**Name of Journal:** *World Journal of Gastroenterology*

**Manuscript NO:** 65273

**Manuscript Type:** MINIREVIEWS

**Machine perfusion of the liver: Putting the puzzle pieces together**

Boteon YL *et al*. Machine perfusion of the liver

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**Received:** March 2, 2021

**Revised:** May 3, 2021

**Accepted:** July 21, 2021

**Published online:**

**Abstract**

The realm of extended criteria liver transplantation created the 'adjacent possible' for dynamic organ preservation. Machine perfusion of the liver greatly expanded donor organ preservation possibilities, reaching before unattainable goals, including the mitigation of ischemia-reperfusion injury, viability assessment, and organ reconditioning prior to transplantation. However, current scientific evidence lacks uniformity between studies, perfusion protocols, and acceptance criteria. Construction of collaborative research networks for sharing knowledge should, therefore, enable the development of high-level evidence and guidelines for machine perfusion utilization, including donor acceptance criteria. Finally, this approach shall guarantee conditions for further progress to occur.

**Key Words:** Machine perfusion of the liver; Liver transplantation; Organ donation; Extended criteria donors; Liver preservation; Clinical trials

Boteon YL, Martins PN, Muiesan P, Schlegel A. Machine perfusion of the liver: Putting the puzzle pieces together. *World J Gastroenterol* 2021; In press

**Core Tip:** The frequent use of extended criteria donor organs unveiled the limits of static cold storage and created the conditions for the renewed interest in dynamic organ preservation. Indeed, several studies have suggested the superiority of this method over static cold storage for these high-risk donors. However, controversy still exists between different machine perfusion modalities and even amongst studies employing the same method, compromising the strength of the available evidence. With the currently fragmented efforts, the development of collaborative networks will produce collective outcomes that will expand the boundaries of current knowledge.

**INTRODUCTION**

Previously disregarded and surpassed by the simplicity of static cold storage (SCS), dynamic organ preservation found the 'adjacent possible' in the realm of extended criteria donors (ECD) liver transplantation. Over the last few years, the liver transplantation community has experienced a surge in studies investigating the safety and efficacy of dynamic preservation[1-5]. This event is mainly a consequence of the increased use of ECD organs in order to attend to the growing demand for donor livers. The use of these high-risk organs exposed the limitations of SCS and posed a threat to recipients due to the risk of early allograft dysfunction and even primary non-function; thus, they are often discarded[6,7]. Therefore, at present, ECD liver transplantation has given the possibility to revisit and develop further machine perfusion techniques, previously hindered by the lack of appropriate conditions (*e.g.*, complexity, specific perfusates, and devices, costs). Figure 1 illustrates the evolution of conditions favouring the renewed interest in machine perfusion of the liver (MPL).

**DYNAMIC ORGAN PRESERVATION AND ISCHAEMIA-REPERFUSION INJURY MITIGATION**

The keystone of dynamic organ preservation is the mitigation of ischemia-reperfusion injury (IRI). IRI is responsible for the inflammatory-mediated tissue injury involved in transplantation and is ultimately associated with adverse results, such as early allograft dysfunction and primary non-function[8]. Dynamic preservation is characterized by a continuous flow of an oxygenated solution, preventing ischemic damage to the cells. In addition, the continuous flow of the perfusate provides nutrients and removes toxic metabolic waste products[7].

Diverse techniques of MPL are currently under research, while a few are currently clinically implemented. These include the *ex-situ* (normothermic, hypothermic, subnormothermic, controlled oxygenated rewarming), the *in-situ* modalities (normothermic regional perfusion, NRP), and the ischemia-free organ transplantation[2-4,6,9-12]. Furthermore, combinations of techniques have more recently gained the attention of the transplant community (*e.g.*, cold-to-warm, hypothermic plus normothermic, NRP plus hypothermic or normothermic *ex-situ* perfusion)[13-18]. Also, *ex-situ* normothermic machine perfusion (NMP) can be performed in a preservation approach during organ transportation or in an end-ischemic method, whereby the organ will be perfused after arrival at the transplant centre[7]. Hypothermic machine perfusion (HMP) can be done using an oxygen pre-charged perfusate or an actively oxygenated perfusate — with perfusion *via* portal vein exclusively (hypothermic oxygenated perfusion, HOPE) or in association with the hepatic artery (dual HOPE, D-HOPE)[3,5]. Although HMP is currently used in an end-ischemic approach, a device for hypothermic perfusion during organ transportation is under clinical investigation in the United States (clinical trials.gov, NCT number 03484455).

Albeit all these techniques hold the potential to benefit ECD livers, clearly controversy exists about the best strategy. Currently, the two most studied modalities are NMP and HMP. Both can be performed *ex-situ* in an end-ischemic approach for organs from donors after brain death (DBD) and donors after circulatory death (DCD). Furthermore, due to the need to increase DCD organ utilization avoiding its associated post-transplant complications, NRP is routinely applied in a few countries, including Spain, France, and Italy. NRP alleviates the warm ischemic injury to the organ and induces initial normothermic reperfusion in the donor. Thereby, it allows assessment of viability markers (most lactate and transaminases) which help to decide whether the organ must be declined or not. Several studies currently evidenced the benefits of this technique for DCD liver transplantation[11,19].

Despite consistently attending the primary aim to mitigate IRI, questions are still emerging about the mechanistic pathways involved in this process within each method[8,20]. In fact, benefits vary slightly between techniques mainly as a consequence of the physical properties employed by the methods and the moment of application. Table 1 describes the potential advantages of different MPL techniques in clinical studies. Notably, only three of the mentioned studies were randomized and the study of Ghinolfi *et al*[21] included only 10 patients[2,21,22].

