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Review

HYPOTHERMIC MACHINE PERFUSION OF THE LIVER. THE REASONS FOR SUCCESS

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Summary

Machine perfusion is an attractive preservation technique to improve outcomes after transplantation. Based on underlying protective mechanisms two main concepts are described today, both with the same goal to provide oxygen to ischemic tissue. While normothermic machine perfusion (NMP) is rather labor intense and conveys its highest effect when performed instead of cold storage, the concept of hypothermic machine perfusion (HMP) is simpler and used after cold storage in the recipient centre. During re-oxygenation mitochondria immediately initiate the inflammatory cascade of ischemia-reperfusion-injury (IRI), although those features appear significantly less pronounced under cold conditions. In contrast to a normothermic perfusion, hypothermic techniques lead to a functional recovery of the respiratory chain and the Krebs cycle, thereby reloading cellular energy and metabolizing toxic metabolites, including Succinate. Although the preconditioning of mitochondria with cold oxygenation prior to transplantation is repeatedly described since the early days of organ transplantation, an increasing body of supportive literature is seen only recently. Newly published randomized controlled trials confirm this protection and demonstrate less organ injury, less complications including non-anastomotic biliary strictures, lower acute rejection rates and better graft survivals with the use of hypothermic oxygenated perfusion (HOPE). Of great interest is also the simplicity of HOPE, particularly when applied through the portal vein only, enabling the performance of a liver split during perfusion without the risk of arterial injury. In addition, there is increasing evidence how to assess liver viability during HMP through real-time spectroscopy of mitochondrial metabolism. Perfusate levels of Flavin mononucleotide (FMN), released from complex-I during reperfusion correlate with posttransplant complications and graft survival. This review article describes all features of HMP and addresses remaining challenges of this beneficial preservation technique.

Key words: hypothermic machine perfusion, extended criteria donors, oxygen, mitochondria, viability assessment

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Abbreviations

ALT: Alanine Aminotransferase; AS: Anastomotic strictures; AST: Aspartate-Aminotransferase; ATP: Adenosine-trisphosphate; CIT: Cold ischemia time; CS: cold storage; Damp's: Danger associated molecular pattern's; DBD: donation after brain death; DCD: Donation after circulatory death; DWIT: donor warm ischemia time; EAD: Early allograft dysfunction; ECD: Extended Criteria Donor; FMN: Flavin-mononucleotide; HA: Hepatic artery; HAT: Hepatic artery thrombosis; HMGB-1: High mobility group box-1 protein; HMP: Hypothermic machine perfusion; HOPE:

Hypothermic oxygenated perfusion; IC: Ischemic cholangiopathy; IRI: Ischemia-reperfusion injury; LT: Liver Transplantation; MELD: Model of end stage liver disease; MPT pore: Mitochondria permeability transition pore; NAD/NADH: nicotine adenine dinucleotide (oxidized/ reduced); NMP: Normothermic machine perfusion; NRP: normothermic regional perfusion; PNF: Primary non function; PV: portal vein; RCT: Randomized controlled trial; RET: reverse electron flow; ROS: reactive oxygen species; SCS: static cold storage; SDH: Succinate dehydrogenase; TLR-4: Toll-like-receptor-4

INTRODUCTION

Since the first liver transplantation, static cold storage (SCS) is a simple and cost-effective method to preserve organs (Fig. 1). With the higher number of marginal organs offered for an increasingly populated waiting list, the limits of SCS became obvious. Dynamic preservation techniques gained therefore renewed interest, thereby slowly modernizing common habits and paradigms. Amongst currently available perfusion techniques, hypothermic machine perfusion (HMP) is an easy and low-cost approach, and does not imply a change in organ procurement or graft implantation. Particularly based on a high oxygen concentration in the perfusate, cold organ perfusion achieves an organ protection through mitochondrial reprogramming and energy recharging before implantation. Subsequently, known features of ischemia-reperfusion-injury (IRI) are reduced. In various experimental and clinical studies, this technique was associated with better outcomes, including less posttransplant complications and better graft survival, when compared to SCS alone.
This review describes first, the mechanisms of IRI with the clinical impact on outcomes after liver transplantation. The role of mitochondria in the injury cascade is then underlined together with the protective effect of hypothermic oxygenated perfusion (HOPE). To highlight the clinical relevance of this preservation technique, the most recent literature is presented with a focus on different graft types, including livers with advanced donor age or from donors after circulatory death (DCD), livers with steatosis and partial grafts. Next, the specific impact of HOPE on the immune system activation is reported, followed by the best possible timing and perfusion duration. Due to the underlying mechanism rooting from mitochondrial complex proteins, further details on modern molecules used for viability assessment are presented. In context of lower recipient complications after HOPE, a potential impact on transplant-related costs will also be discussed. Finally, potential future directions in the field are highlighted including remaining challenges in the field.

