LONG-TERM OUTCOMES AFTER PEDIATRIC LIVER TRANSPLANTATION

Silja Kosola

ACADEMIC DISSERTATION

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To the true heroes and future hopes; to children
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Since survival rates after pediatric liver transplantation (LT) have improved, the focus of interest has shifted to gradually evolving histological changes, long-term complications, and quality of life. Histological changes and complications are closely associated with each other and the immunosuppressive medication used, and may in turn affect the LT recipient’s perception of quality of life.

This thesis investigates the survival and complication rates, usefulness of protocol liver biopsies and long-term histological status of the liver graft, and cholesterol metabolism profile after pediatric LT. The effect of immunosuppression on biopsy-proven changes and cholesterol metabolism is also evaluated. In the quality of life assessment, the effects of different complications are explored.

The study population includes all 99 children <18 years who underwent LT in Finland between 1987 and 2007. Retrospective data on LT indications, surgical procedures, surgical and medical complications, immunosuppression, and annual follow-up visits were collected from patient records and the national LT registry. For 12 patients transplanted for hepatoblastoma (HB) or hepatocellular carcinoma (HCC), also relevant cancer treatment details were gathered.

Cross-sectional data included liver biopsies of 54 LT recipients (82% of survivors), serum levels of noncholesterol sterols (surrogate markers of cholesterol metabolism) and fibroblast growth factor 21 (FGF21) in 49 LT recipients (74%), and health-related quality of life (HRQoL) assessment of 57 LT recipients (86%) at median 10-11 years posttransplant. Serum levels of noncholesterol sterols and FGF21 were compared to 93 controls matched for age and gender, and the HRQoL measurements to 141 randomly picked controls matched for age, gender, and place of residence.

Of the 12 patients with liver malignancies, six had HB and six HCC. All patients received neoadjuvant chemotherapy, but no routine chemotherapy was administered after LT. Median time from diagnosis to LT was 7 (2-133) months. At LT, none of the patients had radiological evidence of extrahepatic disease. The overall survival rates at one, five, and ten years after LT were 100%, 80%, and 67%, respectively. Survival was similar between the tumor types, and two deaths occurred secondary to tumor recurrence, one of each type.

Biopsies of 18 patients (33%) showed near-normal histology with minimal changes. Portal inflammation was present in 14 samples (26%), and was less frequent in patients whose immunosuppression included steroids (14% vs. 47%; p = 0.008). Fibrosis was found in 21 biopsies (39%), and fibrosis staging correlated negatively with serum prealbumin levels (r = -0.364, p = 0.007) and positively with portal inflammation (r = 0.350, p = 0.010) and periportal cytok-
eratin 7 staining ($r = 0.529$, $p < 0.001$). Microvesicular steatosis was detected in 23 biopsies (43%; 5-80% of hepatocytes), and correlated with the patients’ body-mass-index ($r = 0.458$, $p < 0.001$) but not with steroid use. The histological findings were mainly mild and led to treatment changes in ten patients (19%), while only one minor complication was encountered.

Serum lipid levels were similar in LT recipients and controls. LT recipients, however, displayed increased whole-body synthesis and decreased absorption of cholesterol compared to controls (lathosterol to cholesterol ratio $129 \pm 55$ vs. $96 \pm 41$, respectively, $p < 0.001$; campesterol to cholesterol ratio $233 \pm 91$ vs. $316 \pm 107$, respectively, $p < 0.001$). Low-dose methylprednisolone and azathioprine were negatively associated with the lathosterol/sitosterol ratio ($r = -0.492$, $p < 0.001$ and $r = -0.383$, $p = 0.007$, respectively) reflecting a favorable effect on cholesterol metabolism. FGF21 levels were higher in LT recipients than in controls ($248 \text{ pg/mL}$ vs. $77 \text{ pg/mL}$, $p < 0.001$).

Under school-aged LT recipients and controls had similar HRQoL, and 54% of LT recipients aged over 7 scored within the controls’ normal range on all HRQoL domains. Biliary complications, reoperations, and obesity were independently associated with decreased HRQoL ($p < 0.05$ for all). For those LT recipients already attending adult health care, physical functioning and general health yielded reduced scores ($p < 0.05$). Still 64% of adults considered their health excellent. Sexual health was similar to controls although LT recipients may experience problems with their orgasm strength ($p = 0.050$).

In conclusion, continued use of low-dose steroids seems to be associated with milder changes of both liver graft histology and cholesterol metabolism. Normal HRQoL and sexual health are achievable in long-term survivors of pediatric LT.
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:


These articles were reprinted with the kind permission of their copyright holders. Some previously unpublished data are also presented.
ABBREVIATIONS

AFP alpha-fetoprotein
aHUS atypical hemolytic uremic syndrome
AIH autoimmune hepatitis
ALF acute liver failure
ALT alanine transaminase
ARPKD autosomal recessive polycystic kidney disease
AST aspartate transaminase
ATG antithymocyte globulin
AUDIT alcohol use disorders’ identification test
AZA azathioprine
BA biliary atresia
BMI body mass index
CK7 cytokeratin 7
CMV cytomegalovirus
CNI calcineurin inhibitor
CsA cyclosporine A
DISF-SR Derogatis interview for sexual functioning self report
EBV Epstein-Barr virus
FGF21 fibroblast growth factor 21
GFR glomerular filtration rate
GGT gamma-glutamyltransferase
HAT hepatic artery thrombosis
HB hepatoblastoma
HCC hepatocellular carcinoma
HDL high density lipoprotein
HOMA homeostasis model assessment
HRQoL health-related quality of life
IL interleukin
INR international normalized ratio
IQ interquartile range
LDL low density lipoprotein
LT liver transplantation
MARS molecular adsorbent recirculating system
MELD model for end-stage liver disease
MMF mycophenolate mofetil
MP methylprednisolone
mTOR mammalian target of rapamycin
NAFLD non-alcoholic fatty liver disease
PELD pediatric model for end-stage liver disease
PTLD post-transplant lymphoproliferative disorder
SD standard deviation
TC total cholesterol
INTRODUCTION

The birth of a child is a miracle, and the illness of a child a tragedy. Among pediatric illnesses, liver disease account for a small minority. The liver, however, is a vital organ with many digestive, synthetic, endocrine, and exocrine functions. No matter how hard doctors fought, no child with end-stage liver disease could survive before the era of liver transplantation (LT). The first successful LT was performed by Thomas Starzl and colleagues on a 19-month old girl with hepatocellular carcinoma in 1967 (1). Technical problems leading to severe bleeding, thrombosis, or biliary complications and insufficient immunosuppression - based on steroids and azathioprine - leading to rejection limited the survival of LT recipients, and in the 1970’s only 28% lived through the first year after LT (2).

A new immunosuppressant, cyclosporine A, introduced in 1980, started a new era in the field of transplantation by greatly decreasing the rates of rejection and consequent graft loss (3). This change, as well as improvements in surgical technique and patient care, transformed LT from an experimental procedure into a therapeutic operation and led to the establishment of many new transplant centers. Already in 1982, the first Nordic LT was performed in Finland by the pioneer team led by Krister Höckerstedt. In 1987, the first Finnish child underwent LT for biliary atresia, the most common cause for pediatric LT worldwide, at the age of four years.

As children are physically small, the first allografts came from deceased children. Children rarely die under conditions enabling organ donation, however, and in 1985 Bismuth and Houssin described a transplantation technique using reduced-size grafts from adult donors (4). Despite introducing some new technical challenges due to e.g. size-mismatch of blood vessels and bile ducts, reduced-size grafts usually including segments II-III or II-IV became standard practice and increased the chances of survival for children waiting for LT. Abroad, the shortage of donor organs has also been confronted by living-donor LT, first performed by Raia in 1987 (5). As waiting list times for children in Finland have remained reasonable and living donation predisposes healthy family members – whose efforts are needed in caring for the LT recipient – to the potential hazards of major surgery, this option has so far not been used in Finland.

Surgeons may save a child’s life, but this alone is not enough. Children also need to be able to play and study, grow and enjoy their lives, and hopefully develop into independent adults. LT and the immunosuppression involved, however, subject recipients to many potential risks and complications. Besides the immediate risks of surgical issues or rejections, long-term complications include renal dysfunction, cardiovascular disease, growth problems, and even cancer (6-9). In trying to tackle these issues, different immunosuppressive
strategies have been attempted (10-12), but no consensus has been reached. Little is also known about the long-term function and histological state of the graft (13,14), which are poorly depicted by any routine laboratory tests, or the factors involved in sustaining sufficient function. Most alarmingly, quality of life after childhood LT is reported to be even as low as in children with cancer (15,16). This is an unacceptable result for a treatment meant to be a cure, not another illness.

This thesis aims at describing the survival and complication rates as well as causes of death after pediatric LT in Finland as base knowledge. We assessed histological findings, graft function as depicted by markers of cholesterol metabolism, and the quality of life of long-term survivors of childhood LT. All of these and clinical data were combined to find explanations for both satisfactory and disappointing results and to form a comprehensive image of the first 20 years of pediatric LT in Finland. We hope our findings aid in the continued efforts to further improve patient outcomes.
REVIEW OF THE LITERATURE

The beginning

To understand many of the pediatric diseases leading to LT, the challenges of the surgical procedures, and the multitude of issues to be faced after LT, it is vital to comprehend the principles of liver development, anatomy, and functions.

Development and anatomy of the liver

Liver development occurs at an early stage of organogenesis of the embryo. During the 3rd week of development, the liver bud appears at the distal end of the foregut. Rapidly proliferating cells of endodermal origin penetrate the septum transversum. Mesodermal cells of the septum transversum give rise to hepatopoietic cells, Kupffer cells, and connective tissue within the liver. The bile duct is simultaneously formed as a narrowed connection between the liver bud and what is later to become the duodenum. The gallbladder and cystic duct develop from a bulge in the bile duct. (17)

The liver is covered by a capsule of strong connective tissue and the surface of the liver is lined by visceral peritoneum, except on its cranial surface, where it is in direct contact with the diaphragm. The coronary ligament attaches the liver to the diaphragm, while the falciform ligament attaches the liver to both the diaphragm and the anterior abdominal wall. (18)

The liver is functionally divided into right and left lobes (Figure 1). Each lobe has its own circulatory and bile drainage system. The liver can further be divided according to the three main hepatic veins and primary branches of the hepatic artery and portal vein into eight segments, important for the surgical procedure of pediatric LT described later.

