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COMBINED SEQUENTIAL *EX VIVO* PERFUSION OF LIVER GRAFTS FROM EXPANDED CRITERIA DONORS: A CONTEMPORARY PERSPECTIVE

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Despite significant advancements in the field of liver transplantation (LT) over the last 30 years, the gold standard for allograft preservation – static cold storage with pharmacological agents – has remained largely unchanged. The growing disparity between demand for liver transplants and shortage of donor livers, along with a high waiting list mortality rate (potentially up to 20%), has forced transplant teams to broaden donor eligibility criteria. This expansion, however, has inevitably impacted both the immediate and long-term LT outcomes. Dynamic preservation of liver allografts has shown consistently positive outcomes, particularly among expanded criteria donors, including those classified as high-risk donors" Over the past decade, several perfusion techniques, integrating various temperature conditions, have been developed and are under active investigation. A significant advancement in this area is the emergence of combined sequential *ex vivo* machine perfusion, which integrates multiple perfusion strategies. This approach leverages the strengths of each method while mitigating their individual limitations. This paper reviews current experience with combined sequential *ex vivo* perfusion of liver grafts, providing a concise overview of the key stages encompassed within this protocol.

Keywords: ex vivo perfusion, liver perfusion, liver transplantation.

INTRODUCTION

Liver transplantation, a definitive treatment for various end-stage and focal liver diseases, has evolved significantly since its inception, becoming a routine procedure in many medical centers. While the expansion of liver transplant (LT) indications has allowed more patients to be treated, it also results in a growing pool of potential recipients on the waiting list each year [1, 2]. However, the relatively static donor pool limits the availability of liver transplants, prompting the global transplant community to broaden the criteria for liver allograft suitability, despite potential risks associated with such a step [3–5].

MP (MP) has emerged as a leading strategy for improving LT outcomes, particularly when using organs from expanded criteria donors (ECDs). Beyond conditioning and direct "recovery-rehabilitation" of the organ – notably of mitochondrial function – after a period of cold ischemia during hypothermic oxygenated perfusion (HOPE), MP significantly increases the number of usable organs by enabling *ex vivo* viability testing of both hepatocellular and cholangiocellular components during the normothermic machine perfusion (NMP) phase. However, NMP's protective capacity against ischemia-reperfusion-conservation injury remains relatively modest compared to HOPE [6–8]. Controlled oxygenated rewarming (COR) further optimizes graft preservation by minimizing "rewarming injury" [9] when transitioning from hypothermic to physiological perfusion conditions. The combination of all three MP approaches maximizes the protective and predictive capabilities of the method by harnessing the strengths of each. For example, combined perfusion protocols have achieved excellent outcomes with grafts from donation after circulatory death (DCD) – the highest-risk group – historically associated with poorer outcomes compared to donation after brain death (DBD), largely due to an initial period of warm ischemia.

STATIC COLD STORAGE

Static cold storage (SCS), due to its simplicity and affordability, has been the gold standard for solid organ preservation in transplantation for decades [10–12]. However, the use of isolated SCS in ECDs leads to severe ischemia-reperfusion-preservation injury (IRPI) affecting various components of the liver allograft [13, 14]. Hepatocellular injury manifests as graft dysfunction or primary nonfunction, driven by extensive hepatocyte dysfunction and necrosis [15, 16]. Early allograft dysfunction (EAD) can occur in up to 53.6% of recipients and significantly impacts transplant outcomes: analysis of 1,950 liver transplants showed that 1-year and 5-year graft survival rates were markedly lower in recipients

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with EAD compared to those without (84.1% *vs* 92.7% and 73.4% *vs* 83.9%, respectively), as were patient survival rates (86.8% *vs* 95.2% and 67.9% *vs* 79.6%; $p < 0.01$) [17, 18].

The most significant consequence of biliary IRPI is the development of diffuse fibrosis and ischemic non-anastomotic strictures, a condition termed non-anastomotic ischemic cholangiopathy (NAIC), which adversely affects graft survival, recipient quality of life, and overall treatment costs, including the need for retransplantation [19, 20]. In a 29-year analysis of asystolic donor experience by the University of Wisconsin group, a significantly higher rate of biliary complications was observed among recipients of non-heart-beating (NHB) donor grafts (51% *vs* 33.4% in the DBD group; $p < 0.01$), as well as a markedly higher risk of retransplantation due to ischemic cholangiopathy (13.9% *vs* 0.2% in the DBD group; $p < 0.01$).

A cost analysis by Jay et al. revealed a 53% increase in treatment costs at 1 year for patients who developed NAIC ($p < 0.01$), and a 107% increase ($p < 0.001$) if retransplantation was required due to disease progression [21]. LT recipients generally experience a higher incidence of NAIC when receiving grafts from DCD donors compared to those from DBD donors (44% *vs* 1.6%, $p < 0.001$).

The aforementioned complications reach their peak when using DCD allografts, primarily due to the unavoidable period of primary warm ischemia. This significantly limits the utilization of marginal allografts unless supplemented by additional protective strategies.

MP, being a long-established organ preservation technique, has consistently demonstrated excellent outcomes in high-risk allograft cases, achieving outcomes comparable to transplants from optimal donors [22, 23].

HYPOTHERMIC OXYGENATED PERFUSION

HOPE facilitates the restoration of electron flow through the mitochondrial electron transport chain (ETC), promoting ATP resynthesis while simultaneously minimizing consumption under hypothermic conditions. It actively clears ischemic metabolites such as succinate and NAD+, thereby preventing excessive formation of reactive oxygen species during subsequent rewarming and averting damage to critical ETC components, particularly complex I [24–26].

HOPE helps maintain cellular energy by effectively reprogramming mitochondria, the cell's energy hubs, to function under extremely low metabolic conditions. This "reprogramming" allows cells to thrive even when their energy production is 10–15 times lower than during typical SCS.

HOPE offers the advantage of relative technical simplicity: a short-term session (1–2 hours) conducted at the end of SCS – the so-called end-ischemic or back-to-base approach – is sufficient to achieve meaningful graft protection [24]. The perfusate used is typically a standard preservation solution (used routinely in SCS) or a modified variant such as the University of Wisconsin-Belzer Machine Perfusion Solution (UW-Belzer MPS), actively oxygenated to achieve a partial oxygen pressure (pO_2) of 400–600 mmHg under hypothermic conditions (8–12 °C) [25]. The standard HOPE protocol is detailed in Table 3. This technique does not require the addition of oxygen carriers to the perfusate, and the use of hypothermic temperatures reduces the risk of rewarming injury in the event of device malfunction.

Perfusion can also be initiated directly at the donor site (upfront approach); however, this method is limited by the need for specialized portable perfusion devices. Moreover, the absence of active oxygenation in many such systems often results in suboptimal oxygen delivery to the allograft [25]. Nonetheless, several studies have shown promising outcomes with this approach. For instance, Guarrera et al. reported a statistically significant reduction in biliary complications (4 *vs* 13 cases, $p = 0.016$) and shorter hospital stays (3.64 ± 10.9 *vs* 20.14 ± 11.12 days, $p = 0.001$) in the perfusion group compared to standard preservation [27, 28]. Results from the PILOT study indicated a significant reduction in the risk of irreversible graft dysfunction (IQR 3.4% [2.4–6.5] *vs* 4.5% [2.9–9.4], $p = 0.024$). However, differences between the MP and SCS groups in rates of primary nonfunction (0% *vs* 2.2%, $p = 0.10$) and biliary complications (6.3% *vs* 16.4%, $p = 0.18$) did not reach statistical significance [29].

