CHAPTER 8

Summary, discussion and future perspectives

SUMMARY

The studies included in this thesis assist in the development of a more rational approach to prevention and treatment of thrombotic and bleeding complications in pediatric patients undergoing liver transplantation. The studies identified risk factors for thrombotic and bleeding complications and provided evidence for maintenance of a hemostatic balance in children undergoing liver transplantation. Furthermore, our studies suggest that our posttransplant antithrombotic therapy protocol is a valuable strategy in pediatric liver transplantation. Finally, *in vitro* studies suggest important differences in the efficacy of pro- and anticoagulant agents in children with end-stage liver disease (ESLD). This knowledge may facilitate further optimization of perioperative hemostatic management. A general introduction and the outline of this thesis were given in **Chapter 1**.

In **Chapter 2** we evaluated the results of the national pediatric liver transplantation program in the Netherlands. We performed a retrospective cohort study which included all children who underwent a primary liver transplantation between 1995-2016. The results of this study demonstrated that outcomes after pediatric liver transplantation in the Netherlands improved over the past two decades, with an actuarial 5-year patient survival of 83% in the most recent cohort, and a reduction in the need for retransplantation. Living donor liver transplantation is increasingly performed in the Netherlands, with superior outcomes compared to transplantation using organs from deceased donors, including a 5-year patient survival rate of 95%. Based on this study, we can conclude that the Netherlands has a successful national pediatric liver transplant programme.[1]

Chapter 3 describes a retrospective cohort study that evaluates incidences and risk factors of thrombotic and bleeding complications, from the time we have implemented a protocol of routine antithrombotic therapy. In 200 consecutive pediatric liver transplant recipients receiving routine postoperative antithrombotic therapy, consisting of unfractionated heparin for 1 week, followed by 3 months of acetylsalicylic acid, the incidence of posttransplant thrombosis was low. Hepatic artery thrombosis occurred in 15 (7.5%) and portal vein thrombosis in 4 (2.0%) recipients. Clinically relevant bleeding occurred in 37%, but consequences of thrombosis were more severe, as was reflected in graft and patient survival. Identified risk factors for thrombosis were intra-operative vascular interventions during transplantation, low recipient age, low Child-Pugh scores and low donor age. High recipient age, high Child-Pugh scores and intra-operative blood loss increased the risk for posttransplant bleeding. The results of this study suggest that posttransplant antithrombotic therapy is a valuable strategy in pediatric liver transplantation.[2,3] In clinical practice, perhaps a more personalized approach in antithrombotic therapy may have merit, which will be further discussed later in this chapter.

In **Chapter 4** a prospective cohort study is presented which assessed the hemostatic status of children with ESLD undergoing liver transplantation. Whereas adults with ESLD are in a rebalanced hemostatic state due to concurrent changes in pro- and antihemostatic pathways,

hemostasis in pediatric patients, who have a maturing hemostatic system, different disease etiologies and higher incidences of thrombosis, remained unknown.[4-6] Hemostasis was assessed with routine hemostasis tests, thrombomodulin-modified thrombin generation assays, clot lysis times and levels of hemostatic proteins in serial blood samples derived from children undergoing primary liver transplantation. Whereas conventional laboratory tests (platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT)) suggested a hypocoagulable state, state-of-the-art hemostasis tests demonstrated a normal to hypercoagulable hemostatic state in children with ESLD. During transplantation, a temporary heparin-induced hypocoagulable state rapidly converted to a hemostatic balance with distinct hypercoagulable features until at least day 30 posttransplantation. These hypercoagulable features might contribute to the increased risk of posttransplant thrombosis in children.[7-9]

In **Chapter 5** we aimed to analyze the efficacy of clinically available pro- and anticoagulant drugs in plasma from children undergoing liver transplantation. Therefore, *in vitro* effects of pro- and anticoagulant drugs on thrombin generation capacity were tested in plasma samples of pediatric patients undergoing liver transplantation, and compared to the effects in plasma from age-matched controls with intact liver function. Addition of pooled normal plasma had no effect in patients or controls, whereas 4-factor prothrombin complex concentrate (PCC) increased thrombin generation in controls, with an additional augmentation in patients. For anticoagulants, rivaroxaban had comparable effects in patients and controls. Dabigatran and unfractionated heparin had a higher anticoagulant potency in patients at start of transplantation, whereas 30 days after transplantation low molecular weight heparin was slightly less effective in patients, when compared to controls. This study revealed important differences in efficacy of commonly used pro- and anticoagulant drugs in children undergoing liver transplantation. Therefore, dose adjustments of these drugs may be required. The new insights from this study may be helpful in the development of urgently needed protocols for strategies to prevent and treat bleeding and thrombotic complications in pediatric liver transplantation.