The liver has a double blood supply, 25% from the hepatic artery and 75% from the portal vein. The common hepatic artery arises from the celiac trunk, while the portal vein is formed by the union of the superior mesenteric and splenic veins. Together with the bile duct, these two vessels form the portal triad. Blood leaves the liver via the hepatic veins, which join the inferior vena cava just below the diaphragm.
Figure 1. Liver segments and major blood vessels. Segments are numbered according to Couinaud. Segment 1 lies inferoposteriorly to segment 4. The hepatic artery and portal vein supply blood to the liver, while blood from the liver drains to the inferior vena cava via the hepatic veins.

The bile duct is 8-10 cm long and 5-6 mm in diameter. Around its distal end, the circular muscle forms the choledochal sphincter at the connection to the duodenal wall. The bile duct has its own arteries and veins along its walls. Variations in hepatic arteries and extrahepatic bile ducts are common and provide clinical challenges for transplant surgeons (18-20).

The neonatal liver consists of roughly 50,000 liver lobules or functional units of hepatocytes, and this amount increases during growth to the 500,000 found in an adult liver (21). At birth, however, the liver constitutes about 5% of the total body weight. During growth the proportionate liver size decreases to about 2% of the total body weight in adults (18).

**Liver structure**

The liver is composed of hepatocytes (80% of liver volume) arranged as cell plates with sinusoids between them and connective tissue stroma, within which the blood vessels and bile ducts travel. Cells of the immune system (Kupffer cells, natural killer cells, and endothelial cells) are also abundant in the liver (22). The classic description of a hepatic lobule (Figure 2a.) as a hexagonal structure with the central vein in its center and portal canals (including branch-
es of the hepatic artery, portal vein, and a bile duct) at its angles highlights the direction of blood flow. The portal lobule (Figure 2b.), on the other hand, is defined around bile secretion: the bile duct is at the center of a triangle, with central veins at the angles. Finally the liver acinus (Figure 2c.) correlates best with blood perfusion, metabolic activity, and pathological findings. Cells closest to the portal triad have best access to circulation and nutrients provided by it, but also suffer first from obstruction of bile flow. Cells furthest away from the portal areas are most vulnerable to ischemia, first to show fat accumulation, but last to suffer from toxic substances and bile stasis. (23)

![Figure 2. Red circles depict portal canals including branches of the hepatic artery, portal vein, and a bile duct, and blue circles depict central veins. 2a) classic hepatic lobule, 2b) portal lobule, and 2c) liver acinus. Adapted from Ross et al. 1995.](image)

**Functions of the liver**

The liver has important functions already in the embryo, as it participates in **hematopoiesis** by producing erythrocytes and leukocytes. This activity dwindles during the last two months of fetal life, leaving only small hematopoietic islands present at birth (17).

During the 12th week of development, **bile acid synthesis and bile excretion** begin. Bile is stored and concentrated in the gallbladder and enters the duodenum via the common bile duct. Bile is necessary for the emulsification of fats and fat-soluble vitamins and excretion of cholesterol.

The liver stores vitamins A, D, and B12, as well as iron and copper. **Amino acid and protein syntheses** begin already in the fetal liver, α-fetoprotein (AFP) being the fetal counterpart of albumin. After birth, albumin is the most important osmolar component of serum and carrier of many hormones and other substances.
Glycogen synthesis begins at the 9th gestational week. After birth, the liver stores significant amounts of the body’s energy reserves as glycogen, degrading it during fasting and strenuous exercise. The liver is further capable of gluconeogenesis, the synthesis of glucose from lactate and amino acids.

The liver has a pivotal role in fatty acid oxidation as well as cholesterol and lipoprotein metabolism. Lipoproteins transport lipids between their absorption sites, the liver, and tissues utilizing lipids. In healthy individuals, a state of homeostasis is maintained between cholesterol synthesis and intestinal absorption (24,25). Cholesterol is synthesized from acetyl coenzyme A units via a complex metabolic pathway and several precursor molecules including cholestenol, desmosterol, and lathosterol. Measuring the serum levels of these precursors is a way of assessing cholesterol synthesis (26). Approximately 50% of intestinal cholesterol in healthy humans is absorbed, and van der Wulp et al recommend estimating absorption by measuring serum levels of cholestanol and plant sterols campesterol and sitosterol contained in a typical Western diet (27).

The liver is the main site for detoxification of many metabolic substances, mainly by conjugation. The processes include glucuronidation of bilirubin to an excretable form and conversion of ammonia to urea. The detoxification capacity of the liver develops further during childhood and adolescence, in part explaining the vulnerability of children to many substances tolerated by adults.

Hepatocytes produce most of the complement components and also complement regulator proteins (factor I and factor H) and, together with liver endothelial cells, Kupffer cells, and natural killer cells, form the framework of the innate immune system of the body (28).

Hemostasis is primarily regulated by the liver. It produces fibrinogen, prothrombin, and coagulation factors V (solely synthesized by the liver), VII, IX, X, XI; inhibitors of coagulation (protein C, protein S, α1-antitrypsin, and antithrombin III); fibronectin (induces coagulation), fibrinogen (precursor of fibrin) and plasminogen (degrades fibrin clots). The liver also secretes thrombopoietin, which induces platelet production in bone marrow. In addition, the liver secretes insulin-like growth factor I, a molecule vital for childhood growth.
The setting: Pediatric liver diseases and indications for transplantation

Most pediatric liver diseases leading to liver failure and LT are rare, chronic and, with few exceptions, non-recurrent. Sustained liver injury causes activation of hepatic stellate cells which then differentiate into myofibroblastic cells and produce extensive amounts of collagen (28). Collagen accumulation in the liver leads to suppression of hepatocytes and subsequent failure of function, as well as portal hypertension in turn causing splenomegaly and esophageal and gastric varices. As can be deduced from the liver functions, symptoms of liver failure include jaundice and pruritus (due to insufficient bile secretion and elevated serum bile acid and bilirubin levels), edema and ascites (deficiency of osmolar protein synthesis and portal hypertension), nausea and other central nervous system symptoms (increase in serum bilirubin and ammonia levels), infections (immune deficiency), bleeding (deficiency of coagulation factors and platelets), malnutrition and growth failure.

Cholestatic liver disease

Extrahepatic biliary atresia (BA), although rare, is globally the most common cause for pediatric LT. In roughly 20% of patients, the defect occurs already during fetal development, and these infants have associated anomalies including splenic malformation, intestinal malrotation, or cardiovascular anomalies, while 80% of patients present with an isolated destruction of normally formed extrahepatic bile ducts (29-31). The actual cause still remains unknown, but genetic, infectious, inflammatory and even toxic etiologies are suspected (31). BA leads to progressive liver cirrhosis and death within two years without treatment. The primary treatment option is the Kasai hepatoportoenterostomy, and its success rate depends on the timing of surgery and center experience (31,32). Up to 80% of children with sufficient biliary drainage after the Kasai operation can reach adolescence without LT (33), but usually the Kasai operation only postpones LT. LT should be considered if patients suffer from persistent cholestasis, progression to cirrhosis, or portal hypertension with ascites or recurrent variceal bleeding.

Acute liver failure (ALF)

ALF in children is defined as biochemical evidence of liver injury in a patient without prior history of liver disease, associated with coagulopathy uncorrected by vitamin K, and INR > 1.5 in patients with hepatic encephalopathy or INR > 2.0 if no encephalopathy is present (34). Although several diseases may cause
ALF (e.g. infections, toxicities, or metabolic diseases), frequently this condition is still left without definite diagnosis due to the sudden and complete destruction of the liver. Sudden necrosis of hepatic cells leads to the accumulation of toxic substances, vasoactive substances, and metabolic waste products normally metabolized by the liver, which cause multisystem organ failure. If less than 20% of hepatic cells remain viable on liver biopsy, regeneration and recovery are unlikely, and LT offers the only chance of survival. Especially in high-urgent cases, molecular adsorbent recirculating system (MARS) albumin dialysis can sometimes cost-effectively be used to extend the time a patient survives on the waiting list (35,36). Especially in children, ALF of viral origin may infrequently be associated with fulminant aplastic anemia, where hepatitis is followed 2-3 months later by pancytopenia (37,38).

Hepatitis

Neonatal hepatitis is a term historically used to describe cholestasis associated hepatic inflammation of infants. Due to advances in diagnostics, a specific diagnosis can now often be reached, but neonatal hepatitis still includes a heterogeneous group of liver diseases (34). Toxoplasmosis, rubella, cytomegalovirus (CMV), herpes simplex, bacterial infections (e.g. *Escherichia coli* and *Listeria monocytogenes*), hepatitis B, and endocrine and metabolic diseases are all possible causes, but in some patients the etiology remains unrecognized. Histologically portal areas are better preserved than lobular areas, and multinucleated giant cells are often present. Prognosis depends on the underlying etiology and extent of liver necrosis and fibrosis. (39)

A combination of a mononuclear infiltrate of portal tracts, increased serum transaminase levels, and presence of non-organ and liver specific autoantibodies is called autoimmune hepatitis (AIH). In children, AIH often responds well to immunosuppressive medication. Especially acute cases may require LT but disease recurrence after LT is possible (40).

Hepatitis B is a globally polarized disease; a third of the world’s population is infected but prevalence is highest in Asia and Sub-Saharan Africa. Much of the disease burden is carried by children, as most common routes of transmission are vertically from mother to child and horizontally between children. Hepatitis B is also a major risk factor for developing hepatocellular carcinoma. (41)

Metabolic liver diseases

Tyrosinemia is an autosomal recessively inherited deficiency of fumarylacetoacetate hydrolase, which is the last enzyme of tyrosine degradation. The toxicity
of metabolic compounds, especially succinyl acetone, leads to liver crises and cirrhosis in early childhood. Liver dysfunction is associated with varied renal symptoms and painful paresthesias (39). The lifetime risk of developing hepatocellular carcinoma (HCC) is increased. Currently patients with tyrosinemia have dietary restriction of tyrosine and phenylalanine combined with nitisine treatment, which inhibits tyrosine catabolism and reduces formation of toxic metabolites (42). The effect of nitisine treatment on the risk of HCC remains unknown, and LT still offers the only cure.

**α1-antitrypsin deficiency** is a genetic disorder, in which the substitution of a single amino acid leads to an abnormally folded protein that is retained in the endoplasmic reticulum of liver cells. Normally α1-antitrypsin inhibits especially serine proteases released by activated neutrophils. Liver damage is suspected to be caused by the accumulation of abnormal α1-antitrypsin molecules in hepatic cells, and diagnosis may be made at any age from infancy to adulthood depending on the onset of symptoms. (39)

**Wilson’s disease** is an autosomal recessively inherited disease due to accumulation of copper first in the liver and later also in the nervous system, cornea, and other tissues. Symptoms may present at any age, with hepatic symptoms most common before age 10 years and neuropsychiatric symptoms in older patients. Wilson’s disease can be treated with copper chelation if discovered early enough, but often LT is the only cure (39).