HOPE can significantly enhance LT outcomes when using grafts from ECDs, especially those from DCD donors (Table 1), enabling the use of allografts from both optimal and marginal donors, particularly NHB donors. One of the largest randomized trials conducted by the Groningen group confirmed the strong protective capacity of a brief end-ischemic dual HOPE (D-HOPE) session: NAIC occurred in only 6% of the HOPE group versus 18% in the control group (OR 0.36; 95% CI, 0.14–0.94; $p = 0.03$). Similarly, EAD was observed in 26% of the D-HOPE group compared to 40% in the control group (OR 0.61; 95% CI, 0.39–0.96) [30]. However, the specific additional benefit of D-HOPE over single HOPE (mono-HOPE) during the hypothermic phase of MP remains under investigation [31, 32]. For example, Koch et al., in an analysis of 183 liver transplants preserved with either mono-HOPE ($n = 90$) or D-HOPE ($n = 93$), reported no significant difference in the incidence of NAIC (10.96% *vs* 8.22%, $p = 0.574$) or graft survival (91.2% *vs* 93.3%, $p = 0.893$) between the two groups [31].

NORMOTHERMIC MACHINE PERFUSION

Normothermic machine perfusion (NMP) is a more technically demanding and complex method of liver allograft preservation. Operating at normal body temperature (36–37 °C), NMP maintains the organ in a near-normal

metabolic state, necessitating precise and continuous monitoring of perfusion parameters. Any technical failure during this process may result in significant graft injury and potentially require a return to SCS [33, 34]. The standard NMP protocol is summarized in Table 3. This procedure requires the use of an oxygen-carrying perfusate, most commonly red blood cell (RBC) concentrates, due to their hemoglobin content. However, the limited organ availability, along with their unsuitability for hypothermic conditions due to increased membrane fragility and cold-induced agglutination, has prompted efforts to develop alternatives.

In this context, hemoglobin-based oxygen carriers (HBOCs) have emerged as a promising substitute. Van Leeuwen et al. reported comparable graft preservation outcomes between HBOC- and RBC-based perfusates (Table 1). A key advantage of HBOCs is their versatility: unlike RBCs, they are compatible across all MP phases – HOPE, COR, and NMP – allowing for seamless, uninterrupted perfusion protocols without the need to change the perfusate [35].

Conducting NMP sessions in both "upfront" and "end-ischemic" modes results in IRPI, although typically to a lesser degree than observed in the recipient organism, due to the absence of the effector component of the recipient's immune response [36–38]. In the VITTAL study, Mergental et al. reported a 18.2% incidence of NAIC, which was comparable to controls ($p = 0.063$), and a 31.8% incidence of EAD, which was higher than in controls ($n = 4$, $p = 0.034$) [33]. Similarly, Nasralla et al., in their analysis of 220 liver transplants, found no statistically significant differences between the NMP and SCS groups regarding biliary complications, including NAIC, despite a 74% reduction in the incidence of EAD in the NMP group ($p < 0.001$) [39]. These findings support the notion that NMP has limited protective capacity against IRPI.

Nevertheless, as a standalone technique, NMP remains an effective method for preserving liver grafts obtained from ECDs, showing superior outcomes compared to SCS, as confirmed by multiple studies (Table 1), though generally less effective than HOPE [6]. A key advantage of NMP – beyond organ reconditioning – is the ability to assess liver graft viability prior to transplantation. This pre-implantation assessment enables the rejection of organs that would likely result in severe post-transplant complications, such as ischemic cholangiopathy, EAD, or primary non-function (PNF) [40].

On the other hand, considering the largely subjective nature of macroscopic organ assessment by transplant surgeons, the ability to reassess initially "rejected" organs based on objective viability criteria offers a promising opportunity to significantly expand the donor pool. This is especially relevant in light of projections indicating that by 2030, only 44% of liver allografts will be utilized – down from 78% in 2010 – which would translate to approximately 2,230 fewer liver transplants annually [41]. Viability assessment thus emerges as a key strategy to address the growing gap between organ demand and availability. Notably, several studies have included grafts that had been declined by all transplant centers, yet achieved successful transplantation in 30% to 91% of these cases [33, 40], resulting in a donor organ utilization increase of 20% or more.

CONTROLLED OXYGENATED REWARMING (COR)

One of the critical challenges of static cold preservation of liver allografts – besides the accumulation of anaerobic metabolic byproducts and subsequent oxidative damage during warm reperfusion – is the so-called "rewarming injury". This injury is associated with the rapid rise in graft temperature to physiological levels (36–37 °C) [9, 42–44]. The underlying pathophysiology involves a progressive loss of mitochondrial transmembrane potential during cold ischemia. Upon rewarming – either in the recipient or during NMP – this dysfunction manifests as severe mitochondrial injury due to the massive opening of mitochondrial permeability transition pores, calcium ion leakage, apoptosis, free radical formation, and disintegration of the mitochondrial respiratory chain. Collectively, these processes are recognized as key components of ischemia-reperfusion-conservation injury. Interestingly, such thermal damage is not observed in isolated hepatocyte cultures preserved at temperatures above 16 °C [9, 43].

A potential solution to rewarming injury has been proposed by a group of researchers from University Hospital Essen. To minimize thermal damage, they advocate for a brief session of highly oxygenated MP with a gradual increase in temperature to 20 °C, performed in an "end-ischemic" format. The standard COR protocol is detailed in Table 3. In the first clinical validation of this approach, Hoyer et al. evaluated 6 LT recipients and reported a statistically significant reduction in peak transaminase levels – used as surrogate markers of IRPI – in the COR group (AST 563.5 *vs* 1204 U/L, $p = 0.023$) compared to a control group that underwent SCS ($n = 106$). Improvements in coagulation parameters were also observed, with a lower international normalized ratio (INR) in the COR group (1.48 *vs* 1.86, $p = 0.07$), reflecting better synthetic liver function postoperatively. Furthermore, the incidence of EAD and PNF was lower in the COR group (0% *vs* 35.9%, $p = 0.07$ and 0% *vs* 2.8%, $p = 0.68$, respectively). Graft survival in the COR group was 100%, compared to 80.9% in the SCS group ($p = 0.24$) [9, 43]. However, not all outcome differences reached statistical significance.

A subsequent randomized controlled trial involving 40 LT recipients, randomized to receive either COR ($n = 20$) or SCS ($n = 20$) for graft preservation, further

supported the potential benefits of COR. Improved graft function was demonstrated by a higher 13C-methacetin clearance (LiMAx test, 294 ± 106 *vs* 187 ± 121 ng/kg/hour, p = 0.006) and increased synthesis of coagulation factor V on postoperative day 1 (103 ± 34 *vs* 66 ± 26, p = 0.001) [45]. However, the study did not meet its primary endpoint, as the difference in peak AST levels between the groups was not statistically significant (767 ± 1157 *vs* 1371 ± 2871 U/L, p = 0.273).