**ORGAN VIABILITY ASSESSMENT IN LIVER TRANSPLANTATION: CONTINUOUS FLOW FOR REAL-TIME ANALYSIS**

Once inducing reperfusion, whilst NMP abbreviates the cold ischemia time, it results in full activation of the aerobic mitochondrial respiration with reactive oxygen species production and release. Further downstream processes are triggered with subsequent oxidative tissue damage and initiation of the inflammatory cascade[8,23]. The fully physiologically active organ allows real-time assessment of its metabolic functioning, which is currently mostly based on lactate clearance and bile production[6,24]. However, definitive viability criteria are not yet characterized and validated[25].

We are currently transitioning from a combination of hepatocyte function-based criteria to a more comprehensive assessment that incorporates cholangiocyte function. The Birmingham group developed pioneer clinical viability criteria, which included: lactate clearance, evidence of bile production, perfusate pH stability, hepatic artery flow and portal vein flow, and homogeneous graft perfusion with soft consistency of parenchyma[6]. Later, Watson *et al*[24] advocated using changes in lactate, in glucose and transaminases, and the ability to maintain a stable perfusate pH without bicarbonate supplementation to consider an organ viable. Most recently, the Groningen group highlighted the importance of bile composition analysis during viability assessment (biliary bicarbonate, pH, glucose, lactate dehydrogenase); and their viability criteria already included bile flow and its properties on the analysis[26]. Another study, evaluating discarded donor livers, demonstrated a low hepatocellular injury during NMP; with, however, an elevated bile duct injury on histology[27]. Notably, the impact of MPL on the peribiliary glands and peribiliary vascular plexus, rather than solely bile duct injury, deserves attention. This is because if damage to the bile duct cannot be avoided, establishing a healthy environment to the peribiliary glands contributes to the regenerative capacity of this structure, possibly preventing the development of nonanastomotic strictures[28-30]. The relevance of biliary biomarkers to assess cholangiocyte function and bile duct viability is currently a research topic in liver transplantation[31].

The importance of biliary analysis was indirectly reinforced by another study investigating discarded donor livers, the VITTAL (Viability testing and transplantation of marginal livers) trial[6]. Despite excellent synthetic function with isolated bile flow assessment, a significant proportion of organs developed biliary complications, even requiring retransplantation[6]. In accordance, recently, Eshmuminov *et al*[32] questioned the value of bile formation as a marker of a deteriorating liver function. These findings were paralleled by previous studies, concluding that continuous bile production alone appears not sufficient enough to evaluate liver viability during *ex-situ* machine perfusion[27]. In this study, porcine and human livers were perfused for up to 1 wk and the authors demonstrated good bile production once appropriate drug-induced stimuli were offered[27]. Finally, they suggest the response to vasoconstrictors and hormones as viability markers for liver function. Interestingly, part of the viable livers included in the study from Eshmuminov *et al*[27,33] was initially declined for transplantation based on elevated flavin mononucleotide (FMN) levels during HOPE[34].

Conversely, HMP —usually performed at temperatures between 4 °C and 12 °C — keeps the organ at a low metabolic rate, making the assessment of most of its function more challenging. Despite that, actively oxygenated HMP techniques act seemingly on the mitochondria, restoring the electron transport chain and enhancing the mitochondrial oxidative phosphorylation and adenosine triphosphate production[8,23]. Despite the different metabolic status during HMP, more than 2000 molecules can be quantified from perfusates[34]. Recent viability assessment during HOPE focused on mitochondria function and injury. Muller *et al*[34] identified the first biomarker of organ viability during HMP, the FMN. FMN is a molecule in mitochondria complex I, which is released during organ reperfusion concomitantly and from the same pocket as reactive oxygen species[35,36]. The number of released FMN molecules was also assessed during different perfusion modalities and authors found a higher FMN release during normothermic liver perfusion[36]. Therefore, ultimately, it reflects the severity of IRI, the mitochondrial injury and dysfunction[36]. The authors from Zurich found a strong correlation between perfusate FMN levels' — fluorometric readings after 30 min of HMP — and post-transplant outcome parameters (hospital stay, cumulative complications, and 90-d graft loss)[34]. Importantly, based on the autofluorescence properties, FMN can be measured in real-time using a spectrometer[34]. This mitochondrial parameter was further validated during NRP and NMP in kidneys and livers. Data presented by Wang *et al*[36] supported the entire concept of mitochondrial injury quantification through FMN release and concluded that this marker appears useful during various preservation approaches.

Table 2 summarizes parameters tested in clinical studies to assess the viability of donor organs during MPL prior to transplantation. Importantly, current studies included different parameters within their viability criteria. In addition, even when the same parameters were investigated, thresholds and the timepoints analysed varied.

**A WAY BEYOND PRESERVATION: ORGAN RECONDITIONING**

Moving organ preservation forward, MPL extrapolates the simple concept of mitigation of donor organ damage before transplantation; in the interim, the dynamic preservation aims to improve the quality of the donated organs or, in other words, recondition them. This step further is fundamental to increase ECD organ utilization once they were exposed to more severe damage and carry a high risk to receptors if SCS is applied.

