

Machine perfusion of the liver: Putting the puzzle pieces together

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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

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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

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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.

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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

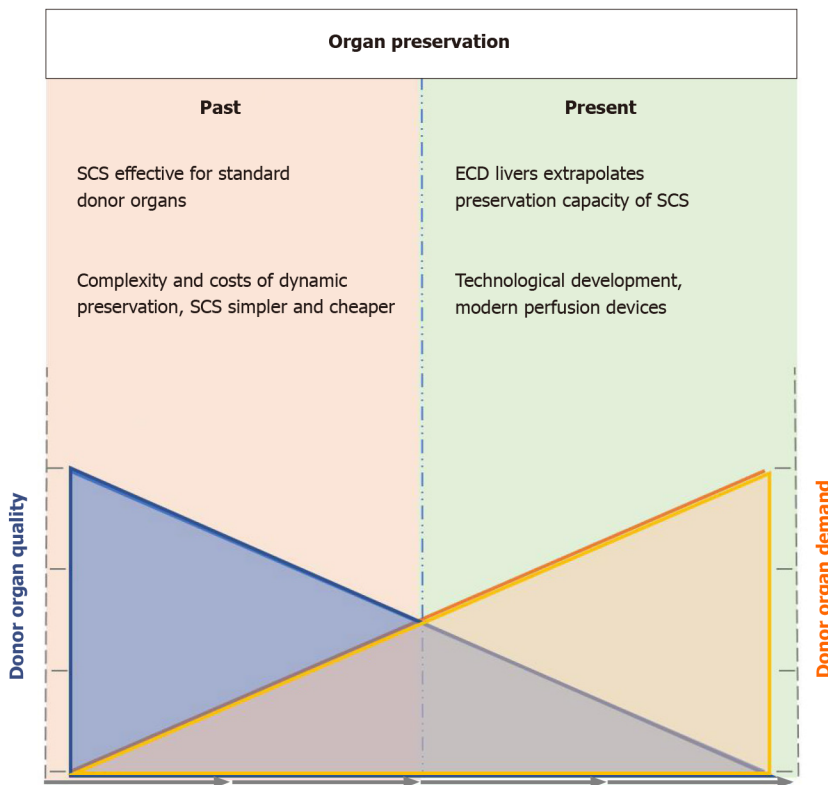


Figure 1 Conditions favouring the development of machine perfusion of the liver. At the start of transplantation, the utilization of good quality donor organs and the low price and simplicity of static cold storage (SCS) favoured its general use. However, the rise in demand for liver transplantation 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.

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

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 perfusion ¹	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 transplantation ³	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.

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, Eshmunov *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 Eshmunov *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,

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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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.

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.

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