In summary, COR shows promise as a supplementary MP strategy for liver graft preservation. Nonetheless, its clinical efficacy requires confirmation through larger-scale studies and direct comparisons with other established techniques such as HOPE.

Despite the current absence of established practice for assessing allograft viability at the COR stage – owing to its inherently end-ischemic application – Hoyer et al. reported a strong correlation between AST levels measured at 120 minutes of COR perfusion at 20 °C and peak AST levels observed in the postoperative period. The coefficient of determination was $R^2 = 0.90$, $p < 0.001$). In comparison, similar correlations observed during HOPE were associated with lower predictive accuracy ($R^2 = 0.73$) [46].

COMBINED SEQUENTIAL MACHINE PERFUSION

With the development and introduction of HOPE and NMP into routine clinical practice, several researchers have attempted to compare the two techniques [6]. The results of a meta-analysis of 7 randomized trials and 1017 patients included demonstrate a statistically significant reduction in the incidence of EAD with both HOPE and NMP (NMP *vs* SCS, OR 0.50, 95% CI 0.30–0.86, p = 0.01, I2 = 39%; HOPE *vs* SCS: OR 0.48, 95% CI 0.35–0.65, p < 0.00001, I2 = 5%). At the same time, a greater protective potential of HOPE has been noted, consisting of a reduction in serious complications (Clavien–Dindo >IIIb, HOPE *vs* SCS: OR 0.76, 95% CI 0.63–0.93, p = 0.006, I2 = 0%), retransplantation rate (HOPE *vs* SCS: OR 0.21, 95% CI 0.04–0.96, p = 0.04; I2 = 0%) and graft loss (HOPE *vs* SCS: OR 0.40, 95% CI 0.17–0.95, p = 0.04; I2 = 0%). Both techniques also had a positive effect on the incidence of biliary complications and non-anastomotic strictures. Over time, the view on the use of MP has changed toward the use of combined protocols that include sequential HOPE and NMP [35, 47]. At the D-HOPE stage, the previously described reconditioning of mitochondria of the liver allograft is performed, which allows approaching the period of warm reperfusion in an optimal energetic and metabolic state. Thus, in an experimental study, Boteon et al. report a 1.8-fold increase in ATP levels during HOPE and decreased expression of markers of oxidative stress and inflammation (p = 0.008 and p = 0.02) during the NMP stage in the combined perfusion group compared to isolated NMP, with 100% of organs achieving viability criteria in the combined perfusion group compared to 40% in the isolated perfusion group [48].

In more recent studies, the combined perfusion protocol has evolved to incorporate the COR stage as a transitional link between hypothermic and normothermic perfusion phases. By enabling a gradual, stepwise rewarming of the organ under conditions of high oxygenation, COR mitigates the abrupt thermal shift associated with direct transitions from hypothermic to normothermic perfusion. Although definitive clinical evidence demonstrating the superiority of this integrated approach is still lacking, the strong pathophysiological rationale and promising outcomes observed with isolated COR have led several researchers to adopt it as part of their combined perfusion strategies [35, 49]. Following the COR phase, D-HOPE is succeeded by NMP, allowing not only continued graft reconditioning through restoration and maintenance of ATP reserves but also real-time assessment of organ viability under near-physiological conditions. Once the graft meets established viability criteria – identical to those used in isolated NMP – it is deemed suitable for transplantation and can be successfully implanted into the recipient [49–51].

The choice of perfusate in the combined perfusion protocol warrants careful reconsideration. The use of oxygen carrier-free perfusate during the normothermic phase is inadequate due to the high metabolic demand of the liver graft and the limited oxygen-carrying capacity of such solutions. Conversely, the most commonly employed perfusate in NMP – based on red cell mass – is unsuitable for hypothermic conditions due to increased fragility of erythrocyte membranes, a heightened risk of hemolysis, and cold-induced RBC agglutination. According to van Leeuwen et al. [35], two primary strategies have been proposed to address this limitation. The first involves replacing the perfusate following the HOPE phase with a RBC-based solution and initiating the subsequent perfusion at a starting temperature of 20 °C. This approach avoids erythrocyte damage while enabling effective oxygen delivery during the COR and NMP phases. Importantly, the perfusate exchange typically requires no more than 20–30 minutes and does not significantly affect overall perfusion outcomes. The second approach employs HBOCs throughout all stages of the combined perfusion protocol. This method has demonstrated safety and efficacy in comparison to both RBC-based perfusates during NMP and conventional UW-MPS during HOPE [35, 49, 50]. However, the clinical application of HBOCs remains limited, primarily due to the absence of regulatory approvals for use in perfusion preservation in the United States and European Union. Nevertheless, given the constrained availability of donor red cell mass, HBOC-based perfusion is a promising area for future research.

The main outcomes of the combined MP protocol are presented in Table 1. It is important to note the relatively small number of studies, the limited patient cohorts, and the near-complete absence of direct comparisons between combined and isolated protocols, which currently makes it difficult to draw definitive conclusions about the advantages of combined perfusion. However, several ongoing clinical trials – particularly the DHOPE-COR-NMP study led by the Groningen group – are expected to provide deeper insights into the potential benefits of integrating multiple perfusion techniques [52].

Table 1

Outcomes of hypothermic oxygenated perfusion (HOPE) and normothermic machine perfusion (NMP) in liver transplantation (Adapted from Jakubauskas et al. [66] and Banker et al. [8], with additions)

Research	Comparison groups	Donation type	Study design	Number of patients	Duration of machine perfusion	Perfusion system and perfusate	Effects observed
1	2	3	4	5	6	7	8
HMP and HOPE, including dual-HOPE (D-HOPE)							
Guarrera et al. [27] 2010	HMP and SCS	Brain-dead donors	Prospective cohort study, case-matched 1 : 1	20 and 20	4.3 ± 0.9 hours	Modified Medtronic PBS (Vasosol)	1. No statistically significant difference: early allograft dysfunction (EAD), primary non-function (PNF), survival 2. In HMP group: significant reduction in length of hospital stay, decreased peak levels of AST, ALT, and total bilirubin, as well as improved renal function (significantly lower serum creatinine levels)
Henry et al. [67] 2012	HMP and SCS	Not indicated	Prospective cohort study, case-matched 1 : 1	18 and 15	4.2 ± 0.9 hours	Modified Medtronic PBS (Vasosol)	In HMP group: decreased expression of proinflammatory cytokines, activation of adhesion molecules, along with reduced ultrastructural damage to the allograft
Guarrera et al. [68] 2015	HMP and SCS	Donation after brain death (DBD)	Prospective non-randomized, case-matched 1 : 1	31 and 30	3.8 ± 0.9 hours	Modified Medtronic PBS (Vasosol)	1. Equal number of EAD, 1-year recipient survival 2. In NMR group: lower incidence of biliary complications within the first year post-transplant 3. Significant reduction in length of stay in the hospital
Dutkowski et al. [69] 2015	HOPE and SCS	Donation after circulatory death (DCD) and DBD	Multicenter study, case-matched analysis 1 : 1	25 and 50	2.2 hours	Liver Assist device, UW gluconate solution (KPS-1)	In HOPE group: significant reduction in peak ALT levels, lower incidence of cholangiopathies and biliary complications, and improved 1-year graft survival
Van Rijn et al. [70] 2017	D-HOPE and SCS	DCD	Non-randomized study, case-matched 1 : 2	10 and 20	2.1 (2.1–2.3) hours	Liver Assist device, UW gluconate solution (Belzer MPS) and glutathione 3 mmol/L	In D-HOPE group: 1. Decreased peak levels of ALT, gamma-glutamyl transferase, alkaline phosphate, total bilirubin at 30 days post-transplant, along with an 11-fold increase in ATP levels 2. ALT and bilirubin at 1 week post-transplant are two times lower
Patrono et al. [71] 2019	D-HOPE and SCS	DBD	Non-randomized study, case-matched 1 : 2	25 and 50	3.1 ± 0.8 hours	Liver Assist device, UW-MP solution (Belzer MPS)	Acute renal injury stage 2–3 and severe postreperfusion syndrome are significantly lower in the D-HOPE group