Non-randomized single-centre clinical studies have confirmed the ability of HMP to enhance the results of ECD liver transplantation[3,10,37]. Dutkowski *et al*[37] suggested that high-risk DCD organs — associated with most severe IRI, poor clinical outcomes, and a high rate of post-operative complications — submitted to the HOPE technique for 1-2 h prior to transplantation have similar outcomes to standard DBD organs, including the length of hospital stay and biliary complications. Later, the same group compared HOPE-treated DCD livers to a matched cohort of DCD organs without further intervention. The perfused livers presented lower post-transplant alanine aminotransferase levels, decreased biliary complication rate, and increased 1-year graft survival[3]. More recently, Schlegel *et al*[38] reported a 5-year graft survival of 94% for transplanted HOPE-treated DCD livers against 78% for untreated DCD organs. Similar benefits were also found in D-HOPE studies led by the Groningen group[30]. van Rijn *et al*[22] recently published a randomized clinical trial on HMP with 160 patients transplanted with DCD organs. The study reached its primary endpoint, D-HOPE-perfused DCD livers had a lower incidence of symptomatic non-anastomotic biliary stricture (6%) compared to 18% in the control group (*P* = 0.03). This study also demonstrated that D-HOPE reduced the occurrence of early allograft dysfunction post-transplantation[22].

Importantly, evolving evidence supports the safety of performing D-HOPE for up to 24 h, which may improve transplant logistics[39]. A recent study suggested that HOPE plays a protective role in preventing hepatocellular carcinoma recurrence after liver transplantation, which is possible due to protection from IRI, downstream inflammation, and subsequent pro-tumour-recurrence environment[40,41].

A large multicentre trial recently evidenced preservation NMP was able to reduce in 50% the hepatocellular enzyme releases post-operatively, increasing organ utilization rate and preservation time[2]. Nevertheless, NMP did not reduce biliary complications rate post-transplant and, also, did not impact on graft or patient survival[2]. Similar results were found in another study wherein end-ischemic NMP was used[42]. The Birmingham group applied NMP to discarded donor livers, 71% of the perfused organs were transplanted. Although this study reported 100% 90-d patient and graft survival, perfusion did not prevent non-anastomotic biliary stricture (incidence of 18%), and four recipients required retransplantation within 542 d[6]. Albeit the near-physiological conditions of donor organs during NMP favours the assessment of their metabolic functions, the ability of NMP per se to recondition ECD organs is still controversial. Whether a severely damaged organ would recover after activation of the damaging IRI machinery during NMP without any further intervention is not clear. Seemingly, additional support must be required[8,13,43,44].

The uncertainty about NMP reconditioning properties is counterbalanced by evident advantages. The organ's full metabolism facilitates its viability assessment, increases the surgeon's confidence, and allows safe prolonged organ preservation[2,6]. This ambiguity opened up an opportunity for further additions to the technique, for example, combinations of perfusion modalities and pharmacological interventions[15,43]. Combinations of perfusion modalities may merge both techniques' benefits; experimental and clinical studies have already suggested the possibility to enhance the reconditioning of the most severely damaged organs with the realization of HMP before NMP[13-15,17]. Similarly, pharmacological interventions may mitigate IRI and improve donor organ metabolic recovery, which has been suggested in pre-clinical studies investigating discarded steatotic donor livers[43]. For a comprehensive review on pharmacological agents used in MPL, please refer to Resch *et al*[45].

**FINAL CONSIDERATIONS**

Although several studies currently have demonstrated the benefits of dynamic organ preservation, limitations of this evidence exist. This is because significant variability of technicalities occurs even between studies employing the same method. For example, for NMP, parameters such as the rewarming period, arterial and portal vein perfusion pressure, and perfusate constitution vary between studies. Similarly, studies diverge on the perfusion duration, either replacing SCS during organ transportation or performed as an end-ischemic approach at the recipient transplant centre. For HMP, similar challenges also occur. So far, most studies focused on organs with a short period of cold ischemia time and, also, the level of perfusate oxygenation differs between them. Adopting standardized protocols might enable the construction of large perfusion databases, which may unveil further advantages of MPL.

In addition, the foundation of large networks to develop studies collaboratively must benefit even further the era of machine perfusion in liver transplantation. The development of large collaborative perfusion groups working collectively may allow reliable comparison between perfusion modalities and, even, identify which technology is most beneficial to each specific organ.

**CONCLUSION**

Over the last few years, machine perfusion in liver transplantation gathered momentum, though it seems the construction of definitive evidence is still needed. This is mainly because of current disjointed individual efforts with non-standardization of methods and publication of single-centre non-randomized small studies. Dynamic organ preservation must thrive on collaborative networks wherein collective outcomes must consolidate its actual benefits (viability assessment, prolonged organ preservation, and organ reconditioning) and create the conditions for further progress to occur. Pharmacological *ex-situ* treatments and investigation of the impact of this technology on specific donor organs are some possibilities within reach, lying in the realm of the possible.

**ACKNOWLEDGEMENTS**

This paper presents independent research supported by the Brazilian Ministry of Health *via* the Support Program for Organizational Development of the SUS (PROADI-SUS) at the Hospital Israelita Albert Einstein. The views expressed are those of the author(s) and not necessarily those of the Ministry of Health, the PROADI-SUS, or the Hospital Israelita Albert Einstein. We are extremely grateful to the staff from the Hospital Israelita Albert Einstein and Hospital Municipal Vila Santa Catarina, whose continued support provides resources and intellectual input that is shaping the thoughts and future strategies for the continuing development of our research.

