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General Introduction and Aims of the Thesis
Introduction

Liver transplantation has emerged over the past decades as a successful treatment option for patients with acute liver failure, end-stage liver disease, and hepatocellular carcinoma (1). After the kidney, the liver is the second most transplanted organ worldwide. Annually, around 6000 liver transplantations are performed in Europe, a number similar to that of the United States of America (1). One-year patient survival rate after transplantation is about 85-90% and the long-term survival rate is approximately 75-85% (1,2). Although the number of transplantations has increased progressively over the last three decades, the expanding demand of liver grafts is not balanced by an increase in donation of livers. The annual report of the Eurotransplant International Foundation 2013 showed a growing difference between available donor livers and the number of patients on the waiting list. Currently, approximately 15% of patients die while waiting for a suitable donor organ (2). In the beginning of clinical liver transplantation, donor livers were recovered from relatively young and healthy donors who had suffered traumatic brain injury, typically in the context of a motor vehicle accident. However, the supply of these reference donors is not enough to expand the pool of available organs today. To minimize the current organ shortage, many centers try to reduce the present misbalance between organ demand and supply, with the use of domino liver grafts, living donations, and so-called extended criteria donor (ECD) livers. Today, around two-thirds of transplanted livers are derived from ECDs (3,4). Characteristics of ECD livers are donor age above 40 years, graft steatosis, grafts with underlying diseases (for example hepatitis B and C), donors derived from donation after circulatory death (DCD) procedures, donors with abnormal liver function tests, and causes of donor death different than trauma (5). ECD livers, previously called “suboptimal grafts”, are associated with an expected inferior tissue quality compared to reference donors. Due to this impaired quality, ECD livers are more prone to preservation and ischemia/reperfusion (I/R) related injuries (5-7). As a result, ECD livers are related to higher incidences of postoperative risks such as an increased rate of primary non-function (PNF), delayed graft function, and biliary complications (8,9). In particular, biliary complications, like non-anastomotic strictures (NAS), are a major reason for long-term morbidity. Patients with NAS may suffer from recurrent periods of cholangitis due to bile duct necrosis and fibrosis. In severe cases of NAS, injury to the bile ducts may cause biliary cirrhosis and unfortunately, a second episode of end-stage liver disease. For that reason, severe NAS can only be cured with a retransplantation (10). Therefore, NAS formation is one of the most troublesome complications after transplantation. In particular, the occurrence of NAS after DCD donation limits transplant centers to routinely accept DCD donor livers for transplantation (11).
Currently, the issue is not whether ECD livers should or should not be used in liver transplantation. ECD livers are immediately needed for increasing the pool of potential donor livers, even if their use is associated with an increased morbidity and mortality (5). One of the current strategies to deal with ECD donor livers is to only accept them for transplantation when additional donor and recipient related risk factors are limited (11,12). On the other hand, some studies have shown that also a survival benefit can be achieved if ECD donors are transplanted in patients with high model for end-stage liver disease (MELD) scores (13,14). Although both strategies will lead to a reduction of waiting list mortality, these strategies are not sufficient enough to make more ECD livers suitable for transplantation. For that reason, the aim of this thesis is to assess other strategies to improve outcome after transplantation of ECD livers. In particular, we studied the potential role of machine perfusion in reducing hepatobiliary preservation injury and its ability to enhance organ quality prior to transplantation.

Part A: Strategies to Improve Outcome after Transplantation of ECD livers in Clinical and Animal Studies

The aim of this section (Part A) is to study the effects of new potential strategies in handling ECD livers, as conducted in clinical and animal studies. One of the easiest strategies to lower the decline in quality of an ECD liver is reduction of the cold ischemia time. The cold ischemia time during a transplant procedure is defined as the time between cooling of a liver graft, subsequent cold storage on ice, and the start of implantation in the recipient.

As mentioned previously, advanced age of the donor is an important ECD liver variable. Due to the aging process, the older liver has an impaired regenerative capacity and decreased synthetic function (15). Moreover, these livers have a reduced capacity to adequately respond to oxidative stress, to which the liver is exposed during preservation (16). Transplantation of livers from older aged donors has been associated with an impaired postoperative function and a higher incidence of biliary complications (17,18). On the other hand, one could hypothesize that acceptable postoperative outcomes can still be achieved if the cold ischemia time is kept short when elderly donor livers are transplanted. In chapter 2 we tested this hypothesis in a study focussing on postoperative outcomes, in particular incidences of biliary complications, after transplantation of elderly donor livers (≥ 65 years) in a cohort of patients transplanted in the three transplant centers in the Netherlands.

Another ECD liver variable is donor liver steatosis. From a histological perspective, steatosis can be categorised qualitatively and quantitatively. Qualitatively, there are two different histological varieties of hepatic steatosis: macrovesicular and microvesicular steatosis. Quantitatively, hepatic steatosis can be categorized
according to the percentage of hepatocytes affected by fat vacuoles; < 30% mild, 30-60% moderate, and > 60% severe steatosis (19). To what extent steatosis affects postoperative outcome depends on the type and grading. Macrovesicular steatosis is in general associated with higher susceptibility to I/R injury and poorer postoperative graft function, whereas microvesicular steatosis does not negatively influence outcome (20). In addition, mild macrovesicular hepatic steatosis is thought not to negatively affect outcome, on the other hand, the general consensus is that severe macrovesicular steatotic grafts should not be used for transplantation (20,21). Moderate steatosis is an important risk factor for poorer postoperative outcome. It is, however, unclear what the effects of moderate steatosis are on postoperative outcomes when the cold ischemia time is kept short (12). The aim of chapter 3 was therefore to examine if short cold ischemia times during transplantation of moderate (30-60%) macrovesicular steatotic donor livers can lead to similar postoperative outcomes compared to donor livers without steatosis.

