# Prolonged preservation of donor livers: the benefits of hypothermic machine perfusion

Brüggenwirth, Isabel Margaretha Antoinette

*DOI:*
10.33612/diss.214594430

# Chapter 10

## Summary, general discussion & future perspectives

## SUMMARY

Traditional preservation of donor livers by static cold storage (SCS) offers a simple and effective approach to preserve good quality donor livers. However, a number of limitations are associated with SCS, including inevitable ischemia-reperfusion injury, inability to assess hepatobiliary viability, limited opportunity for organ repair, tissue damage after prolonged preservation, and time pressure to limit cold ischemia time. A brief period of hypothermic oxygenated machine perfusion (HOPE) after SCS protects livers against reperfusion injury and improves post-transplantation outcomes. In this thesis, we investigated whether HOPE could also be used to prolong preservation time. The studies in the first part of this thesis show that prolonged SCS is a risk factor for graft failure after liver transplantation. In the second part of this thesis, we demonstrate that dynamic preservation by HOPE is a promising technique to prolong preservation time.

A general introduction is presented in **chapter 1**. In **chapter 2**, we analyzed to which extend donor diabetes mellitus (DM) modifies the effect of prolonged (>8 hours) cold ischemia time (CIT) on the risk of graft failure after liver transplantation. Using data from the Scientific Registry of Transplant Recipients between 2002-2015, a total of 58,226 donor liver recipients were analyzed of whom 6,478 (11.1%) patients received a liver from a donor with DM. Donor DM and prolonged CIT were each associated with an increased risk of graft failure (hazard ratio [HR] 1.19; 95% confidence interval [CI] 1.06-1.35 and HR 1.42 [95% CI 1.32-1.53, respectively]. However, the combination of donor DM and prolonged CIT was associated with a higher risk of graft failure than either factor alone (HR 1.79, 95% CI 1.55-2.06) and had a synergy index of 1.30. These results suggest that liver grafts from donors with DM are more susceptible to the adverse effects of prolonged CIT than livers from non-DM donors.

In **chapter 3**, we developed a risk model for survival after liver retransplantation (reLT) using data from the European Liver Transplantation Registry. Between 2006-2016, 85,067 liver transplants were recorded, including 5,581 reLTs (6.6%). After uni- and multivariable analysis, seven predictors of graft failure were identified, including recipient age, model for end-stage liver disease score, indication for reLT, recipient hospitalization, time between primary liver transplant and reLT, donor age, and CIT. A simplified risk score was created ranging from 0-10 points. Low-risk (0-3 points), medium-risk (4-5 points), and high-risk (6-10) categories were identified

with significantly different 5-year survival rates of 56.9% (95%CI 52.8%–60.7%), 46.3% (95%CI 41.1%–51.4%), and 32.1% (95%CI 23.5%–41.0%), respectively (P<0.001). The reLT risk score may serve as prognostic guidance for transplant physicians in clinical decision making on matching donor livers with recipients.

A review of preclinical and clinical machine perfusion studies is presented in **chapter 4.** After extensive preclinical work, the first nonrandomized clinical trial comparing hypothermic machine perfusion with SCS was performed in 2010. Years later, in 2016, the first clinical trial on normothermic machine perfusion was performed. The first clinical experiences with machine perfusion suggest the technique is safe and feasible, reduces ischemia-reperfusion injury, and is associated with improved hepatobiliary function after transplantation.

In the study presented in **chapter 5**, we investigated a novel and relatively simple approach to prevent adenosine triphosphate (ATP) depletion during preservation of DCD donor livers. In a porcine *ex situ* reperfusion model, we assessed the effects of active oxygenation of the preservation fluid used for *in situ* donor cold flush out and subsequent static cold storage (SCS), compared to using a non-oxygenated solution. It was demonstrated that oxygenation of the preservation solution improved hepatic ATP content at the end of SCS compared to non-oxygenated controls. However, better ATP preservation was not associated with improved hepatobiliary function nor reduced hepatobiliary injury after reperfusion.