**REFERENCES**

1 **Serifis N**, Matheson R, Cloonan D, Rickert CG, Markmann JF, Coe TM. Machine Perfusion of the Liver: A Review of Clinical Trials. *Front Surg* 2021; **8**: 625394 [PMID: 33842530 DOI: 10.3389/fsurg.2021.625394]

2 **Nasralla D**, Coussios CC, Mergental H, Akhtar MZ, Butler AJ, Ceresa CDL, Chiocchia V, Dutton SJ, García-Valdecasas JC, Heaton N, Imber C, Jassem W, Jochmans I, Karani J, Knight SR, Kocabayoglu P, Malagò M, Mirza D, Morris PJ, Pallan A, Paul A, Pavel M, Perera MTPR, Pirenne J, Ravikumar R, Russell L, Upponi S, Watson CJE, Weissenbacher A, Ploeg RJ, Friend PJ; Consortium for Organ Preservation in Europe. A randomized trial of normothermic preservation in liver transplantation. *Nature* 2018; **557**: 50-56 [PMID: 29670285 DOI: 10.1038/s41586-018-0047-9]

3 **Dutkowski P**, Polak WG, Muiesan P, Schlegel A, Verhoeven CJ, Scalera I, DeOliveira ML, Kron P, Clavien PA. First Comparison of Hypothermic Oxygenated PErfusion Versus Static Cold Storage of Human Donation After Cardiac Death Liver Transplants: An International-matched Case Analysis. *Ann Surg* 2015; **262**: 764-70; discussion 770-1 [PMID: 26583664 DOI: 10.1097/SLA.0000000000001473]

4 **op den Dries S**, Karimian N, Sutton ME, Westerkamp AC, Nijsten MW, Gouw AS, Wiersema-Buist J, Lisman T, Leuvenink HG, Porte RJ. Ex vivo normothermic machine perfusion and viability testing of discarded human donor livers. *Am J Transplant* 2013; **13**: 1327-1335 [PMID: 23463950 DOI: 10.1111/ajt.12187]

5 **Graham JA**, Guarrera JV. "Resuscitation" of marginal liver allografts for transplantation with machine perfusion technology. *J Hepatol* 2014; **61**: 418-431 [PMID: 24768755 DOI: 10.1016/j.jhep.2014.04.019]

6 **Mergental H**, Laing RW, Kirkham AJ, Perera MTPR, Boteon YL, Attard J, Barton D, Curbishley S, Wilkhu M, Neil DAH, Hübscher SG, Muiesan P, Isaac JR, Roberts KJ, Abradelo M, Schlegel A, Ferguson J, Cilliers H, Bion J, Adams DH, Morris C, Friend PJ, Yap C, Afford SC, Mirza DF. Transplantation of discarded livers following viability testing with normothermic machine perfusion. *Nat Commun* 2020; **11**: 2939 [PMID: 32546694 DOI: 10.1038/s41467-020-16251-3]

7 **Ceresa CDL**, Nasralla D, Jassem W. Normothermic Machine Preservation of the Liver: State of the Art. *Curr Transplant Rep* 2018; **5**: 104-110 [PMID: 29564207 DOI: 10.1007/s40472-018-0186-9]

8 **Gilbo N**, Catalano G, Salizzoni M, Romagnoli R. Liver graft preconditioning, preservation and reconditioning. *Dig Liver Dis* 2016; **48**: 1265-1274 [PMID: 27448845 DOI: 10.1016/j.dld.2016.06.031]

9 **Hoyer DP**, Mathé Z, Gallinat A, Canbay AC, Treckmann JW, Rauen U, Paul A, Minor T. Controlled Oxygenated Rewarming of Cold Stored Livers Prior to Transplantation: First Clinical Application of a New Concept. *Transplantation* 2016; **100**: 147-152 [PMID: 26479280 DOI: 10.1097/TP.0000000000000915]

10 **Guarrera JV**, Henry SD, Samstein B, Reznik E, Musat C, Lukose TI, Ratner LE, Brown RS Jr, Kato T, Emond JC. Hypothermic machine preservation facilitates successful transplantation of "orphan" extended criteria donor livers. *Am J Transplant* 2015; **15**: 161-169 [PMID: 25521639 DOI: 10.1111/ajt.12958]

11 **Watson CJE**, Hunt F, Messer S, Currie I, Large S, Sutherland A, Crick K, Wigmore SJ, Fear C, Cornateanu S, Randle LV, Terrace JD, Upponi S, Taylor R, Allen E, Butler AJ, Oniscu GC. In situ normothermic perfusion of livers in controlled circulatory death donation may prevent ischemic cholangiopathy and improve graft survival. *Am J Transplant* 2019; **19**: 1745-1758 [PMID: 30589499 DOI: 10.1111/ajt.15241]

12 **He X**, Guo Z, Zhao Q, Ju W, Wang D, Wu L, Yang L, Ji F, Tang Y, Zhang Z, Huang S, Wang L, Zhu Z, Liu K, Zhu Y, Gao Y, Xiong W, Han M, Liao B, Chen M, Ma Y, Zhu X, Huang W, Cai C, Guan X, Li XC, Huang J. The first case of ischemia-free organ transplantation in humans: A proof of concept. *Am J Transplant* 2018; **18**: 737-744 [PMID: 29127685 DOI: 10.1111/ajt.14583]

13 **Westerkamp AC**, Karimian N, Matton AP, Mahboub P, van Rijn R, Wiersema-Buist J, de Boer MT, Leuvenink HG, Gouw AS, Lisman T, Porte RJ. Oxygenated Hypothermic Machine Perfusion After Static Cold Storage Improves Hepatobiliary Function of Extended Criteria Donor Livers. *Transplantation* 2016; **100**: 825-835 [PMID: 26863473 DOI: 10.1097/TP.0000000000001081]

