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Transplantation of extended criteria donor livers

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Chapter 9

Summary, Discussion, and Future Perspectives



SUMMARY AND GENERAL DISCUSSION

The studies described in this thesis have resulted in a better understanding of strategies to improve the outcome of liver transplantation with extended criteria donor (ECD) grafts. Specific subgroups of ECD liver grafts with acceptable outcome and cost-effectiveness were identified. Furthermore, the clinical findings confirmed experimental findings that hypothermic machine perfusion has the potential to attenuate ischemia-reperfusion injury and prevent further damage of ECD liver grafts. With machine perfusion rapidly gaining a central role in ECD organ transplantation, a clinical-grade facility for organ preservation and resuscitation (OPR) has been developed in our center.

In this chapter, the results of this PhD thesis are summarized and discussed, followed by a section on future perspectives. An introduction to this thesis and the aims of this thesis were provided in **Chapter 1**.

In **chapter 2** we analyzed the long-term outcome of liver transplantation with pediatric DCD liver grafts in a retrospective cohort study including all Dutch pediatric (≤ 16 years old) DCD grafts ever transplanted since the start of a liver transplant program in the Netherlands. We assessed the 10-year graft and patient survival and incidence of NAS and compared it with that of pediatric DBD liver grafts in the same time period. We demonstrated that transplantation of livers from pediatric DCD donors resulted in good long-term outcome (10-year graft survival rate of 81%) when the donor warm ischemia time was kept ≤ 30 min. Furthermore, the incidence of non-anastomotic biliary strictures after transplantation of pediatric DCD livers was remarkably low. Although NAS is recognized as the most relevant and prevalent complication of adult DCD livers, this does not seem to be the case for pediatric DCD livers. Possibly, young donors have better preserved regenerative capacity of the bile ducts. This finding is in line with previous studies demonstrating that an impaired biliary regenerative capacity is associated with the development of NAS.^{1,2}

In **chapter 3** we examined the financial impact and clinical outcome of transplantation of high risk DBD liver grafts. We performed a 5-year prospective observational study together with the two other liver transplant centers in the Netherlands. The Eurotransplant donor risk index (ET-DRI) was used to determine graft quality. Patients were divided into four quartiles based on the ET-DRI. We demonstrated that the low quality of a DBD liver graft was associated with higher incidence of biliary complications and associated costs. However, the graft quality did not affect the total one-year costs, survival, or cost-effectiveness. A possible explanation is that the ET-DRI is designed for a population including DCD liver grafts, while this study included solely DBD liver grafts. To support this hypothesis, an additional analysis with both DBD and DCD grafts was performed and indeed we found that lower graft quality, based on ET-DRI, was associated with higher one-year costs and lower graft survival. Nevertheless, the ET-DRI is up to present the best available risk model with a continuous scoring system incorporating donor variables only.

Chapter 4 provided a general overview of the pathogenesis of NAS and the promising role of machine perfusion in preventing NAS. In summary, the key process in the pathogenesis of NAS is now considered to be the impaired regenerative capacity of the bile duct due to injury of essential

components such as the peribiliary vascular plexus and the peribiliary glands which contain progenitor cells. The different endpoints for assessment of the bile duct injury and function were discussed. Also, the wide spectrum of different approaches to machine perfusion was presented. An overview was given of the animal studies and preclinical human studies on machine perfusion in relation to biliary injury. In conclusion, these studies provide promising evidence that machine perfusion does not only reduce the amount of bile duct injury but also helps restore the regenerative capacity of the bile ducts. Therefore, machine perfusion may lead to attenuated ischemia-reperfusion injury in ECD livers and reduced risk of NAS.

In **chapter 5** we assessed the safety and feasibility of two hours of end-ischemic dual hypothermic oxygenated machine perfusion (DHOPE) in a first-in-man, prospective, case-control study. Between April 2014 and November 2014, ten consecutive patients (age ≥ 18 years) undergoing DCD liver transplantation in our center were included in the study. The study demonstrated that DHOPE was safe and feasible, as seen by 100% graft and patient survival at 1 year, no technical problems or device malfunctioning. The hepatic adenosine 5'-triphosphate (ATP) level increased 11-fold during DHOPE, postoperative markers for hepatobiliary function (bilirubin) were better and markers for ischemia-reperfusion injury (serum alanine aminotransferase, alkaline phosphatase, and γ -glutamyl transferase) were lower in the DHOPE group compared to the matched control group. Although DHOPE is a relatively simple and safe technique, it does not allow for *ex situ* functional assessment as is the case in normothermic machine perfusion. Hypothermic machine perfusion rather aims at attenuating ischemia-reperfusion injury and thereby reducing posttransplant complications. Whether DHOPE can actually reduce the risk of complications has yet to be assessed in randomized controlled clinical trials.