In the study in **Chapter 6** a novel prediction model for patient survival after pediatric liver retransplantation was developed and validated by performing a multicenter cohort study including 1361 children who underwent a liver retransplantation and were included in the European Liver Transplant Registry. Six clinically relevant risk factors for mortality were identified: recipient age < 5 years, primary transplantation because of acute liver failure, primary non-function of the primary graft, recipient admitted to the intensive care unit, donor age > 18 years and late retransplantation (> 1 month). A simplified risk assessment tool was developed, the Pediatric Liver Retransplantation Risk Score, in which three risk groups were defined: low-risk (4-7 points), medium-risk (8-9 points), and high-risk (10-13 points) with significantly different patient survival rates. The Pediatric Liver Retransplantation Risk Score thus appears a reliable tool to predict survival by combining donor and recipient factors, and provides an easy-to-use scoring system for clinical practice to predict survival before acceptance of a donor organ. This score indicates that good outcomes after pediatric liver retransplantation can be achieved after careful selection of recipient, donor, and transplant factors.

In **Chapter 7** the first successful transplantation of a pediatric liver graft after hypothermic oxygenated machine perfusion is presented. A full-size liver graft, derived from a 13-year-old female donor, who died after circulatory death, was end-ischemically resuscitated with dual hypothermic oxygenated machine perfusion for 2 hours. Arterial and portal pressures were adjusted to the size of the liver graft. The pretreated liver graft was successfully transplanted into a 16-year-old girl with progressive familial intrahepatic cholestasis. With this, we demonstrated hypothermic machine perfusion of a pediatric liver graft, with adjusted perfusion pressures, to be feasible and safe. In the future, *ex situ* machine perfusion might be a valuable tool in expanding the donor pool and improving outcome after pediatric liver transplantation.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Although important questions have been answered in this thesis, which brings us one step closer to a rational approach in the prevention and treatment of thrombotic and bleeding complications in pediatric patients undergoing liver transplantation, still many problems remain unsolved. Even new questions and challenges arise. In the last section of this chapter, some of these challenges will be discussed and placed in a future perspective.

Hemostasis in pediatric and adult liver transplantation

As outlined in this thesis, hemostasis is a complex process of blood clot formation at the site of blood vessel injury to stop bleeding. This process can be subdivided into platelet adhesion and aggregation (primary hemostasis), coagulation/fibrin deposition (secondary hemostasis) and fibrinolysis (tertiary hemostasis). Children are characterized by a developmental hemostasis, wherein functional levels of hemostatic proteins change in a predictable way with age.[5]

Regarding primary hemostasis, in healthy children both platelet counts and von Willebrand Factors (VWF) levels are increased, when compared to adults, especially below the age of one year. Levels of a disintegrin and metalloproteinase with thrombospondin motifs type 13 (ADAMTS13) are comparable in children and adults. Notably, platelets in children are hypo reactive, which result in a net decreased functionality of primary hemostasis in healthy children.[10] In children with ESLD, primary hemostasis appears to be rebalanced and maintained. Their liver disease-associated thrombocytopenia is compensated for by high VWF levels, that appear to preserve platelet adhesion, and reduced levels of ADAMTS13, which probably reduce VWF proteolysis within a growing thrombus.[11] This is in line with previous observations in adults with chronic liver disease.[12] However, no functional analyses of platelet adhesion and aggregation have been performed in our studies in pediatric patients, which would be an interesting topic for future studies.

In secondary hemostasis, levels of prothrombin, factor V, VII, IX, X, XI and XII are lower during childhood when compared to adults, as are natural anticoagulants including antithrombin,

protein C and S.[13] Most factors are at a minimum level at birth and gradually increase with age. Factor VIII levels in contrast, are increased in children. Conventional hemostasis tests including PT and APTT are prolonged in children and thrombin generation capacity decreased. Together, this suggests a hypocoagulable state in healthy children. As presented in **Chapter 4** of this thesis and as previously demonstrated by Magnusson et al, hepatic synthetic function is impaired in children with ESLD resulting in decreased plasma levels of procoagulant clotting factors (II, V, VII, IV, X, XI and fibrinogen).[14] A similar reduction in anticoagulant proteins (protein C & S, antithrombin II) is present, whereas factor VIII levels are high. However, thrombin generation capacity appeared to be intact, suggesting a normal, balanced secondary hemostasis. A similar pattern has been demonstrated in adults undergoing liver transplantation whereas patients have a normal to hypercoagulable secondary hemostasis.[7]