Urea cycle disorders, such as ornithine transcarbamylase deficiency, are inherited defects in liver enzymes responsible for protein and nitrogen metabolism. These defects result in the accumulation of ammonia, which in turn leads to cerebral edema and neurocognitive dysfunction (39). Central nervous system injury is irreversible, and thus early diagnosis, aggressive support, and timely LT are essential.

**Primary hyperoxaluria** is due to deficiency of alanine-glyoxylate transaminase, a peroxisomal enzyme found only in the liver, which is needed in amino acid metabolism. Elevation of oxalate in urine induces progressive formation of kidney stones and nephrocalcinosis (39). The recommended treatment for patients with hyperoxaluria induced end-stage renal disease is combined liver-kidney transplantation (43), but also pre-emptive LT is possible (44).

**Cystic fibrosis** is a genetic disorder affecting chloride channels of secretory epithelia. Patients consequently suffer from elevated sweat chloride concentration, pancreatic insufficiency, chronic lung disease, and variable liver disease (39). Liver disease occurs mainly in the first decade (45), is unassociated with the severity of pulmonary disease, and is only cured by LT (46). Due to the genetic isolation of Finland, cystic fibrosis is extremely rare.
Fibropolycystic liver disease

Fibropolycystic liver disease refers to a heterogenous group of liver diseases characterized by ductal plate malformation, variable intrahepatic bile duct dilatation, and periportal fibrosis (34,47).

Autosomal recessive polycystic kidney disease (ARPKD) is a rare autosomally inherited disease and, despite its name, it presents with two characteristics: fusiform dilatation of renal collecting tubules leading to bilateral enlarged kidneys and bile duct abnormalities rarely visible as macroscopic cysts (34,39). Both renal and hepatic fibrotic lesions progress over time, and thus both kidney transplantation and LT may be needed for long-term survival (48).

Congenital hepatic fibrosis is an autosomal recessive disorder characterized by hepatomegaly and portal hypertension with intact lobular architecture, superimposed periportal fibrosis, and dilated intrahepatic bile ducts. In addition, portal vein branches may be hypoplastic and hepatic artery branches excessive (34). Primary treatment aims at preventing cholangitis episodes and the sequelae of portal hypertension, while LT offers the final solution.

Liver malignancies

Liver malignancies comprise roughly 1% of childhood cancers. The most common cancer type is hepatoblastoma (HB), with peak incidence in children under age three (49). HB is usually found in an otherwise healthy liver, although increased risk is associated with very low birth weight, Beckwith-Wiedemann syndrome, and familial adenomatous polyposis (50-52). Hepatocellular carcinoma (HCC) is the second most common tumor type, and usually affects older children from age 10 to 14 years (49). HCC is often preceded by hepatic viral infections or another liver disease leading to cirrhosis, and occasionally tumors in children have features of both HB and HCC (53). Current recommended treatment for both tumor types includes chemotherapy followed by complete surgical resection of the liver tumor (54,55). Chemotherapy is often enough to eradicate extrahepatic metastases of HB. Unresectable tumors may be cured by LT despite tumor size beyond the criteria set for adult patients, if no extrahepatic metastases of HB are present, while extrahepatic spread of HCC at any time point is a contraindication for LT (54-56). Pediatric and adult HCC may in fact be two different diseases (57). As survival after LT is excellent and tumor recurrence often leads to inferior treatment results, heroic resections should be avoided (55). Prognosis depends on several factors including pretreatment extent of disease (PRETEXT), histological subtype, and response to chemotherapy (53,54,58).
Other rare conditions

Noncardiogenic hepatic venous outflow obstruction that results in ascites and liver enlargement is called **Budd-Chiari syndrome**. A congenital caval stricture may be the underlying cause, and in bigger children and adults Budd-Chiari syndrome is often caused by conditions predisposing to thrombosis, such as protein C resistance due to the factor V Leiden mutation (59). Therapy is initially conservative, but patients with progressive liver disease are candidates for LT (60).

**Atypical hemolytic uremic syndrome (aHUS)** is the combination of thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure most often due to mutations in the complement factor H gene (61). Since factor H is mostly synthesized in the liver, LT offers a cure, sometimes combined with kidney transplantation.

The climax: Pediatric liver transplantation

Pretransplant assessment

Any child suffering from end-stage liver disease associated severe complications or impaired quality of life should be considered for LT. The LT candidate must be well enough to survive the operation and recover from it. The candidate and their family must also be able to adhere to both medications and follow-up, and thus both children and their parents will undergo psychological evaluation. Evaluations of cardiopulmonary and renal functions, comorbidities, nutritional status and growth, and vaccination status; screening for infections, and thorough cleansing of infection foci (e.g. teeth) are also part of the pretransplant assessment (62).

Organ donors

Most liver allografts come from heart-beating **deceased donors** after brain death due to stroke or trauma. In addition, death should occur in a hospital setting where optimizing the donor’s hemodynamics until organ procurement is feasible. Contraindications for liver donation include hepatobiliary disease, severe abdominal trauma, hepatitis B, hepatitis C, and human immunodeficiency virus or other systemic infections, and cancer. Age is a relative contrain-
dication, as higher donor age has been associated with decreased graft survival in children (63,64).

**Non-heart beating donors** theoretically provide more organs for transplantation. After careful donor selection this approach has also been tried in children. Despite good short-term results (65,66), however, non-heart beating donors remain a marginal option.

**Living related donors** are used especially in countries where deceased donors are too scarce (67,68). In children, segments II and III or the whole left liver lobe are most often procured according to the size of the recipient. Due concern remains, however, about the morbidity and even mortality caused to previously healthy donors (69,70). Living donation has to date never been performed in Finland because of ethical issues and relatively short waiting lists.

### Waiting lists and organ allocation

Organ allocation from deceased donors should be objective, standardized, and fair, and provide the scarce donor organs to those with highest urgency. The organ allocation policies of different countries vary greatly.

The model for end-stage liver disease (MELD) score was chosen as a guideline in the United States in 2002 (71,72). MELD is based on INR, creatinine, and bilirubin and predicts disease severity and waiting list mortality (73). The MELD score is also used for children aged 12 or older. For younger children, the pediatric model for end-stage liver disease (PELD) score is used (72,73), which adds growth retardation and age below or above 1 year as allocation criteria. In the United States, however, great variation exists between local allocation policies, exceptions to the set rules are common, only about half of pediatric deceased donor liver grafts are allocated according to PELD scores and the official organ allocation system also allows multiple listing (19,74,75) thus obscuring the objectivity and transparency of the process.

In Europe, organ allocation programs operate nationally in Spain, France, and Italy. Eurotransplant is the coalition of Austria, Belgium, Germany, Luxembourg, the Netherlands, Croatia, and Slovenia, and has used a MELD-based allocation system since 2006 (76). The UK and Ireland use their own algorithm (UKELD), a modified MELD system incorporating serum sodium level which is an independent predictor of survival after LT (77). Scandiatransplant prioritizes allocation of first available liver grafts to high-urgency candidates (defined as either acute liver failure patients at risk of death within 72 hours or LT recipients in need of re-LT within two weeks after LT) of Finland, Norway, Sweden,
Denmark, and Iceland, while elective cases are primarily allocated locally (78). All children in need of left lateral segments in the Nordic countries are listed through Scandiatransplant.

In Finland all LTs are centralized in Helsinki. Putting a patient on the waiting list and organ allocation are based on careful clinical judgment by a multidisciplinary team. Liver function is estimated with emphasis on the following parameters: serum prealbumin level, serum cholesterol level (in noncholestatic patients), factor V (due to independence of vitamin K), and thromboplastin time. Although LT is postponed as long as possible to allow the child to grow, the clinical condition of the patient is crucial. Disturbance of growth, refractory pruritus, increasing fatigue, recurrent cholangitis episodes, and portal hypertension leading to ascites, cytopenias (especially anemia), or variceal bleeding prompt evaluation for LT. In acute liver failure, the percentage of viable liver present, possible encephalopathy, and hepatorenal syndrome are taken into account. For all patients, histological findings and measured galactose half-life (79) provide additional support for decision making.

**Surgical procedure**

Correct size of the liver graft is very important. Grafts too large make wound closure more difficult, and closure by force will compromise the blood flow to the graft. Transient swelling of the graft after reperfusion can be tackled by using a temporary surgical mesh. Grafts too small (graft to recipient weight ratio roughly \( \leq 0.8\% \)), on the other hand, are associated with increased morbidity and impaired graft survival (80). The estimation of the actual liver volume at a certain age, however, is a complicated issue. Measurements based on imaging studies give up to 8% lower estimates of liver volume than measurements made at autopsy (81). In general, segments II and III are adequate for a recipient up to 20kg, segments II-IV for medium-sized children, and full adult grafts for teenagers (82).

The recipient operation in children is performed with a subcostal bilateral incision. Even if a whole liver is transplanted, the operation requires more creativity than in adults, due to frequent size mismatch of vessels and bile ducts. The liver is carefully mobilized to expose the vena cava. The hilum is dissected meticulously and vessels are divided as high as possible to assure uncomplicated anastomosing (82). After the simultaneous preparation of the donor liver on the back table, the recipient's portal vein is clamped, marking the beginning of the anhepatic phase, and the native liver is excised. The liver graft is placed orthotopically and the caval anastomoses are performed. The portal vein is anastomosed most commonly to the portal vein, but also the superior mesenteric or other convenient vein may be used in case of portal thrombosis. Arterial
anastomosis is performed usually anatomically, but sometimes using arterial conduits from the aorta, in which case the conduits are sutured prior to graft removal (83). The clamps may be removed to allow reperfusion either prior to the arterial anastomosis or after it. Finally, biliary reconstruction is performed either directly duct-to-duct or with a Roux-en-Y loop of jejunum in cases of biliary diseases and some reduced grafts (83).

The first and still widely used technical variant liver graft is the reduced graft of a deceased donor (4). Most often segments II and III or the whole left liver lobe are resected on the back table according to the size of the recipient, and the segments not transplanted are discarded (84). As the shape of a reduced graft differs from a whole liver, the graft should be positioned in the peritoneal cavity before performing anastomoses to avoid kinking of vessels. Some centers always use the piggy-back technique with reduced grafts preserving the recipient’s vena cava because of improved hemodynamic stability (85). In all technical variant grafts, hemostasis of the resection surfaces may sometimes prove challenging.

