CHAPTER 1

General introduction & outline of this thesis
PEDIATRIC LIVER TRANSPLANTATION

Children that require a pediatric liver transplantation (pLT) are generally chronically ill and have end-stage liver disease (ESLD). Orthotopic pLT is the only available treatment for ESLD in children (1). Indications for pLT include various cirrhotic and non-cirrhotic liver diseases. Under the age of 12 years the most common indication is biliary atresia, whereas from 12 years and older the main indications are acute liver failure and metabolic liver disease (2).

During a pLT, the diseased liver is removed, and replaced with a healthy donor liver in the same place the diseased liver was located (3). The donor liver can be a full-sized liver from a heart beating or non-heart beating deceased person. Alternatively, it can be a partial liver, in which a living donor donates part of their left liver lobe (living donor LT; LDLT) (3, 4). In case of an LDLT, the donor’s remaining liver will subsequently undergo hypertrophy to the physiological need of the donor (5). The use of LDLTs in children has increased in recent years, with concurrent decreased pretransplant mortality and waitlist duration (2, 6).

The half-life of pLTs (as a measure of graft survival) in children of all ages is estimated to be 31 years, with a higher half-life in children below 12 years old compared to older children because of different underlying diseases (2). Most graft loss occurs in the first 12 months after pLT, and this is related to intraoperative and postoperative surgical complications, vascular complications such as hepatic artery thrombosis (HAT), sepsis, and graft dysfunction (7-9). LDLTs are reported to have a slightly higher graft loss rate compared to full size LTs, which is thought to be the result of technically more complex surgery (2, 4). Although graft survival has increased over time, in the last two decades further improvement of survival has been small (2). Potential further areas for improvement may be the centralization of LTs (2), and improved machine perfusion of explanted pLTs prior to implantation (10).

However, even in an optimal setting of a specialized pLT center with a multidisciplinary team, with surgical expertise and implemented technical refinements, peri- and postoperative complications may still occur. Radiological imaging is important for planning surgery, detect complications, and guide therapy. While radiological imaging is already widely implemented, there appears to be significant variation between pLT centers on how radiological imaging is used. For example, the protocol for Doppler ultrasound (DUS) surveillance during and after surgery varies greatly (11).

One of the issues with diagnostic imaging in children is that research in children is challenging. Children, both sick and healthy, are justifiably well protected by medical ethical committees from time consuming and invasive studies. In this way, risks related to a scientific study such as incidental (usually benign) findings, complications related to anesthesia or contrast, and distress are reduced (12). Although these constraints are necessary to safeguard children, the downside is that prospective pediatric studies are relatively scarce because they are difficult to establish. When pediatric studies are available, they are often retrospective, which are generally considered to be scientifically inferior to prospective studies and clinical trials. Consequently, pediatric radiologists must frequently rely on studies in adults to decide on the best imaging strategy in children.

SCOPE OF THIS THESIS

This thesis aims to critically assess the already established diagnostic pathway of children with ESLD and acute liver disease that undergo pLT, from preoperative workup, to perioperative imaging, and short and long-term postoperative follow-up. This thesis also aims to identify and subsequently correct gaps in the current understanding of the usefulness of radiological imaging, and provide clinically useful tools where possible. Furthermore, it aims to provide an improved scientific base for future studies, and identify areas of research that may help to improve graft survival after pLT.

PREOPERATIVE IMAGING

When the indication for pLT has been made by a pediatric gastroenterologist, all children undergo DUS as part of the preoperative workup. DUS provides information on patency of vessels, speed and direction of blood flow, detects focal lesions, and informs on general anatomy and its variations. Depending on the pLT center, children may also undergo computed tomography (CT) of the abdomen. The main purpose of CT is to obtain a further detailed analysis of the vascular anatomy, in particular the hepatic artery anatomy, for which CT was shown to be superior to DUS (13). Magnetic resonance imaging (MRI) in the preoperative phase is generally reserved for characterization of focal liver lesions. The aim of preoperative imaging is to provide an optimal preparation for the transplant surgeon who will perform the LT.