In **chapter 6** we investigated the effect of DHOPE on bile duct injury in DCD liver transplantation performed in chapter 5. We demonstrated that the degree of bile duct injury increased after graft reperfusion compared to at the end of static cold storage in the control group (stroma necrosis and deep peribiliary glands). However, in the DHOPE group, the level of injury did not increase. Also, the level of injury after reperfusion was lower in the DHOPE group compared to the control group (deep peribiliary glands). In contrast to previous studies by our group, we did not observe an effect of DHOPE on the peribiliary vascular plexus and arteriolonecrosis.³ A possible explanation is the short time interval of 1-2 hours after graft reperfusion in this study compared to 4 hours in the previous study. Altogether, the results in this chapter suggest that DHOPE prevented further worsening of bile duct injury after graft reperfusion. As injury to the peribiliary glands is a known risk factor for development of NAS, the protective effect of DHOPE on the peribiliary glands can (hypothetically) lead to reduced risk of the NAS.² The key mechanism of DHOPE is probably the restoration of cellular ATP content by "resuscitation" of the mitochondria. The downstream effects are reduced ROS-production, decreased Kupffer cell activation and decreased activation of the innate immune system.⁴⁻⁸ Although the aforementioned effects of hypothermic machine perfusion have previously been demonstrated with regard to hepatocellular injury, this study demonstrated such an effect on

bile duct injury for the first time.

In **chapter 7** we described the study protocol of the DHOPE-DCD Trial which aims to determine the efficacy of DHOPE in reducing the incidence of NAS in DCD liver transplantation. In summary, it is a multicenter, international, prospective parallel-arm, randomized, controlled trial comparing DHOPE with SCS. It was initiated in October 2015 and has included 50% of 156 planned trial patients so far. At present, five academic centers in the Netherlands and Belgium are participating in the trial (Groningen, Rotterdam, Leiden, Leuven, and Gent) and two centers in the United Kingdom will be joining soon (Birmingham and King's College in London).

In **chapter 8** we described the development of the organ preservation and resuscitation (OPR) unit for clinical-grade machine perfusion of livers, lungs, and kidneys. With the OPR unit, optimal conditions were created to facilitate the increasing use of machine perfusion. The implementation of machine perfusion in clinical care was not only enabled by building a location such as an OPR unit. Also, financial means were required for materials, machines, disposables, and additional dedicated personnel such as 'organ perfusionists' which may become a new profession.

Future Perspectives

Although the studies in this thesis have contributed to new strategies to improve the outcome in ECD liver transplantation, there are still many new challenges and questions to be addressed. In the last section of this chapter, these challenges will be discussed and new directions for future research will be given.

The transplant community has been continuously investigating approaches to safely expand the donor liver pool. Assessment of risk factors for graft dysfunction and posttransplant complications has led to better understanding of the boundaries. Also, risk models such as the balance-of-risk (BAR), donor risk index (DRI), and Eurotransplant-DRI (ET-DRI) have been developed to estimate the risk of a specific liver graft. However, these models should be used cautiously for individual patients as they have been developed and validated within large retrospective studies.⁹⁻¹² Chapter 3 illustrates that such models have limited value in smaller cohorts and are less suitable for study populations with exclusively DBD or DCD grafts. As the graft type (DCD or DBD) weighs heavily in the ET-DRI score, the model retains poor predictive value after excluding this factor from the study population. Separate risk models for DCD and DBD liver grafts would add to the understanding of the risk factors for each graft type. Eliminating graft type from the model will allow novel risk factors to become apparent, such as donor warm ischemia time, a well-known and important risk factor for DCD liver transplantation, which is not incorporated in current risk models such as the ET-DRI.^{9,13}

Data presented in this thesis suggest that improved outcome after ECD liver transplantation could be achieved with end-ischemic DHOPE. Excellent results after DHOPE have been demonstrated for bile ducts and liver graft quality in general. Whether this technique can actually reduce the incidence of NAS remains to be determined with randomized controlled trials, such as the trial described in chapter 7. Although DHOPE is a promising technique, there is no consensus on

active oxygenation of the perfusion fluid as well as dual perfusion of the portal and arterial vessels. Data from experimental studies suggest that active oxygenation significantly improves the effect of hypothermic machine perfusion on ischemia-reperfusion injury.^{5,8,14-18} However, there are no studies that investigated dual perfusion via both the hepatic artery and the portal vein compared to single perfusion of the portal vein only. Theoretically, dual perfusion is superior to single portal perfusion as the biliary vascular plexus is mainly dependent on the arterial system.^{19,20} With NAS being the most feared complications after DCD liver transplantation, it seems sensible to perform dual perfusion despite the possible risk of causing mechanical injury to the fragile hepatic artery, although this has not been seen in any clinical study reported until now. Moreover, recent studies by our group demonstrate that DHOPE has a positive effect on endothelial function.²¹ Nonetheless, the superiority of dual versus single perfusion should be investigated in future experimental and clinical studies.

