CHAPTER 1

Introduction and Aim of This Thesis

Liver transplantation is a life-saving treatment for patients with end-stage liver disease or hepatocellular carcinoma. Outcome after liver transplantation has substantially improved since the first human liver transplantation in 1963 (1,2). Improved surgical techniques, anesthesiology, intensive care facilities, and immunosuppressant therapy have reduced morbidity and mortality rates during the years thereafter. While 1- and 5-year patient survival rates are currently around 90% and 75%, respectively, other challenges have emerged (3). The success of liver transplantation has broadened the indication for liver transplantation, thereby increasing the time on the waiting list for liver transplantation. The number of patients on the waiting list now far exceeds the number of available donor livers (4). As a consequence, patients may become either too sick to be transplanted or die before a suitable donor liver becomes available. The waiting list mortality in the Netherlands during the period 2006 – 2017 was 17% (3).

Increased Use of High-Risk Donor Livers

To increase the number of donor livers, extended criteria donor (ECD) livers are increasingly used for transplantation. ECD livers are characterized by graft steatosis, high donor age, donation after circulatory death (DCD), abnormal laboratory tests of liver function or -injury, prolonged ischemia times or a combination of these (5-9). ECD livers are more susceptible to ischemia reperfusion injury (IRI) (5,10). As a result, complications such as primary non-function (PNF), early allograft dysfunction (EAD) and post-transplant cholangiopathy (including non-anastomotic strictures (NAS) of the biliary tree) are more often seen after transplantation of ECD livers (6,7,11). Today, ECD livers account for >50% of all transplanted donor livers in the Netherlands (12). However, a considerable part of ECD livers is still discarded because of fear for complications. In 2016 in the Netherlands 235 donor procedures were effectuated, yet 75 livers (32%) remained unused (13). These discard rates are similar in the UK and the USA (14). One could imagine that at least some of these 'discarded livers' could potentially be used for transplantation. If we could identify suitable livers from the discarded pool, we could increase the number of liver transplantations.

Complications after Transplantation of High-Risk Donor Livers

The incidence of previously mentioned complications PNF, EAD and NAS are 2-6%, 2-25% and 15-30%, respectively (15-18). The incidence of NAS is particularly high in DCD livers, due to the inevitable period of warm ischemia prior to organ procurement (18-22). NAS are difficult to treat because of the multifocal localization of strictures throughout the biliary tree. If severe, NAS may lead to graft loss necessitating re-transplantation (23-26). Although the pathogenesis of NAS is not fully understood, several studies have shown that IRI is one of the main contributors to the development of NAS (27-29).

There is currently no reliable method to predict whether donor livers, especially ECD livers, will develop NAS, PNF or EAD. A reliable method is necessary to predict which ECD liver could safely be transplanted, requires treatment prior to transplantation, or should be discarded.

Aim of This Thesis

The aim of this thesis is to increase the knowledge on the pathogenesis of NAS and to identify ECD livers that can be safely transplanted despite initial decline, by using *ex situ* machine perfusion. Bile duct viability during *ex-situ* machine perfusion and the development of post-operative (biliary) complications play a central role in this thesis.

Outline of This Thesis

Chapter 2 provides an historical overview of liver transplantation in Groningen. From 1979 to 2016, 1478 liver transplantations were performed in Groningen, of which 459 were in children. Important developments and new challenges and opportunities in liver transplantation are described in this report. **Chapter 3** describes the pathogenesis of post-transplant cholangiopathy. This chapter explains that post-transplant cholangiopathy must be considered a spectrum of bile duct pathologies. Furthermore, four proposed main risk factors for post-transplant cholangiopathy are critically reviewed.

Machine Perfusion

Dynamic preservation of donor livers by using machine perfusion can be performed with different goals, i.e., resuscitation, *ex situ* viability assessment, and administration of therapeutic agents for potential optimization and/or repair. Oxygenated hypothermic machine perfusion, performed at 4 – 12 °C resuscitates the mitochondria and increases adenosine triphosphate (ATP) content, thereby mitigating IRI to the donor liver upon transplantation (30,31). Normothermic machine perfusion (NMP), performed at 37 °C allows for *ex situ* viability assessment of donor livers as the liver is fully metabolically active at this temperature (32,33). Furthermore, at (sub)normothermic temperature therapeutic agents can be administered to the perfusion solution. With regards to *ex situ* viability assessment of donor livers several research groups have established potential viability criteria which a livers should meet before being deemed transplantable (32-34).

Chapter 4 aims to establish biliary viability criteria. Thus far, viability testing during NMP mainly focused on hepatocellular function and injury, but not on cholangiocyte (the epithelial cells lining bile duct) injury or function (32-34). In this study biopsies of extrahepatic bile ducts of discarded human donor livers that underwent NMP were histologically assessed. Biochemical parameters in bile, reflecting cholangiocyte injury and function, were correlated with histological appearance of the bile ducts. **Chapter 5** describes the DHOPE–COR–NMP study protocol. Initially nationwide declined livers were subjected to a combined protocol of sequential *ex-situ* dual hypothermic oxygenated machine perfusion (DHOPE), controlled oxygenated rewarming (COR) and NMP, for resuscitation followed by viability assessment. When an initially nationwide declined donor liver met the predefined viability criteria, the liver was transplanted. A novel perfusion solution based on a hemoglobin-based oxygen carrier (HBOC) was used for this study. In **Chapter 6**. the first transplantations based on the DHOPE–COR–NMP protocol are described. This chapter mainly aims to describe the safety and efficacy of the newly proposed machine perfusion protocol, using an HBOC-based perfusion solution. We describe the machine perfusion procedures of both the non-transplanted and transplanted livers as well as post-operative results of the transplanted livers. **Chapter 7** describes the final results of the DHOPE–COR–NMP protocol as outlined in chapter 6. The aim of **Chapter 8** was to investigate whether dual hypothermic oxygenated perfusion (DHOPE) is superior to hypothermic oxygenated perfusion (HOPE) with respect to preservation of the bile ducts and vasculature of porcine livers. Because HOPE of donor

livers is exclusively via the portal vein, we hypothesized that DHOPE perfusion via both the portal vein and the hepatic artery would be superior because the biliary tree is predominantly vascularized via the arterial tree. **Chapter 9** includes a letter to the editor on the use of HBOC-based perfusion solutions for machine perfusion. This thesis will conclude with a 'Discussion and Future Perspectives' in **Chapter 10**.

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