Split liver techniques were invented to expand the donor pool, as one deceased donor liver provides liver grafts for two recipients: one receiving the right lobe, the other the left lobe or left lateral segments (86,87). The surgical procedure utilizes the piggy-back technique since the donor vena cava has to be divided between the two grafts. The division of the hilum often leads to multiple biliary anastomoses and requirement of vascular conduits, which in part explain the complexity of the procedure and high reported incidence of surgical complications (83). Living donation is actually a direct extension of splitting, as a partial liver graft is taken from a living donor.
Figure 3. Description of a liver transplantation operation. In this case, the arterial anastomosis is directly to the aorta and the donor vena cava has been tapered to better match the size of the recipient vena cava. These details depict the individual choices required of a transplant surgeon.

The immune response

Already in the reperfusion phase of LT the liver develops a pro-inflammatory phenotype due to cytokines and complement components released by injured cells. Cytokines make the liver a target for cells of the innate immune response, e.g. macrophages and dendritic cells (28,88). After phagocytosis, macrophages and dendritic cells also serve as antigen presenting cells to the adaptive immune system. Presentation of antigens to CD4+ T lymphocytes may lead to direct killing of antigen-expressing cells, recruitment and activation of cytotoxic CD8+ T lymphocytes, or recruitment of B lymphocytes which produce antigen-specific antibodies (89). Recognition of donor antigens occurs via two pathways. In the
direct pathway, the recipient T lymphocytes recognize major histocompatibility complex molecules on antigen presenting cells transferred from the donor during LT. In the indirect pathway, recipient T lymphocytes recognize donor-derived intracellular peptides expressed on recipient antigen presenting cells (22,90). The direct pathway predominates in the early post-LT period but as graft-derived donor cells gradually die, the indirect pathway becomes pivotal. This sequence of antigen recognition pathways may also explain the decreasing rejection risk during follow-up (22)(Figure 4). The ability of T lymphocytes to respond to foreign histocompatibility antigens is termed allorecognition. Allorecognition is necessary in combat against infections but after transplantation this process ultimately leads to graft rejection in most LT recipients, unless the immune reaction is suppressed.

Figure 4. The time dependence of immunosuppressive medication.

**Immunosuppression**

The main principle in attaining successful immunosuppression is keeping a balance between effective prevention of rejection and overimmunosuppression leading to opportunistic infections, malignancies, and organ toxicities. To achieve this balance, Tredger et al recommend combining immunosuppressive agents with different actions for a synergistic effect: minimal dosage and subsequent minimized toxicity without compromised efficacy of immunosuppression (91). Avoiding the cumulative burden of immunosuppression and large doses of a single agent is especially important in children due to their long life expectancy. Some centers, however, currently aim at monotherapy to increase adherence to medication and overall minimization of immunosuppression (92,93). As reported by Americans, even in centers officially aiming at IS monotherapy, 37% of patients actually required at least two different medicines (94).
Table 1. Mechanisms of function and adverse effect profiles of the most commonly used immunosuppressants.

<table>
<thead>
<tr>
<th>Immunosuppressant</th>
<th>Principal immunological effects</th>
<th>Adverse effects</th>
</tr>
</thead>
</table>
| Corticosteroids   | Non-specific inhibition of IL production  
|                   | Reduction of T lymphocyte proliferation  
|                   | Suppression of Ab production          | Dose dependent: weight gain, diabetes, hypertension, dyslipidemia, osteoporosis, skin atrophy, impaired growth |
| Cyclosporine      | Calcineurin inhibition > inhibition of IL2 gene transcription > decreased T lymphocyte activation | Nephrotoxicity, neurotoxicity, hypertension, dyslipidemia, hirsutism, gingival hyperplasia |
| Tacrolimus        | Calcineurin inhibition > inhibition of IL2 gene transcription > decreased T lymphocyte activation | Nephrotoxicity, neurotoxicity, hypertension, diabetes, GI toxicity |
| Azathioprine      | Purine synthesis antagonization     | Myelosuppression, hepatotoxicity |
| Mycophenolate     | Suppression of de novo purine synthesis | Myelosuppression, GI toxicity |
| Sirolimus/Everolimus | Inhibition of mTOR pathway > signal blocking of cell surface receptors > suppression of T lymphocyte proliferation | Dyslipidemia, impaired wound healing, oral ulcerations, acne, myalgia/arthritis, proteinuria |
| ATG               | Polyclonal Ab binds to cell-surface antigens > lymphocyte depletion | Cytopenias, cytokine release syndrome or anaphylaxia |
| OKT3              | Monoclonal Ab binds to CD3 antigen > lymphocyte depletion | Possibility of anti-OKT3 antibody development |
| Basiliximab/ Daclizumab | IL-2 receptor antibodies > depletion of activated T lymphocytes | Specific; side effects rare |

IL= interleukin, Ab= antibody, mTOR= mammalian target of rapamycin, ATG= antithymocyte globulin, OKT3= muromonab-CD3

Corticosteroids are the oldest known immunosuppressants and are still widely used both in the prevention and treatment of rejection. They act through intracellular receptors present in almost every cell of the body and through several mechanisms, which explains the wide variety of adverse effects (22,95) (Table 1). Because of the multitude of potential adverse effects, many centers aim to withdraw steroids completely (10,29,93). While short-term results of steroid withdrawal and steroid-free regimes are acceptable, surprises may arise in later graft histology and function (13).

Calcineurin inhibitors (CNIs) form the basis of current immunosuppressive regimes. As their name implies, CNIs inhibit the phosphatase activity of calcineurin. This inhibition leads to blocked transcription of cytokines vital for T lymphocyte activation (22,95). Cyclosporine (CsA) revolutionized the field of transplantation and remains widely used still today, although the shift is toward increased tacrolimus use because of higher efficacy in prevention of rejections and the slightly different profile of adverse effects (10,96). The most
serious adverse effects of both CNIs are nephrotoxicity and neurotoxicity. CsA may also cause hirsutism and gingival hyperplasia and is more frequently associated with dyslipidemia than tacrolimus, while tacrolimus is more diabetogenic and may induce severe gastrointestinal symptoms (22,95,97,98). Children require higher doses of CNIs than adults due to faster metabolism (95). CNIs are metabolized by the enzymes of cytochrome P450 3A family, which potentially leads to significant drug interactions with several common antibiotics, non-steroidal anti-inflammatory drugs, and statins (95). To reduce organ toxicities, CNIs are often used in combination with steroids and either azathioprine or mycophenolate.

**Azathioprine** (AZA) and **mycophenolate** mofetil (MMF) are antimetabolites which interfere with purine nucleotide synthesis, consequently hinder DNA replication, and thus block the proliferation of lymphocytes (95,97). AZA is metabolized to 6-mercaptopurine and subsequently acts as a purine analogue unselectively antagonizing purine synthesis especially in rapidly proliferating cells, such as lymphocytes and hematopoietic stem cells of bone marrow (22,97). MMF is hydrolyzed into active mycophenolic acid which inhibits synthesis of guanosine monophosphate nucleotides thus suppressing the de novo purine synthesis vital for lymphocyte activation (95,99). Of the two antimetabolites, AZA is toxic to the liver, while MMF is more selective and effective in immunosuppression. MMF, however, costs significantly more and some patients may be intolerant of its side effects.

**Sirolimus** inhibits the mammalian target of rapamycin (mTOR) pathway resulting in suppression of cytokine dependent T lymphocyte proliferation (99). When introduced, sirolimus raised high hopes of a new era in transplant immunology. The antiproliferative effects are especially useful in patients treated for cancer or post-transplant lymphoproliferative disorder. Disappointingly, mTOR inhibitors have lead to impaired wound healing, possibly a higher rate of hepatic artery thrombosis, and cases of severe interstitial lung disease (99,100). Little data is also available on the usefulness of **everolimus** in children, which has so far been used as a rescue therapy when other immunosuppressants have failed (101).

Intravenously administered **antibodies** may be used in the immediate post-LT period in induction of immunosuppression or for the treatment of steroid-resistant rejection (95,102). Antibodies may be associated with increased cardiovascular disease risk (103), but reports on long-term results after antibody induction therapy in children are still rare.

At our center, patients have received basiliximab induction therapy since 1999 in two doses, the first intraoperatively and the second on the fourth postoperative day. Children weighing less than 30 kg receive 10 mg boluses, while those weighing more than 30 kg receive 20 mg. Besides induction, the principles of
medication remained constant during the 20-year period. CsA is started as a continuous infusion of 2 mg/kg/day and increased daily to 4-6 mg/kg/day aiming at a concentration of approximately 500 μg/L. At one week, CsA is switched to oral dosing aiming at a trough concentration 250-300 μg/L. At six months, the target concentration is reduced to 150 μg/L, and at one year to 100 μg/L. In long-term survivors, target concentrations may individually be reduced to 60-70 μg/L. Concentrations two hours after CsA administration are also measured to estimate absorption rates and to evaluate the individual validity of trough levels. Children under school-age receive CsA in three daily doses and school-aged children twice daily. Patients receive 1.4 mg/kg of AZA in two boluses intraoperatively, and continue on 2 mg/kg postoperatively. At two weeks, the AZA dose is reduced to 1 mg/kg/day. Intraoperatively 100mg of methylprednisolone (MP) is given in three doses, and the postoperative dosage is 1 mg/kg/day. At two weeks, MP is tapered to 0.25 mg/kg/day, and switched to alternate-day dosing at six months (0.1 mg/kg/day). If the patient is immunologically unstable or suffers from side effects, CsA may be switched to tacrolimus and AZA to MMF individually.
The anticlimax: Complications

Surgical

Liver dysfunction in the early postoperative period should lead to suspicion of surgical complications, which are the most significant factors predicting patient and graft loss at 6 months after LT. Due to immunosuppression, however, reaching the correct diagnosis is often complicated. Bleeding from the cut surface of a reduced-size graft is one important cause for reoperation.

To diagnose hepatic artery thrombosis (HAT), surveillance ultrasound evaluation of the hepatic artery in the post-operative period is crucial (104). Any suspicion of thrombosis should prompt urgent angiography, which remains the gold standard for diagnosis of arterial complications. If HAT is diagnosed before significant liver dysfunction occurs, operative treatment is still feasible (104,105). HAT followed by liver failure and biliary necrosis is an indication for emergency re-LT. The reported incidence of HAT at experienced centers is 7-8% (63,64,83,105,106). HAT is the single most important risk factor for patient and graft loss during the first six months after LT (107). Late HAT, however, is a completely separate entity since collaterals have had time to grow, especially in reduced grafts (108).

Early portal vein thrombosis may present with ascites, liver dysfunction, or recurrent varices, and requires surgical repair by direct revascularization or by means of a Rex shunt (109,110). If revascularization is unsuccessful, patients are listed for urgent re-LT. Reported incidence in children varies between 2-11% (64,83,111,112), and reduced grafts and venous reconstructions are associated with increased risk (83).

Also venous outflow obstruction due to stenosis or thrombosis of the vena cava or hepatic vein may jeopardize liver graft function, although these complications are much less frequent than HAT and portal vein thrombosis and mostly published as case reports.