In the study described in **chapter 6**, we compared hypothermic oxygenated machine perfusion (HOPE) through the portal vein alone with HOPE through both the portal vein and hepatic artery (dual HOPE or DHOPE) in a porcine *ex situ* reperfusion model. We hypothesized that DHOPE would provide additional benefits over HOPE by biomechanical endothelial cell stimulation and better perfusion of the peribiliary vasculature. Based on this study, this hypothesis was refuted. We did not observe differences in hepatobiliary function and injury between livers preserved by HOPE versus DHOPE, except for a two-fold lower alanine aminotransferase concentration in blood and a three-fold lower lactate dehydrogenase concentration in bile after reperfusion in the DHOPE group. Long-term outcomes after preservation by HOPE versus DHOPE have to be confirmed in a transplantation model particularly focusing on the development of non-anastomotic biliary strictures (NAS).

In the preclinical study in **chapter 7**, we investigated whether end-ischemic DHOPE could extend *ex situ* preservation time of porcine livers up to 24 hours. Livers preserved by DHOPE remained viable for at least 24 hours, whereas 24-hour preservation by SCS resulted in non-viable grafts. Additionally, livers preserved by DHOPE for 24 hours had similar hepatobiliary function and injury markers compared to livers perfused for 2 and 6 hours DHOPE. As a proof of principle, we showed preserved hepatocellular and cholangiocyte function and histology after extended DHOPE preservation of two discarded human livers. The results from this study suggest that DHOPE could be used to extend preservation time, which may facilitate allocation and transplantation logistics.

To further investigate the effects of prolonged HOPE **chapter 8** includes a retrospective, multicenter, cohort study to evaluate outcomes after transplantation of donor livers preserved by prolonged (≥4 hours) (D)HOPE. Twelve centers from 6 countries contributed to the study, and the total study cohort included 93 patients. The median perfusion duration was 4:42 hrs (range 4:00–8:35 hrs) with a total preservation time of 10:50 hours (range 5:50–20:50 hrs). We showed that excellent outcomes may be achieved after transplantation of donor livers preserved by prolonged (D)HOPE.

After promising results were obtained from the studies in chapters 7 and 8, we initiated a prospective clinical trial to investigate the safety and feasibility of prolonged DHOPE preservation in liver transplantation (DHOPE-PRO trial). Preliminary results of the first 6 livers in the intervention group are presented under embargo in **chapter 9.** The trial protocol is included in **appendix I**.

## GENERAL DISCUSSION & FUTURE PERSPECTIVES

The ambition to extend the *ex situ* preservation time of donor livers has been present since the early days of liver transplantation. In the studies described in this thesis we have shown that HOPE is a promising technique to prolong the time a donor liver can be kept viable outside the body, which is likely to have substantial clinical implications. Yet, we all apprehend that science is an ongoing endeavor. Finding answers to previously raised questions, frequently inspires more questions for further research. In this final chapter, I would like to formulate hypotheses and discuss perspectives for future research.

Preservation of donor livers by SCS used to be the gold standard for decades, but by using more suboptimal donor livers, the limits of static preservation have been recognized. Whereas organs were traditionally derived from young donors declared brain dead after traumatic injury, organ scarcity has necessitated extended donor acceptance criteria.[1] At the same time, the life expectancy of the general population increases, as well as the incidence of comorbidities, such as obesity, cardiovascular disease, and DM. As a consequence, transplant surgeons accept organs from increasingly older donors and often with comorbidities. Livers from donors with extended criteria are more prone to prolonged periods of cold ischemia, compared to standard criteria donors.[2] In chapter 2 we have shown that prolonged (>8 hours) SCS and donor DM synergistically increase the risk of graft failure. It may be hypothesized that prolonged cold ischemia aggravates the preexisting microvascular dysfunction of these diabetic donor livers.