HOPE MITIGATES THE ISCHEMIA-REPERFUSION INJURY: THE ROLE OF MITOCHONDRIA

The IRI-cascade represents an accumulation of various processes, that start with tissue hypoxia during warm or cold ischemia and become visible when grafts undergo reperfusion under normothermic conditions [1]. While certain protective effects occur naturally due to the impact of organ cooling and lower oxygen requirements, the following detrimental metabolic effects were also identified. During any type of ischemia, a shift towards an anaerobic metabolism occurs, which is based on mitochondrial dysfunction with a lack of Adenosine-trisphosphate (ATP), a calcium overload and the accumulation of certain metabolites in the cell and mitochondria [2]. The lack of oxygen puts the electron flow throughout the respiratory chain on hold with two main metabolic consequences, first the lack of ATP and secondly the accumulation of NADH at a non-functional complex I. Additionally, the impaired function of the Krebs cycle leads to an accumulation of Succinate together with a Complex II dysfunction [3,4]. Of note, such metabolic changes occur invisibly and progress throughout a prolonged hypoxia at all temperatures (Fig. 2). When oxygen becomes reintroduced (reperfusion) at normothermic temperatures, mitochondria immediately aim to reestablish the interrupted electron flow, which is however initially undirected and retrograde, and causes the production and release of reactive oxygen species (ROS) mainly from Complex I. Another consequence of the anaerobic metabolism during ischemia is the impaired mitochondrial calcium (Ca^{2+}) buffering capacity, which leads to elevated cytosolic Ca^{2+} levels, resulting in a higher release of produced ROS through the mitochondrial permeability transition pore (MPTP) at reperfusion. ROS molecules in turn trigger the release of further pro-inflammatory molecules grouped as danger-associated molecular patterns (DAMPs), including mitochondrial DNA and many more [5]. The immediate downstream consequence of ROS- and DAMPs release appears with activation of other residential liver cells, including Kupffer- and endothelial cells with subsequent release of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-alpha) and various interleukins (IL) [6,7]. This early critical status of the newly reperfused organ creates a proinflammatory environment with additional activation of circulating recipient immune cells (e.g., neutrophils) with intercellular crosstalk through chemotaxis. Importantly, these events escalate to an ongoing inflammation and organ dysfunction right after reintroduction of oxygen. Above-described mechanisms appear significantly less when mammalian tissues undergo reperfusion with oxygen at hypothermic temperatures below the Arrhenius breakpoint of 15degrees Celsius (Fig. 2) [8]. Each additional hour of CIT is associated with a 3.4%

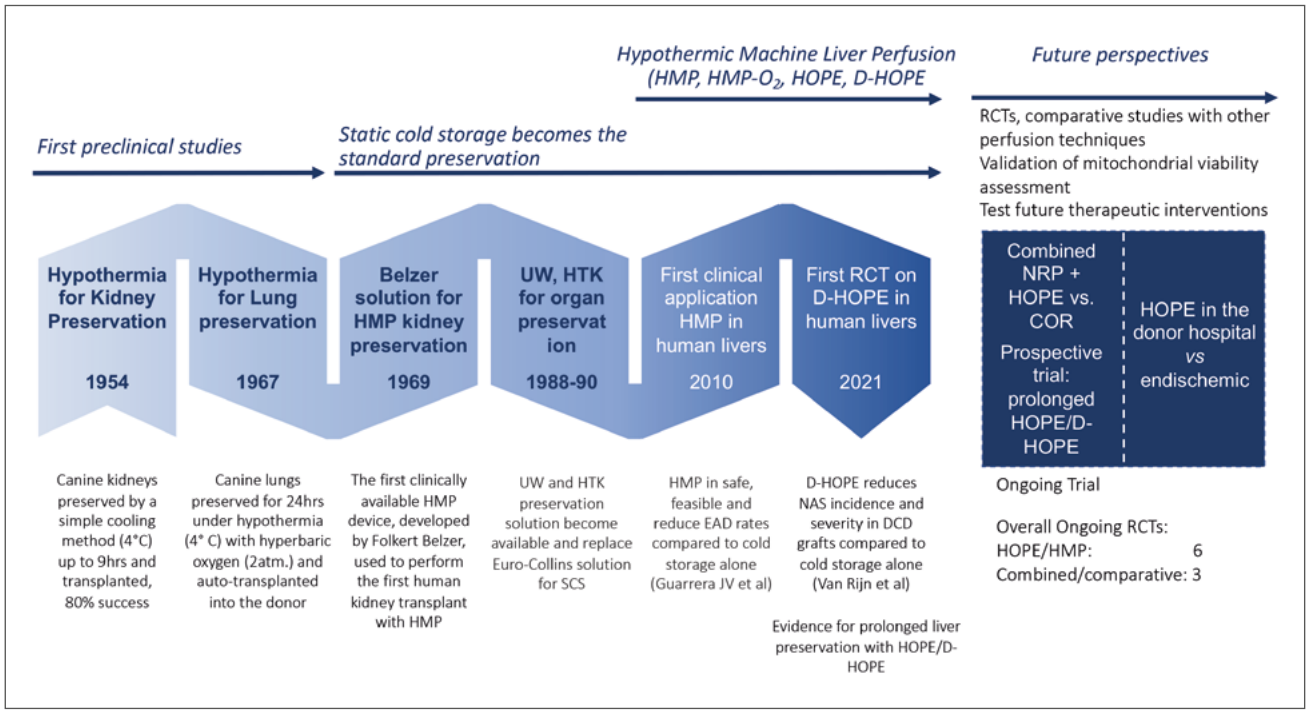

Figure 1. The history of cold organ preservation and hypothermic machine perfusion. This figure summarizes the development of organ preservation during the last 100 years with a focus on cold storage and hypothermic perfusion techniques.

