, 7 days post-transplant Secondary: primary graft failure at 10 days, patient and graft survival at 1, 6, 12 months, post-reperfusion syndrome, post-transplant bilirubin, GGT, AST, ALT and INR at 1, 3, 5, 7 days and 1, 6, 12 months, EAD at 7 days, ICU and hospital stay at 30 days, need for RRT at 1, 6, 12 months, intra-op thromboelastogram and reperfusion injury, biliary stenosis	April 2019	OrganOx metra (OrganOx Ltd., Oxford, UK)	NCT03930459	Hospital Universitario y Politécnico La Fe Valencia, Spain
APHP Plateform of Normothermic Perfusion for Rehabilitation of Hepatic Grafts (PENOFOR)	Single group assignment	20	Primary: portion of grafts that can be evaluated after evaluation by NMP with a 3 year survival rate >90% Secondary: proportion of grafts considered as not initially transplantable, proportion of non-eligible grafts eligible for NMP, proportion of grafts perfused, proportion transplanted, time until liver function recovery on NMP and post-transplant, EAD at 1 month, 1 month overall survival, 1 year graft survival, wait time, incidence of biliary stenosis	October 2019	Not specified	NCT04154696	AP-HP, Paul Brousse Hospital Villejuif, France
OCS Liver PROTECT Continued Access Protocol (CAP) Continuation Post-Approval Study	Observational	74	Primary: graft survival at 24 months post transplant	February 2020	OCS™ Liver System (TransMedics, Andover, MA, USA)	NCT05096754	TransMedics, Andover, MA, USA
Safety and Feasibility of Normothermic Machine Perfusion to Preserve and Evaluate Orphan Livers	Single group assignment	30	Primary: rate of patient survival and primary non function (PNF) at 30 days after transplantation Secondary: Early Allograft Dysfunction (EAD), 6 months patient and graft survival, peak liver function tests at 7 days after transplantation, surgical outcomes (operative time, transfusion requirement etc.), rate of post-transplant kidney failure, assessment of histological ischemia reperfusion (liver and bile duct), rate of vascular complications, rate of biliary complications, hospital and ICU length of stay, rejection rate, infection rate, the ability to predict function based on “on-pump” viability markers, and the incidence of adverse effect	March 2020	Not specified	NCT03456284	Cleveland Clinic Cleveland, Ohio, United States
OCS Liver DCD Trial	Single group assignment	9	Primary: graft survival 6 months post-transplant Secondary: rate of donor liver utilization after OCS perfusion, incidence of ischemic biliary cholangiopathy at 6 months, EAD or PNF at 7 days, patient and graft survival at 1, 6, 12, 24, 36, 48, 60 months	July 2020	OCS™ Liver System (TransMedics, Andover, MA, USA)	NCT04194437	TransMedics, Andover, MA, USA

Table 2. Cont.