The studies described in this thesis concerned transplantation of liver grafts which were transplanted within the acceptance criteria. However, many livers fall outside these criteria and are declined for transplantation. A considerable number of these donor livers are currently being declined due to an increased risk of NAS. The exact number is not known, but in total 30% of livers of donors are accepted for transplantation.^{22,23} The remaining 70% of donor livers could potentially be used if protective methods, such as machine perfusion, are able to reduce the risk of NAS, primary non-function, and other posttransplant complications. Remarkably, adequate liver function in the donor is no guarantee for successful liver transplantation.²⁴ Processes during donation and transplantation cause injury to the graft and can lead to posttransplant complications. Such processes include death of the donor, ischemic conditions during organ procurement, preservation, and transplantation.²⁴⁻²⁶ The universal hypothesis is that the function of declined livers should be assessed with machine perfusion after the injury mentioned above is acquired. As pointed out previously, there are different temperatures for machine perfusion such as hypothermia (0-12°C), subnormothermia (25-34°C), and normothermia (35-38°C).²⁷ The liver function cannot be tested at hypothermic conditions because cellular metabolism is initiated at $\geq 12^\circ\text{C}$.^{27,28} Therefore, the role of DHOPE in declined livers is probably limited but remains to be investigated. For declined livers, subnormothermic and especially normothermic machine perfusion can be used to assess the hepatobiliary cellular function by analyzing biomarkers (real-time) in perfusate and bile.²⁹⁻³² Recently, excellent results of transplantation after normothermic perfusion have been reported in livers initially declined for transplantation.³³⁻³⁵ Whether this approach leads to an acceptable incidence of NAS has yet to be determined as the incidence of NAS was high (27%) in the first case series by Watson *et al.*³⁵ Also, the long-term survival remains to be investigated.

Another advantage of an intact cellular metabolism during (sub)normothermic machine perfusion is the ability to administer drugs or perform interventions aiming to improve graft function. As underlined by the low incidence of NAS in pediatric DCD liver grafts (chapter 3), the regenerative capacity is a key component in the development of NAS. Enhancement of the

regenerative capacity of the bile duct can potentially be mediated by adding mesenchymal stem cells to the perfusion fluid. Mesenchymal stem cells are known to be able to differentiate into cell types other than their tissue of origin, have unique immunomodulatory features and secrete growth factors and anti-apoptotic cytokines.³⁶ In kidney transplantation, pre-clinical results are promising and first clinical trials have demonstrated that MSCs are safe and feasible.^{37,38} Follow-up studies are awaited. Another interesting new research field in normothermic machine perfusion is liver graft imaging. Real-time functional testing of the mitochondrial resuscitation and measurement of ATP levels during machine perfusion have been successfully performed using MRI in porcine kidneys.^{39,40} This technology may provide valuable information on a reliable marker (ATP) of organ viability in real-time.

Regardless of the potential benefits of normothermic machine perfusion, the optimal temperature for machine perfusion has yet to be determined. Experimental research from our group has suggested that machine perfusion reduces the degree of ischemia-reperfusion injury regardless of the temperature.⁴¹ Moreover, temperature changes can be achieved promptly or gradually with machine perfusion. Based on excellent results in animal studies, Minor *et al* have initiated a clinical trial with controlled oxygenated rewarming of ECD liver grafts, of which the results are awaited with interest.^{42,43}

The studies in this thesis have focused on improving the outcome of ECD livers without increasing the total number of available donor livers. The total number of livers can potentially be increased with the application of (sub)normothermic machine perfusion or controlled oxygenated rewarming. These types of machine perfusion enable the functional assessment of high-risk donor livers that would otherwise not be accepted for transplantation. Another method to increase the total number of donor livers is increasing the informed consent rate for organ donation. The impact of a higher consent rate could be substantial. In the Netherlands the informed consent rate is as low as 35% while it is 66% and 85% in the United Kingdom and Spain respectively.^{22,44} An increase of 5% would result in 40 extra donors in the Netherlands while about 40 patients awaiting liver transplantation yearly die on the liver waiting list.²² Therefore, in my opinion, nation-wide investigations should be initiated into factors involved in the low consent rate in the Netherlands, similar to the research carried out by the United Kingdom's Organ Donation Taskforce 2008.⁴⁵ This taskforce demonstrated that the most easily modifiable factor affecting consent rate was the involvement of the Specialist Nurses in Organ Donation (SN-OD) in the family approach.^{45,46} Similarly in Spain, beneficial effects have been seen after the introduction of trained requestors.⁴⁷ Identification of factors associated with increased informed consent rate specific to the Dutch population would be valuable for any patient awaiting transplantation.

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