Factors increasing the risk of biliary complications include reduced-size grafts and multiple bile ducts in the split liver graft (19,83), and reported rates are around 15-24% (83,111,112). Biliary leaks usually present within the first weeks after LT and predispose to sepsis. Leaks from the resection surface of a reduced graft are preferably treated by percutaneous drainage, while leaks from the biliary reconstruction may require a reoperation. Endoscopic retrograde cholangiopancreatography is often not feasible, especially in patients with Roux-en-Y biliary anastomosis. Biliary strictures, on the other hand, lead to cholestasis with cholangitis episodes or graft dysfunction several months or even years after LT, and percutaneous transhepatic cholangiography with or
without stent placement is the treatment of choice for the majority combined with operative repair if necessary (83).

**Bowel perforation** occurs in 6-10% of pediatric LT operations, and the risk is increased by previous liver surgery (20,113). Diagnosing perforation is difficult, as patients may experience surprisingly little pain and only half present with fever or leukocytosis due to the high doses of immunosuppressive agents required in the immediate post-LT period (113). Bowel perforation is associated with a 4-fold risk of patient and graft loss (107).

**Primary nonfunction**, as defined by the European Liver Registry, means listing for re-LT or death within a week of LT. It is characterized by progressive encephalopathy and multisystem organ failure, and reported incidence in pediatric LT recipients is roughly 6% (64,92,96,112). Urgent re-LT offers the only chance of survival. Reported re-LT rates for all causes range from 11-22% (63,92,112,114), but unfortunately re-LT is associated with decreased patient survival (107).

**Medical**

**Rejection**

Sudden rise in liver function tests (especially ALT) is often the first sign but histological findings are required for definite diagnosis of **acute rejection**. Acute rejection may occur already on day 3 after LT, and is most common during the first two months (115). Up to 70% of patients experience at least one episode of acute rejection within the first year after LT (92,112). Acute rejection may, however, occur even years after LT if changes are made in immunosuppression. Tacrolimus is more effective than CsA in the prevention of acute rejections, especially steroid-resistant rejections (116). Acute rejection is usually managed by increasing steroid doses, but steroid-resistant cases may require antibody treatment. Usually early rejection episodes have no significant effect on graft survival (22), and no difference in patient survival has been found between tacrolimus and CsA treatment groups (116,117). Chronic rejection is described later (see chapter Late graft histology).

**Infections**

Besides immunosuppression, factors predisposing children to infections after LT include patient age and pretransplant malnutrition, foreign material, dura-
tion of the surgical operation and its complications. As the requirement of efficient immunosuppression is the greatest in the first months after LT, so is also the risk of infection (118). Infections within 30 days of LT are usually associated with pre-existing conditions or surgical complications. In a large report from North America, 38% of pediatric LT recipients developed serious bacterial or fungal infections during the first postoperative month (119), while 14% had viral infections within 15 months of LT. The time period between one and six months of LT is typical for opportunistic infections, especially cytomegalovirus (CMV) either as a primary infection or reactivation (118). To decrease mortality and morbidity caused by infections, most centers currently use prophylactic antibiotic and antiviral agents during the described times of greatest infection risk. At our center, LT recipients use trimethoprim with sulfadiazine for one year post-LT for the prevention of *Pneumocystis jiroveci* and *Toxoplasma gondii* infections and CMV-seronegative LT recipients receive valganciclovir for 3-6 months as anti-CMV prophylaxis. In Finnish adult LT recipients, the overall rates of late infections (more than a year post-LT) have increased significantly since the 1990s (120). Possible explanations include older LT candidates, increase in comorbidities, or more efficient immunosuppression.

**Renal dysfunction**

A most unfortunate side effect of CNIs – and a major factor in invention of immunosuppressive regimes free of them – is renal dysfunction. Insensitive serum creatinine-based formulas overestimate glomerular filtration rate (GFR) by at least 16% (121,122), and renal dysfunction is present in 25-32% of pediatric LT recipients at 3-10 years with measured GFR (7,123). Kivelä et al measured GFR in 57 pediatric LT recipients at our center (86% of long-term survivors), and reported stage 3 chronic kidney disease (GFR 30–59 mL/min/1.73 m²) in 31% at ten years post-LT (122). The decline in GFR is similar in patients treated with CsA and tacrolimus (116). Renal function may further be challenged by CNI-induced hypertension and diabetes. In addition, renal function may have suffered already prior to LT as a consequence of end-stage liver disease (hepatorenal syndrome), or due to prolonged ischemia during the LT operation (124,125).

**Cardiovascular problems**

Although adult LT recipients initially represent a population with low risk for cardiovascular disease due to careful pretransplant assessment, the relative risk of cardiovascular events is 3-fold and that of cardiovascular deaths 2.6-fold when comparing adult LT recipients to an age-matched population (126). In children, no studies exist on the actual cardiovascular disease risk as it usu-
ally presents at an older age. Gathering evidence, however, confirms that risk factors for cardiovascular disease and the metabolic syndrome accumulate in pediatric LT recipients. Variation in methodology and definitions complicate making reliable estimates of the prevalence of different risk factors.

**Elevated blood pressure** was recently reported in 39-47% of European children at one year post-LT (116) and in 18-28% of American children 5-10 years after LT (127). Prevalence of hypertension tends to decrease over this time period as immunosuppression is tapered to long-term maintenance levels, although increasing rates of renal dysfunction have the contrary effect (128).

**Dyslipidemia** is common after pediatric LT, with hypertriglyceridemia reported in 26-30% and hypercholesterolemia in 6-26% of patients (94,129). Cholesterol synthesis is stimulated by obesity, insulin resistance, and nonalcoholic fatty liver disease (NAFLD) (130-132), and low cholesterol absorption during adolescence predicts later cardiovascular disease risk (25). Cholesterol metabolism in LT recipients is further influenced by immunosuppressive drugs. CsA reduces the transport of cholesterol into intestine and impairs the clearance of low-density lipoprotein. Tacrolimus shows similar effects but causes less dyslipidemia than CsA (116,128), and the effect of corticosteroids on serum lipid and glucose levels and insulin resistance is dose-dependent (133). Drug interactions are a concern in treating dyslipidemic LT recipients, as CsA is primarily metabolized by cytochrome P450 3A4, the same enzyme responsible for metabolism of statins. Low doses of statins, however, are considered safe after LT (128).

Fibroblast growth factor 21 (FGF21) regulates hepatic lipid metabolism and glucose production in rodents (134,135). The exact functions of FGF21 in humans remain unclear, but blood levels are increased in humans with obesity, insulin resistance, biochemical evidence of liver injury, non-alcoholic fatty liver disease (NAFLD), and chronic kidney diseases (136-138). FGF21 has been proposed to be a novel predictor of cardiovascular risk (138,139).

**New onset diabetes mellitus** is a serious complication of LT, which increases susceptibility to infectious and cardiovascular complications and has a clear impact on the quality and length of life (140). Reported rates of diabetes in pediatric LT recipients with long-term follow-up range from 9-13% (141,142). Hathout et al also reported a significant association of diabetes and tacrolimus-based immunosuppression (diabetes diagnosed in 14% of tacrolimus users vs. 6% of CsA users; p<0.0001) (141). A meta-analysis of randomized studies with comparable doses of CsA, tacrolimus, and steroids in adults reported the odds ratio to be 4.21 (2.26-7.85) in favor of CsA (140).

Intertwined with the above risk factors is obesity. According to age adjusted body mass index (BMI), 31.5% of American long-term survivors of pediatric LT are overweight and 11% obese (141,143), similar to the general American
population (144). Obesity may, however, carry greater risks for LT recipients than for healthy children. We found no studies reporting prevalence of obesity in European centers.

**Growth and bone health**

End-stage liver disease leads to growth failure in the majority of children due to growth hormone alterations, malabsorption of fat and fat-soluble vitamins, and abnormal metabolism of proteins, cholesterol, and carbohydrates (145,146). Weight in the pretransplant period is an unreliable figure, since effects of ascites and peripheral edema due to chronic liver disease and portal hypertension are difficult to estimate. After LT, most children exhibit catch-up growth, especially during the second year (147,148). They are still often left shorter than their peers, partly due to immunosuppression including steroids (149), but also due to renal dysfunction and the severity of growth impairment prior to LT (148). Evans et al reported that patients who waited more than a year for LT were smaller at LT than patients who waited less than a year and, while most improved growth velocity after LT, they still had negative z-scores (150). This highlights the importance of including growth failure as a criterion for LT allocation.

Valta et al found severely reduced bone mineral density and asymptomatic vertebral fractures in 15% and 18%, respectively, of pediatric LT recipients at median 7 years after LT (151). Older age at LT increased the risk of both signs of impaired bone health.

Published studies from different centers give somewhat varying figures on the frequency of growth retardation. In London, 87% of long-term survivors of pediatric LT reached normal height, while 13% were stunted (z-score below -2) (152). In Chicago, 34% of LT recipients were below the 10th percentile and 24% below the 5th percentile at two years after LT, and increased risk of growth impairment was associated with metabolic diseases as original cause for LT and prednisone use for longer than 18 months (148). Low-dose steroids are still used by 92% of LT recipients 10 years after LT in France, but despite this, the patients’ mean height z-score reaches -0.47 ± 1.35 (112). Adequate catch-up growth is achievable on an alternate-day steroid regimen (29), as most of the adverse effects of steroids are dose-dependent. Also, recombinant human growth hormone is effective and safe in the treatment of the most severely stunted LT recipients (153).
Malignancies

Posttransplant lymphoproliferative disorder (PTLD) is a hematologic malignancy which in most patients is due to Epstein-Barr virus (EBV) infection induced changes in B lymphocyte DNA and subsequent clonal proliferation. EBV primary infection after LT is associated with greater risk of developing PTLD than latent infection. As most children are EBV negative at the time of LT, children have a greater risk of PTLD than adults. The risk of PTLD is further increased by the intensity of immunosuppression (154). Ng et al reported the incidence of PTLD to be 5.3% at 10 years after LT (94) but Goss et al reported a 10% incidence in children receiving tacrolimus (111). Tacrolimus has recently been associated with a greater PTLD risk also in adult kidney recipients (154). Frequent EBV monitoring and reduced immunosuppression depending on the EBV viral load may decrease incidence of PTLD (155). LT recipients with acute rejection episodes have a lower overall cancer risk than those without rejections (9). One possible explanation may be that in case the immune system is only partially suppressed by medication, it remains more vigilant also in detecting malignant cells. In adults, the overall cancer risk is 2.6-fold in comparison to the general population. Risks are especially elevated for non-melanoma skin cancer and basal cell carcinoma of the skin, but these malignancies occur at a significantly older age than PTLD (9).