increase in the risk of graft loss [9] and a cold storage of more than 7 hours was shown to be linked to an increased odds of prolonged recipient length of stay [10]. Importantly, the negative effect of IRI becomes more evident in grafts from extended criteria donors (ECD), which include elderly donors, prolonged cold ischemia times (CIT) of more than 12 hours, moderate to severe macrosteatosis and DCDs [11]. The use of DCD livers with a CIT of more than 6 hours is associated with a longer post-transplant hospital stay, higher rates of primary non-function (PNF), and higher serum bilirubin six months after transplantation [12]. To revert or avoid hypoxia is the overall goal of all dynamic preservation methods. The early introduction of oxygen with recirculating donor blood after initial donor warm ischemia time (DWIT) is practiced with *in-situ* normothermic regional perfusion (NRP), the primary method to assess and procure DCD livers in various European countries, including Italy [13,14]. *Ex-situ* normothermic liver preservation clearly reduces the overall injury when applied instead of cold storage, rather than after SCS in the recipient centre [15,16]. This is in sharp contrast to hypothermic techniques, which enable the modulation of mitochondrial metabolisms before reperfusion at warm conditions during liver implantation. Hypothermic techniques were described, more than 50 years ago, and first in context of kidney transplantation and the required time for donor-recipient type and screening [17]. Unit now more than 700 articles were published on perfusion techniques, frequently applied after organ transport to the recipient centre. Various experimental and clinical studies have demonstrated the protective effect in all solid human organs [6,18-21].

The HOPE-technique was found to reduce IRI-associated inflammation by protecting mitochondria. Cold, dynamic preservation with a high perfusate oxygen of > 60 kPa induces metabolic changes in the mitochondrial respiratory chain with steady, slow and forward electron flow without significant ROS release (Fig. 2). Based on this, the Krebs cycle returns to normal function and previously accumulated succinate is metabolized together with ATP reloading at complex-V [7]. Additional effects of a homogenous, pressure-regulated HOPE appear with the clearance of catabolites and toxic molecules [22,23]. Related to the initial level of organ injury (donor quality), this mitochondrial recovery requires 90-120 min of HOPE-treatment and results in significantly reduced ROS levels at later reperfusion under normothermic conditions with less downstream inflammation and posttransplant complications [6]. Such relevant metabolic changes prepare cells of all origins for the later reintroduction of oxygen under warm conditions with lower complications and better posttransplant outcomes [24-26]. Of importance is however a high perfusate oxygen concentration during HMP. As shown by Lazeyras et al, a partial oxygen pressure of only 20kPa (as seen in free fluids) or even 50 kPa does not induce the best possible ATP reloading [27]. Results from this kidney study were paralleled