Poor hepatic microcirculation has been associated with severe ischemia-reperfusion injury.[3] Yet, only a few studies investigated the influence of microvascular dysfunction of the donor liver on outcome after liver transplantation. The microvasculature supplying the bile ducts in particular is a topic which deserves further research. In an earlier study from our group, it was shown that damage to the peribiliary vascular plexus is a risk factor for the development of non-anastomotic biliary strictures (NAS) after liver transplantation.[4] Every liver will be subjected to some degree of ischemic bile duct injury prior to transplantation, and the process of biliary regeneration requires an adequate supply of oxygen and nutrients. Impaired blood supply to the donor organ, therefore, seems a critical mechanism underlying the development of NAS. Although the DHOPE-DCD trial showed that end-ischemic DHOPE significantly reduces the incidence of NAS

compared to SCS alone, 6% of machine-perfused grafts still developed biliary strictures within 6 months after transplantation (versus 18% in the SCS group).[5] It is likely that, in these grafts, regeneration of the biliary tree was insufficient, leading to strictures and eventually requiring retransplantation in some cases. Disrupted microvascular function of the donor organ might play a role here and could be subject of future research. Therapeutic interventions that target microvascular dysfunction, such as platelet inhibitors, anti-inflammatory agents, or vasodilators could be advantageous. Such therapies would ideally be administered at the donor site before warm ischemic injury occurs (although this is currently not feasible for DCD donors in the Netherlands, due to national regulations).

In recent the years, clinical experience with machine perfusion has grown tremendously and the technique has been implemented in an increasing number of transplant centers worldwide. In the Netherlands, dual HOPE is now standard of care in the transplantation of livers obtained from DCD donors. Although end-ischemic HOPE can be considered a relatively simple technique (e.g., compared to normothermic machine perfusion or in situ normothermic regional perfusion) perfusion protocols vary between centers and consensus about several aspects of the technique is still lacking. With transplant groups worldwide pushing the boundaries to prolong machine perfusion time and the likelihood that more centers will implement HOPE as standard care, the need to further optimize and standardize the technique is ever increasing.

The route of perfusion has been an ongoing topic of debate.[6] The preclinical study in chapter 6 only showed a marginal positive effect for livers preserved by dual HOPE compared to single HOPE. However, this study was performed using an *ex situ* reperfusion model, which prevented us from drawing any conclusions regarding the development of NAS after longer follow-up. A prospective clinical study comparing dual versus single HOPE is not likely to be performed, because large numbers are needed to draw solid conclusions. It may be hypothesized that the beneficial effects of additional arterial perfusion become more evident during prolonged machine perfusion, which has not yet been studied. If single and dual HOPE are equally effective, this could facilitate transplant logistics in such a way as to allow less experienced surgeons, or perhaps even senior residents, to perform backtable preparations and connect the donor liver to the perfusion device. This way, if machine perfusion is prolonged to enable transplantation at daytime, the on-call surgeon does not necessarily need to connect the liver to the machine.

Another critical topic is the tight balance between perfusion pressure and occurrence of endothelial injury. Data collected from the multicenter study in chapter 8 substantiates that machine perfusion settings vary widely across centers. For example, portal venous pressures used during HOPE ranged between 3-9 mmHg with corresponding flow rates of 50-900 mL/min. In our center, we tend to use as low as possible pressures to ensure adequate arterial (>20 mL/min) and portal venous (>80 mL/min) flows. However, while a minimum perfusion pressure is less dangerous for sinusoidal endothelial cells, parts of the liver may remain under-perfused, especially in DCD grafts.[7] Homogenous perfusion is important to ensure adequate flushing of the organ and deliver oxygen to all cells, including those in the liver periphery, to restore mitochondrial function. In this light, I would propose a study using magnetic resonance imaging (MRI) to assess perfusion homogeneity during different perfusion pressures in real-time. Magnetic resonance techniques can visualize regional distribution patterns of hepatic perfusate flow, which was previously shown during kidney perfusion.[8]

