Hemostatic system activation and reperfusion injury in liver machine preservation and transplantation of extended criteria donor livers

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DOI:
10.33612/diss.161905515

CHAPTER 1

General introduction and outline of this thesis

For patients with end-stage liver disease and certain types of hepatic malignancies, liver transplantation is universally accepted as the most effective and only curable treatment available.[1] This is reflected in the excellent 1- and 5-year patient survival rates which in the last decade, have been reported to be 90% and 80%, respectively[2]. The success of liver transplantation has ironically however, become one of the greatest challenges faced by transplant health professionals worldwide as a great discrepancy continues to exist between the supply of suitable donor livers for transplantation and the demand. According to the annual report by the Eurotransplant international foundation in 2018, whilst slightly over 1500 liver transplantations were performed in the Eurotransplant zone, over 2400 patients remained on the waiting list[3]. Unfortunately for many of these patients, the risk of mortality or drop-out within 2 years of placement without undergoing transplantation is slightly over 10% for both[4].

In an effort to tackle the donor organ scarcity, avenues to expand the donor organ pool are increasingly being sought after. In the recent decades, several governments and health ministries have launched public campaigns and lobbied for changes in legislation to alter the donation systems from “opt-in” to “opt-out” in order to boost the number of registered donors[5,6]. Moreover, rates of living-donor and split-liver transplantations have steadily increased[7-9]. However, a significant proportion of additional donor organs has resulted from the increased reliance on the use of extended criteria donor (ECD) livers. Such livers include; livers from older donors, livers that exceed the traditionally accepted degree of steatosis (or in lay terms; “fatty livers”) and livers donated after circulatory death (DCD). In fact, in 2018 in the Netherlands, more than 50% of deceased donor liver transplants were derived from DCD donors[10]. Several studies have shown that selective use of ECD livers results in successful transplantation procedures and acceptable survival rates following transplantation[11]. Nevertheless, a higher incidence of post-transplant morbidity such as early allograft dysfunction, (severe) intraoperative bleeding and life threatening biliary complications, have been reported after transplantations with ECD livers[12,13]. Studies have

shown that these are largely attributed to by ischemia-reperfusion injury (IR injury) incurred by these organs during the procurement, preservation and implantation processes[14].

An important and well-described aspect of ischemic-reperfusion (IR) injury is the hemostatic dysfunction mediated by endothelial-cell activation. This often triggers a profound inflammatory response (reperfusion injury) upon the restoration of blood flow through the hepatic vasculature after a period of ischemia, leading to (severe) blood loss[15,16]. So much so that liver transplantation was, and in some cases, still is frequently associated with high rates of intraoperative blood loss often resulting in a need for substantial amounts of red blood cell (RBC), fresh frozen plasma (FFP), and platelet concentrate transfusions[17,18].

The current standard of care in the preservation of donor livers for transplantation is static cold storage (SCS). Despite being capable of adequately impeding the metabolic processes in the organ and thus minimizing ischemic injury, SCS is limited by the duration in which donor organs can be preserved. Unlike low-risk livers that are capable of tolerating moderate ischemia, ECD donor livers possess an impaired tolerance to ischemia[19]. Therefore, it is crucial that preservation of these ECD livers is optimized in order to reduce the risk of intraoperative and post-transplantation-related complications.

Machine perfusion (MP) is a promising alternative preservation modality that allows for the storage of donor organs under conditions simulating *in vivo* physiology. Therefore, ischemia is minimized. During MP, a continuous circulation of oxygen, nutrients, and other (metabolic) substrates can be provided for a given period of time. In contrast to the traditional SCS, this dynamic preservation modality is capable of resuscitating the liver whilst flushing out toxins and waste products. MP also allows for the possibility of assessing graft quality as well as provides the opportunity to extend the preservation time of an organ, prior to its implantation in the recipient[20]. The hope for the future is to incorporate therapeutic interventions during MP to improve the quality and function of an (ECD) donor liver prior to transplantation.