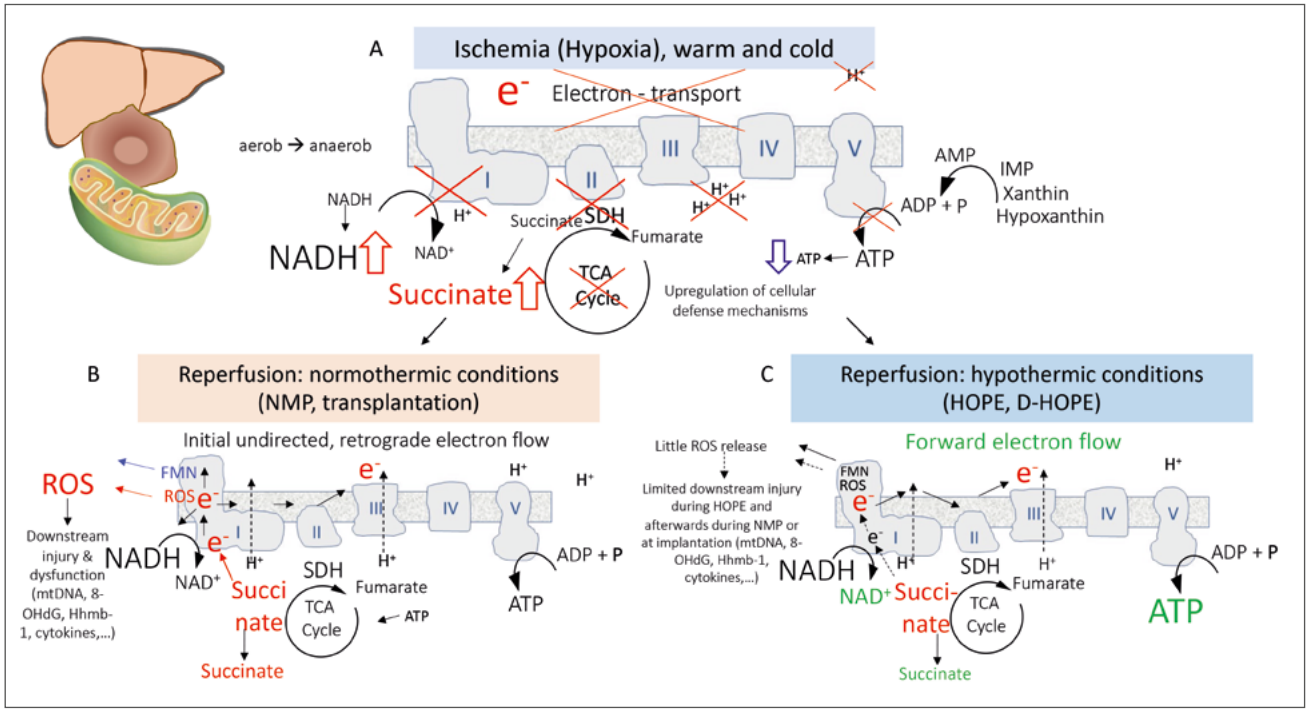

Figure 2. Mechanisms of Ischemia-Reperfusion-Injury and protection through hypothermic oxygenated perfusion of liver grafts. Three different templates describe underlying mechanisms of injury and protection during ischemia (A), reperfusion under normothermic (B) and hypothermic (C) conditions. During warm and cold ischemia, mammalian tissues suffer a loss of ATP, based on the interruption of the mitochondrial electron flow with succinate and NADH accumulation (A). The immediate consequence during reoxygenation under normothermic conditions is a release of reactive oxygen species (ROS) from complex I with subsequent downstream inflammation and organ dysfunction (B). When oxygen is however reintroduced under hypothermic conditions, mitochondria work more efficient to restore ATP, and to metabolize accumulated succinate and NADH. Such mechanisms are crucial before rewarming to avoid the extensive proinflammatory signal, triggered by ROS release (C). HOPE treatment avoids this feature and prepares cells for reperfusion under normothermic conditions, as seen during implantation or on normothermic perfusion devices.

by the Minor group in livers [28]. Another parameter of importance is the perfusion duration. Mitochondria were found to recover from the ischemic insult during a 2hr HOPE-treatment [29]. Spectroscopic quantification of NADH in perfusates demonstrated the reduction of previously accumulated NADH during warm and cold ischemia within 90-120 min of HOPE, the time needed for mitochondria to switch and being prepared to undergo normothermic reperfusion at implantation [30]. The following subchapters describe clinically relevant literature and discuss further aspects.

HOPE PROTECTS FROM BILIARY COMPLICATIONS

Biliary complications include a broad spectrum of complications with different grades of severity. While biliary leaks usually occur in the early post-operative period, biliary strictures are evident early and later, months after transplantation and are linked to graft injury and quality. Such strictures can be divided into anastomotic (AS) and non-anastomotic biliary strictures (NAS) [31]. While most AS can be successfully treated with endoscopic stenting, the NAS management is more challenging requiring frequent interventions at the biliary tree with a higher risk for graft loss. According to a large series report, early NAS develops within one year from transplant, with a median of 4.1 months after surgery [32]. Prolonged warm and cold ischemia time are critical factors related to early NAS, whereas later NAS are associated with other risk factors, including recurring biliary tract infections, the primary recipient disease (e.g., primary sclerosing cholangitis) or a poor HLA match [33]. The development of NAS appears as one consequence of a too high IRI-associated inflammation, conveyed through severely injured mitochondria, unable to recover their function early after reperfusion. Indeed, due to the initial lack of ATP, hepatocytes secrete fewer bile acids resulting in an accumulation of toxic bile and cholestasis, which further injures hepatocytes through the accumulation of hydrophobic bile salts [34]. In addition, hepatocytes are unable to metabolize cholesterol and secrete

phospholipids, resulting in an impaired bile secretion, that is toxic to cholangiocytes in the biliary tree downstream to hepatocytes [33].

The impact of HOPE on the biliary tree has been a highly studied in the last years. In a randomized controlled trial (RCT), Czigany et al. [35] compared HOPE and SCS outcomes, reporting better results in HOPE-treated grafts with a reduced number of overall biliary complication (17%, n = 4/23) compared to cold stored controls (26%, n = 6/23). Such clinical findings are based on the metabolic reprogramming of mitochondria during hypothermic reoxygenation. Importantly, the protective HOPE-effect was even more pronounced with grafts from donors with warm ischemia donated after circulatory death (DCD). Such livers are prone to develop more complications after transplantation, including NAS, when compared to organs from brain dead donors (DBD) [36]. A pre-clinical study on a rodent model of DCD liver transplantation reported a significantly decreased hepatocyte injury, Kupffer and endothelial cell activation with less biliary injury after implantation [37]. Those results were further supported by clinical evidence. In 2015, Dutkowski et al. [38] reported the first larger cases series of 25 DCD livers. The Authors found a significant reduction of graft injury compared to matched cold-stored control DCD livers. The peak alanine-aminotransferase (ALT peak was 1239 *vs* 2065 U/L, p = 0.02) was reduced and a lower rate of NAS (0 *vs* 22%, p = 0.015), overall biliary complications (20 *vs* 46%, p = 0.042), and an improved 1-year graft survival (90 *vs* 69%, p = 0.035) was found after HOPE-treatment. The results achieved were not only superior to non-perfused DCD grafts, but also comparable to a control DBD livers cohort.

Additional clinical evidence was presented in 2017 by the Groningen group [39]. Ten DCD livers which underwent dual-HOPE (performed through hepatic artery and portal vein) were compared with 20 non-perfused controls, resulting in an 11-fold increase of ATP-content and lower postoperative transaminases. Importantly, the incidence of NAS was 10% (n = 1/10) in the HOPE group and 45% (9 = 20) in the SCS group. Notably, NAS were more clinically severe in non-perfused, cold stored controls, when compared to HOPE (Tab. I). Another larger multicentre study compared the outcome of fifty HOPE-treated DCD livers with DBD and DCD livers, both with simple SCS [40]. Of note, the group of DCD grafts with subsequent HOPE had prolonged DWIT times due to the obligatory 10-min stand-off period in Switzerland. Interestingly, the non-tumor related graft loss in the HOPE group (8%; n = 4/50) was comparable to standard DBD livers (10%; n = 5/50). In contrast, one-third of recipients of cold stored DCD livers (16/50) experienced a graft loss (p = 0.005), despite significantly (p < 0.001) shorter DWIT. Importantly, the five-year graft survival rates, were significantly higher for HOPE-treated DCD liver transplants (94%) compared to untreated DCD controls (78%) (p = 0.024) [40]. The overall NAS rate was significantly lower after HOPE (8 *vs* 22%). This study highlighted the HOPE-effect after SCS in high-risk livers before implantation.