14 **de Vries Y**, Matton APM, Nijsten MWN, Werner MJM, van den Berg AP, de Boer MT, Buis CI, Fujiyoshi M, de Kleine RHJ, van Leeuwen OB, Meyer P, van den Heuvel MC, de Meijer VE, Porte RJ. Pretransplant sequential hypo- and normothermic machine perfusion of suboptimal livers donated after circulatory death using a hemoglobin-based oxygen carrier perfusion solution. *Am J Transplant* 2019; **19**: 1202-1211 [PMID: 30588774 DOI: 10.1111/ajt.15228]

15 **van Leeuwen OB**, de Vries Y, Fujiyoshi M, Nijsten MWN, Ubbink R, Pelgrim GJ, Werner MJM, Reyntjens KMEM, van den Berg AP, de Boer MT, de Kleine RHJ, Lisman T, de Meijer VE, Porte RJ. Transplantation of High-risk Donor Livers After Ex Situ Resuscitation and Assessment Using Combined Hypo- and Normothermic Machine Perfusion: A Prospective Clinical Trial. *Ann Surg* 2019; **270**: 906-914 [PMID: 31633615 DOI: 10.1097/SLA.0000000000003540]

16 **Friend PJ**, Dengu F. Expanding the Scope of Donation After Circulatory Death Liver Transplantation. *Liver Transpl* 2021; **27**: 325-326 [PMID: 33098747 DOI: 10.1002/lt.25924]

17 **De Carlis L**, De Carlis R, Lauterio A, Di Sandro S, Ferla F, Zanierato M. Sequential Use of Normothermic Regional Perfusion and Hypothermic Machine Perfusion in Donation After Cardiac Death Liver Transplantation With Extended Warm Ischemia Time. *Transplantation* 2016; **100**: e101-e102 [PMID: 27495774 DOI: 10.1097/TP.0000000000001419]

18 **Ghinolfi D**, Dondossola D, Rreka E, Lonati C, Pezzati D, Cacciatoinsilla A, Kersik A, Lazzeri C, Zanella A, Peris A, Maggioni M, Biancofiore G, Reggiani P, Morganti R, De Simone P, Rossi G. Sequential Use of Normothermic Regional and Ex Situ Machine Perfusion in Donation After Circulatory Death Liver Transplant. *Liver Transpl* 2021; **27**: 385-402 [PMID: 32949117 DOI: 10.1002/lt.25899]

19 **Savier E**, Lim C, Rayar M, Orlando F, Boudjema K, Mohkam K, Lesurtel M, Mabrut JY, Pittau G, Begdadi N, Cherqui D, Adam R, Dondero F, Sepulveda A, Soubrane O, Bucur P, Barbier L, Salame E, Jasseron C, Antoine C, Riou B, Scatton O. Favorable Outcomes of Liver Transplantation from Controlled Circulatory Death Donors Using Normothermic Regional Perfusion Compared to Brain Death Donors. *Transplantation* 2020; **104**: 1943-1951 [PMID: 32639402 DOI: 10.1097/TP.0000000000003372]

20 **Weissenbacher A**, Vrakas G, Nasralla D, Ceresa CDL. The future of organ perfusion and re-conditioning. *Transpl Int* 2019; **32**: 586-597 [PMID: 30980772 DOI: 10.1111/tri.13441]

21 **Ghinolfi D**, Rreka E, De Tata V, Franzini M, Pezzati D, Fierabracci V, Masini M, Cacciatoinsilla A, Bindi ML, Marselli L, Mazzotti V, Morganti R, Marchetti P, Biancofiore G, Campani D, Paolicchi A, De Simone P. Pilot, Open, Randomized, Prospective Trial for Normothermic Machine Perfusion Evaluation in Liver Transplantation From Older Donors. *Liver Transpl* 2019; **25**: 436-449 [PMID: 30362649 DOI: 10.1002/lt.25362]

22 **van Rijn R**, Schurink IJ, de Vries Y, van den Berg AP, Cortes Cerisuelo M, Darwish Murad S, Erdmann JI, Gilbo N, de Haas RJ, Heaton N, van Hoek B, Huurman VAL, Jochmans I, van Leeuwen OB, de Meijer VE, Monbaliu D, Polak WG, Slangen JJG, Troisi RI, Vanlander A, de Jonge J, Porte RJ; DHOPE-DCD Trial Investigators. Hypothermic Machine Perfusion in Liver Transplantation - A Randomized Trial. *N Engl J Med* 2021; **384**: 1391-1401 [PMID: 33626248 DOI: 10.1056/NEJMoa2031532]

23 **Hofmann J**, Otarashvili G, Meszaros A, Ebner S, Weissenbacher A, Cardini B, Oberhuber R, Resch T, Öfner D, Schneeberger S, Troppmair J, Hautz T. Restoring Mitochondrial Function While Avoiding Redox Stress: The Key to Preventing Ischemia/Reperfusion Injury in Machine Perfused Liver Grafts? *Int J Mol Sci* 2020; **21**: 3132 [PMID: 32365506 DOI: 10.3390/ijms21093132]

24 **Watson CJE**, Kosmoliaptsis V, Pley C, Randle L, Fear C, Crick K, Gimson AE, Allison M, Upponi S, Brais R, Jochmans I, Butler AJ. Observations on the ex situ perfusion of livers for transplantation. *Am J Transplant* 2018; **18**: 2005-2020 [PMID: 29419931 DOI: 10.1111/ajt.14687]

25 **Watson CJE**, Jochmans I. From "Gut Feeling" to Objectivity: Machine Preservation of the Liver as a Tool to Assess Organ Viability. *Curr Transplant Rep* 2018; **5**: 72-81 [PMID: 29564205 DOI: 10.1007/s40472-018-0178-9]