MP can be performed in different ways; each with a specific objective. Briefly, MP can be performed at low temperatures (4-12 °C), also known as hypothermic machine perfusion (HMP) whereby metabolism within the liver graft is slowed, cellular energy stores are restored and ATP reserves within the graft are replenished. In so doing, IR injury is minimized upon *in situ* reperfusion during transplantation. Alternatively, MP can also be performed at physiological core body temperature (37 °C). This is known as normothermic machine perfusion (NMP). During NMP, liver grafts are reconditioned by circulating nutrients and oxygen at 37°C which enables aerobic metabolism to continue during the preservation phase, limiting ischemic injury and allowing for the opportunity to assess the viability of the graft prior to transplantation. These different modalities of MP with their distinct objectives and methodologies are discussed in depth further in this thesis.

Outline of this thesis

This thesis focuses on the activation of the hemostatic system and reperfusion injury during machine perfusion and transplantation of DCD livers in particular. The first part of this thesis will pay specific attention to the hemostatic system. Herein, we investigated fibrinolysis and coagulation activation during machine preservation and transplantation of DCD donor livers. The second part of this thesis delves into reperfusion injury in DCD liver transplantation, its role in the development of post-transplant biliary complications and the role machine perfusion plays in circumventing reperfusion injury, is further investigated.

PART I: COAGULATION AND FIBRINOLYSIS IN LIVER MACHINE PERFUSION AND TRANSPLANTATION

Liver transplantation can be associated with heavy intraoperative blood loss. Even though this phenomenon has been reported on in depth in the past, majority of these studies mainly involved donation after brain death (DBD) livers[21,22]. Given the additional ischemia-reperfusion (IR) injury DCD livers incur as compared to DBD livers, we hypothesized that hemostatic dysfunction upon reperfusion is more severe in DCD liver transplantation. Taking into account that DCD livers are increasingly making up the majority of donor livers available for transplantation in the Netherlands, the objective of **Chapter 2** was to investigate whether DCD liver transplantation was indeed associated with an increased bleeding risk resulting in higher intraoperative blood loss and a greater need for intraoperative blood product transfusions, in comparison to DBD transplantation.

E*x-situ* normothermic machine perfusion involves perfusion of donor livers at normal body core temperature. In so doing, *in vivo* graft reperfusion is mimicked. One of the features of reperfusion of a donor liver *in vivo* during transplantation is the activation of both the coagulation and fibrinolytic systems. Although the changes in blood coagulation and fibrinolysis after graft reperfusion during liver transplantation have been described in great detail[22,23], little is known about activation of coagulation and fibrinolysis during end-ischemic *ex situ* NMP and what the implications may be. The aim of **Chapter 3** was to therefore determine whether activation of coagulation and/or fibrinolysis occur during end-ischemic *ex situ* NMP of human donor livers and whether this could be used as a marker for graft IR injury and/or function.

Pre-clinical and clinical studies on *ex situ* NMP have shown metabolic and synthetic functions of the liver to resume during perfusion[24,25]. As NMP increasingly makes the transition into clinical care, the duration for which livers can be preserved has reached 24+ hours and beyond. These extended perfusion periods likely result in the synthesis of liver-

derived hemostatic proteins, however the exact rate at which this may occur, remains unknown. In **Chapter 4,** we investigated the production of hemostatic proteins during six hours of NMP of human donor livers. Furthermore, we evaluate the ways to optimize anticoagulant management during NMP in order to prevent the occurrence of potential thromboembolic events. Lastly, **Chapter 5** provides a review of the literature on current anticoagulant management during liver machine perfusion, the synthesis of hemostatic proteins during *ex situ* NMP and the clinical implications hereof.