Such promising results were further supported by the first RCT, published in 2021. Outcomes after transplantation with and without D-HOPE were analyzed in randomly assigned DCD livers [41]. D-HOPE was associated with a significant reduction of NAS (p = 0.03), lowering its development from 17.9% (n = 14/78) in the SCS arm to 6.4% (n = 5/78) within a 6-month follow-up. None of the recipients with NAS required retransplantation in the D-HOPE group, whereas two patients needed a retransplant in the SCS group. A recent large cohort study from Turin, confirmed such findings. Patrono et al. demonstrated a reduction in major complications including the severity of NAS after D-HOPE treatment in more than 100 perfused grafts. The findings were confirmed in a subset of transplantations from old DBD donors [42]. Overall, there is increasing evidence that a simple endischemic HOPE-treatment is effective in the reduction of biliary complications in various grafts types, including DCD livers and other extended criteria donor grafts.

An example of extreme donor risk is seen in Italy with an obligatory 20 min stand-off period in DCD donors. The preservation approach includes the immediate oxygen reintroduction through normothermic regional perfusion (NRP) in the donor, combined with standard organ procurement with cold flush, followed by SCS and transport plus endischemic HOPE-treatment in the recipient centres. This combination of NRP and HOPE was demonstrated as beneficial with low complications, within a median follow-up of 3 years, despite this high donor risk [13,43,44]. Only a very small number of recipients developed minor non-anastomotic biliary strictures, which were treated conservatively.

Another example of a combined preservation approach, where the anti-inflammatory HOPE-effect is of relevance, appears with the combination of hypothermic and normothermic perfusion. The group from Groningen tested the benefit of D-HOPE combined with controlled oxygenated rewarming (COR) and NMP in DCD livers from donors > 60 y of age, usually declined in the Netherlands based on the national guidelines. Such grafts were perfused with the D-HOPE-COR-NMP protocol and transplanted with a reported utilization of 63% [25]. While comparative studies are currently performed, the use of additional NMP after HOPE is explained by the authors with the need for viability assessment. Although there is an increasing body of evidence regarding the viability assessment during HOPE, a higher number of studies is required for a wider acceptance. The last subchapter of this review will provide further details of the underlying mechanisms and current technologies.

HOPE AND STEATOTIC LIVER GRAFS

Steatotic liver grafts are another source of potentially

high-risk grafts. Not only the challenges in reliable fat quantification, but also the highly variable metabolic situation makes an outcome prediction and the safe utilization difficult. With their unfavorable metabolism, livers with advanced steatosis are at high risk of early functional failure and severe complications. For example the microcirculation is significantly impaired, thus fatty livers are vulnerable to the decreased synthesis of protective molecules (e.g. thrombomodulin, nitric oxide) determined by the lack of mechanical shear stress over the endothelium during static cold storage [45,46]. Above all, the previously described impaired mitochondrial metabolism can be more severe when compared to DCD livers with high levels of succinate and NADH and at the same time tremendously low ATP levels [47]. The reduction of such metabolites, combined with a mitochondrial reprogramming and ATP reloading is therefore critical to achieve acceptable outcomes. The HOPE procedure was shown to provide such benefits not only to DCD grafts but also in macrosteatotic grafts.

Croome et al. [48] reported a propensity match analysis of 96 grafts with three different levels of steatosis, including < 5%, mild and moderate steatosis. Recipients of moderately macrosteatotic graft had a higher rate of post-reperfusion syndrome (PRS) (37.5 *vs* 18.8%; p = 0.004), early allograft dysfunction (EAD) (76.4 *vs* 25.8%; p < 0.001), renal dysfunction with the need for renal replacement therapy after transplant (18.8 *vs* 8.3%; p = 0.03) and presented a higher rate of surgical revisions within 30 days (24.0 *vs* 7.3%; p = 0.002), compared to the group of livers without relevant steatosis. Importantly, a recipient cardiac arrest at the time of reperfusion was reported in eight (8.3%) recipients in the moderate macrosteatosis group, compared to one (1.0%) in the patients with mild macrosteatosis (p = 0.02).

Given the association between graft steatosis, post-reperfusion syndrome and impaired outcomes, several authors were interested to improve such posttransplant results and have assessed the impact of HOPE. Kron et al. [47] induced a severe macrosteatosis in the rat model and compared 12 h cold storage with a second study group with an additional 1hr of HOPE-perfusion. Expectedly, HOPE treatment significantly decreased IRI by less oxidative stress, less Kupffer and endothelial cells activation and less parenchymal fibrosis one week after liver transplantation. Importantly, the protective effect was lost with hypothermic but non-oxygenated perfusion, highlighting crucial role of a high oxygen concentration in the HOPE perfusate. Such findings were then translated into clinical practice, where the same authors demonstrated a protective effect in a small, matched cohort of human livers with macrosteatosis. HOPE treatment after SCS resulted in better early and late outcomes after transplantation. Despite such promising results, which were also paralleled by the group from Turin in 2020, more translational studies are required to demonstrate potential differences with HOPE and the underlying mechanism in such risky grafts.