Reference values for Doppler ultrasound in healthy children

Depending on the etiology of the disease, a liver may become cirrhotic or appear morphologically normal. In both cirrhotic and non-cirrhotic disease, ultrasound is frequently performed to detect abnormal vascular flow, focal lesions, and anatomical features including liver size and splenomegaly. Ultrasound uses high frequency (approximately 3 to 18MHz) soundwaves which travel through soft tissue, and are reflected...
back to visualize any soft tissue (14). DUS is a specific technique that can be combined with conventional ultrasound to detect, quantify and illustrate blood flow by using the Doppler effect (15). Medical ultrasound is harmless, generally not painful, and may be performed at the bedside. In liver disease, DUS is of particular importance for the assessment of portal hypertension, which is an increased pressure in the portal vein (PV), splenic vein, and mesenteric vein, and may progress to several chronic morbidities including variceal hemorrhage (16). DUS may suggest early portal hypertension by demonstrating decreased portal flow velocity, which will later progress to pendulating flow and then reversed flow or thrombosis (13). Clinical findings of portal hypertension include splenomegaly, for which ultrasound is also helpful, in addition to thrombocytopenia (<150 x 10^9/L platelets).

For any measurement a reference value is necessary to decide if the obtained measurement is normal or abnormal. However, as children grow, aspects of their physiology may change, so at every age reference values may be different. Therefore, for every phase of pediatric life, measurements from healthy children are required to establish age corrected reference values. For optimal preoperative assessment of a pediatric liver, one of the aims of this thesis is to determine DUS reference values in healthy children.

Hepatic artery anatomy imaging

Preoperative imaging of the hepatic artery anatomy is thought to be especially important in children because of the small caliber of the native artery. This smaller anatomy combined with (usually) a size mismatch with the adult donor artery results in a more challenging creation of the hepatic artery anastomosis than in adults. Consequently, there is a higher prevalence of HAT in pLT compared to adult LT (17). To reduce the risk of HAT, plastic surgeons may be asked to make this anastomosis using a surgical microscope. With the aim of optimal preparation and thereby reducing the occurrence of HAT, optimal preoperative hepatic artery imaging is necessary.

CT angiography (CTA) has been reported as superior to DUS for the purpose of hepatic artery imaging (13). However, CT is preferably avoided in pediatric radiology because the technique utilizes ionizing radiation to acquire images. This radiation is considered to increase the lifetime risk of malignancy, and children are thought to be especially sensitive to radiation (18). Therefore, in pediatric radiology, alternatives such as magnetic resonance imaging (MRI) should be considered whenever possible (19). However, CTA may not be readily replaced. MRI is more susceptible to movement artefacts and requires children to lie still for up to 25 minutes. Consequently, anesthesia is performed (with associated risks and costs) between 6 months and 6 years, whereas children under 6 months can often still be examined using the feed and wrap method (20, 21). In contrast, modern CTs can scan quickly at 73.7 cm/s (22), providing such high temporal resolution that even in breathing (or crying) movement artefacts are negligible, and anesthesia can be avoided. Another advantage of modern CT compared to MRI is a very high resolution (down to 0.24 mm (22)) and the availability of isotropic data, for which MRI may take up to 10 minutes to approach but not replicate this. Soft tissue contrast, however, is superior in MRI, and MRI has clear benefits in this respect. For hepatic artery imaging in children, the advantages and disadvantages of CTA and MRI angiography (MRA) should be weighed for their ability to accurately determine the preoperative vascular anatomy. In order to decide if the radiation exposure of CT can be justified, one of the aims of this thesis is to determine if MRA can replace CTA for the preoperative assessment of the hepatic artery anatomy.

Nutrition and sarcopenia

Many children with liver disease suffer from malnutrition because of a higher metabolism and altered nutrient processing and absorption (23). Malnutrition is closely related to frailty, which is a multisystemic physiological decline associated with chronic illness (24). Sarcopenia, representing low muscle quality and quantity, is considered a measurable component of frailty and for which the etiology overlaps with malnutrition (25). Pediatric radiologists already look at muscles for atrophy associated with muscle and neurological disease, assess fat compartments for their effect on image quality, and study bone for osteopenia in osteogenesis imperfecta or rickets. However, radiologists are usually not aware of malnutrition in a patient, or of malnutrition as a factor affecting muscle and fat in imaging. In general, investigating malnutrition is relevant because it is independently associated with longer hospital stays, more severe and more frequent infections, and poor wound healing in children (26, 27). Clinical assessment of malnutrition consists of measuring weight and length for age, determining body metrics such as the body mass index, mid-upper arm circumference, and triceps skin fold thickness (28). Imaging may contribute to the workup of malnutrition by assessing sarcopenia, in addition, fat compartments can also be measured (29, 30). In adults undergoing LT, sarcopenia can be measured on CT to predict waitlist mortality and posttransplant morbidity and mortality (31, 32). In children, measuring sarcopenia has the same potential, as it may help predict post-surgical infection and sepsis, and direct nutrition policy (33, 34). However, the body composition of children varies from that of adults, and is also age dependent (30). Therefore, the usefulness of CT-based body metrics in adults cannot be directly translated to children. One of the aims of this thesis is to investigate if preoperative CT-based body metrics in pLT children are useful for postoperative outcome prediction.

