Studies on bile duct Injury and the protective role of oxygenated machine perfusion in liver transplantation
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CHAPTER 1

General Introduction and Aims of This Thesis
End stage liver disease (ESLD) due to chronic liver disease and cirrhosis is the 12th leading cause of death with an estimated 65,000 deaths per year in the United States (1). While liver transplantation has proven to be the only successful treatment for patients suffering from ESLD, only about 10,000 patients per year are listed for liver transplantation in the US (2). Yet despite the improvements in the awareness about organ donation there is not enough organ supply for patients on the waiting list. There are currently over 123,000 patients according to the United Network for Organ Sharing (UNOS) and 15,000 patients in the Eurotransplant region on the waiting list for organ transplantation; however, during 2014 only 14,000 donors in the UNOS and about 2,000 deceased donors in the Eurotransplant region were registered and used for transplantation. This discrepancy in the supply and demand for organ transplantation causes high rates of mortality on the waiting list and inferior post-transplantation survival in the patients who are on the waiting list for a long time (3).

In an effort to overcome the organ shortage crisis, the selection criteria for accepting livers for transplantation has been extended towards using donor liver grafts with suboptimal quality when compared with standard criteria donors (SCD). This group of donor grafts, so called “extended criteria donor” (ECD) livers, include grafts with mild to moderate steatosis, livers from older donors or donors with a higher body mass index (BMI), and livers donated after circulatory death (DCD) (4-6). However, despite the increase in the number of donors over the last decade the utilization of donor livers for transplantation has declined, mostly due to an increasing number of DCD liver grafts (7). The reasons to decline a DCD graft include a higher incidence of early or delayed graft dysfunction, graft failure, and biliary complications resulting in inferior post-transplantation outcomes (8-10).

Biliary complications remain a major concern among all the various post-transplantation complications. Biliary complications occur in 10-40% of recipients and affect the patient survival, hospital readmissions, and overall costs of care (11-13). Bile duct strictures are the most troublesome of all biliary complications. They are categorized into anastomotic strictures (AS) and non-anastomotic strictures (NAS). AS are solitary strictures at the anastomosis site with an incidence rate of 9-12% (14-16). NAS on the other hand are usually multifocal strictures occurring at any location of the biliary tree (extrahepatic and intrahepatic). NAS occur in up to 10% of patients receiving a liver donated after brain death (DBD) (16-18), while an incidence rate of up to 30% has been reported in recipients of DCD livers (18-22). The underlying mechanisms causing AS and NAS have been under investigation for many years. Major predictive risk factors for development of AS were shown to be donor age, previous anastomotic leak, duct to duct anastomosis, and sex mismatch with a female donor/male recipient (13,23,24). The etiology of NAS is thought to be multifactorial and can be categorized into four types of biliary injury: 1) ischemia/reperfusion injury; 2) immune-mediated injury; 3) cytotoxic injury caused by hydrophobic bile salts; and 4) insufficient regeneration capacity of progenitor cells located in peribiliary glands (24-26). NAS is associated with high rates of morbidity and mortality due to its therapy-resistant nature and that makes NAS a major burden in liver transplantation, in particular DCD liver transplantation (24).

Despite the higher rates of post-transplantation complications after DCD liver transplantation,
this type of grafts seems to be one of the most important solutions to increase the donor pool. The number of DCD liver transplantation is continuously increasing (7) and circulatory death protocols for organ donation are becoming authorized in more countries (27), leading to increased overall costs of transplantation (28). Therefore it is crucial to understand the underlying mechanisms of post-transplantation complications, most importantly NAS, and investigate the potential preventive or therapeutic strategies to improve the quality of organs before transplantation. Machine perfusion has attracted increasing attention over the last decade as an alternative organ preservation method to conventional static cold storage (SCS). However, the role of different modalities of machine perfusion in improving the quality of liver grafts and protection of bile ducts is not fully known yet. Therefore, the aim of this thesis is to provide a better understanding of the etiologies of bile duct injuries that lead to NAS after transplantation and to study the role of different modalities of machine perfusion to improve the quality of organs prior to transplantation and potentially improve organ selection for transplantation. This thesis concludes with the first clinical experience of machine perfusion prior to transplantation of DCD liver grafts as a way to improve the quality of the organ before transplantation.

Part A: Bile Duct Injury in Liver Transplantation

The aim of this section is to provide a literature-based overview on the occurrence of bile duct injury after liver transplantation and to further investigate the underlying etiologies of NAS. Different types of biliary complications that can occur after liver transplantation are discussed in chapter 2. The prevalence and clinical presentation of these complications, diagnostic and therapeutic options as well as potential preventive strategies are discussed in this chapter. The exact mechanisms leading to development of NAS after transplantation are still poorly known. A better understanding of pathophysiology of NAS may lead to improvements of preventive and therapeutic strategies. The aim in chapter 3 is to understand the origin of biliary injury during liver transplantation. The traditional belief is that the development of NAS after transplantation depends on the amount of biliary epithelial cell injury that occurs after transplantation due to ischemia-reperfusion injury, immune-mediated injury, or bile salt cytotoxicity. However, two studies showed that severe biliary epithelial cell injury exists already before transplantation in the vast majority of liver grafts (29,30,31) but only 10-15% of liver grafts develop NAS after transplantation. A new perspective on the extent and origin of bile duct injury during liver transplantation is discussed in this chapter. Clinical investigations on bile duct injury during liver transplantation have been performed on the biopsies taken from the distal end of the donor extrahepatic bile duct (26). However, it is not known whether the amount of injury detected in the biopsies taken from the extrahepatic bile duct represents the injury throughout the biliary tree including intrahepatic bile ducts. The aim of study described in chapter 4 is to determine whether the injury in the extrahepatic bile duct is representative for the rest of the biliary tree. Therefore, in this study, histological injury of extrahepatic bile duct of human donor livers that were declined for transplantation for various reasons were assessed based on a systematic scoring system (26) and was
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compared with the histological injury detected in the various levels of intrahepatic bile ducts, including sectoral and segmental bile ducts.