For tertiary hemostasis (fibrinolysis) in healthy children, both plasminogen and antiplasmin levels are reduced, with plasminogen activator inhibitor type 1 levels comparable to levels in healthy adults. Clot lysis times are prolonged, resulting in a net effect of hypofibrinolysis.[15] A similar effect is seen in children with ESLD. During liver transplantation, however, clot lysis times are short with a clear hyperfibrinolytic state after reperfusion. After transplantation increased clot lysis times and increased plasminogen activator inhibitor type 1 levels are demonstrated, which combined with low plasminogen levels contribute to a subsequent profound hypofibrinolytic state at the end of transplantation. Again, these observations are in line with previous studies in adult liver transplantation.[16]

Monitoring of hemostasis

In vivo, the processes of primary, secondary and tertiary hemostasis are interconnected, occur simultaneously, and stimulate and subsequently deactivate each other. Besides that, hemostasis is dependent on multiple other components and processes, including blood cells, endothelium and blood flow, to initiate blood clot formation. To monitor hemostasis, tests analyzing clot formation in whole blood, taking blood flow and other components such as blood-endothelial cell interactions would be ideal, yet, such a test is not available.[17] In clinical practice, hemostasis is mainly measured with PT and APTT tests. Although these tests do demonstrate some of the coagulation capacity, they are only sensitive for plasma levels of procoagulant proteins and thus, not helpful in patients with ESLD in whom plasma levels of anticoagulant proteins are also reduced. In adult patients with ESLD, the rebalanced hemostasis is reflected by changes in pro- and anticoagulant proteins, and therefore the PT and APTT are inadequate to represent hemostasis in these patients. In **Chapter 4** we demonstrated that the same is true in children with ESLD. Whereas conventional hemostasis tests, including PT and APTT, indicate a high bleeding tendency, a normal to hypercoagulability is shown when assays that are sensitive for both pro- and anticoagulant proteins are used. So, the PT and APTT give an inaccurate representation of the *in vivo* hemostatic functions in both adult and pediatric patients with ESLD.

Upcoming strategies for measurement of hemostasis and coagulation in patients with hemostatic alterations are viscoelastic tests (thromboelastography, rotational thromboelastometry, or sonorheometry), which are point-of-care whole blood tests. These tests are better and more complete indicators of hemostasis than PT or APTT and may be useful to assess coagulation, although no cut-off values have been established yet. An important drawback of these viscoelastic tests is that, although assessing whole blood, they do not include the effects of flow rates and they are insensitive for VWF and the protein C system, which are of great importance in the rebalanced hemostasis in patients with ESLD.[18] Consequently, even when thromboelastography demonstrates a normal hemostatic state in patients with ESLD, this is probably still an underestimation of their actual hemostatic potential.[19,20]

As demonstrated in this **Chapter 4** of this thesis, thrombomodulin-modified thrombin generation tests probably better represent hemostasis in patients with ESLD. This is in line with previously demonstrated results in adults with liver disease.[7] The calibrated automated thrombogram quantifies the generation of thrombin in clotting plasma using a microtiter plate reading fluorometer. In this assay, plasma levels of both pro- and anticoagulant proteins are measured under conditions in which the natural anticoagulant systems are fully activated. Unfortunately, this test is not clinically available and not yet suitable for clinical use. However, a whole blood thrombin generations point-of-care test is in development, and this test may also have merit in patients with liver disease.[21,22] Thrombomodulin-modified thrombin generations tests seem adequate in assessing the rebalanced hemostasis in patients with ESLD, but clinical studies are required to assess their clinical utility.

Anticoagulant therapy in pediatric liver transplantation

Type of anticoagulant drug

As outlined in this thesis in **Chapter 3**, anticoagulant therapy seems to be of great value in the prevention and treatment of posttransplant thrombosis. Although a high incidence of bleeding complications is reported under this regimen, it seems worth taking this risk since bleeding complications can be treated well, in contrast to the devastating consequences of thrombosis including graft failure and death. Moreover, the rebalanced hemostatic state in children undergoing liver transplantation, as demonstrated in **Chapter 4**, adds to the concept for anticoagulant therapy. Based on literature and the studies presented in this thesis, for now unfractionated heparin seems to be the treatment of choice for pediatric liver transplantation. There is a lot of experience with this drug, and it has been demonstrated to be effective in reducing the rate of thrombotic complications.[23]