Literature on the role of nutrients in hypothermic machine perfusion solutions is scarce. Currently, the UW Machine Perfusion Solution (MPS) as formulated by Belzer and colleagues in the 1980s is the most commonly used solution, and no significant modifications have been made to its original composition.[9] Vasosol solution is also used and is based on the chemistry of UW MPS, but with added antioxidants, metabolic substrates and vasodilators.[10] Optimization of the preservation solution for HOPE could be a topic for future research, especially in light of prolonged machine perfusion. Although graft metabolism is reduced to around 10% of normothermia, we observed several electrolyte-shifts and altered glucose levels in the perfusion solution during prolonged HOPE (own data, chapter 7, 9). These observations were made previously during short term HOPE, but seem to be more pronounced when machine perfusion is prolonged.[11] After approximately 10 hours of HOPE, perfusate glucose levels had tripled compared to baseline. Increasing glucose levels can either be attributed to (ongoing) glycogenolysis because of preservation injury, or, less likely, gluconeogenesis. Moreover, sodium levels decreased and potassium levels increased during machine perfusion. Under hypothermic temperatures, the enzymatic function of the Na+/K+-ATPase is likely to be impaired, which facilitates potassium release and impairs sodium uptake. After reperfusion in the recipient, opposite potassium shifts are observed with a risk of clinically relevant hypokalemia (whereas recipients receiving static cold

stored grafts used to be at risk for hyperkalemia). Theoretically, insulin added to the perfusate could enhance intracellular potassium and glucose uptake, but it is unknown if this mechanism functions under hypothermic conditions.

In an attempt to improve the preservation solution for (prolonged) HOPE, the effect of different oncotic agents is of interest. Whereas the oncotic component of UW solution and Vasosol is hydroxyethyl starch (HES), other solutions contain polyethylene glycol 35 (PEG35) as a main constituent. Viscosity is lower for solutions containing PEG as compared to HES, which has been associated with a more efficient washout of the organ and less shear stress.[12] The risk of rouleaux formation is reduced because hyper-aggregation of red blood cells with HES in livers flushed with UW is prevented. Moreover, the presence of PEG35 in a rinse solution seems to provide more mitochondrial protection and enhance vasodilation as compared to a rinse solution without PEG35.[13] In a preclinical study comparing 24-hour HOPE of rat livers with Polysol (a solution containing PEG35) versus UW, there was less cell swelling and preservation injury in the Polysol group.[14] In a retrospective study from Belgium, the effect of different SCS preservation solutions on outcome after transplantation was investigated.[15] Livers preserved with Institute George Lopez solution (IGL, containing PEG35) tended to less often develop biliary strictures after transplantation when compared to livers preserved in UW, but the difference was not statistically significant. However, the proportion of DCD donors was 3% in the UW group versus 37% in the IGL group, which is likely to have influenced the results. In all, it seems worthwhile to further investigate the effects of different oncotic agents in the perfusion solution, especially when HOPE is prolonged or for the preservation of fatty livers.

Safe prolongation of the *ex situ* preservation time is likely to have a substantial impact on current liver transplantation practice. To date, there are a few reported cases of liver transplantation after 24-hour preservation using normothermic machine perfusion (NMP).[16] In an impressive study from the Zürich group, preservation of porcine and discarded human livers up to 1 week with NMP was reported. Another study showed 27-hour preservation of discarded human livers with supercooling at -4°C. Yet, the results from chapters 7-9 strongly suggest that HOPE may realize similar results, but with a less complicated and labor-intensive technique. Since the organ is maintained in a hypometabolic state, there is little production of waste products and the need to make adjustment to perfusate is reduced. With rapid technical advances, such as artificial intelligence, it is not unlikely that, within a

couple of years, HOPE could be performed fully automated. In case of technical errors during NMP, the risk of graft failure is much higher, making it unlikely that prolonged NMP will be performed without anyone monitoring the perfusion.