PERIOPERATIVE IMAGING

Ultrasound image quality is optimal when the ultrasound probe is as close to the studied object as possible. This, along with the mobility of an ultrasound machine, makes it ideal for intraoperative imaging, in which the probe can be placed directly on the pLT. During
surgery, DUS is used to assess patency of the hepatic vessels, with particular attention to the vascular anastomoses. The usefulness of intraoperative DUS is self-evident; if a thrombosis is detected with the abdomen still open it is easier to restore patency than when the abdomen is closed. Early removal of thrombosis improves graft survival and decreases the chances of cholangiopathy such as anastomotic and non-anastomotic biliary stenosis (AS and NAS) (35). In addition to intraoperative DUS, DUS may also be performed for the same reason in the operating room after abdominal closure, and ultrasound immediately at arrival in the ICU. In this thesis we consider these three timepoints as peri-operative imaging.

**Perioperative thrombosis**

Although HAT is the most common vascular thrombosis in pLT, and the primary reason to perform frequent DUS both peri- and postoperatively, the time of occurrence of HAT is unclear (36, 37). The most specific review reported a range of detection of HAT between day 1 and 9.6 after pLT (17). This lack of more exact data is possibly due to a variety of peri- and postoperative DUS protocols employed in pLT centers around the world. A better understanding of the occurrence of HAT may help justify intraoperative DUS, with the aim of improving graft survival. It may also provide scientific base for the costs of an on-call service of dedicated radiologists or sonographers that come to the operating theater during the pLT. In addition, PV and hepatic vein thrombosis may be detected and corrected similarly to HAT.

**Assessment of perioperative vascular complications**

DUS not only detects thrombosis, but it can also identify vascular complications such as anastomotic stenosis, vascular kinking, and extrinsic compression (38). Morphologically, a stenosis is considered significant in case of a 50% narrowing compared to the pre-stenotic vessel caliber (39, 40). However, ultrasound is insufficient to accurately measure the diameter of small vascular stenosis because of a limitation of spatial resolution, obscuration by gas and metal clip artefacts, and the difficulty of producing the right image planes for such measurements (41). Instead, DUS investigates anastomotic stenosis by measuring flow velocity both in the pre-anastomotic native vessel, and in the vascular anastomosis (4). According to hydrodynamic physics, a 50% anastomotic stenosis would result in a fourfold flow velocity increase between pre- and anastomotic flow velocity measurements (42). Although this does not take into account factors such as turbulent flow, it is useful as an estimate during DUS (43). For the PV an anastomotic threshold of 120-125cm/s peak systolic velocity (PSV) is suggested (44, 45). Alternatively, a 1:3-4 increase pre- to anastomotic PSV ratio may be considered diagnostic of a PV anastomotic stenosis (4, 42). For the hepatic artery exact DUS pre- and anastomotic measurements are often not possible, and so an anastomotic PSV of >200 cm/s is reported to be significant (4, 40, 44, 46-48). For the assessment of the hepatic vein(s) anastomotic PSV acceleration or thresholds are generally not used. Instead, assessment of the phasicity of the flow curve is performed, which represents flow direction changes resulting from cardiac pressure changes (49). For a more objective expression of hepatic outflow the phasicity has incidentally been expressed as the venous pulsatility index (VPI) (44, 50).

Although the gold standard for diagnosing any vascular stenosis is the demonstration of a pressure gradient via an intravascular catheter, DUS represents the best non-invasive alternative (51, 52).

A recently transplanted liver undergoes hemodynamical changes which are not fully understood, a hypercoagulable state, and the pLT and its vessels may be influenced by surrounding soft tissue swelling and collections (38, 53, 54). These peri- and postoperative factors may impact flow velocities. Consequently, it is likely that the abovementioned reported DUS thresholds and methods for diagnosing stenosis are less valid in the perioperative and early postoperative setting. Therefore, reference values of uncomplicated vessels after pLT are necessary. One of the aims of this thesis is to investigate how often vascular thrombosis and other vascular complications occur during pLT. Another aim is to determine perioperative DUS reference values in children without vascular complications.