IMPACT OF HOPE ON PARTIAL LIVERS

Liver machine perfusion is not only a preserving and assessment tool, but also a platform that allows the performance of a surgical procedure including backtable preparation and liver splitting. Rossignol et al described their experience with 8 left lateral/extended right lobe split liver grafts, performed during HOPE. Importantly, all HOPE-split livers were successfully transplanted. Splitting the graft during HOPE significantly shortened the time of hypoxia through a reduction of SCS (472 *vs* 544 min; p = 0.001) and reduced IRI-features including neutrophil infiltration assessed through post-reperfusion biopsies. Further smaller case series with cold perfusion during split procedure or afterwards were presented in recent years in Italy, Netherlands and Germany [49-51]. Colleagues from France are currently performing a prospective study in this special liver cohort to assess the benefit of HOPE further. Surgical procedures during HOPE are still under evaluation. Even in countries with a policy of *in-situ* split such as Italy, HOPE could represent a valid tool to safely extend preservation time in challenging logistical scenarios to avoid longer cold ischemia in context of a proinflammatory status due to the liver transection.

HOPE MODULATES THE IMMUNOLOGICAL RESPONSE AND IMPACTS ON ACUTE GRAFT REJECTION

The insult caused by IRI and surgery promotes a pro-inflammatory status, that upregulates the innate immune system. Therefore, IRI-modulation by HOPE also translates into a modification of the immune response with a lower number of acute rejections, as demonstrated by a reduced number of graft-infiltrating T-cells and circulating cytokines in recipients of HOPE-treated allogeneic livers [52]. Such rodent grafts demonstrated less histological signs of acute rejection and fibrosis even after transplantation without immunosuppression. This experimental study underlined further the key role of oxygen during HMP, because recipients of allogeneic livers treated with de-oxygenated HMP were not protected from this severe immune response [52]. Similar results were seen in a parallel study with HOPE in an allogeneic model of kidney transplantation [53]. Importantly, pre-clinical findings were further supported by various recent clinical studies, including RCTs reporting a significant reduction of acute rejection rates from 20.5% in untreated human livers compared to 11.5% after D-HOPE treatment (Tab. I) [35,40,41].

WHAT IS AN OPTIMAL TIMING FOR HOPE?

While there is an increasing body of evidence that hypothermic oxygenation before implantation reduces clinically relevant complications, the best possible timing and required perfusion duration are less well explored [54]. The benefit for recipient outcomes should be balanced with required costs and logistics. In most studies, the benefits of HOPE being a simple approach, that allows standard procurement and organ transport with back-table and subsequent endischemic perfusion in the recipient centre, was demonstrated. In their ongoing randomized controlled trial (RCT), Guarrera et al use the Lifeport Liver Transporter® to bring the organ from the donor centre to the recipient. While results of this study are eagerly awaited, the need of a perfusion during the entire organ preservation with subsequent device transport remains unclear, particularly when looking at the underlying mechanisms with mitochondrial switch within 90-120 min. The endischemic HOPE-technique in the recipient centre has additional logistical benefits. Clinicians are free to perform parts of the backtable preparation or arterial reconstructions during HOPE, which may even reduce the cold ischemia time [12,40]. In a recent study, Lantinga et al. reported that starting the backtable during HOPE could further reduce the cold ischemia time from 279 minutes (IQR 254-297) to 214 minutes (IQR 132-254; $p < 0.01$) [55]. The performance of HOPE through the portal vein enables an almost immediate perfusion start at organ arrival in the recipient centre, because this approach allows arterial reconstruction.

Another important perfusion parameter currently discussed is the ideal duration of HOPE. While in context of the required energy reloading and mitochondrial preparation for later implantation, a duration of 2 hours is beneficial, the maximal perfusion time is less well assessed [29,30]. Colleagues from Germany and Brazil have described the beneficial effects of a prolonged HOPE after SCS in livers from extended criteria donors [56,57]. HOPE was performed for more than 4 and 5hrs after SCS, enabling transplantation of such ECD grafts after more than 11, 15 and 17 hrs of total preservation [56,57]. A recent multicenter study has further paralleled these findings, showing a safe prolongation of the overall preservation with endischemic HOPE in DBD and DCD livers [58]. More than 4hrs of HOPE with a total median preservation time of 10 hrs led to equally good outcomes as seen with shorter preservation times. The Groningen Group currently explores this aspect further in their prospective study.