When HOPE devices become transportable, prolonged preservation could be used to transport organs across large countries (e.g., United States) or between countries (e.g., Eurotransplant). This will also improve donor-recipient matching. Transportation costs will be reduced when livers are shipped with commercial flights instead of chartered jets, or by cars instead of ambulances. Storage time can also be prolonged in case of logistical issues at the recipient center (to accept two livers at the same time or in case there is a lack of operating rooms). Above all, prolonged HOPE may increase the number of suitable livers for transplantation by reducing organ discard for logistical reasons. Another major benefit could be to schedule transplantation at daytime instead of during the night, as proposed in chapter 9. Extended work hours and high intensity of workloads of medical personnel have become an increasingly important topic in medicine. The relationship between fatigue from working beyond regular working hours and occurrence of adverse events has been reported continuously and is of concern. More complex transplant cases, such as retransplantation, are particularly arduous when performed at night. Some studies point out that, with expertise, some surgeons have developed physiologic adaption to chronic sleep deprivation and, through conditional learning, practiced enough to reduce errors under these circumstances. Although in these cases the patient may not be at increased risk of complications, the concerning effects of inadequate sleep and circadian rhythm disruption for the attending surgeon should not be underestimated.[17] Epidemiological and genetic studies link disruption of circadian clock functions to an increased risk of several types of cancer. There is also evidence of a greater risk of metabolic syndrome, including increased body mass index and insulin resistance, in shift workers with sleep deprivation.[18] Besides its role in disease pathogenesis, there has been growing evidence of the interaction between disruption of the sleep-wake cycle and ageing, including ageing-related diseases, such as Alzheimer.[19,20] Intriguingly, the severity of ischemia-reperfusion injury is also likely to have a circadian rhythm pattern.[21] For example, most studies report larger infarct sizes after myocardial infarction in the early morning compared to later times during the day.[22] Considering the above, I believe nighttime surgery should be avoided whenever possible if prolonged HOPE is a safe and feasible technique to prolong preservation time and enable surgery at daytime.

Whilst safe prolongation of HOPE up to 24 hours will have a significant impact on transplantation logistics, I anticipate that preservation times can be extended beyond this timeframe. Future studies investigating HOPE up to several days or a week are awaited with excitement. From a more basic science point of view, it seems relevant to study the pathophysiological, cellular, or molecular pathways, which define the limits for the duration of HOPE. It will be of high value to study which organelles are damaged first and by which mechanisms of injury. More advanced techniques, such as multi-omics, could be used for this purpose.

To conclude, machine perfusion has evolved from bench to bedside and it is exciting to see HOPE being implemented as standard care for DCD livers in the Netherlands. The opportunity to prolong storage time of donor livers by machine perfusion is invaluable and the benefits for the transplant community are multifold. In the coming years, further optimization of several perfusion components is necessary to standardize the technique and enable safe clinical implementation worldwide. Above all, the limits of machine perfusion do not seem to have been reached just yet and I am looking forward to see where the field is going in the coming years.