Study Title	Study Type	Number Enrolled	Outcomes	Start Date	Device	Identifier	Group
RESTORE Declined Livers Study	Prospective, non-randomized	25	Primary: 6 month graft survival rate, patient survival rate at 6 months Secondary: graft function and survival at 3 months–1 year, 90 day and 1 year graft and patient survival, morbidity at 3 months–1 year, quality of life score, proportion of declined livers eligible for NMP	December 2020	OrganOx metra (OrganOx Ltd., Oxford, UK)	NCT04483102	Washington University School of Medicine Saint Louis, Missouri, United States
Comparison of Hypothermic Versus Normothermic Ex-vivo Preservation. (DCDNet)	Prospective randomized	60	Primary: graft loss at 6 months, ischemic type biliary lesions at 6 months Secondary: 1 year graft and patient survival, BCL-2/BAX livers after 2 h of perfusion, levels of soluble keratin 18 and HMGB1 in perfusate at 2 h	December 2020	Not specified	NCT04744389	UO Chirurgia Epatica e del Trapianto di Fegato Pisa, Italy
Hypothermic Oxygenated (HOPE) Versus Normothermic Machine Perfusion (NMP) in Human Liver Transplantation (HOPE-NMP)	Randomized control trial	213	Primary: post-op complications at 90 days Secondary: peak ALT, AST at 7 days, EAD and PNF anad 7 days, biliary complications at 6 months, organ utilization rate, total preservation time, duration and cost of ICU and hospital stay, post-op complications at 1 year, recipient and graft survival at 1 year	January 2021	OrganOx metra (OrganOx Ltd., Oxford, UK)	NCT04644744	Bridge to Life Ltd., Northbrook, IL USA
Sequential Hypo- and Normo-thermic Perfusion to Preserve Extended Criteria Donor Livers for Transplantation	Single group assignment	15	Primary: patient and graft survival at 1 month post-transplant Secondary: EAD at 7 days, patient and graft survival at 6 months, estimated blood loss during surgery, peak ALT and AST at 7 days, total bilirubin and INR at 7 days, hospital and ICU stay	May 2021	Institutional-developed perfusion device	NCT04023773	Cleveland Clinic Cleveland, Ohio, United States
OCS Liver Perfusion (OLP) Post-Approval Registry	Observational	160	Primary: patient survival at 1 year Secondary: graft and patient survival at 6 months, 1 and 2 years post-transplant	October 2021	OCS™ Liver System (TransMedics, Andover, MA, USA)	NCT05074160	TransMedics, Andover, MA, USA

8. Conclusions

Despite the ability to assess liver viability and extend preservation times in liver transplantation, it is unclear whether these advantages warrant its greater cost and complexity. Standard criteria livers have demonstrated good outcomes, and the use of NMP is unlikely to improve them. However, at a time when there is a 15% mortality rate while waiting for an LT, the need to increase available livers is a global priority. We believe that NMP could provide great benefit from the ability to assess the viability of suboptimal organs and increase the utilization of organs that are likely to be discarded by current standards. NMP can be used as a supplement to current preservation methods, particularly when using high-risk organs. Additionally, we recommend the use of NMP to evaluate liver function prior to transplant, as this can help develop universal parameters that determine liver suitability. Table 3 breaks down ECD donors into subcategories and describes when NMP use may be indicated. Higher-risk ECD livers could benefit from the use of NMP. Nonetheless, future clinical trials are required to assess long-term outcomes and maximize the potential of this technology. Impact on other complications, such as ischemic cholangiopathy and PRS, that would limit the utilization of marginal organs awaits additional randomized trials.

Table 3. Risk categories in ECD livers with suggested actions.

Graft Risk	Definitions	Suggested Actions
Low-risk ECD grafts	DBD: donor age \leq 80 yo, CIT \leq 10 h, graft macrosteatosis \leq 30% DCD: donor age \leq 60 yo, CIT \leq 6 h, WIT \leq 20 min, graft macrosteatosis \leq 5%	SCS is first line, can consider machine perfusion on a case-by-case basis
Intermediate-risk ECD grafts	DBD: donor age $>$ 80 yo, CIT $>$ 10 h, graft macrosteatosis $>$ 30% DCD: donor age 60–80 yo, CIT 6–8 h, WIT 20–30 min, graft macrosteatosis 5–20%	Machine perfusion
High-risk DCD grafts and declined overextended livers	Donor age $>$ 80 yo, CIT $>$ 8–10 h, WIT $>$ 30 min, graft macrosteatosis $>$ 30%, poor in situ perfusion, prolonged retrieval, significantly elevated LFTs, declined for reason other than nonvascular reason	Not possible without machine perfusion, requires viability assessment

ECD, extended donor criteria; CIT, cold ischemia time; DBD, donation after brain death; CIT, cold ischemia time; DCD, donation after cardiac death; WIT, warm ischemia time. Modified from Schlegel et al. [101] and Czgany et al. [18], ECD liver grafts can be sub-classified into three categories: low-, intermediate- and high-risk. These classifications can help guide clinical decisions. Machine perfusion is recommended for intermediate- and high-risk ECD grafts.

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