Continuation of Table 1

1	2	3	4	5	6	7	8
Schlegel et al. [72] 2019	HOPE and SCS	DCD and DBD	Prospective study, case-matched 1 : 1 : 1 (HOPE-DCD and SCS-DCD and brain-dead SCS donor	50 and 50	2 (1.6–2.4) hours	Liver Assist device, UW gluconate solution (Belzer MPS)	Significant increase in graft survival in the HOPE-DCD group compared to the DCD-SCS group
Ravaioli et al. [73] 2020	HOPE and SCS	DBD	Non-randomized study, case-matched 1 : 3	10 and 30	2.2 (1–3.5) hours	Center-developed perfusion device, UW-MP solution (Belzer MPS)	Mean AST at postoperative day 7 significantly lower in the HOPE group
Rayar et al. [74]	HOPE and SCS	DBD	Non-randomized study, case-matched 1 : 3	25 and 69	2 (1.3–4.2) hours	Liver Assist device, UW-MP solution (Belzer MPS)	Mean leant of stay in the hospital and in ICU significantly lower in the HOPE group
Van Rijn et al. [30] 2021	D-HOPE and SCS	DCD	Multicenter prospective randomized controlled clinical study	78 and 78	2.2 (2–2.5) hours	Liver Assist device, UW gluconate solution (Belzer MPS) and glutathione 3 mmol/L	In HOPE group: significant reduction in symptomatic non-anastomotic stricture (NAS), EAD, incidence of postreperfusion syndrome
Czigany et al. [75] 2021	HOPE and SCS	DBD	Prospective, randomized controlled study	23 and 23	–	Liver Assist device, UW gluconate solution (Belzer MPS)	In HOPE group: decreased peak ALT levels, along with shorter ICU stays and overall hospitalization time
NMP							
Ravikumar et al. [76] 2016	NMP and SCS	DCD and DBD	Non-randomized study, case-matched 1 : 2	20 and 40	9.3 (3.5–18.5) hours	OrganOx metra, colloidal solution (Gelofusine)	1. Similar 30-day patient and graft survival 2. Significant reduction in peak AST levels within the first 7 days in the NMP
Selzner et al. [77] 2016	NMP and SCS	DCD and DBD	Pilot study, case-matched 1 : 3	10 and 30	8 (5.7–9.7) hours	OrganOx metra, dextran-based perfusate (Steen solution), red cell mass (RCM), albumin	1. Similar 30-day recipient and graft survival 2. No cases of graft loss 3. Similar postoperative graft function, hospitalization duration, and ICU stay between groups
Bral et al. [78]	NMP and SCS	DCD and DBD	Non-randomized study, case-matched 1 : 2	9 and 27 (intention-to-treat 10 and 30)	11.5 (3.3–22.5) hours	OrganOx metra, perfusate based on erythrocyte-RCM and colloidal solution	1. Similar 30-day and 6-month graft and patient survival 2. Hospitalization and ICU stays significantly longer in the NMP group 3. Similar postoperative graft function
Nasralla et al. [39] 2018	NMP and SCS	DCD and DBD	Multicenter, randomized controlled study, case-matched 1 : 2	120 and 101	9.1 (6.2–11.8) hours	OrganOx metra, colloidal solution (Gelofusine), RCM	1. In NMP group: lower incidence of EAD, less organ injury (by 50%, based on peak transaminase levels), lower rate of postreperfusion syndrome 2. Increased number of allografts transplanted 3. Similar 1-year recipient and graft survival
Ghinolfi et al. [28] 2019	NMP and SCS	DBD donors aged >70 years	Single-center controlled study	121 and 101	4.2 (3.3–4.7) hours	Liver Assist, colloidal solution (Gelofusine), albumin, RCM	Similar 6-month patient and graft survival Similar complication rates and length of hospitalization in both groups Less organ injury in the NMP group according to histological analysis

Continuation of Table 1

1	2	3	4	5	6	7	8
Mergental et al. [33, 79] 2020, 2024 – the VITTAL trial	NMP	DCD and DBD	Single-center, non-randomized study, case-matched 1 : 2	31	587 (450–705) minutes	OrganOx metra, colloidal solution (Gelofusine), RCM	1. 71% of organs (n = 22) reached viability criteria and were successfully transplanted 2. 90-day graft survival was 100% 3. Ischemic strictures requiring retransplantation occurred in 18% of patients (n = 4, mean follow-up 542 days), comparable to 2.3% (n = 1) in the control group (p = 0.063) 4. No cases of PNF 5. EAD in 31.8% of cases (n = 7), more frequent than in the control group (n = 4, p = 0.034) 6. 1-year graft and recipient survival of 86.4% and 100% (comparable to control group: 86.4% and 95.5%) 7. 5-year graft and recipient survival: 72% and 82% 8. All deceased recipients had a functioning graft
Liu et al. [80] 2020	NMP and SCS	DCD and DBD	Non-randomized study, case-matched 1 : 4	21 and 84	5 ± 1.1 hours	Custom-built perfusion device; perfusate composed of fresh frozen plasma, RCM, and albumin.	Frequency of EAD, peak ALT and AST levels significantly lower in NMP group
Quintini et al. [81] 2022	NMP of liver allografts declined by other transplant centers	DCD and DBD	Non-randomized clinical study	21	3.49–10.29 hours	Custom-built perfusion device	Ability to rehabilitate and successfully transplant 15 of 21 organs initially deemed unsuitable
Markmann et al. [82] 2022	NMP and SCS	DCD and DBD	Prospective randomized study	153 and 147	2.0–5.5 hours	Transmedics Organ Care Systems, albumin-based perfusate (Steen solution), RCM	1. In NMP group: reduced incidence of EAD, ischemic biliary complications 2. Decreased intensity of ischemia-reperfusion-preservation injury (according to histologic study) 3. Increased number of utilized organs from DCD donors in the group where NMP was used
Combined *ex-vivo* machine perfusion protocols							
Boteon et al. [48] 2018	NMP and HOPE-NMP	DCD and DBD	Prospective cohort study, case-matched 1 : 1 (pilot study)	5 and 5	6 hours NMP (NMP group). 2 hours HOPE and 4 hours NMP (HOPE-NMP group)	Liver Assist device, perfusate: hemoglobin-based oxygen carrier (HBOC) with albumin (for NMP); UW-MP solution (Belzer MPS) for HOPE	*Birmingham (Mergental, 2016) criteria were used to assess allograft viability at the NMP stage (see Table 2)* 1. 60% (n = 3) in the NMP group and 100% (n = 6) in the HOPE-NMP group were deemed viable and successfully transplanted 2. In the combined perfusion group: statistically reduced expression of oxidative stress markers (4-hydroxynonenal, CD14), inflammatory markers (CD11b, VCAM)