## REFERENCES


1. Tector AJ, Mangus RS, Chestovich P, et al. Use of extended criteria livers decreases wait time for liver transplantation without adversely impacting posttransplant survival. Ann Surg. 2006;244(3):439-450.
2. Lozanovski VJ, Döhler B, Weiss KH, Mehrabi A, Süsal C. The Differential Influence of Cold Ischemia Time on Outcome After Liver Transplantation for Different Indications-Who Is at Risk? A Collaborative Transplant Study Report. Front Immunol. 2020;11:892.
3. Kelly DM, Shiba H, Nakagawa S, et al. Hepatic blood flow plays an important role in ischemia-reperfusion injury. Liver Transpl. 2011;17(12):1448-1456.
4. op den Dries S, Westerkamp AC, Karimian N, et al. Injury to peribiliary glands and vascular plexus before liver transplantation predicts formation of non-anastomotic biliary strictures. J Hepatol. 2014;60(6):1172-1179.
5. van Rijn R, Schurink IJ, de Vries Y, et al. Hypothermic Machine Perfusion in Liver Transplantation - A Randomized Trial. N Engl J Med. February 2021 Apr 15;384(15):1391-1401.
6. Brüggenwirth IMA, Burlage LC, Porte RJ, Martins PN. Is single portal vein perfusion the best approach for machine preservation of liver grafts? J Hepatol. 2016;64(5):1194-1195.
7. Schlegel A, de Rougemont O, Graf R, Clavien P-A, Dutkowski P. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. J Hepatol. 2013;58(2):278-286.
8. Schutter R, Lantinga VA, Hamelink TL, et al. Magnetic resonance imaging assessment of renal flow distribution patterns during ex vivo normothermic machine perfusion in porcine and human kidneys. Transpl Int. 2021;34(9):1643-1655.
9. Wahlberg JA, Southard JH, Belzer FO. Development of a cold storage solution for pancreas preservation. Cryobiology. 1986;23(6):477-482.
10. Guarrera J V, Henry SD, Samstein B, et al. Hypothermic machine preservation in human liver transplantation: the first clinical series. Am J Transplant. 2010;10(2):372-381.
11. Burlage LC, Hessels L, van Rijn R, et al. Opposite acute potassium and sodium shifts during transplantation of hypothermic machine perfused donor livers. Am J Transplant. 2019;19(4):1061-1071.
12. Panisello Rosello A, Teixeira da Silva R, Castro C, et al. Polyethylene Glycol 35 as a Perfusate Additive for Mitochondrial and Glycocalyx Protection in HOPE Liver Preservation. Int J Mol Sci. 2020;21(16).
13. Bardallo RG, da Silva RT, Carbonell T, et al. Role of PEG35, Mitochondrial ALDH2, and Glutathione in Cold Fatty Liver Graft Preservation: An IGL-2 Approach. Int J Mol Sci. 2021;22(10).
14. Bessems M, Doorschodt BM, van Marle J, Vreeling H, Meijer AJ, van Gulik TM. Improved machine perfusion preservation of the non-heart-beating donor rat liver using Polysol: a new machine perfusion preservation solution. Liver Transpl. 2005;11(11):1379-1388.
15. Van den Eynde J, Achtergaele J, Fieuws S, et al. The effect of organ preservation solutions on short-term outcomes after liver transplantation: a single-center retrospective study. Transpl Int. 2021;34(2):327-338.
16. Cardini B, Oberhuber R, Fodor M, et al. Clinical Implementation of Prolonged Liver Preservation and Monitoring Through Normothermic Machine Perfusion in Liver Transplantation. Transplantation. 2020;104(9):1917-1928.

17. Ruan W, Yuan X, Eltzschig HK. Circadian rhythm as a therapeutic target. Nat Rev Drug Discov. 2021;20(4):287-307.
18. Knutson KL, Spiegel K, Penev P, Van Cauter E. The metabolic consequences of sleep deprivation. Sleep Med Rev. 2007;11(3):163-178.
19. Hood S, Amir S. The aging clock: circadian rhythms and later life. J Clin Invest. 2017;127(2):437-446.
20. Wu H, Dunnett S, Ho Y-S, Chang RC-C. The role of sleep deprivation and circadian rhythm disruption as risk factors of Alzheimer's disease. Front Neuroendocrinol. 2019;54:100764.
21. Peek CB, Levine DC, Cedernaes J, et al. Circadian Clock Interaction with HIF1 Mediates Oxygenic Metabolism and Anaerobic Glycolysis in Skeletal Muscle. Cell Metab. 2017;25(1):86-92.
22. Muller JE, Stone PH, Turi ZG, et al. N Engl J Med. 1985;313(21):1315-1322.