26 **Matton APM**, de Vries Y, Burlage LC, van Rijn R, Fujiyoshi M, de Meijer VE, de Boer MT, de Kleine RHJ, Verkade HJ, Gouw ASH, Lisman T, Porte RJ. Biliary Bicarbonate, pH, and Glucose Are Suitable Biomarkers of Biliary Viability During Ex Situ Normothermic Machine Perfusion of Human Donor Livers. *Transplantation* 2019; **103**: 1405-1413 [PMID: 30395120 DOI: 10.1097/TP.0000000000002500]

27 **Eshmuminov D**, Schuler MJ, Becker D, Bautista Borrego L, Mueller M, Hagedorn C, Häusler S, Steiger J, Tibbitt MW, Dutkowski P, Rudolf von Rohr P, Stieger B, Hefti M, Clavien PA. Bile formation in long-term ex situ perfused livers. *Surgery* 2021; **169**: 894-902 [PMID: 33422346 DOI: 10.1016/j.surg.2020.11.042]

28 **de Jong IEM**, Matton APM, van Praagh JB, van Haaften WT, Wiersema-Buist J, van Wijk LA, Oosterhuis D, Iswandana R, Suriguga S, Overi D, Lisman T, Carpino G, Gouw ASH, Olinga P, Gaudio E, Porte RJ. Peribiliary Glands Are Key in Regeneration of the Human Biliary Epithelium After Severe Bile Duct Injury. *Hepatology* 2019; **69**: 1719-1734 [PMID: 30506902 DOI: 10.1002/hep.30365]

29 **Weeder PD**, van Rijn R, Porte RJ. Machine perfusion in liver transplantation as a tool to prevent non-anastomotic biliary strictures: Rationale, current evidence and future directions. *J Hepatol* 2015; **63**: 265-275 [PMID: 25770660 DOI: 10.1016/j.jhep.2015.03.008]

30 **van Rijn R**, van Leeuwen OB, Matton APM, Burlage LC, Wiersema-Buist J, van den Heuvel MC, de Kleine RHJ, de Boer MT, Gouw ASH, Porte RJ. Hypothermic oxygenated machine perfusion reduces bile duct reperfusion injury after transplantation of donation after circulatory death livers. *Liver Transpl* 2018; **24**: 655-664 [PMID: 29369470 DOI: 10.1002/lt.25023]

31 **Gaurav R**, Atulugama N, Swift L, Butler AJ, Upponi S, Brais R, Allison M, Watson CJE. Bile Biochemistry Following Liver Reperfusion in the Recipient and Its Association With Cholangiopathy. *Liver Transpl* 2020; **26**: 1000-1009 [PMID: 32108995 DOI: 10.1002/lt.25738]

32 **Watson CJE**, Kosmoliaptsis V, Randle LV, Gimson AE, Brais R, Klinck JR, Hamed M, Tsyben A, Butler AJ. Normothermic Perfusion in the Assessment and Preservation of Declined Livers Before Transplantation: Hyperoxia and Vasoplegia-Important Lessons From the First 12 Cases. *Transplantation* 2017; **101**: 1084-1098 [PMID: 28437389 DOI: 10.1097/TP.0000000000001661]

33 **Eshmuminov D**, Becker D, Bautista Borrego L, Hefti M, Schuler MJ, Hagedorn C, Muller X, Mueller M, Onder C, Graf R, Weber A, Dutkowski P, Rudolf von Rohr P, Clavien PA. An integrated perfusion machine preserves injured human livers for 1 week. *Nat Biotechnol* 2020; **38**: 189-198 [PMID: 31932726 DOI: 10.1038/s41587-019-0374-x]

34 **Muller X**, Schlegel A, Kron P, Eshmuminov D, Würdinger M, Meierhofer D, Clavien PA, Dutkowski P. Novel Real-time Prediction of Liver Graft Function During Hypothermic Oxygenated Machine Perfusion Before Liver Transplantation. *Ann Surg* 2019; **270**: 783-790 [PMID: 31592808 DOI: 10.1097/SLA.0000000000003513]

35 **Chouchani ET**, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord ENJ, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa ASH, Brookes PS, Davidson SM, Duchen MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T, Murphy MP. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature* 2014; **515**: 431-435 [PMID: 25383517 DOI: 10.1038/nature13909]

36 **Wang L**, Thompson E, Bates L, Pither TL, Hosgood SA, Nicholson ML, Watson CJE, Wilson C, Fisher AJ, Ali S, Dark JH. Flavin Mononucleotide as a Biomarker of Organ Quality-A Pilot Study. *Transplant Direct* 2020; **6**: e600 [PMID: 32904032 DOI: 10.1097/TXD.0000000000001046]

37 **Dutkowski P**, Schlegel A, de Oliveira M, Müllhaupt B, Neff F, Clavien PA. HOPE for human liver grafts obtained from donors after cardiac death. *J Hepatol* 2014; **60**: 765-772 [PMID: 24295869 DOI: 10.1016/j.jhep.2013.11.023]

38 **Schlegel A**, Muller X, Kalisvaart M, Muellhaupt B, Perera MTPR, Isaac JR, Clavien PA, Muiesan P, Dutkowski P. Outcomes of DCD liver transplantation using organs treated by hypothermic oxygenated perfusion before implantation. *J Hepatol* 2019; **70**: 50-57 [PMID: 30342115 DOI: 10.1016/j.jhep.2018.10.005]