Continuation of Table 1

1	2	3	4	5	6	7	8
de Vries et al. [49] 2019	DHOPE-COR-NMP	DCD	Prospective single-center	7	283–517 minutes	Liver Assist device, perfusate: HBOC with albumin used throughout all perfusion stages	*The Groningen (van Leeuwen, 2019) criteria were used to assess allograft viability at the NMP stage (see Table 2)* 1. 5 out of 7 liver grafts used 2. No cases of EAD 3. No cases of PNF 4. 3-month graft and recipient survival – 100%
van Leeuwen et al. [58] 2019	DHOPE-COR-NMP	DCD	Prospective (stage 1), retrospective cohort study (stage 2)	16	Total perfusion time is not specified. Standard protocol: 1 hour D-HOPE, 1 hour COR, 150 minutes NMP. If the graft is deemed viable, prolong perfusion until ready for implantation. Total preservation time: 868 (IQR 805–924) min	Liver Assist device, perfusate: HBOC with albumin used throughout all perfusion stages	*The Groningen (van Leeuwen, 2019) criteria were used to assess allograft viability at the NMP stage (see Table 2)* 1. 69% of organs (n = 11) were deemed viable and were transplanted 2. Recipient and graft survival at 3, 6, and 12 months was 100% 3. 1 case (9%) of ischemic cholangiopathy (4 months post-transplant) 4. No cases of PNF 6. Transplant activity increased by 20% following implementation of the protocol
van Leeuwen et al. [35] 2022	DHOPE-COR-NMP	DCD and DBD (2%, n = 1)	Prospective observational cohort study	54	Total perfusion time is not specified. Standard protocol: 1 hour D-HOPE, 1 hour COR, 150 minutes NMP. If the graft is deemed viable, prolong perfusion until ready for implantation	Liver Assist device, perfusate: HBOC with albumin (n = 12) used for all perfusion stages; or UW-MPS for HOPE and red blood cell mass with albumin for COR-NMP stages (n = 22)	*The Groningen (van Leeuwen, 2019) criteria were used to assess allograft viability at the NMP stage (see Table 2)* 1. 63% of organs (n = 34) were deemed viable and successfully transplanted 2. 1-year survival rates were 100% for recipients and 94% for grafts 3. Non-anastomotic cholangiopathy developed in 3% of cases (n = 1) 4. Comparable results when using HBOC and RCM in the perfusate 5. No cases of PNF 6. Two retransplantations for reasons unrelated to perfusion (venous outflow obstruction and chronic rejection)
Liu et al. [82] 2023	HOPE-NMP	DCD and DBD	Prospective, observational, single-center	17	1–2 hours at NOPE stage and 4–9 hours at NMP stage	Perfusion device: center-developed custom perfusion system. UW-MPS (HOPE stage) and RCM-based perfusate (NMP stage)	*The Quintini et al. (2022) criteria was used to assess allograft viability at the NMP stage (see Table 2)* 1. 76.5% of organs (n = 13) were deemed viable and successfully transplanted 2. Graft and recipient survival rates were 100% (follow-up period 6–13 months) 3. EAD occurred in 5 patients (35%) 4. Non-anastomotic ischemic cholangiopathy developed in 2 patients (15%) 5. No statistically significant differences in post-transplant outcomes when compared to the retrospective NMP group without HOPE

End of Table 1

1	2	3	4	5	6	7	8
Thorne et al. [83] 2023	DHOPE-COR-NMP	DCD	Prospective, observational, single-center	55	Total perfusion time not specified. Standard protocol: 1 hour D-HOPE, 1 hour COR, 150 minutes NMP. If the graft is deemed viable, prolong perfusion until ready for implantation	Liver Assist device, RCM- and albumin-based perfusate (for NMP and COR stages), UW-MP solution (Belzer MPS) (for D-HOPE)	*The Groningen (van Leeuwen, 2019) criteria were used to assess allograft viability at the NMP stage (see Table 2)* 1. 70% of allografts (n = 35) were deemed viable and successfully transplanted 2. Recipient and graft survival (death-censored): 97% and 94%, respectively 3. Two retransplantations were required (due to chronic rejection and hepatic artery thrombosis) 4. One patient death occurred (due to interstitial lung disease) 5. One case of ischemic cholangiopathy was reported
Magistri et al. [84] 2025	D-HOPE – NMP	DCD and DBD	Retrospective, observational, single-center	33	90 minutes D-HOPE NMP time: 427 (260–559) in the viable allograft group and 240 (120–375) in the non-viable allograft group	Liver Assist device, PerLife (Aferetica) RCM-based perfusate (NMP)	*The "traffic light" criteria developed by the Groningen group were used to assess allograft viability at the NMP stage, modified (by excluding biliary tree viability assessment due to logistical and laboratory limitations) in the center (see Table 4)* 1. 48.5% (n = 16) of grafts were deemed viable and successfully transplanted 2. One case of EAD (with the patient surviving 30 months post-transplant) and one case of PNF (resulting in death on postoperative day 46)

Note: HMP, hypothermic machine perfusion; SCS, static cold storage; HOPE, hypothermic oxygenated perfusion; D-HOPE, dual hypothermic oxygenated perfusion; NMP, normothermic machine perfusion; COR, controlled oxygenated rewarming; AST, Aspartate Transaminase; ALT, Alanine Transaminase.

VIABILITY ASSESSMENT

Assessment of liver allograft viability is a critical aspect of machine perfusion: identifying and discarding non-viable allografts during preservation can prevent most complications associated with transplanting a non-viable organ. The main methods used for viability assessment are summarized in Table 2.

The ability to evaluate viability during hypothermic perfusion is considerably limited due to the considerably reduced allograft metabolism. Although several studies have described correlations between various perfusate parameters (such as glucose, lactate, AST, and ALT) during HOPE and post-transplant outcomes [53], the only validated marker for viability assessment is the measurement of flavin mononucleotide (FMN, a component of mitochondrial complex I) levels in the perfusate at 30 minutes of perfusion [31, 72].

A multicenter, cross-national study analyzing 473 HOPE/D-HOPE perfusate samples confirmed the strong predictive value of FMN levels for graft loss (due to NAIC or PNF), showing ROC values between 0.7733 and 0.8418 depending on the determination method ($p < 0.0001$), for NAIC (ROC 0.7456–0.7686, $p < 0.0001$), and for the risk of acute kidney injury (ROC 0.7616–0.7144, $p < 0.0001$ and $p < 0.0014$) [54].