The finale: Outcomes

Survival and causes of death

Both patient and graft survival figures demonstrate a significant learning curve from the beginning of the transplant era until today. In the largest transplant centers of North America, current 5-year patient and graft survival rates are 87% and 79%, respectively (107). In France and Belgium, reported 10-year patient survival rates reach 79-82% and 70-71% (92,112). Patient age at LT, LT era, and number of LTs performed are associated with patient survival (111), while increased donor age, technical variant grafts, LT urgency, and earlier LT era predict decreased graft survival (63,64,112). Most deaths occur within months after LT, but another drop in patient and graft survival rates occurs during adolescence.

Most common causes of death world-wide include infections (accounting for roughly 30% of deaths), followed by rejection, cardiopulmonary causes, central nervous system complications, malignancies, and surgical complications (63,92,119,156).
Late graft histology

Core needle biopsies are used to diagnose various conditions early after LT. In this early phase, harvesting-related injury, hepatic venous outflow obstruction, and acute rejection are the most common diagnoses (157). A fairly strong international consensus prevails about the importance of core needle biopsies in the immediate post-transplant period.

Table 2. Histological changes in recent studies on long-term survivors of pediatric liver transplantation.

<table>
<thead>
<tr>
<th>Author</th>
<th>LT recipients</th>
<th>Years after LT</th>
<th>Normal</th>
<th>Fibrosis</th>
<th>Cirrhosis</th>
<th>Inflamm.</th>
<th>AIH</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fouquet 2005 (ref 112)</td>
<td>67</td>
<td>10</td>
<td>27 %</td>
<td>22 %</td>
<td>6 %</td>
<td>NA</td>
<td>NA</td>
<td>42 %</td>
</tr>
<tr>
<td>Evans 2006 * (ref 13)</td>
<td>64</td>
<td>10</td>
<td>2 %</td>
<td>64 %</td>
<td>15 %</td>
<td>87 %</td>
<td>6 %</td>
<td>NA</td>
</tr>
<tr>
<td>Ekong 2008 (ref 163)</td>
<td>63</td>
<td>≥3</td>
<td>NA</td>
<td>97 %</td>
<td>0 %</td>
<td>70 %</td>
<td>22 %</td>
<td>10 %</td>
</tr>
<tr>
<td>Scheenstra 2009 (ref 14)</td>
<td>55</td>
<td>10</td>
<td>NA</td>
<td>69 %</td>
<td>29 %</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

LT = liver transplantation, Inflamm. = inflammation, AIH = autoimmune hepatitis, CR = chronic rejection
* only data on biopsies acquired at 10 years after LT presented
NA = data not reported

In long-term symptom-free survivors, even adults, protocol liver biopsies remain a subject of heated debate. Some centers believe in the usefulness of protocol liver biopsies since sensitivity and specificity of normal liver function tests for predicting histological changes are poor (158,159), and immunosuppression can be adjusted individually according to histology (160). Because of potential complications causing morbidity and mortality (estimated at 0.6% and 0.02%, respectively) and the costs of the procedure, others recommend abandoning protocol biopsies (161,162).

Liver biopsies of pediatric LT recipients after at least three years of follow-up have demonstrated surprisingly high rates of histological changes (13,14,112,163) (Table 2). In several reports (13,14,163) the most frequent finding was fibrosis. During cell injury, stellate cells become activated and differentiate into myofibroblastic cells that produce excessive amounts of collagen (28). The underlying causes for this subtly creeping change remain largely unexplained, although known predisposing factors include ischemia (64), biliary complications (164), and inflammation (13,115,165).

Inflammation per se was graded in studies by Evans and Ekong et al (13,163), and was also strikingly common. Definitions and grading of inflammation may vary slightly, but the concordance of these two studies increases credibility.

REVIEW OF THE LITERATURE
While Ekong et al found no clinical correlates for the histological changes, Evans et al noted the incidence of fibrosis to be especially high in biopsies with definite chronic hepatitis and a tendency of these changes to increase in severity over time.

Chronic hepatitis in combination with liver dysfunction, presence of autoantibodies, and raised serum immunoglobulin levels leads to the diagnosis of de novo AIH in patients transplanted for other causes. De novo AIH is more common in children than adults as up to 22% of pediatric LT recipients may receive this diagnosis and its features may arise if immunosuppression is too light (115,163). Both of these findings suggest that, instead of a different entity, de novo AIH may in fact be part of a spectrum of immune-mediated damage (115).

Traditionally the most feared diagnosis revealed by biopsies was chronic rejection. Principal findings of chronic rejection are loss of bile ducts and cholestasis, which sometimes make differentiation from biliary complications difficult (166). Fortunately chronic rejection has become less frequent due to better immunosuppressive regimes, and currently less than 2% of grafts fail as a consequence of chronic rejection (167).

Steatosis is a well-recognized risk in adults after LT (168,169). Scoring systems have been developed for the advancement of NAFLD (170), which may be used also in the LT population. Despite the prevalence of obesity in pediatric LT recipients (143) and the effects of fat accumulation on cholesterol metabolism (132), steatosis is unmentioned in reports of histological findings of pediatric LT recipients.

**Psychological and cognitive aspects**

Malnutrition and growth failure prior to LT are risk factors for delayed cognitive development. This developmental delay and hepatic encephalopathy may affect cognitive capacity and learning after LT (171-173). In France, 31% of 10-yr survivors showed delayed school performance (112). In North America, 23% of a highly selected patient population had repeated a grade or been held back at least one year in school, while attention deficit-hyperactivity disorder was diagnosed in 9% and learning disability in 26% (94). Headaches are a common complaint in pediatric LT recipients (152,174), but their frequency has not been plausibly compared to a healthy population. Taylor et al also reported that 22% of teenagers with mean follow-up of 7.5 years have received psychiatric or psychological support after LT (152).
Quality of life

Increased survival rates and the growing population of LT survivors emphasize the importance of long-term health-related quality of life (HRQoL). HRQoL includes subjective evaluations of multidimensional positive and negative aspects of life and their effect on perceived health. Validated questionnaires can be used in HRQoL measurement either from the patient’s perspective or from the perspective of a close proxy, such as a parent. Proxy respondents’ answers may vary greatly from the child’s own perceptions especially in emotional and social dimensions of HRQoL (175,176). Even if the child’s improved HRQoL is the official aim of LT, it is the parents’ perception which influences the extent of health care utilization of a growing child. The older the child becomes, the more his/her own impression of HRQoL should weigh.

Several recent studies have suggested that HRQoL in pediatric LT recipients is suboptimal (15,16,177,178) (Table 3). Alonso reported HRQoL to be comparable to children undergoing cancer treatment (16), while Duffy stated HRQoL in 20-year survivors to be better than in patients with chronic liver disease, chronic heart failure, or diabetes (178). Surprisingly, emotional functioning is the dimension most frequently reported to be similar to healthy peers (177-179). Controls in all the recent studies, however, are from previously published population norms or previous publications on HRQoL in chronically ill children. In no study has a control group been matched specifically for the LT recipients either according to age or gender. Despite the gathering evidence on HRQoL after pediatric LT, the exact reasons for HRQoL impairment remain unknown. Younger age at LT, longer follow-up after LT, and high self-esteem have been associated with better HRQoL, whereas hospitalizations during the last year led to inferior evaluations (15,152). Except for decreased renal function which showed no correlation (15), and biliary complications which impacted family function (177), the effect of long-term complications on HRQoL has never been evaluated.
Adherence and transition to adult services

If everything has gone more or less according to plan, a time comes when the pediatric LT recipient is no longer a child. Since immunosuppressive medication and follow-up visits are still necessary, the care for the young patient will be transferred to adult health care. This, however, is easier said than done.

International articles report alarming rates of poor adherence to medication, graft loss, and even patient deaths during adolescence (178,180,181). Unfortunately often the official age limit of pediatric hospitals, also ours, is 16 years. In adult care, patients are infrequently guarded and protected as in pediatric centers, and patients are expected to show initiative and take responsibility. Systematic thinking and true understanding of cause and effect are late events in mental development, occurring roughly at age 20-25 years, and are at their weakest during mid youth (182-184). In chronically ill adolescents, both physical and psychological development are often delayed (185), and thus the chronological age is a poor measure of maturity. Youth is characterized by impulsivity, feelings of invulnerability, and risk-taking. Risk-taking behaviour is even more common among chronically ill adolescents, who smoke more, begin sexual relationships at a younger age, and act more violently than healthy teens (186-188). Adolescents with chronic illness, however, may suffer more serious consequences of risk-taking than their healthy peers. As possible ex-
planations for risk-taking, Suris et al (187) suggested an increased desire to be accepted by their peers and insufficient knowledge about the possible effects on the youth's condition.

Just as youth is a process spanning many years, so should also be the transition of care. The transition period and the first years after it are especially critical to the survival of transplant patients and their organs (181,189). Experts have written recommendations on the organization of the transition process taking into consideration both the perspectives of the patient and their family as well as the pediatric and adult teams (190-192). So far, no prospective studies on transition and associated factors are available.
AIMS OF THE STUDY

Despite being a valid therapeutic option, pediatric LT is still a rare procedure. Obtaining sufficient patient populations for evaluating outcomes can thus only be achieved through unselected data collection covering long follow-up periods. We therefore conducted this population-based study which combines data from the first two decades of the LT era in our country with cross-sectional analyses to obtain a comprehensive view of the patients’ current situation.

Our specific aims were:

1. To describe patient survival and causes of death after pediatric LT, especially in a subset of patients transplanted for liver malignancies

2. To investigate the histology of liver transplants in long-term survivors of LT and to find clinical correlates for histological findings

3. To evaluate the long-term metabolic effects of pediatric LT by assessing cholesterol metabolism as depicted by non-cholesterol sterols and FGF21

4. To measure HRQoL and sexual health in long-term survivors of pediatric LT in comparison to healthy controls and to find explanations for possible reduction in HRQoL

5. To discuss ways in which patient outcomes could be further improved.
**PATIENTS, CONTROLS AND METHODS**

**Patients and controls**

This thesis includes all 99 patients who underwent LT at less than 18 years of age in Finland between 1987 and 2007. All Finnish patients are transplanted at the Helsinki University Central Hospital to which they return for regular follow-up visits. Patients living in other hospital districts also undergo follow-up at their local centers. Insurance type is insignificant as health care in Finland is based on national social security ensuring equal access to medical care for all citizens.

Retrospective data were collected on all LT recipients, including the deceased patients. Study I assessed these retrospective data in a subgroup of patients transplanted for hepatic malignancies.