PART II: REPERFUSION INJURY IN LIVER MACHINE PERFUSION AND TRANSPLANTATION

Ischemia-reperfusion (IR) injury is a well-described phenomenon whereby damage imposed on an organ following a hypoxic or anoxic period is further aggravated upon the restoration of continuous blood flow and concomitant re-oxygenation[26]. DCD donation is particularly affected by additional ischemia sustained during the agonal phase following withdrawal of life supporting treatment, as well as the period of warm ischemia upon circulatory arrest and the verification of death. These successive ischemic events contribute to the suboptimal quality of DCD livers upon in situ reperfusion during transplantation and result in an increased risk of developing primary non-function (PNF), early allograft dysfunction (EAD), post-transplant cholangiopathy and a lower graft survival overall[4].

Post-transplant cholangiopathy continues to be a major problem in DCD liver transplantation, with an overall incidence varying between 10-40%[27,28]. The occurrence of biliary complications critically affects patients' long-term survival, results in an increased likelihood of re-transplantation and significantly impacts quality of life and cost of care[29,30]. The most prevalent and troublesome post-transplant cholangiopathy are the non-anastomotic biliary strictures (NAS), also known as ischemic-type biliary lesions (ITBL). Livers derived from DCD donors are particularly more susceptible to developing NAS with an incidence ranging from 13-35% as opposed to a mere 1 to 24% in DBD-derived donor livers[31]. The etiology of

NAS is yet to be fully understood, however studies have shown that IR injury, especially to the peribiliary glands and the peribiliary vascular plexus, plays a major role[32-34]. The hepatic artery is responsible for >90% of the vascularization of the biliary tree[35].Therefore, minimizing ischemia to the biliary tree by minimizing the time to arterial reperfusion may reduce the risk of developing NAS. In the absence of randomized controlled trials evaluating the effect of the order of reperfusion on the development of NAS in DCD liver transplantation, the aim of the multi-center retrospective study in **Chapter 6** was to assess whether the time between portal and arterial revascularization influences the development of NAS in DCD liver grafts in the Netherlands.

In the past two decades, incredible advances have been made in both experimental and clinical research into machine preservation of donor livers. With numerous research groups worldwide working on MP, various techniques are being explored, often applying different nomenclature and methodology. Therefore in **Chapter 7,** a systematic literature review was performed in order to catalog the differences observed in the nomenclature used to denote various MP techniques and in the manner in which methodology is reported. Moreover, we proposed standardized nomenclature and a standardized set of guidelines for the reporting of methodology for future studies on liver MP. The main objective for the standardization was to facilitate comparison of studies on liver MP as well as facilitate clinical implementation of liver MP procedures in the future.

Chapter 8 provides the reader with in-depth insight into hypothermic machine perfusion (HMP). This review discusses the protective and resuscitative effect HMP has on DCD liver grafts. Additionally, the role HMP plays in minimizing the incidence of NAS is addressed.

It has been demonstrated that ECD liver grafts, particularly DCD livers, have an impaired tolerance to ischemia and thus incur greater IR injury. Compared to DBD liver grafts, DCD livers have shown to release more of pro-inflammatory cytokines and danger-associated molecular patterns (DAMPs) during MP[36]. Inherent to the model of isolated *ex situ* liver

(N)MP, these pro-inflammatory cytokines and DAMPS remain in the circulating perfusate. These injurious cytokines and DAMPs may therefore potentially further perpetuate IR injury during MP. Favorably, *ex situ* NMP offers the opportunity to potentially apply repair strategies to improve graft quality. Therefore, in **Chapter 9** a pilot study was performed to investigate whether addition of a cytokine adsorber that allows for continuous filtration of the perfusate during NMP could safely and effectively remove such cytokines thereby mitigating IR injury and optimizing graft function of DCD porcine livers upon transplantation.

Chapter 10 is a summary of all the chapters in this thesis followed by a general discussion of the main findings. Insight into the future perspectives in these fields of study is given and the thesis is concluded with a summary in the Dutch language in **Chapter 11.**

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PART I

Activation of the hemostatic system