However, FMN assessment requires mass spectrometry or fluorometry, necessitating specialized equipment and limiting the widespread adoption of this method. The role of FMN measurement in combined perfusion protocols remains undefined. Of particular interest is the combined perfusion protocol developed at the Zurich clinic, where a decision to proceed with or abandon subsequent NMP is guided by FMN levels: NMP is withheld for severely damaged allografts with high FMN values, whereas optimal allografts with elevated FMN levels proceed to NMP. [55].

A comprehensive and detailed assessment of organ viability becomes possible during the normothermic perfusion stage. As previously mentioned, by recreating near-physiological conditions during normothermic perfusion, it is feasible to evaluate key metabolic parameters and identify signs of dysfunction or injury, which may indicate organ non-viability or future graft dysfunction.

Table 2

Assessment of liver graft viability during hypothermic oxygenated perfusion (HOPE) and normothermic machine perfusion (NMP): current criteria and practical outcomes (adapted from Groen et al. [56] and Jeddou et al. [40], with additions). Criteria related to cholangiocellular viability are italicized in the NMP section

Study	Number of organs / salvage rate (%) *Use of initially rejected organs*	DCD / DBD	Viability criteria	Perfusion device	Perfusate base	Outcomes
1	2	3	4	5	6	7
HOPE, D-HOPE						
Eden et al. [85] (2023)	Perfused: not indicated Transplanted: 158	Not indicated	Flavin mononucleotide (FMN) at 30 minutes HOPE (<6000 A.U.) NADH at 30 minutes HOPE (<8000 A.U.)	ECOPS, Liver Assist, VitaSmart	UW-MPS (Belzer MPS)	1-year graft survival: 89% Primary non-function (PNF): 7 cases Ischemic cholangiopathy (IC): 11 cases Anastomotic strictures (AS): 53 cases Biliary congestion (BC): 9 cases
Patrono et al. [53] (2020)	Perfused: 50 Transplanted: 50 100% utilization rate	0/50	Perfusate during HOPE: lactate, AST, ALT, LDH, glucose, pH	Liver Assist device	UW-MPS (Belzer MPS)	Graft loss: 1 case Early allograft dysfunction (EAD): 13 cases
Schlegel et al. [26] (2020)	Perfused: 50 Transplanted: 50 100% utilization rate	32/18	Perfusate, liver parenchyma and mitochondria during HOPE: FMN at 30 minutes (<8000 A.U.) NADH (<10000 A.U.)	Liver Assist device	UW-MPS (Belzer MPS)	Graft loss (unspecified causes): 7 cases
Muller et al. [86] (2019)	Perfused: 54 Transplanted: 54 Utilization rate: 100%	35/19	FMN at 30 minutes HOPE	Liver Assist device	UW-MPS (Belzer MPS)	Graft loss: 7 cases PNF: 4 cases IC: 1 case
NMP						
Olumba et al. [87] (2023)	Perfused: 22 Transplanted: 16 Utilization rate: 72.7%	10/12	Within the first 2 Hours of NMP: – Perfusate lactate <2.2 mmol/L – Hepatic artery (HA) flow >100 mL/min and portal vein (PV) flow >500 mL/min *Plus at least two of the following*: – Evidence of glucose metabolism – Perfusate pH >7.25, with sodium bicarbonate requirement <70 mL – Evidence of bile production – Perfusate AST <10,000 U/L and ALT <7,000 U/L – Homogeneous parenchymal perfusion and soft allograft consistency	OrganOx metra device	Red cell mass (RCM)	PNF: none Graft-related death: none Non-anastomotic stricture (NAS): none
van Leeuwen et al. [35] (2022)	Perfused: 54 Transplanted: 34 Utilization rate: 63%	53/1	After 2.5 hours of NMP : – Perfusate lactate <1.7 mmol/L – Perfusate pH 7.35–7.45 without repeated bicarbonate administration – Bile output >10 mL, with >4 mL in the last hour – Bile pH >7.45 – ΔpH (bile – perfusate) >0.10 – Δ bicarbonate (bile – perfusate) >5.0 – Δ glucose (bile – perfusate) <–5.0	Liver assist device	Cases 1–8: hemoglobin-based oxygen carrier (HBOC), Cases 19–54: RCM	1-year graft survival: 94% NAS: 1 case AS: 12 cases Bile leaks: 4 cases

Continuation of Table 2

1	2	3	4	5	6	7
Seidita et al. [88] (2022)	Perfused: 19 Transplanted: 17	3/16	– Normalization or ≥50% reduction of lactate by the end of perfusion – Perfusate pH >7.3 without repeated sodium bicarbonate infusions – Evidence of bile production – HA and PV flows	Not indicated	RCM, fresh frozen plasma	1-year graft survival: 94% EAD: 1 case
Quintini et al. [80] (2022)	Perfused: 21 Transplanted: 15 Utilization rate: 71%	13/8	Within 6 hours of NMP – at least two of the following criteria: – Perfusate lactate <4.5 mmol/L or 60% reduction from peak value within the first 4 hours – Bile output >2 mL/hour – Stable perfusion flows: HA >0.05 mL/min/g and PV >0.4 mL/min/g – Homogeneous parenchymal perfusion and soft allograft consistency	OrganOx metra device	RCM	EAD: 7 cases Ischemic strictures: 1 case (due to biliary wall obstruction) PNF: none
Zhang et al. [89] (2020)	Perfused: 4 Transplanted: 4 (retrospective) Utilization rate: 100%	3/1	Within the first 4 hours of NMP: – Perfusate lactate <2.5 mmol/L – Evidence of bile production – Stable HA and PV perfusion flows (>150 mL/min and >500 mL/min) – Perfusate pH >7.3 without a need for repeated sodium bicarbonate infusions	Liver Assist device	RCM leukoreduced, washed red blood cells	6-month graft survival: 100% EAD: 1 case 1 AS PNF or NAS: none
Reiling et al. [90] (2020)	Perfused: 10 Transplanted: 10 Utilization rate: 100%	5/5	After 4 hours of NMP: – Perfusate lactate <2 mmol/L within the first 2 hours – Glucose metabolism trending downward within 4 hours – Physiologic perfusate pH without need for continuous sodium bicarbonate infusion – Stable HA and PV flows – Homogeneous parenchymal perfusion and soft allograft consistency – Evidence of bile production	OrganOx metra device	RCM	6-month graft survival: 100% EAD: 5 cases AS: 1 case Bile leaks: 1 case (originating from the biliary anastomosis) PNF or NAS: none
Mergental et al. [33, 78] – the modified **Birmingham criteria** (2020–2024)	Perfused: 31 Transplanted: 22 Utilization rate: 71%	14/17	Within the first 4 hours of NMP: – Perfusate lactate ≤2.5 mmol/L *Plus at least two of the following criteria:* – Evidence of bile production – Perfusate pH ≥7.3 without a need for repeated sodium bicarbonate injections – Evidence of glucose metabolism – Stable PV and HA perfusion flows (≥150 mL/min and ≥500 mL/min, respectively) – Homogeneous parenchymal perfusion and soft allograft consistency	OrganOx metra device	RCM	1-year graft survival: 86.4% EAD: 7 cases NAS (requiring retransplantation): 4 cases AS: 2 cases PNF: none
Cardini et al. [91] (2020)	Perfused: 34 Transplanted: 25 Utilization rate: 73%	4/30	After 2 hours of NMP – Rapid decrease and sustained low lactate levels during the first 2 hours of perfusion – Continued bile production, bile pH – Maintenance of physiologic perfusate pH without the need for continuous sodium bicarbonate infusion – "Danger signals": Exceptionally high and rapidly rising levels of AST, ALT, LDH	OrganOx metra device	RCM	20-month graft survival: 88% AS: 7 cases Bile leaks: 3 cases PNF or NAS: none