However, there are a few drawbacks and considerations related to this anticoagulant drug. Firstly, heparin initiation and dosing are based on laboratory tests including platelet counts, PT, and APTT. However, as outlined above, PT and APTT values are unreliable in patients with ESLD. Since PT and APTT are already prolonged in children with ESLD, and further increase during liver transplantation, target ranges are unclear. Secondly, the APTT has been shown

to overestimate drug levels in patients with liver disease.[24] An alternative monitoring strategy of unfractionated heparin is using anti-Xa tests, which have been shown to underestimate levels in adults with liver disease related to their decreased antithrombin levels. Indeed, as shown in **Chapter 4**, children also have decreased antithrombin levels, especially children with ESLD, and consequently unfractionated heparin levels will be underestimated by anti-Xa tests and overestimated when using APTT.[25] Thirdly, it was previously demonstrated that an equal to enhanced anticoagulant effect of heparin was seen in plasma from adult patients with cirrhosis, compared to healthy adults.[26] As described in **Chapter 5**, a comparable effect was seen in children with ESLD. Heparin showed a higher anticoagulant potency in children with liver disease, when compared to healthy children with intact liver function when using thrombin generations tests. Altogether, monitoring anticoagulant effects of heparin with APTT is unreliable in patients with ESLD and therapeutic effects of heparin can be unpredictable. With this, dose escalations may potentially lead to a substantial bleeding risk. Again, point-of-care hemostasis tests, taking both pro- and anticoagulant effects into account are required. Whether current development of point-of-care thrombin generation tests will provide a solution needs to be determined with further research.

Perhaps in the future, there is a role for direct oral anticoagulants (DOACs) in pediatric liver transplantation, since these agents have several advantages over heparin. Especially the elimination of injections, and the lack of requirement for intensive monitoring and dietary restrictions are of extra importance for pediatric patients.[27-29] The effects of DOACs in children with intact liver function have been examined in a few studies, and were indicated as safe and of similar potency as in adults.[27,29] Moreover, it was previously demonstrated that developmental hemostatic changes had only minimal influence on responses to dabigatran, in contrast to heparin and low molecular weight heparin.[27] As presented in **Chapter 5** dabigatran showed profoundly enhanced anticoagulant *in vitro* effects in patients with ESLD, as was previously seen in adults as well.[26] This increased *in vitro* potency in patients with ESDL, when compared to healthy patients with intact liver functions may indicate dose-adjustments are required. Of course, it remains unknown whether this increased potency is also present *in vivo*, and if so, whether the extent of potency difference is clinically relevant. In a recent study by our group, it was demonstrated that administration of the Xa-targeting DOAC edoxaban to adult patients with cirrhosis resulted in a decreased *in vivo* inhibition of coagulation, when compared to effects in healthy adults, with similar drug concentrations, confirming earlier *in vitro* observations.[30] The clinical relevance of the differences in *in vivo* potency of DOACs has not been established yet, but future studies comparing regular with increased edoxaban doses with clinically relevant endpoints (prevention/treatment of thrombosis and bleeding) seem indicated. Similar *in vivo* studies examining potency and efficacy of various DOACs are also of interest for the pediatric population. Until now, DOACs were never tested in pediatric patients with liver disease and further studies are necessary, to explore this therapeutic option as strategy to prevent and treat thrombosis in pediatric liver transplantation.

Timing of anticoagulant drug

Thrombotic complications mainly occur in the first week after transplantation. This is the result of multiple transplantation-related factors that initiate coagulation and platelet adhesion, including surgical damage, stasis due to intraoperative clamping of vessels, release of activators from the donor liver and the systemic inflammatory response. Despite the fact that most pediatric liver transplant centers administer a form of anticoagulant therapy from posttransplantation day 1 onwards, thrombosis remains an important complication. There may be several explanations for this. First, the optimal timing of initiation of antithrombotic therapy is difficult to determine. Antithrombotic therapy is mainly started based on clinical parameters combined with conventional coagulation tests as PT and APTT. However, as mentioned, whereas PT and APTT might suggest inappropriate coagulation capacity, advanced coagulation tests show an intact thrombin generation capacity. In practice, coagulation capacity is probably underestimated and therefore antithrombotic therapy is started relatively late. Besides this, administered antithrombotic therapy is probably under-dosed since for most therapies no reliable monitoring tests are available and heparin concentrations are often underestimated.

Perhaps a more personalized approach in antithrombotic therapy may have merit, to optimize its risk/benefit ratio in pediatric liver transplantation, based on personal risk factors. A more proactive use of antithrombotic therapy might be beneficial in young recipients with metabolic liver diseases, especially in case of intraoperative vascular interventions. A more careful approach might be justified in older recipients with high Child-Pugh scores or significant intraoperative blood loss, but this requires clinical confirmation.[2,31,32]

Antiplatelet therapy

One might suggest to provide double antithrombotic therapy in the first week after transplantation, including antiplatelet therapy, since one of the main goals of antithrombotic therapy is to prevent hepatic artery thrombosis. Although the mechanism of hepatic artery thrombosis in incompletely understood, this is probably the result of several factors, including technical aspects (surgical technique, type of anastomosis, use of conduits, small vessel size, vessel kinking), organ damage (due to ischemia and reperfusion) and the rebalanced hemostatic state of the recipients, which includes clear hypercoagulable features. It has been very well established that platelets are main players in arterial thrombotic events (such as myocardial infarction and stroke) whereas fibrin formation is the main driver of venous thrombotic events. As previously described, although platelet counts are low in children with ESLD, primary hemostasis seems to be preserved.