**POSTOPERATIVE IMAGING**

**DUS surveillance and reference values for vascular stenoses**

In the postoperative phase, DUS remains the primary imaging modality for vascular complication surveillance. Reported DUS short-term postoperative surveillance protocols vary from daily for 3 to 7 postoperative days, to twice a day for 14 subsequent days (17, 55). To what extent long-term DUS surveillance after hospital admittance is performed in the various pLT centers is unknown.

Vascular complications such as thrombosis and stenosis are often initially clinically occult, which is why DUS surveillance is performed to ensure timely diagnosis and treatment (7, 36, 56). When present, the clinical manifestations of vascular complications vary per vessel. PV stenosis may result in portal hypertension, associated variceal hemorrhage, and subsequent occlusion with parenchymal damage (57). Hepatic artery thrombosis and stenosis may present with biliary damage such as AS and/or NAS, as well as clinical itch and jaundice. Lastly, hepatic vein stenosis results in hepatic congestion, ascites and abnormal liver tests (54, 58). These vascular anastomotic stenoses may occur in the early or long-term postoperative phase, but the precise rates of occurrence and incidences are unknown.
Only DUS is suitable for frequent surveillance of vascular patency after LT because of its harmless nature compared to CT (which entails radiation) and MRI (which often requires anesthesia and is particularly costly). However, for successful surveillance a test with sufficient sensitivity and specificity should be used. For the detection of vascular complications, the test performance of DUS is unclear. To determine this, comparison of each DUS with a reference standard such as CT with intravenous contrast would be needed, but this would not be feasible for each DUS. Despite the lack of established test performance, DUS is already widely employed. In this setting, while an abnormal DUS will receive follow-up by imaging such as a CT or DUS, surgery, or careful clinical observation, a negative DUS will generally not be followed-up by a reference standard. Consequently, false-negatives and true-negatives are not registered in clinical practice. Therefore, it is challenging to determine its test performance in the current setting of widely employed DUS surveillance. One of the aims of this thesis is to investigate the incidence of all types of vascular complications after pLT as detected by DUS, and to determine the positive predictive value of DUS.

In adults after LT, intraoperative measured DUS values of the hepatic artery and PV were shown to be higher than preoperatively, followed by a significant decrease over time after LT (56). Because a pLT likely undergoes the same changes as an adult LT, it is likely that DUS values also change over time in children. However, one study investigating a pediatric population noted higher measurements compared to adults on day 1 (59). Consequently, change over time in pLT might be similar to adults, but not necessarily the same. If there is indeed change over time, the static thresholds as described previously are probably insufficient, and there is a need for timepoint dependent DUS reference values after pLT. This will be useful for the early postoperative phase, but also at later timepoints such as 1 or 2 years after pLT. Improved timepoint dependent reference values may improve diagnostic accuracy, and improve outcomes. One of the aims of this thesis is to determine DUS reference values at timepoints up to 2 years after pLT.

Fasting for a DUS and improving the assessment of the portal vein anastomosis

In both adult and pediatric radiology, abdominal ultrasound is preferably performed with the patient fasted to minimize bowel gas that may reduce visibility. Fasting is also thought to decrease discomfort from pressure from the ultrasound transducer (60). However, the consequence of this may be a hungry and unhappy child. This may be especially burdensome for children and parents that frequently need to travel long distances to attend their DUS surveillance appointments. In healthy adults, a study of postprandial ultrasounds showed a postprandial increase of portal venous blood flow (61). As in adults, it is likely that eating will increase mesenteric perfusion in children, and this may disturb the DUS measurements.

Increased postprandial mesenteric flow with subsequent increased pre- and anastomotic PV PSV on DUS may result in a false-positive diagnosis of a PV stenosis when the anastomotic PSV method is measured (44, 45). To correct for postprandial changes, the pre- to anastomotic PSV ratio of 1:3-4 may be more suitable (4, 42). Namely, although increased mesenteric flow after eating may effect both methods, the latter method should be less (or not at all) because it is an index that corrects for pre-anastomotic PSV increase. For children to stop fasting for a surveillance DUS of their pLT, DUS measurements must remain feasible and reliable, and measurements must still be possible despite potential increased intra-abdominal gas. In addition, the child should still be sufficiently comfortable to allow the DUS to take place. One of the aims of this thesis is to investigate if fasting is necessary for a routine pLT DUS, and if the pre- to anastomotic PSV ratio is preferred over the anastomotic PSV for the assessment of the PV anastomosis.