Continuation of Table 2

1	2	3	4	5	6	7
van Leeuwen et al. [58] (2019) – the **Groningen criteria**	Perfused: 16 Transplanted: 11 Utilization rate: 69%	16/0	After 2.5 hours NMP: – Lactate clearance to <1.7 mmol/L – Perfusate pH between 7.35–7.45, without repeated sodium bicarbonate infusions – Total bile production >10 mL, with at least 4 mL produced in the last hour – *Bile pH >7.45*	Liver Assist Device	HBOC	PNF: none NAS: 1 case
Bral et al. [92] (2019)	Perfused: 46 Transplanted: 43 Utilization rate: 93%	10/33	– Lactate level at the start of perfusion – Lactate clearance – Need for pH correction with sodium bicarbonate – Hourly bile production	OrganOx metra device	RCM	3-month graft survival: 100% EAD: 11 cases NAS: 2 cases AS: 6 cases
Modified Groningen criteria	Preclinical study: 23 Clinical study: 4/6 Utilization rate: 66%	6/0	After 2.5 hours of NMP: – Lactate clearance to <1.7 mmol/L – Perfusate pH between 7.35–7.45, without repeated sodium bicarbonate infusions – Total bile production >10 mL – *Bile pH >7.48*	Liver assist device	HBOC	PNF or NAS: none
Ceresa et al. [93] (2019)	Perfused: 34 Transplanted: 31 Utilization rate: 91%	8/23	Within the first 4 hours of NMP: – Lactate clearance – Glucose metabolism – Maintenance of pH without repeated sodium bicarbonate infusions – Evidence of bile production – Perfusate transaminase levels – HA and PV perfusion flows	OrganOx metra device	RCM	PNF or NAS: none
De Vries et al. [49] (2019)	Perfused: 7 Transplanted: 5 Utilization rate: 71%	7/0	After 2.5 hours of NMP: – Lactate clearance to <1.7 mmol/L – Perfusate pH between 7.35–7.45, without repeated sodium bicarbonate infusions – Bile output >10 mL total – *Bile pH >7.45*	Liver Assist Device	HBOC	PNF: none NAS: 1 case
Watson et al. [94] – the **Cambridge-criteria** (2018)	Perfused: 47 Transplanted: 22 Utilization rate: 47%	35/12	Within the first 2 hours of perfusion: – Maximal decrease in lactate level ≥4.4 mmol/L/kg/hour – ALT at 2 hours: <600 U/L – Perfusate pH >7.2, while sodium bicarbonate requirement ≤30 mmol/L – *Maximal bile pH >7.5* – *Bile glucose: ≤3 mmol/L, or at least 10 mmol/L lower than perfusate glucose* – Decrease in glucose levels after 2 hours of perfusion, or perfusate glucose <10 mmol/L with further decrease following administration of 2.5 g of glucose – ALT perfusate <6000 U/L	OrganOx Metra device	RCM leukoreduced, washed red blood cells	PNF: 1 case NAS: 4 cases EAD: 1 case
Watson et al. [84] (2017)	Perfused: 12 Transplanted: 12 Utilization rate: 100%	9/3	– Lactate clearance – Perfusate glucose level – Maintenance of pH without repeated sodium bicarbonate infusions – Perfusate transaminase levels	OrganOx metra device	RCM	PNF: 1 case NAS: 3 cases

End of Table 2

1	2	3	4	5	6	7
Birmingham criteria	Perfused: 6 Transplanted: 5 Utilization rate: 83%	4/1	Within the first 3 hours of NMP: Lactate clearance <2.5 mmol/L OR bile production plus at least two of the following criteria: – Perfusate pH >7.3 without repeated sodium bicarbonate injections – Perfusion flow >150 mL/min for HA and >500 mL/min for PV – Homogeneous parenchymal perfusion and soft allograft consistency	1–5 allografts: Liver assist device 6 allografts: OrganOx Metra device	RCM	PNF or NAS: none Transplant related complications: none

Note: HMP, hypothermic machine perfusion; SCS, static cold storage; HOPE, hypothermic oxygenated perfusion; D-HOPE, dual hypothermic oxygenated perfusion; NMP, normothermic machine perfusion; AST, Aspartate Transaminase; ALT, Alanine Transaminase; LDH, lactate dehydrogenase.

Table 3

Recommended perfusion protocols for hypothermic oxygenated perfusion (HOPE), normothermic machine perfusion (NMP), and controlled oxygenated rewarming (COR)

Parameter	HOPE [20, 63, 40]	NMP [23, 28, 40]	COR [9, 42, 46]
Perfusate temperature (°C)	8–10	36–38	8–10 °C (start) 12 °C (30 min) 16 °C (45 min) 20 °C (60 min) Sustained perfusion at 20 °C for functional assessment for up to 90 minutes, then flush with 1 L of preservative solution to reduce the temperature to 4–16 °C before implantation
Oxygenation level (pO_2, mmHg)	400–600	90–200	500
Flow – hepatic artery (mL/min)	40–70	>150–300	Not indicated
Flow – portal vein (mL/min)	300–400 (≤500)	>500	Not indicated
Pressure – hepatic artery (mmHg)	20–25	60–70	25
Pressure – portal vein (mmHg)	3–5	10–13	4

Research groups worldwide have long employed various combinations of parameters to assess the viability of both the hepatocellular and cholangiocellular compartments of the allograft [40, 56]. The main viability criteria currently used by different centers are summarized in Table 2.

A universal marker of hepatocellular viability is the measurement of perfusate lactate levels and their dynamics over the course of perfusion. Notably, current trends favor assessing the trend of lactate clearance rather than relying on absolute lactate values at fixed time points. Relying solely on static thresholds may lead to unnecessary rejection of potentially viable allografts. For example, in their study, Panconesi et al. demonstrated that when applying a lactate clearance criterion at 6 hours of NMP, only 13 (6.1%) out of 213 allografts were classified as non-viable. However, when the same cohort was evaluated using viability criteria from other centers, 14.6% of grafts would have been deemed non-viable based on the Groningen criteria and 11.2% based on the Brisbane criteria. The authors also highlighted comparable results among so-called "lactate-high" allografts [57].

An interesting observation was reported by Mergental et al.: in three allografts, lactate levels, although initially reaching the target threshold of less than 2.5 mmol/L, began to rise after 2 hours of perfusion. Two of these organs were subsequently classified as non-viable based on elevated lactate levels, while the third was transplanted due to a hepatectomy having already been performed. Notably, the recipient of this graft was alive at the time of reporting, despite experiencing EAD (ALT 2,074 U/L and AST 3,031 U/L) [33]. This case illustrates that, while lactate clearance is a key marker of viability, the criteria for interpreting lactate dynamics still require further refinement.