39 **Brüggenwirth IMA**, van Leeuwen OB, de Vries Y, Bodewes SB, Adelmeijer J, Wiersema-Buist J, Lisman T, Martins PN, de Meijer VE, Porte RJ. Extended hypothermic oxygenated machine perfusion enables *ex situ* preservation of porcine livers for up to 24 hours. *JHEP Rep* 2020; **2**: 100092 [PMID: 32195456 DOI: 10.1016/j.jhepr.2020.100092]

40 **Mueller M**, Kalisvaart M, O'Rourke J, Shetty S, Parente A, Muller X, Isaac J, Muellhaupt B, Muiesan P, Shah T, Clavien PA, Schlegel A, Dutkowski P. Hypothermic Oxygenated Liver Perfusion (HOPE) Prevents Tumor Recurrence in Liver Transplantation From Donation After Circulatory Death. *Ann Surg* 2020; **272**: 759-765 [PMID: 32889870 DOI: 10.1097/SLA.0000000000004258]

41 **Li CX**, Man K, Lo CM. The Impact of Liver Graft Injury on Cancer Recurrence Posttransplantation. *Transplantation* 2017; **101**: 2665-2670 [PMID: 28665890 DOI: 10.1097/TP.0000000000001844]

42 **Ceresa CDL**, Nasralla D, Watson CJE, Butler AJ, Coussios CC, Crick K, Hodson L, Imber C, Jassem W, Knight SR, Mergental H, Ploeg RJ, Pollok JM, Quaglia A, Shapiro AMJ, Weissenbacher A, Friend PJ. Transient Cold Storage Prior to Normothermic Liver Perfusion May Facilitate Adoption of a Novel Technology. *Liver Transpl* 2019; **25**: 1503-1513 [PMID: 31206217 DOI: 10.1002/lt.25584]

43 **Goumard C**, Turco C, Sakka M, Aoudjehane L, Lesnik P, Savier E, Conti F, Scatton O. Ex-Vivo Pharmacological Defatting of the Liver: A Review. *J Clin Med* 2021; **10**: 1253 [PMID: 33803539 DOI: 10.3390/jcm10061253]

44 **Harada N**, Yoshizumi T, Mori M. Current review of machine perfusion in liver transplantation from the Japanese perspective. *Surg Today* 2021 epub ahead of print [PMID: 33754175 DOI: 10.1007/s00595-021-02265-x]

45 **Resch T**, Cardini B, Oberhuber R, Weissenbacher A, Dumfarth J, Krapf C, Boesmueller C, Oefner D, Grimm M, Schneeberger S. Transplanting Marginal Organs in the Era of Modern Machine Perfusion and Advanced Organ Monitoring. *Front Immunol* 2020; **11**: 631 [PMID: 32477321 DOI: 10.3389/fimmu.2020.00631]

46 **Oniscu GC**, Randle LV, Muiesan P, Butler AJ, Currie IS, Perera MT, Forsythe JL, Watson CJ. In situ normothermic regional perfusion for controlled donation after circulatory death--the United Kingdom experience. *Am J Transplant* 2014; **14**: 2846-2854 [PMID: 25283987 DOI: 10.1111/ajt.12927]

47 **Hoyer DP**, Benkö T, Manka P, von Horn C, Treckmann JW, Paul A, Minor T. Long-term Outcomes After Controlled Oxygenated Rewarming of Human Livers Before Transplantation. *Transplant Direct* 2020; **6**: e542 [PMID: 32309628 DOI: 10.1097/TXD.0000000000000987]

48 **Zhang Z**, Tang Y, Zhao Q, Wang L, Zhu C, Ju W, Wang D, Yang L, Wu L, Chen M, Huang S, Gao N, Zhu Z, Zhang Y, Sun C, Xiong W, Shen Y, Ma Y, Hu A, Zhu X, Rong J, Cai C, Guo Z, He X. Association of Perfusion Characteristics and Posttransplant Liver Function in Ischemia-Free Liver Transplantation. *Liver Transpl* 2020; **26**: 1441-1454 [PMID: 32542994 DOI: 10.1002/lt.25825]

49 **Chen M**, Chen Z, Lin X, Hong X, Ma Y, Huang C, He X, Ju W. Application of ischaemia-free liver transplantation improves prognosis of patients with steatotic donor livers - a retrospective study. *Transpl Int* 2021 [PMID: 33484201 DOI: 10.1111/tri.13828]

50 **Cardini B**, Oberhuber R, Fodor M, Hautz T, Margreiter C, Resch T, Scheidl S, Maglione M, Bösmüller C, Mair H, Frank M, Augustin F, Griesmacher A, Schennach H, Martini J, Breitkopf R, Eschertzhuber S, Pajk W, Obwegeser A, Tilg H, Watson C, Öfner D, Weissenbacher A, Schneeberger S. Clinical Implementation of Prolonged Liver Preservation and Monitoring Through Normothermic Machine Perfusion in Liver Transplantation. *Transplantation* 2020; **104**: 1917-1928 [PMID: 32371845 DOI: 10.1097/TP.0000000000003296]

51 **Reiling J**, Butler N, Simpson A, Hodgkinson P, Campbell C, Lockwood D, Bridle K, Santrampurwala N, Britton L, Crawford D, Dejong CHC, Fawcett J. Assessment and Transplantation of Orphan Donor Livers: A Back-to-Base Approach to Normothermic Machine Perfusion. *Liver Transpl* 2020; **26**: 1618-1628 [PMID: 32682340 DOI: 10.1002/lt.25850]

**Footnotes**

**Conflict-of-interest statement:** The authors have no conflicts of interest to disclose.

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**Manuscript source:** Invited manuscript