Acetylsalicylic acid, which is a platelet function inhibitor, may have an important role in the prevention of hepatic artery thrombosis.[33] As mentioned, at the site of vessel injury and with this endothelial damage, which occur after conducting an anastomosis, platelet adhesion and aggregation will be activated. Sustained platelet activation and aggregation perhaps in combination with fibrin formation, potentially contribute to hepatic artery thrombosis.

Furthermore, with acetylsalicylic acid inhibition of platelet activation and platelet-mediated inflammation after liver transplantation may be reduced, resulting in prevention of hepatic artery thrombosis. Therefore, perhaps anti-platelet therapy in combination with anticoagulation may be an effective antithrombotic strategy without an unacceptably high bleeding risk.

A survey under pediatric transplant centers previously demonstrated that nearly 80% of centers initiated antiplatelet therapy in the first week after transplantation in absence of bleeding and when platelet counts are > 50-100 x10^9/L.[23,31] In adult liver transplantation, immediate initiation of acetylsalicylic acid after liver transplantation reduced the rate of hepatic artery thrombosis leading to early graft loss, without increasing the bleeding risk.[33] It might be of great relevance, since the main indication for early retransplantation in pediatric liver transplantation are vascular complications including hepatic artery thrombosis. Yet, only a few studies with limited patients included studied the effects of acetylsalicylic acid immediately posttransplantation and further research is required.

Procoagulant therapy in pediatric liver transplantation

At the onset of liver transplantation, procedures were very often accompanied by massive transfusion with "blind" prophylactic transfusions of red blood cells (RBC), fresh frozen plasma (FFP) and platelet concentrates. Fortunately, these days, with more surgical and anesthesiological expertise in hemostasis, this approach has been largely abandoned. As mentioned in **Chapter 2** of this thesis, it is of great importance to assess whether prohemostatic treatment, or blood component transfusion is really necessary, since blood transfusion comes with significant morbidity and mortality.[34] Still, the threshold for blood transfusion in patients with ESLD is low, particularly since some centers still asses the need for blood component transfusion with conventional coagulation tests as PT and APTT.

In case of actual need of transfusion, different prohemostatic therapy strategies are practiced. Along with red blood cell transfusion, platelets, and FFP, 4-factor prothrombin complex concentrates (PCC) and fibrinogen concentrates are frequently administered as prohemostatic agents in clinical practice.[35-37] It remains unclear what strategies are preferable in children with ESLD, respecting their complex maturing hemostatic state.

As mentioned, although platelet counts are often decreased in patients with liver disease, elevated levels of VWF seem to compensate for the number of platelets, preserving primary hemostasis. Clinically, it is unclear whether a minimal platelet count is required for optimal and adequate hemostasis in patients with ESLD. It was previously suggested that thrombocytopenia increases the bleeding risk in these patients, however, it is unsure whether transfusion of platelet decrease their bleeding risk.[38] Notably, platelet transfusion is associated with increased mortality in liver transplantation patients.[39] Disadvantages of platelet transfusion include the risk on infectious and immunologic complications, and besides that, transfused platelets may become entrapped in the transplanted liver and cause severe complications including acute lung injury.[39] Given the detrimental effects of platelet

transfusion, triggers of platelet transfusion in liver transplant recipients should not be based on platelet counts alone.[17] We think that, in clinical practice a restrictive use of platelet transfusion is recommended as long as perioperative hemostasis is secured, and platelet transfusions should only be given in case of severe bleeding complications.

FFP contains all coagulation factors, anticoagulant and fibrinolytic proteins in physiological concentrations. However, to increase plasma activation of these factors, large volumes are necessary to reach this effect, which may lead to volume overload. This is clinically relevant in patients with ESLD, since restrictive fluid infusion policy, to maintain a low central venous pressure is an important strategy to minimize blood loss in these patients. In **Chapter 5** it was demonstrated that pooled normal plasma (to simulate FFP), had no effect on hemostatic capacity in patients at start of transplantation, but did slightly improve coagulation during liver transplantation. This is probably the result of a high consumption of coagulation factors during an invasive procedure as transplantation. Importantly, the *in vitro* potency of PCC was clearly higher in children with liver diseases.