Among the underlying etiologies of NAS, immune-mediated bile duct injury is the least well studied. The aim of chapter 5 is to determine the role of immune system in the development of bile duct injury. NAS has been associated with various immunological processes including ABO blood type incompatibility between the donor and the recipient (32,33), recipients' preexisting autoimmune disease such as primary sclerosing cholangitis (PSC) (34), cytomegalovirus infection (35), and chronic rejection (36). Moreover, it has been reported that recipients carrying a loss-of-function mutation in the chemokine receptor CCR5 (CCR5 Δ32) have a higher rate of NAS development after transplantation (37). CCR5 functional deficiency may lead to impaired recruitment of regulatory T cells (TRegs), which have potent anti-inflammatory properties (38,39). In this chapter we assessed the role of CCR5 functional deficiency on recruitment of TRegs upon obstructive bile duct injury in CCR5 knock out mice and compared the effect with their corresponding wild-type mice.

Part B: Oxygenated Machine Perfusion: A Potential Strategy to Improve Organ Quality Prior to Transplantation

In this section the role of oxygenated machine perfusion in improving the quality of liver grafts before transplantation, its potential protective effect on bile duct injury, the safety and feasibility of machine perfusion in human livers, and the potential effect of machine perfusion on organ selection for transplantation is studied. Machine perfusion has been suggested as an alternative organ preservation tool to SCS since many years ago (40,41). However, over the last decade with improvements in the machine perfusion technologies it has attracted a lot of attention because of its potential in preservation of DCD and other ECD livers before transplantation (42,43). Machine perfusion can be performed at different temperatures categorized as hypothermic (0-10°C), subnormothermic (±21°C) and normothermic (37°C) (24,44). During machine perfusion the liver grafts are perfused with an oxygenated or non-oxygenated perfusion fluid (45-48).

Hypothermic machine perfusion (HMP) has shown promising results in preclinical and the first two clinical studies, offering a better preservation of liver and peribiliary vascular plexus (49,50). However, HMP did not provide protection of the bile duct epithelium, when compared to SCS, in a porcine DCD model (50). On the other hand, normothermic machine perfusion (NMP) of donor livers might offer potential to meet the higher requirements of suboptimal liver grafts by providing an environment that is closer to physiological conditions. Therefore, the aim of chapter 6 is to evaluate the impact of NMP on preservation of bile duct in both DCD and non-DCD rat livers.

In chapter 7 the technique of ex-situ NMP of human donor livers is described as a detailed protocol combined with a video demonstration of the technique (51). A newly developed device is used for perfusion, which allows dual pressure-controlled perfusion of liver grafts providing a pulsatile arterial flow through the hepatic artery and a continuous portal vein flow.
The feasibility of oxygenated ex-situ NMP of human ECD livers using this perfusion device has been shown before (52). NMP provides a near physiological environment for the livers allowing the grafts to function with full metabolic activity. This might offer the opportunity to evaluate the function of the grafts before transplantation unlike SCS in which the liver does not show any detectable function. A better evaluation of graft function might improve organ selection for transplantation. Therefore it is necessary to identify markers during NMP that can predict liver viability after transplantation. For this reason in chapter 8 we aimed to develop criteria to evaluate liver function and viability early during the course of NMP that would allow us to predict liver function and viability during 6 hours of NMP (53).

Oxygenated machine perfusion can be performed at different phases during organ preservation. One approach is normothermic regional perfusion (NRP) to re-oxygenate DCD organs during a short period of in situ perfusion (54,55). Another approach is to use NMP instead of SCS during the whole period of organ preservation and transportation (48). Although NMP may provide better viability testing and preservation of livers, it requires a more complex and challenging setting to provide a near-physiological environment for the graft (48,56). Introducing the technique of machine perfusion to the clinical setting requires logistic simplicity and safety. A relatively simple approach to revitalize the organs prior to transplantation is end-ischemic hypothermic oxygenated machine perfusion. Experimental studies have shown that end-ischemic hypothermic oxygenated machine perfusion allows organ recovery by boosting the liver tissue ATP content (46,57,58). This approach allows safe organ transportation without any chances of technical failure and organ resuscitation occurs in the transplantation center immediately prior to transplantation. In chapter 9 we evaluated the efficacy of this approach as a proof of concept in discarded human donor livers. Human donor livers that were not accepted for transplantation for various reasons were subjected to hypothermic oxygenated machine perfusion (HMP) for 2 hours followed by normothermic viability assessment using NMP for 6 hours. Given the very promising results of this study, we initiated the first clinical feasibility study on dual hypothermic oxygenated machine perfusion (DHOPE) to revitalize DCD liver grafts prior to implantation. This study is described in chapter 10.

**Part C: Addendum**

In this section, this thesis is concluded by providing a summary of the results, followed by a general discussion and future perspectives in chapter 11.
REFERENCES


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PART A

Bile Duct Injury in Liver Transplantation