DUS surveillance of cholangiopathy and fluid collections

Although not the primary goal, surveillance ultrasound also provides information on peritoneal fluid collections such as ascites, blood and pus, in addition to cholangiopathy such as bile duct dilatation in AS and NAS (62). Fluid collections often present clinically with abdominal distension, or via intra-abdominal drains. However, sometimes hematomas can give external compression on fresh vascular anastomoses without abdominal distension or drain production. Biliary complications may present with abnormal biochemicaly followed by jaundice and itch. Although most biliary complications such as AS and NAS present in the late postoperative phase, a tight biliary anastomosis may already cause biliary duct dilatation shortly after the pLT, and this may warrant timely surgical correction (63).

The role of CT and MRI

As was already briefly mentioned before, there is a role for CT and MRI in pediatric liver transplantation. The main benefit of CT in children is the fast acquisition of high-resolution images, which can be performed without anesthesia. The downsides are poor intrinsic soft tissue resolution for which intravenous Iodine-based contrast is necessary, multiple scans for different contrast phases (e.g. arterial, portal, late), and the use of radiation. In the postoperative phase, CT can be used to confirm significant DUS findings such as thrombosis or significant stenosis, or assess for abscesses (4). Assessment of biliary ducts is difficult on CT, and for this magnetic resonance cholangiopancreatography (MRCP) is performed on MRI (63).

Magnetic resonance imaging for symptomatic cholangiopathy

The physics behind MR image acquisition is complex. In short, the MRI machine creates a magnetic field stronger than that of the earth. The angular momentum of hydrogen protons (as part of the patient) within that field align with the gradient of the produced magnetic field. Next, the protons are excited with a radiofrequency pulse, and the protons...
absorb this energy while being knocked out of alignment. Depending on various tissue characteristics, as they return into alignment they release the energy as radiofrequency waves which are received by MR coils and translated into T1 and T2 information. The degree of T1 and T2 information depends on several aspects of an MR sequence and this decides which tissue characteristics are visible in the image. The most important MR variables to adapt are repetition time (TR) and echo time (TE). Fluid appears very bright on sequences based on T2 information, whereas fluid is dark on a T1-weighted sequence. MRCP makes use of TR and TE in such a way that almost only T2 information is incorporated in the sequence, this creates images in which only fluid compartments, such as bile ducts, are depicted (64). MRCP allows for the assessment of bile ducts throughout the liver.

Performing an MRCP in a child is a high threshold decision because it requires anesthesia between ages 6 months to 6 years, a feed and wrap procedure in children <6 months, or requires a child older than 6 years to lie still for approximately 30 minutes (21). Whereas adults can be instructed to hold their breath to reduce movement artefacts, this is difficult for children. Even under anesthesia breath holds are usually not possible. Therefore, in addition to children having a smaller (more difficult to image and interpret) anatomy than adults, there are often more movement artefacts. Consequently, research from MRCP studies after LT from adults may not apply to children.

With the biliary ducts being primarily vascularized by the hepatic artery, NAS is primarily associated with biliary ischemia related to HAT (65). However, NAS may be also be caused by ischemia-reperfusion injury after transplantation, immune-mediated injury, bile salt toxicity, cholangitis, and arise in longstanding or complicated AS (66, 67). AS may be the result of an anastomosis that is too tight, but may also be associated with hepatic artery complications. In adults NAS on MRCP is not always symptomatic (10). Due to the variation in imaging, anatomy, and a higher incidence of HAT it is important to investigate MRCP in children with symptomatic cholangiopathy, and to investigate the clinical significance of those findings. One of the aims of this thesis is to investigate the yield of MRCP after pLT in the case of symptomatic cholangiopathy, and to correlate MRCP findings to clinical outcomes.

OUTLINE OF THE THESIS

In chapter 2, DUS reference values of the hepatic artery and PV at the hepatic hilum of 100 children without liver pathology are presented, which were retrospectively gathered to provide reference values and to determine if these are age dependent.


64. Schild HH. MRI Made Easy [...] Schering AG; 1990.