Cross-sectional studies II-IV included all surviving LT recipients, who participated in data collection for each respective study (Figure 5). Cross-sectional data were analyzed together with retrospective data. Non-participants formed a heterogeneous group, and only three LT recipients didn’t participate in any sub studies. Non-participants were mainly males (10/12 in study II, 10/17 in study III, and 8/9 in study IV), but no significant differences in comparison to participants were found in diagnoses leading to LT, complication rates, or length of follow-up.

Figure 5. Number of participants in each respective study.
For the cholesterol metabolism study (III), we recruited 93 age- and sex-matched controls from out-patient surgery clinics of the Children’s Hospital and Peijas Hospital. Eligible patients had no suspicion of gastrointestinal disease, metabolic diseases affecting lipid metabolism (e.g. diabetes, celiac disease or thyroid diseases) nor were they using lipid lowering medication.

The Finnish Population Register Centre randomly picked six individuals matched for age, sex, and place of residence for each of the 66 survivors to serve as controls in the quality of life study (IV). Of these 396 persons, 141 (36%) replied. Respondents were considered healthy, as they reported no other illnesses besides mild allergies.

Methods

Retrospective data collection (I-IV)

Retrospective data were collected from patient records and the Finnish LT registry and analyzed until death, end of follow-up for each respective study, or the completion of the whole study program on September 1, 2012. Patient records of 94 patients (95%) were available for data collection for this study.

Data included diagnosis of liver disease and other possible illnesses, LT date, all surgical and endoscopic procedures, both surgical and medical complications and their treatment, immunosuppression and changes made, other medications, and hospitalizations. Annual follow-up visits included height and weight measurements, blood pressure, GFR measured using $^{51}$Cr-EDTA clearance or creatinine clearance, and laboratory tests. Laboratory test results collected for this study include those presented in Table 4.

Table 4. Laboratory test results collected from annual follow-up visits.

<table>
<thead>
<tr>
<th>Test Category</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete blood count</td>
<td>Hemoglobin, hematocrit, red cell count, white cell count, platelet count</td>
</tr>
<tr>
<td>Liver function tests</td>
<td>Total and conjugated bilirubin, ALT, AST, GGT, prealbumin</td>
</tr>
<tr>
<td>Blood coagulation markers</td>
<td>Activated partial thromboplastin time, factor V, INR</td>
</tr>
<tr>
<td>Serum lipid levels</td>
<td>Total cholesterol, HDL-C, LDL-C, triglycerides</td>
</tr>
<tr>
<td>Serum glucose homeostasis</td>
<td>Fasting glucose and insulin, oral glucose tolerance test</td>
</tr>
<tr>
<td>Inflammation marker</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>Tumor marker*</td>
<td>α-fetoprotein</td>
</tr>
</tbody>
</table>

ALT= alanine transaminase, AST= aspartate transaminase, GGT= gamma-glutamyltransferase, INR= international normalized ratio, HDL-C= high density lipoprotein cholesterol, LDL-C= low-density lipoprotein cholesterol

* = only measured in patients who received LT for liver malignancies
Insulin resistance was evaluated using HOMA (homeostasis model assessment) according to the formula: fasting glucose (mU/mL) x fasting insulin (mmol/L) /22.5 (193). The cutoff of 2.5 for the diagnosis of insulin resistance was used (194).

BMI was calculated (kg/m²), and for LT recipients under 18 years, adjusted for age (BMI-for-age) according to the new Finnish growth charts (195) to enable evaluation of obesity in the total patient population.

**Histopathology (II)**

Ultrasound guided liver biopsies were taken by experienced radiologists (II). Biopsies were then fixed in formalin, embedded in paraffin, and cut for staining. Conventional stains at our center include hematoxylin-eosin, periodic acid-Schiff (PAS), diastase-PAS, trichrome, reticuline, iron, and copper. For the purpose of this study, immunostaining for cytokeratin 7 (CK7) and complement component 4d (C4d) were also performed. All biopsies were reviewed by the primary researcher (S.K.) and two experienced liver pathologists to reach a consensus.

Inflammation, fibrosis, CK7-immunopositivity, and C4d-immunopositivity were graded semi-quantitatively. Grading of inflammation was performed according to Banff criteria (196) and according to histological areas (portal, lobular, interface, endothelial, and bile duct). The distribution of inflammatory cell types was estimated from hematoxylin-eosin stains whenever inflammation was encountered. Fibrosis was graded primarily from trichrome and reticuline stains according to the Metavir system on a scale of 0 to 4 (197). Liver fat accumulation was divided into micro- and macrovesicular forms and estimated as percentage of hepatocytes affected. For statistical analyses, also a dichotomized variable of steatosis was used. CK7 stains were analyzed for both immunopositivity of periportal hepatocytes and presence of bile duct proliferation. For periportal CK7-immunopositivity, a scale of 0 to 3 (0= none, 1= mild, 2= moderate, 3= marked) was used. C4d-immunopositivity was reported as present or absent.

**Non-cholesterol sterols and FGF21 (III)**

Blood was drawn after an overnight fast. Levels of total cholesterol (TC), triglycerides, and HDL-C were measured by standard enzymatic methods of our institution. LDL-C levels were calculated (198). Density gradient ultracentrifugation was used to separate LDL and HDL fractions from serum. The non-cholesterol sterols (cholestanol, cholestenol, desmosterol, lathosterol, campesterol, sitosterol, and avenasterol) were determined from serum and the LDL and HDL
fractions by gas-liquid chromatography (199). Each run was manually approved by the primary researcher (S.K.) and repeated if necessary. Besides measured noncholesterol sterol concentrations, also ratios to cholesterol (10^2 x μg/mg of cholesterol) measured in the same gas-liquid chromatography run were calculated to eliminate the effect of variance in cholesterol levels.

For FGF21 analysis, serum samples were centrifuged after blood collection and stored at -80°C until analysis. Serum FGF21 concentrations were measured by ELISA kit (BioVendor GmbH, Heidelberg, Germany) following the manufacturer’s instructions. For statistical analyses, the values below the sensitivity of the assay were assigned the lowest assay value of 7 pg/mL. In addition to actual measured levels, also log-transformed FGF21 values were used to account for some exceptionally high levels.

**Quality of life measures (IV)**

**PedsQL**

PedsQL 4.0 is a generic, standardized questionnaire, which is internationally widely used and has been validated in both Finnish and Swedish (200,201). PedsQL 4.0 offers proxy forms for the youngest age group (children <7) and both patient and proxy forms for school-aged children (176). HRQoL is measured in four domains: physical, emotional, social, and school functioning, each by a scale of 0-100. Higher scores reflect better HRQoL.

**SF-36**

SF-36 is a generic, standardized, internationally well-known HRQoL measure, which has also been validated in both Finnish and Swedish. SF-36 is composed of eight domains, which are scored from 0-100: vitality, physical functioning, bodily pain, general health perceptions, mental health, and physical, emotional, and social role functioning (202).

**Derogatis interview for sexual functioning self report (DISF-SR)**

DISF-SR was used to quantify the adult patients’ quality of sexual functioning (203). The DISF-SR includes 25 questions arranged into five domains: sexual
cognition/fantasy, sexual arousal, sexual behaviour, orgasm, and sexual drive/relationship. Higher scores of the orgasm domain indicate better functioning, whereas higher scores of other domains imply a higher activity level.

**AUDIT**

Participating adult patients and controls also filled the Alcohol Use Disorders Identification Test (AUDIT) in their native language to allow for estimates of alcohol consumption, possible alcohol abuse and its effects on HRQoL. A total of ≥ 8 points of maximum 40 is associated with increased health risks (204).

**Statistical analyses**

Study I is descriptive in nature due to the small amount of patients. Statistical analyses for studies II-IV were performed using SPSS Statistics software versions 17.0-19.0 (IBM, Somers, NY, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). Frequencies, percentages, means (with standard deviations, SD), and in case of skewed distribution medians (with interquartile ranges, IQ), were used as descriptive statistics. For differences between patient subgroups, the Mann-Whitney U, chi-square, and Fischer’s exact test were used. Correlations were calculated using the Spearman rho two-tailed test. In studies III and IV, differences in continuous variables between patients and controls were tested with the Wilcoxon signed-rank test, and the McNemar test was used for categorical variables. The false discovery rate with an expected rate for false positives set at 5% was used as a step-up procedure to adjust for multiple comparisons. A final p-value of ≤0.05 was considered statistically significant.

**Ethical considerations**

This study was conducted following the guidelines of the Declaration of Helsinki. The study protocol was approved by the Ethics committee for Pediatrics, Adolescent medicine, and Psychiatry of the Helsinki and Uusimaa Hospital District. All participating patients and controls, and in case of minors, also their parents, signed an informed consent form. Liver biopsies were obtained under local or general anesthesia depending on the patient’s age. The patients’ blood samples were drawn together with routine samples, and the controls’ samples were drawn under general anesthesia of their respective procedures to avoid unnecessary pain.
RESULTS

Survival and causes of death

At the end of follow-up (September 1, 2012), 66 patients (67%) are alive (Figure 6). Characteristics of all 99 patients are described in table 5. MARS treatment was given to nine patients prior to LT, of whom five had ALF, one iron poisoning, and three an acute-on-chronic situation.

Table 5. Characteristics of all 99 liver transplant recipients.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>All patients (n=99)</th>
<th>Alive at follow-up (n=66)</th>
<th>Deceased (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (m/f)</td>
<td>48/51</td>
<td>34/32</td>
<td>14/19</td>
</tr>
<tr>
<td>Age at LT, years</td>
<td>3 (0.4-17)</td>
<td>4 (0.4-17)</td>
<td>3 (0.4-17)</td>
</tr>
<tr>
<td>Follow-up, years</td>
<td>9 (0-25)</td>
<td>14 (5-25)</td>
<td>1 (0-16)</td>
</tr>
<tr>
<td>Biliary atresia</td>
<td>34</td>
<td>21 (32%)</td>
<td>13 (40%)</td>
</tr>
<tr>
<td>Acute liver failure</td>
<td>5</td>
<td>1 (2%)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>12</td>
<td>8 (12%)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Metabolic</td>
<td>17</td>
<td>14 (21%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>ARPKD</td>
<td>5</td>
<td>4 (6%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Tumor</td>
<td>14</td>
<td>8 (12%)</td>
<td>6 (18%)</td>
</tr>
<tr>
<td>Other</td>
<td>12</td>
<td>10 (15%)</td>
<td>2 (6%)</td>
</tr>
</tbody>
</table>

LT= liver transplantation, ARPKD= autosomal recessive polycystic kidney disease.
Age at LT and length of follow-up presented as median (range).

1 Diagnosis group "Metabolic disease" includes: tyrosinemia (9), Wilson's disease (2), hyperoxaluria (2), α1-antitrypsin deficiency (1), ornithine transcarbamylase deficiency (1), and familial hypercholesterolemia (1).