HOPE ALLOWS REAL-TIME VIABILITY ASSESSMENT OF LIVER GRAFTS

Historically, the assessment of the donor's liver was related to surgeons' experience and several weak predictors such as laboratory tests, liver biopsy or anamnestic records [59]. In the last decades, there was a call for new assessment tools, which can make the organ evaluation more objective. Although different parameters could already be explored in the donor and measured in flush solutions, an active oxygenation is usually required for the release of certain molecules from organs presenting features of IRI [60]. Since MP has regained wider interest within the liver preservation field, many efforts were made to identify better viability criteria [61,62]. Parameters used for liver assessment during NMP were mainly derived from clinical practice with patients admitted to intensive care unit or those with liver failure or after hepatectomy [59]. Liver transaminases, perfusate pH or Lactate are only a few to be listed. Various case series describe at least 4 different thresholds for Lactate during NMP [59]. A similar picture arises with bile parameters. Although there is no doubt regarding the importance of bile composition measured during NMP to assess cholangiocytes and the risk for later biliary complications, multiple parameter combinations were recently suggested by many [59]. Van Leeuwen et al from Groningen recently suggested a complex "traffic light system" with 7 parameters assessed in perfusates and bile during NMP. Three characteristics (green, yellow, red) were possible for each parameter, leading to a high number of potential parameter combinations, which requires a high case load to eventually know when to safely accept a liver [25,63].

Another hurdle appears with the definition of a reliable parameter threshold, which requires the transplantation of high-risk organs beyond a certain parameter value, which appears ethically challenging. Capturing a marker close to the root of IRI is of importance. More than 40 years ago, the marker flavin mononucleotide (FMN) was identified to be released from mitochondria being the primary lesion during IRI [64]. Research on ischemic brain injury has further supported the importance of FMN as injury marker during reoxygenation [65]. In addition to various other molecules released into HOPE perfusates, FMN was identified as the most predictive parameter for posttransplant outcomes, including liver function, complications and graft survival [7,66]. Authors from Zurich have retrospectively defined a perfusate FMN threshold in a series of 50 human DCD livers transplanted in their centre. This FMN cutoff is followed since 2017 with FMN quantification within the first hour of HOPE and decision making during that time[7]. With accumulating mitochondrial succinate during ischemia, ROS molecules are subsequently released during reperfusion from Complex I, together with $FMNH_2$ dissociation, followed by rapid non-enzymatic oxidation to FMN [67]. Of note, reoxygenation after ischemia is crucial for the release of ROS and FMN, both at however lower levels under hypothermic conditions when compared to normothermic techniques [7]. FMN appears therefore as attractive biomarker to assess mitochondrial injury during any type of machine perfusion, including normothermic

perfusion and in any organ transplanted [68]. Other mitochondrial parameters, including NADH which is equally auto-fluorescent and detectably with spectroscopy appear of interest as viability marker. Perfusate NADH levels relate to mitochondrial complex I function and are assessed together with FMN in perfusates [7,69]. NADH metabolism and subsequent reduction in perfusates corresponds to a functional complex I, demonstrating recovering mitochondria [7,30]. More studies required to confirm the value of mitochondrial markers, not only during HOPE, but during other preservation approaches.

IS HOPE COST-EFFICIENT?

Any additional technology is prone to increase healthcare costs. Although perfusion technology is projected to increase hospital costs, there is strong potential to achieve cost-benefit due to the impact of HOPE and the reduction of posttransplant complications. Shorter patient hospital stay, better early function and less complications will also lead to an improved quality of life with a faster return to work to potentially counterbalance overall costs related to the introduction of machine perfusion. However, cost-effectiveness and cost-utility analysis are challenging and require data over the transplant patient's lifetime. The economic evaluation of HMP or HOPE should also consider that this technology enables the utilization of previously unsuitable grafts, which reflects in the reduced burden of chronic therapy of patients with end-stage liver disease awaiting transplantation [70].

In a recent study, Rayar et al. [71] highlighted that recipients receiving extended criteria grafts from DBD donors treated with HOPE have a significant reduction in intensive care unit stay (3 *versus* 5 days; p = 0.01) and hospitalization (15 *versus* 20 days; p = 0.01) compared to SCS. Interestingly, the additional cost of HOPE was estimated at € 5`298 per patient. Considering the clinical advantages associated with HOPE, the overall costs and revenues were not different between the HOPE and the control group with SCS (€ 3`023 *versus* € 4`059).

Czigany et al. [35] reported a 25% decrease in the treatment costs over the first three months in HOPE-treated patients compared to SCS. Despite additional costs of € 5`000 for one HOPE-procedure, overall, this perfusion technique created a mean cost benefit of 13,000 Euro per case, also due to the shorter ICU and hospital stay and less complications. Although hypothermic perfusions cost significantly less than normothermic techniques [72,73]

Table I. Clinical studies assessing the impact of hypothermic machine perfusion on graft function, biliary complication and immune system modulation during the last 5 years.