PCCs, which can be subdivided in three and four factors concentrates, have several advantages over FFP, including low infusion volume, ambient temperature storage conditions, rapid reconstitution and lack of blood group specificity. As presented in **Chapter 5**, 4-factor PCC was very potent in boosting thrombin generation capacity in plasma of both healthy children and children with ESLD. Therefore, this might be a suitable procoagulant agent in case of bleeding in children with liver disease. In adult liver transplantation, PCC use is associated with a significantly decrease in need for RBC or FFP transfusion. Although in most studies no increase in thrombotic complications were seen, there is a risk of inducing a clear hypercoagulable state that might contribute to thrombotic risk.[35] In a future perspective, *in vitro* effects of PCC in pediatric liver transplantation seems promising, but further exploration and clinical studies are indicated first.

Living donor liver transplantation

An important finding in the study presented in **Chapter 2**, are the superior outcomes after living donor liver transplantation, reporting a 5-year survival rate of 95%, which is in line with literature.[40,41] The superior outcomes of living donor liver transplantation are probably the result of a combination of factors, including use of an optimal healthy donor, minimal cold ischemia times, elective surgery and optimal timing to the recipients need. One might suggest to focus on living donor liver transplantation, and cease liver transplantation with grafts from deceased donors. However, living donor liver transplantation comes with important risks for the donor, and besides that, not all potential donors are technically suitable for donation an some children with ELSD do not have a suitable living donor.

Living donor liver transplantation has reported mortality of 0.2-0.5% and morbidity from 20-40% for the donor.[41,42] A healthy person undergoes a surgical procedure, with all its risks, which is against the persons own benefits. With this, also an ethical concern is relevant. For

the transplant team it is important to look after the need, donor safety and chance for good recipient outcomes. Donor autonomy should be respected and non-maleficence should be practiced. In the Netherlands, the donor is usually one of the parents or relatives that donates a part of their liver to the child. Although many parents volunteer to donate, an extensive screening is performed, to examine the health of the donor to minimize its risks, to examine the anatomy of hepatic vessels to ensure donation is technically possible, and to see if their psychological state and social system is firm enough do deal with this major impact surgery.

An important upcoming strategy is laparoscopic donor liver procurement, with significant lower morbidity and mortality rates.[43] We expect that with more experience and further clinical studies, the technique of living donor liver transplantation will be further optimized and fulfill an increasing role in pediatric liver transplantation. With this, the waiting list mortality may decrease and overall outcomes for children with ESLD will further improve.

New technologies in pediatric liver transplantation

In adult liver transplantation, an important and upcoming technique is *ex situ* oxygenated machine perfusion preservation of donor grafts to improve graft preservation and reduce risk of graft failure. In the near future, this technique might be introduced in pediatric liver transplantation as well. In **Chapter 7** of this thesis we presented the first successful transplantation of a pediatric liver graft after hypothermic oxygenated machine perfusion, which with adjusted perfusion pressures, seems to be feasible and safe. Currently, mainly liver grafts derived from brain death donors are used in pediatric liver transplantation. Machine preservation might reduce part of the risks of transplantation of liver grafts derived from donors after circulatory death. With this, *ex situ* machine perfusion might be valuable in expanding the donor pool and improving outcomes after pediatric liver transplantation, as it previously did in adult liver transplantation.

Besides this, *ex situ* oxygenated machine perfusion of liver grafts may offer an important solution for split liver procedures, in which the liver graft is split and offered to two recipients, mainly an adult and a child. A major drawback of split liver procedures is the prolonged ischemia time, and with this poor graft quality and increased risks for both recipients. Machine perfusion can facilitate continuous oxygenated perfusion during the *ex situ* split procedure. This was recently shown by Stephenson et al., who successfully performed a split procedure of an adult liver graft under continuous oxygenated machine perfusion, resulting in a left lateral lobe graft and an extended right lobe graft.[44] Technically, machine perfusion of liver grafts is typically performed via the portal main stem, which will be interrupted during split procedure. To enable a split procedure on the pump, another entrance to the portal circulation is needed. Therefore we recently demonstrated the concept of machine perfusion with portal perfusion via the umbilical vein, during which portal venous flows similar to those obtained after cannulation of the portal vein main stem were obtained.[45] In this study also a left lateral split procedure was performed under continuous normothermic machine perfusion with portal perfusion via the umbilical vein. During the split procedure, the left lateral segment

as well as the extended right lobe remained equally perfused, as demonstrated by Doppler-ultrasound. These recent successes offer a platform for further exploring the possibilities of *ex situ* machine perfusion techniques and its values and implications in pediatric liver transplantation, which seem very promising.