Assessment of bile parameters provides valuable insight into the viability of the biliary tree. It is important to distinguish that bile production primarily reflects hepatocyte function, whereas bile composition is determined by the activity of the biliary epithelium. Under normal conditions, cholangiocytes reabsorb glucose from bile and secrete bicarbonate anions into the ductal lumen, forming a protective layer known as the "bicarbonate umbrella" along the bile ducts [49]. Elevated bile glucose

Table 4

The Groningen group liver transplant viability criteria (van Leeuwen, 2022), based on the "traffic light" system. The criteria are divided into green, yellow, and red zones. In the yellow zone, the liver is borderline viable, meaning the organ might still be used depending on other indicators. In the green zone, the liver is considered optimal for transplantation with high viability, and the organ is ready for use. The red zone signifies that the liver does not meet essential viability criteria and is considered unsuitable for transplantation

	Parameter	Green zone (viable)	Yellow zone (borderline)	Red zone (unviable)
Hepatocellular link	Bile production (mL)	≥10 total (≥4 mL in the last hour)	5–10	<5
	Perfusate lactate (mmol/L)	<1.7	1.7–4	>4
	Perfusate pH	7.35–7.45	7.25–7.35	<7.25
Cholangiocellular link	Bile pH	>7.45	7.40–7.45	<7.40
	ΔpH (bile – perfusate)	>0.10	0.05–0.10	<0.05
	ΔHCO_3^- (mmol/L)	>5.0	3.0–5.0	<3.0
	ΔGlucose (bile – perfusate) (mmol/L)	<–5.0	–3.0…–5.0	>–3.0

and lactate levels, alongside decreased pH and bicarbonate concentrations, indicate biliary epithelial damage and a high risk of subsequent ischemic complications. Similar to lactate assessment, viability evaluation should focus not on absolute parameter values but rather on their relationship to corresponding perfusate parameters. For instance, van Leeuwen et al. reported a case in which a patient developed NAIC four months after liver transplantation, despite the bile pH reaching the cholangiocellular viability threshold (bile pH 7.45) [58].

In a retrospective analysis, the authors noted that the perfusate pH was 7.46. This finding led them to modify their viability criteria to include the calculation of the pH difference between bile and perfusate (Table 4). In several protocols, bile composition was not assessed, which, for example, resulted in cholangiopathy and the need for retransplantation in 4 recipients in the VITTAL study [33, 59]. Retrospective analysis further showed that 3 cases of non-anastomotic biliary strictures occurred in recipients of asystolic donor livers, where the bile was characterized by a low pH (<7.65) and low bicarbonate concentration (<25 mmol/L) [33]. In their study, Mateon et al. highlighted the critical role of bile analysis in preventing biliary complications. Based on perfusion analysis of 23 allografts and subsequent histological evaluation of bile ducts, the authors concluded that bile bicarbonate levels >18 mmol/L ($p = 0.002$), bile pH >7.48 ($p = 0.019$), bile glucose <16 mmol/L ($p = 0.013$), and a bile-to-perfusate glucose ratio <0.67 ($p = 0.013$) were associated with reduced bile duct injury. The use of these parameters as criteria for cholangiocellular viability could help prevent NAIC [60].

ISCHEMIC-FREE IMPLANTATION

A critical concern with normothermic preservation methods has been the need for re-cooling the organ via SCS following the completion of NMP. This re-cooling process results in repeated IRPI, which theoretically could exacerbate organ damage. Various strategies have been proposed to mitigate this risk by avoiding re-cooling altogether ("non-recooling"), allowing implantation of the allograft during ongoing NMP [61, 62]. However, the limited available data – based on small sample sizes ($n = 1$ and $n = 7$) and the lack of statistically significant differences between standard implantation and non-recooling groups ($p = 0.462$) – do not support a definitive recommendation for either approach. These findings may also suggest that the impact of re-cooling on overall preservation-related injury is relatively minor.

CONCLUSION

Despite its relatively recent introduction into routine clinical practice, MP of liver transplants is steadily emerging as the preferred method for preserving allografts from ECDs. According to UNOS data from 2016 to 2023, of 52,626 deceased liver donors, only 1,799 (3.5%) underwent MP. However, the use of MP has increased markedly, from 0.3% in 2016 to 15.5% in 2023 [63].

The currently available outcomes show that perfusion preservation of high-risk liver grafts yields results comparable to those achieved with standard-risk organs, significantly expanding the donor pool without a corresponding increase in complications or a decline in graft and recipient survival. For instance, in the United States, half of all allografts from DCD donors in 2023 were preserved using MP, demonstrating improved graft survival rates (HR 0.50, 95% CI 0.35–0.70, $p < 0.001$) [63, 64].

Nevertheless, most current studies evaluating MP are limited by relatively small sample sizes and lower levels of evidence. Future research is focused on multicenter randomized controlled trials, which are expected to establish perfusion preservation as the new gold standard for marginal allografts.

Choosing the optimal perfusion method for each specific allograft remains a pressing challenge. In most centers, the choice is restricted to a single type of MP,

meaning that comparisons are typically made between one perfusion modality and SCS, with few studies directly comparing different perfusion techniques.

The "upfront" use of MP, initiated at the donor site, has not demonstrated clear benefits over standard cold ischemia protocols and remains a complex, logistically demanding process. HOPE and D-HOPE, in contrast, are relatively easy to implement, safe, and have demonstrated excellent outcomes across multiple studies, including randomized controlled trials. While viability assessment during hypothermic perfusion has been advanced by the introduction of FMN measurement in the perfusate, the role of this biomarker within the broader context of perfusion preservation remains to be fully determined.

NMP alone does not offer sufficient protection against IRPI, but it enables detailed viability assessment. Importantly, studies comparing upfront and end-ischemic NMP approaches have not demonstrated significant differences in outcomes [65].

Controlled oxygenated rewarming (COR), although less widely adopted, is a physiologically sound method. When used in an end-ischemic setting, COR has been associated with improved LT outcomes, although the supporting evidence is limited by small sample sizes and single-center study designs.

Viability assessment during NMP stage has become the focus of extensive research within the field of MP. Emerging evidence suggests that the path toward establishing "ideal" viability criteria lies not in relying on isolated absolute values of individual parameters, but rather in evaluating the dynamics of their changes and the relationships between them – for example, comparing bile pH and glucose levels relative to those of the perfusate. Importantly, evaluation of cholangiocellular viability must be an integral part of viability assessment to reduce the risk of biliary complications. There is a clear need for the development of standardized, universal viability criteria, a goal that can only be achieved through large-scale, multicenter collaborative studies.

By combining the main perfusion techniques, the individual limitations of each method are effectively counterbalanced, making combined machine perfusion an especially promising approach for organ preservation. The excellent outcomes achieved with the DHOPE-COR-NMP protocol offer significant potential for further research, particularly in the use of marginal allografts. Integrating sequential MP into protocols for prolonged perfusion (lasting more than 12–24 hours) aimed at organ rehabilitation may further expand the donor pool by enabling the transplantation of initially severely damaged organs that demonstrate viability after several days of perfusion [55]. The transplant community eagerly awaits more data on the indications, broader applications, and long-term outcomes of combined perfusion strategies.

The authors declare no conflict of interest.

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