Type of study (level)	Authors & year	Number and type of livers	Main findings	Biliary complications		Main findings on immune system modulation	Duration of follow-up
				Anastomotic biliary complications (n/%)	Non-anastomotic biliary strictures (n/%)		
Randomized controlled trial (II)	Ravaioli et al., 2022 [74]	55 ECD livers each arm (HOPE vs CS)	HOPE reduced the EAD rate from 35% (35/55) to 13% (7/55) – primary endpoint	Not separately presented, non-significant reduction of biliary and vascular complications, study not powered for this endpoint		Lower rates of acute rejection after HOPE (9/55 vs 4/55 cases, ns)	12 months
	Van Rijn et al., 2021 [41]	78 DCD livers each arm (D-HOPE vs CS)	D-HOPE significantly reduces non-anastomotic biliary strictures (p=0.03) and the number of required interventions	Anastomotic Strictures: CS 28.2% (n = 22/78); D-HOPE: 29.5% (n = 23/78)	CIT 17.9% (n = 14/78); 2 retransplantations, D-HOPE: 6.4% (n = 5/78), no retransplantation	D-HOPE reduces acute rejections: D-HOPE 11.5% vs cold storage control 20.5%	6 months
	Czigany et al, 2021 [35]	23 DBD livers each arm (HOPE vs CS)	HOPE treatment reduced the Peak ALT levels within 7 days (p = 0.03), the ICU (p = 0.045) and hospital stay (p = 0.002), the major complications (p = 0.036), the cumulative complications (CCI: p = 0.021) and the estimated costs (p = 0.016) after transplantation	Biliary complications (clinical; radiological): CS: 26% (n = 6/23); HOPE: 17% (n = 4/23), no specific information on NAS		HOPE treatment reduced the acute rejection rate from 26% (CS control) to 17% (HOPE group)	12 months
Case-control cohort study (IV)	Rayar et al., 2021 [71]	25 extended DBD grafts, 69 unperfused controls	Less peak transaminases, less EAD, less ICU and hospital stay	Anastomotic strictures or leaks: CIT: 10.1% (n = 7/69), HOPE: 8% (n = 2/25)	CS: 1.4% (n = 1/69; ischemic necrosis), HOPE: n = 0/25	Not reported	12 months
	Schlegel et al. 2019 [40]	50 DCD (HOPE), 50 DBD (control), 50 unperfused DCD	Less PNF, HAT and ischemic cholangiopathy result in an improved five-year survival of HOPE treated extended DCD liver grafts	Anastomotic strictures: CS DCD: 18% (n = 9/50), HOPE: 24% (n = 12/50), 1 biliary leak each group (2%)	CS: 22% (n = 11/50) with 10% (n = 1/69) graft loss, HOPE: 8% (n = 4/50) with 0% graft loss	cDCD/HOPE with less acute rejection; 4% HOPE group, 28% CS group, p = 0.0019	5 years
	Ravaioli et al., 2019 [75]	Extended DBD livers (n = 10, HOPE), unperfused controls (n = 30)	No PNF and significantly lower rate of EAD, significantly lower recipient transaminases after HOPE treatment and 100% graft survival compared to the cold storage control group	Anastomotic biliary complications: CS: 10% (n = 3/30), HOPE: 10% (n = 1/10),	No specific information provided	Tendency toward a lower ACR rate: 10% HOPE group and 13.3% CS control	12 months
	Patrono et al., 2019 [46]	Extended DBD livers, macrosteatotic, n = 25 (D-HOPE), 50 unperfused extended DBD (matched)	Lower rate of post-reperfusion syndrome, acute kidney injury grade 2-3, and lower EAD due to lower recipient transaminases.	Anastomotic complications: CS: 12% (n = 6/50); D-HOPE: 16% (n = 4/25)	CS: 8% (n = 4/50), 2 symptomatic patients; D-HOPE: 8% (n = 2/25), both asymptomatic	Lower rate of acute rejections with 8.6% (HOPE group) and 16% CS control	6 months
	Van Rijn et al,. 2017 [39]	DCD livers, n = 10 (D-HOPE), 20 unperfused controls	Restoration of ATP, lower transaminases and protection of the biliary tree from reperfusion injury and complications through D-HOPE	Anastomotic strictures: CS: 15% (n = 3/20); D-HOPE: 20% (n = 2/10)	CS: 45% (n = 9/20) with 2 biliary necroses, 5 retransplantations; D-HOPE: 10% (n = 1/10), no retransplantations	Not reported	12 months
Systematic Review (V)	Jia et al., 2020 [76]	12 studies, 2 randomized, HOPE/HMP and NMP, 1 each	HMP/HOPE improved outcomes in contrast to NMP	Lower incidence of overall biliary complications in HMP-treated patients compared to CS (OR = 0.45; CI: 0.25-0.80; p = 0.007), no differences between NMP and CS	Lower incidence of NAS (IC) in HMP-treated patients compared to CS (OR = 0.25; CI: 0.08-0.73; p = 0.01)	Not reported	12 months
	Zhang et al., 2019 [77]	6 studies, 144 perfused grafts, 178 unperfused controls	HOPE/HMP was associated with a better liver function, lower EAD rate, lower number of biliary complications and better survival	Lower incidence of biliary complications with HMP compared to CS (OR 0.47, CI: 0.28-0.76, p = 0.003)	No specific information provided	Not reported	12 months

and the type of perfusate used does not impact blood availability, more studies on the cost-benefit are needed. While in countries, including Switzerland and Italy the HOPE technique is already commissioned for specific organs, many other regions worldwide still require larger cost studies to convince their local hospital managers and commissioners. In the Netherlands, a specific diagnosis-related group category for MP was recently introduced, allowing hospital reimbursement. Future research should focus on strategies to offer equitable access to MP across different liver transplant centers and on potential patient harm from the non-implementation of a MP program that facilitates transplantation.

WHAT ARE FUTURE DIRECTIONS?

Hypothermic perfusion is a promising preservation technique, which improves patient outcome after transplantation. An increasing body of literature confirms the protective effect through reprogramming of mitochondria and energy reloading before implantation. Despite this success in the last 10 years, further studies are required to demonstrate the benefit of HOPE over other perfusion approaches and the cost-benefit. Additional research is required to identify the optimal and maximal duration of cold ischemia before or after HOPE treatment in organs from different extended criteria donors. The concept of viability testing based on mitochondrial function requires further attention and should also be explored in steatotic grafts.

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Conflict of interest statement

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