Nowadays, no tools are available to quickly and accurately evaluate the degree of hepatic steatosis (22). Assessment of the donor liver is mostly performed in a short period of time. Although histopathological assessment by the pathologist is still the gold standard, this technique is prone to intra- and interobserver variability and pathological expertise is not always available during off hours, especially not in a local donor hospital (23). In addition, inspection and palpation by the surgical team during the donation procedure has low predictive value and remains subjective, especially in grafts with higher degrees of steatosis (> 30%) (24,25). Moreover, ultrasound, computed tomography, magnetic resonance imaging, and magnetic resonance spectroscopy can visualize the hepatic fat content accurately, but none of these techniques can distinguish between different degrees of macrovesicular steatosis and they are all relatively time-consuming (22,26). Due to this lack of techniques, which can assess hepatic steatosis accurately and in real-time, there is a risk that the degree of steatosis is overrated and mild steatotic grafts are incorrectly declined for transplantation. In chapter 4 and 5 we, therefore, studied whether a new technique, diffuse reflectance spectroscopy (DRS) developed by Philips Healthcare, could measure the degree of hepatic steatosis accurately and in real-time. In particular, we assessed if the DRS system could discriminate between mild and moderate/severe steatosis, to help the transplantation team with a better evaluation of the degree of macrovesicular steatosis in potential donor livers.

Intraoperative blood loss and transfusion requirements during liver transplantation have also been identified as risk factors for inferior postoperative outcome (27-29). The possible negative effects of blood transfusions include alloimmunization, transmission of viral diseases, graft-versus-host disease, and an increased postoperative infection rate (30). ECD livers are related to inferior graft quality and increased vulnerability for preservation injury (5). It is therefore conceivable that transplantation with ECD livers is associated with more blood
loss and higher amounts of transfusion requirements. The purpose of the study described in Chapter 6 is to determine whether ECD livers, defined as a donor risk index score of 1.7 or higher, are associated with increased intraoperative blood loss and transfusion requirements during transplantation.

**Part B: Machine Perfusion as a Strategy to Improve Hepatobiliary Viability in ECD Livers**

The aim of this section (Part B) is to study the effects of machine perfusion in its ability to reduce hepatobiliary preservation injury and improve function of ECD livers. Recently, machine perfusion during organ preservation has regained clinical interest for optimizing ECD liver organs prior to transplantation (31-33). The advantages of machine perfusion over static cold storage (the current preservation method) include continuous perfusion of the liver with perfusion fluid enriched with oxygen and nutrients. As a result, the liver will less suffer from anaerobic conditions, which normally occurs during static cold storage (34,35). By providing oxygen and nutrients, the mitochondria can maintain a better functional state and more importantly, the effects of hepatobiliary preservation injury and I/R injury will be minimized compared to static cold storage alone (36). It is known that ECD livers are more prone to hepatobiliary preservation and I/R injury. As a consequence, the general hypothesis is that ECD livers will benefit the most from machine perfusion during organ preservation (37).

In Chapter 7 we provide an overview of the literature on the pathophysiology of NAS and the potential protective role of machine perfusion to lower biliary preservation injury. The aim of Chapter 8 is to examine the role of the peribiliary glands in the pathophysiology of NAS and to find new starting points for future research with machine perfusion.

Although various studies have been shown that with machine perfusion postoperative outcomes of ECD livers can be enhanced, knowledge is still limited about its working mechanism. The aim of Chapter 9 is therefore, to examine the effects of machine perfusion at the end of the period of static cold storage (also called end-ischemic machine perfusion) on a variety of human (discarded) ECD livers. In this study, machine perfusion was conducted at a perfusion temperature of 10 °C (hypothermic machine perfusion). However, the optimal perfusion temperature and temperature strategy for end-ischemic machine perfusion is still under debate (4). In particular, the optimal perfusion temperature for protection of the vulnerable bile ducts in DCD donor livers is not known. It is the purpose of Chapter 10 to examine the ideal perfusion temperature and temperature strategy during end-ischemic machine perfusion in DCD rat livers.
Machine perfusion at 37 degrees (normothermic machine perfusion) has the benefit that the liver is fully metabolically active. As a result, during normothermic machine perfusion pharmacological compounds could be added to the perfusion solution to optimize graft quality and lower preservation injury (38,39). In chapter 11 we report on the results of a study on the pharmacological pre- and postconditioning effects of metformin during normothermic machine perfusion in rat donor livers.

In chapter 12 the results as described in the thesis are summarized and future perspectives are discussed.

Finally, this thesis ends with a Dutch summary (chapter 13).
References


PART A

Strategies to Improve Outcome after Transplantation of Extended Criteria Donor Livers in Clinical and Animal Studies