REFERENCES

1. Adam R, Karam V, Cailliez V, et al. 2018 annual report of the european liver transplant registry (ELTR), 50-year evolution of liver transplantation. *Transpl Int*. 2018;31(12):1293-1317.
2. Ziaziaris WA, Darani A, Holland AJA, et al. Reducing the incidence of hepatic artery thrombosis in pediatric liver transplantation: Effect of microvascular techniques and a customized anticoagulation protocol. *Pediatr Transplant*. 2017;21(4):10.1111.
3. Heffron TG, Pillen T, Welch D, Smallwood GA, Redd D, Romero R. Hepatic artery thrombosis in pediatric liver transplantation. *Transplant Proc*. 2003;35(4):1447-1448.
4. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. *N Engl J Med*. 2011;365(2):147-156.
5. Monagle P, Ignjatovic V, Savoia H. Hemostasis in neonates and children: Pitfalls and dilemmas. *Blood Rev*. 2010;24(2):63-68.
6. Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease: Evidence and clinical consequences. *Blood*. 2010;116(6):878-885.
7. Lisman T, Bakhtiari K, Pereboom IT, Hendriks HG, Meijers JC, Porte RJ. Normal to increased thrombin generation in patients undergoing liver transplantation despite prolonged conventional coagulation tests. *J Hepatol*. 2010;52(3):355-361.
8. Meltzer ME, Lisman T, de Groot PG, et al. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. *Blood*. 2010;116(1):113-121.
9. Pereboom IT, Adelmeijer J, van Leeuwen Y, Hendriks HG, Porte RJ, Lisman T. Development of a severe von willebrand factor/ADAMTS13 dysbalance during orthotopic liver transplantation. *Am J Transplant*. 2009;9(5):1189-1196.
10. Hvas AM, Favaloro EJ. Platelet function testing in pediatric patients. *Expert Rev Hematol*. 2017;10(4):281-288.
11. Raffini L, Witmer C. Pediatric transplantation: Managing bleeding. *J Thromb Haemost*. 2015;13 Suppl 1:S362-9.
12. Caldwell S, Lisman T. The cirrhotic platelet: Shedding light on an enigma. *Hepatology*. 2017;65(2):407-410.
13. Kremers RM, Wagenvoord RJ, de Laat HB, Monagle P, Hemker HC, Ignjatovic V. Low paediatric thrombin generation is caused by an attenuation of prothrombin conversion. *Thromb Haemost*. 2016;115(6):1090-1100.
14. Magnusson M, Berndtsson M, Fischler B, et al. Thrombin generation test in children and adolescents with chronic liver disease. *Thromb Res*. 2015;135(2):382-387.
15. Monagle P, Chan AK, Albisetti M, Vegh P, Andrew M, Mitchell L. Fibrinolytic system in adolescents: Response to venous occlusion stress tests. *Pediatr Res*. 2003;53(2):333-337.
16. Ruitenbeek K, Meijers JC, Adelmeijer J, Hendriks HG, Porte RJ, Lisman T. Intact thrombomodulin-mediated regulation of fibrinolysis during and after liver transplantation, despite a profoundly defective thrombomodulin-mediated regulation of coagulation. *J Thromb Haemost*. 2010;8(7):1646-1649.
17. Biancofiore G, Blasi A, De Boer MT, et al. Perioperative hemostatic management in the cirrhotic patient: A position paper on behalf of the liver intensive care group of europe (LICAGE). *Minerva Anestesiol*. 2019;85(7):782-798.
18. Lisman T. Interpreting hemostatic profiles assessed with viscoelastic tests in patients with cirrhosis. *J Clin Gastroenterol*. 2020;54(4):389-391.
19. De Pietri L, Bianchini M, Montalti R, et al. Thrombelastography-guided blood product use before invasive procedures in cirrhosis with severe coagulopathy: A randomized, controlled trial. *Hepatology*. 2016;63(2):566-573.
20. Mallett SV. Clinical utility of viscoelastic tests of coagulation (TEG/ROTEM) in patients with liver disease and during liver transplantation. *Semin Thromb Hemost*. 2015;41(5):527-537.
21. Wan J, Roberts LN, Hendrix W, et al. Whole blood thrombin generation profiles of patients with cirrhosis explored with a near patient assay. *J Thromb Haemost*. 2020;18(4):834-843.
22. Ninivaggi M, Apitz-Castro R, Dargaud Y, de Laat B, Hemker HC, Lindhout T. Whole-blood thrombin generation monitored with a calibrated automated thrombogram-based assay. *Clin Chem*. 2012;58(8):1252-1259.