**Corresponding Author's Membership in Professional Societies:** International Liver Transplantation Society; Academia Nacional de Medicina; The Transplantation Society

**Peer-review started:** March 2, 2021

**First decision:** April 17, 2021

**Article in press:**

**Specialty type:** Transplantation

**Country/Territory of origin:** Brazil

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Baiocchi L, Zhou S **S-Editor:** Liu M **L-Editor:** Filipodia **P-Editor:**

**Figure Legends**



**Figure 1** **Conditions favouring the development of machine perfusion of the liver.** At the start of transplantation, the utilization of good quality donor organs and the low price and simplicity of static cold storage (SCS) favoured its general use. However, the rise in demand for liver transplantation and the donor organ demographic change imposed a frequent utilization of extended criteria donor (ECD) organs. ECD liver transplantation presented inferior results, exposing the limitations of SCS. This scenario, allied to the technological development, created a favourable environment and renewed interest in dynamic organ preservation.

**Table 1 Potential benefits of the different modalities of machine perfusion of the liver on clinical studies**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Safety and feasibility** | **Prevention of early allograft dysfunction** | **Prevention of biliary complications** | **Prolonged preservation time** | **Viability assessment** | **Therapeutic interventions** |
| Normothermic regional perfusion1 | Yes[11,19,46] | Yes[11,19,46] | Yes[11,19,46] | No data available yet | Yes[11,46] | No data available yet |
| Normothermic machine perfusion | Yes[2,6,21,42,50,51] | Yes[2,6,42] | No data available yet | Yes[2,6] | Yes[6,24,26,50,51] | Yes[43]2 |
| Subnormothermic machine perfusion | No data available yet | No data available yet | No data available yet | No data available yet | No data available yet | No data available yet |
| Hypothermic machine perfusion | Yes[3,37] | Yes[3,37] | Yes[3,22,37] | Yes[39] 2 | Yes[34] | No data available yet |
| Controlled oxygenated rewarming | Yes[9,47] | Yes[9,47] | No data available yet | No data available yet | No data available yet | No data available yet |
| Combined modalities (HMP + NMP) | Yes[14,15]  | No data available yet | No data available yet | Yes[39] 2 | Yes[14,15]  | No data available yet |
| Ischaemia-free liver transplantation3 | Yes[12,48] | Yes[48,49] | No data available yet | No data available yet | No data available yet | No data available yet |

1Only suitable for donors after circulatory death; 2Only pre-clinical studies available so far; 3Only suitable for donors after brain death. HMP: Hypothermic machine perfusion; NMP: Normothermic machine perfusion.

**Table 2 Parameters employed in clinical studies to assess the viability of donor organs before transplantation**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ref.** | **Method** | **Perfusate parameters** | **Bile parameters** | **Perfusion parameters** |
| Watson *et al*[11] | NRP | Within 2 h of NRP: Alanine transaminase up to 500 IU/L, providing no continued rise between the first and second hours |  |  |
| Muller *et al*[34] | HMP | At least 1 h of HMP: Threshold at 10000 A.U. (100 ng FMN/mL perfusate) to refuse livers regardless of the use of a DCD or DBD liver |  |  |
| Watson *et al*[32] | NMP | No timepoint defined: Changes in lactate, glucose, and transaminase concentrations and the ability of the liver to maintain perfusate pH without supplemental bicarbonate |  |  |
| Matton *et al*[26] | NMP |  | Within 2.5 h of NMP: Bicarbonate concentration > 18 mmol/L, pH > 7.48, glucose concentration < 16 mmol/L, bile/perfusate glucose concentration ratio < 0.67, and LDH concentration < 3689 U/L |  |
| de Vries *et al*[14] | NMP | Within 150 min of NMP: Lactate concentration within “normal” values (0.5-1.7 mmol/L); pH within normal values (7.35-7.45), without continuing sodium bicarbonate supplementation | Within 150 min of NMP: Cumulative bile production of ≥ 10 mL and ≥ 4 mL in the preceding hour. pH > 7.45 |  |
| Mergental *et al*[6] | NMP | Within 4 h of NMP: Lactate ≤ 2.5 mmol/L; and two or more parameters: pH ≥ 7.30; Evidence of glucose metabolism | Within 4 h of NMP: Evidence of bile production | Within 4 h of NMP: HA flow ≥ 150 mL/min and PV flow ≥ 500 mL/min; and homogenous perfusion of the parenchyma |
| Cardini *et al*[50] | NMP | Within 2 h of NMP: Rapid decrease and maintenance of lactate to physiological levels, maintenance of physiological pH values without sodium bicarbonate supplementation, and glucose consumption. Remarkably high transaminases and LDH levels and a severe rise of these parameters are deemed warning signals | Within 2 h of NMP: Bile production and bile pH are indicators for bile duct viability and function |  |
| Reiling *et al*[51] | NMP | Within 2 h of NMP: Lactate clearance to < 2 mmol/L. Within 4 h of NMP: Evidence of glucose metabolism. Maintenance of physiological pH without continuing administration of sodium bicarbonate | Within 4 h of NMP: Bile production (no lower limit) | Within 4 h of NMP: Stable hepatic arterial and portal venous flows. Homogeneous graft perfusion with soft consistency of parenchyma |

DCD: Donation after circulatory death; DBD: Donation after brain death; HA: Hepatic artery; HMP: Hypothermic machine perfusion; LDH: Lactate dehydrogenase; NMP: Normothermic machine perfusion; NRP: Normothermic regional perfusion; PV: Portal vein.