23. Calinescu AM, Karam O, Wilde JCH, Ansari M, McLin VA, Wildhaber BE. International survey on anticoagulation and antiplatelet strategies after pediatric liver transplantation. *Pediatr Transplant*. 2018:e13317.
24. Potze W, Arshad F, Adelmeijer J, et al. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. *Br J Haematol*. 2013;163(5):666-673.
25. Potze W, Lisman T. Issues with monitoring of unfractionated heparin in cirrhosis. *Ther Drug Monit*. 2015;37(2):279-280.
26. Potze W, Arshad F, Adelmeijer J, et al. Differential in vitro inhibition of thrombin generation by anticoagulant drugs in plasma from patients with cirrhosis. *PLoS One*. 2014;9(2):e88390.
27. Maas H, Gropper S, Huang F, et al. Anticoagulant effects of dabigatran in paediatric patients compared with adults: Combined data from three paediatric clinical trials. *Thromb Haemost*. 2018;118(9):1625-1636.
28. Halton JML, Albisetti M, Biss B, et al. Phase IIa study of dabigatran etexilate in children with venous thrombosis: Pharmacokinetics, safety, and tolerability. *J Thromb Haemost*. 2017;15(11):2147-2157.
29. Dietrich K, Stang L, van Ryn J, Mitchell LG. Assessing the anticoagulant effect of dabigatran in children: An in vitro study. *Thromb Res*. 2015;135(4):630-635.
30. Bos S, Schreuder T, Blokzijl H, Adelmeijer J, Lisman T, Kamphuisen P. Anticoagulant activity of edoxaban in patients with cirrhosis. *Blood*. 2020; 005319.
31. Voulgarelis S, Vitola B, Lerret SM, Hong JC, Scott JP. Perioperative anticoagulation practices for pediatric liver transplantation. *Pediatr Transplant*. 2018;22(4):e13193.
32. Borst AJ, Sudan DL, Wang LA, Neuss MJ, Rothman JA, Ortel TL. Bleeding and thrombotic complications of pediatric liver transplant. *Pediatr Blood Cancer*. 2018;65(5):e26955.
33. Shay R, Taber D, Pilch N, et al. Early aspirin therapy may reduce hepatic artery thrombosis in liver transplantation. *Transplant Proc*. 2013;45(1):330-334.
34. de Boer MT, Molenaar IQ, Hendriks HG, Slooff MJ, Porte RJ. Minimizing blood loss in liver transplantation: Progress through research and evolution of techniques. *Dig Surg*. 2005;22(4):265-275.
35. Srivastava P, Agarwal A, Jha A, et al. Utility of prothrombin complex concentrate as first-line treatment modality of coagulopathy in patients undergoing liver transplantation: A propensity score-matched study. *Clin Transplant*. 2018;32(12):e13435.
36. Nacoti M, Corbella D, Fazzi F, Rapido F, Bonanomi E. Coagulopathy and transfusion therapy in pediatric liver transplantation. *World J Gastroenterol*. 2016;22(6):2005-2023.
37. Kirchner C, Dirkmann D, Treckmann JW, et al. Coagulation management with factor concentrates in liver transplantation: A single-center experience. *Transfusion*. 2014;54(10 Pt 2):2760-2768.
38. Drolz A, Horvatits T, Roedl K, et al. Coagulation parameters and major bleeding in critically ill patients with cirrhosis. *Hepatology*. 2016;64(2):556-568.
39. Pereboom IT, de Boer MT, Haagsma EB, Hendriks HG, Lisman T, Porte RJ. Platelet transfusion during liver transplantation is associated with increased postoperative mortality due to acute lung injury. *Anesth Analg*. 2009;108(4):1083-1091.
40. Olthoff KM, Smith AR, Abecassis M, et al. Defining long-term outcomes with living donor liver transplantation in north america. *Ann Surg*. 2015;262(3):465-75; discussion 473-5.
41. Hackl C, Schlitt HJ, Melter M, Knoppke B, Loss M. Current developments in pediatric liver transplantation. *World J Hepatol*. 2015;7(11):1509-1520.
42. Ghobrial RM, Freise CE, Trotter JF, et al. Donor morbidity after living donation for liver transplantation. *Gastroenterology*. 2008;135(2):468-476.
43. Soubrane O, Eguchi S, Uemoto S, et al. Minimally invasive donor hepatectomy for adult living donor liver transplantation: An international, multi-institutional evaluation of safety, efficacy and early outcomes. *Ann Surg*. 2020.
44. Stephenson BTF, Bonney GK, Laing RW, et al. Proof of concept: Liver splitting during normothermic machine perfusion. *J Surg Case Rep*. 2018;2018(3):rjx218.
45. van Leeuwen OB, Fujiyoshi M, Ubbink R, et al. *Ex situ* machine perfusion of human donor livers via the surgically reopened umbilical vein: A proof of concept. *Transplantation*. 2019.