2 Diagnosis group "Tumor" includes: hepatoblastoma (6), hepatocellular carcinoma (6), giant adenoma (1), and hemangioendothelioma (1).

3 Diagnosis group "Other" includes: Budd-Chiari syndrome (2), atypical hemolytic uremic syndrome (2), familial congenital cirrhosis (2), iron poisoning (2), primary sclerosing cholangitis (1), Alagille syndrome (1), unknown cirrhosis (1), mitochondrial recessive ataxia syndrome (1), and Farber syndrome (1).

Patient survival rates were 84% (95% CI 77-91%) at one year, 78% (70-86%) at three years, and 71% (60-79%) at ten years (Figure 6), while graft survival rates were 76% (95% CI 68-83%) at one year, 69% (60-77) at three years, and 62% (53-72%) at ten years (Figure 7).
Figure 6. Patient survival after pediatric liver transplantation in Finland between 1987 and 2007.

Figure 7. Graft survival for first liver grafts and retransplants after pediatric liver transplantation in Finland between 1987 and 2007.
Reduced grafts were associated with poorer graft survival than full grafts (50% vs. 73%, *p* = 0.026; Table 6). Cold ischemia times showed a significant difference between survivors and the deceased (444 ± 136 minutes vs. 510 ± 161 minutes, respectively; *p* = 0.024). The mean anhepatic time was 65 ± 30 minutes and the median donor-to-recipient weight ratio was 2.5 (range, 0.6-16), and neither variable was associated with patient or graft survival. Graft weights were unavailable for analysis. Sixty-nine patients had a Roux-en-Y biliary anastomosis, but data on anastomosis type was missing in five cases. In only four patients, the surgical wound was temporarily closed using surgical mesh followed by delayed closure after settling of edema. Diagnosis of liver disease and retransplantations showed no significant differences between survivors and the deceased.

Table 6. Data on all liver grafts and those functioning at the end of follow-up.

<table>
<thead>
<tr>
<th></th>
<th>All grafts (n=115)</th>
<th>Functioning at follow-up (n=66)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft type (full/reduced)</td>
<td>37/78</td>
<td>27/39</td>
<td>0.026</td>
</tr>
<tr>
<td>Segments II-III (%)</td>
<td>45 (39)</td>
<td>22 (33)</td>
<td></td>
</tr>
<tr>
<td>Segments II-IV (%)</td>
<td>22 (19)</td>
<td>11 (17)</td>
<td></td>
</tr>
<tr>
<td>Segments V-VIII (%)</td>
<td>11 (10)</td>
<td>6 (9)</td>
<td></td>
</tr>
<tr>
<td>Retransplants (%)</td>
<td>16 (14)</td>
<td>6 (9)</td>
<td>0.105</td>
</tr>
<tr>
<td>Cold ischemia time (min)</td>
<td>480 ± 147</td>
<td>444 ± 137</td>
<td>0.024</td>
</tr>
<tr>
<td>Anhepatic time (min)</td>
<td>65 ± 30</td>
<td>61 ± 26</td>
<td>0.328</td>
</tr>
<tr>
<td>Donor age, years</td>
<td>23 (1.0-62)</td>
<td>21 (1.0-62)</td>
<td>0.313</td>
</tr>
<tr>
<td>DRWR</td>
<td>2.5 (0.6-16)</td>
<td>2.3 (0.6-16)</td>
<td>0.834</td>
</tr>
</tbody>
</table>

DRWR = donor to recipient weight ratio
Donor age and DRWR presented as median (range).

Causes of death for the 33 deceased patients are summarized in Table 7. In some cases, several factors contributed to the sequence of events leading to death. Table 7 presents only the primary causes of these vicious circles. Young children (age <3) mostly died during the first year after LT (Figure 8) and suffered most from surgical complications and infections. Surgical complications accounted for 18% of deaths. All patients who died as a consequence of non-adherence were in the transition years from pediatric to adult care.
Table 7. Causes of death and follow-up times for 33 deceased liver transplant recipients.

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>n (%)</th>
<th>LT to death (years)</th>
<th>Age at death (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary nonfunction</td>
<td>3 (9)</td>
<td>0.0-0.1</td>
<td>1-15</td>
</tr>
<tr>
<td>Hepatic artery thrombosis</td>
<td>3 (9)</td>
<td>0.0-0.1</td>
<td>0.5-6</td>
</tr>
<tr>
<td>Vena cava thrombosis</td>
<td>1 (3)</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>GI hemorrhage</td>
<td>2 (6)</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>Infection</td>
<td>7 (21)</td>
<td>0.2-3.1</td>
<td>1-9</td>
</tr>
<tr>
<td>Cerebral edema</td>
<td>4 (12)</td>
<td>0.0-0.1</td>
<td>1-15</td>
</tr>
<tr>
<td>Chronic rejection</td>
<td>4 (12)</td>
<td>2-16</td>
<td>2-29</td>
</tr>
<tr>
<td>Tumor recurrence</td>
<td>3 (9)</td>
<td>2-6</td>
<td>10-19</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>1 (3)</td>
<td>0.5</td>
<td>11</td>
</tr>
<tr>
<td>Cardiac myopathy</td>
<td>1 (3)</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Nonadherence</td>
<td>4 (12)</td>
<td>5-16</td>
<td>17-24</td>
</tr>
</tbody>
</table>

Figure 8. Deaths after pediatric liver transplantation according to patient age and length of follow-up.

RESULTS
Survival after LT for liver malignancies

All 12 patients in study I who were transplanted for HB and HCC received neo-adjuvant chemotherapy, most often according to the protocols of SIOPEL trials 1 and 2 (205,206) (Table 8, see also study I, Tables 1 and 2). In seven patients, serum AFP levels decreased more than 99% from their peak values indicating effectiveness of chemotherapy. Median time from diagnosis to LT was seven (range 2-133) months. None of the patients had radiological evidence of extrahepatic disease at LT. After LT, no routine chemotherapy was used. Vascular tumor invasion was found in the explant livers of one patient of each tumor group. Both of these patients had an incomplete AFP response to chemotherapy (decrease of less than 99%) and more than 80% viable tumor at LT, and both died of disease recurrence. One HCC patient died of a fungal infection affecting the central nervous system, and one HB patient died of cardiac myopathy which developed as a complication of chemotherapy. Overall survival for both tumor types combined was 67% at 10 years after LT, which is equal to the overall survival of all pediatric LT recipients in Finland. Of the patients with LT as their primary surgical procedure, six of eight survived, compared with two of four who underwent both resection and LT.

Table 8. Patient and tumor characteristics and outcome.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at LT</th>
<th>Histology</th>
<th>PRETEXT</th>
<th>Resection</th>
<th>AFP response</th>
<th>Viable tumor</th>
<th>Recurrence</th>
<th>Outcome</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>HB, fetal</td>
<td>3M</td>
<td>Lung</td>
<td>&gt;99 %</td>
<td>30 %</td>
<td>No</td>
<td>Died</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>HB, fetal</td>
<td>3P</td>
<td>No</td>
<td>&gt;99 %</td>
<td>50 %</td>
<td>No</td>
<td>Alive</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>HB, mixed</td>
<td>3V,E</td>
<td>Liver x2</td>
<td>98 %</td>
<td>80 %</td>
<td>Yes</td>
<td>Died</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>HB, fetal</td>
<td>4</td>
<td>No</td>
<td>NA</td>
<td>50 %</td>
<td>No</td>
<td>Alive</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>HB, fetal</td>
<td>3</td>
<td>No</td>
<td>&gt;99 %</td>
<td>50 %</td>
<td>No</td>
<td>Alive</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>HB, mixed</td>
<td>4V,M</td>
<td>No</td>
<td>&gt;99 %</td>
<td>50 %</td>
<td>Yes</td>
<td>Alive</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>HCC</td>
<td>4</td>
<td>No</td>
<td>&gt;99 %</td>
<td>50 %</td>
<td>No</td>
<td>Alive</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>HCC</td>
<td>3P</td>
<td>Liver x2</td>
<td>NA</td>
<td>50 %</td>
<td>No</td>
<td>Alive</td>
<td>14</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>HCC</td>
<td>3V,E</td>
<td>No</td>
<td>59 %</td>
<td>95 %</td>
<td>Yes</td>
<td>Died</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>HCC</td>
<td>4V,P</td>
<td>No</td>
<td>98 %</td>
<td>30 %</td>
<td>No</td>
<td>Alive</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>HCC</td>
<td>3P</td>
<td>Liver</td>
<td>&gt;99 %</td>
<td>70 %</td>
<td>No</td>
<td>Died</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>HCC</td>
<td>3P</td>
<td>Liver</td>
<td>&gt;99 %</td>
<td>5 %</td>
<td>No</td>
<td>Alive</td>
<td>11</td>
</tr>
</tbody>
</table>

LT= liver transplantation, PRETEXT= pretreatment extent of tumor (M= metastases, P= portal vein, V= vena cava, E= extrahepatic spread), AFP response= α-fetoprotein response to chemotherapy, HB= hepatoblastoma, HCC= hepatocellular carcinoma, NA = not available.

Age at LT and follow-up presented in years.
Complications

Complication rates are summarized in Table 9. HAT was the most common vascular complication (10 cases), and it led to death in three and re-LT in four patients, while two patients underwent successful operative reconstruction and one thrombolysis. Biliary strictures occurred in ten patients, and were managed by percutaneous transhepatic cholangiography in five patients and by reconstructive surgery in five patients. The four gastrointestinal occlusions were all due to adhesions and required operative release but no bowel resections.

Table 9. Complication rates among 94 pediatric LT recipients.

<table>
<thead>
<tr>
<th>Complication</th>
<th>n (%)</th>
<th>LT to complication (years)</th>
<th>Age at complication (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vascular</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAT</td>
<td>10 (11)</td>
<td>0.06 (0.00-0.5)</td>
<td>3 (0.5-16)</td>
</tr>
<tr>
<td>Portal vein thrombosis</td>
<td>3 (3)</td>
<td>5 (1.3-23)</td>
<td>7 (6-27)</td>
</tr>
<tr>
<td>Portal hypertension</td>
<td>4 (4)</td>
<td>0.15 (0.02-1.5)</td>
<td>7 (0.7-11)</td>
</tr>
<tr>
<td>Vena cava thrombosis</td>
<td>2 (2)</td>
<td>0.02-0.1</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Biliary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaks</td>
<td>3 (3)</td>
<td>0.08 (0.07-0.1)</td>
<td>8 (3-14)</td>
</tr>
<tr>
<td>Strictures</td>
<td>10 (11)</td>
<td>1.0 (0.03-12)</td>
<td>16 (2-26)</td>
</tr>
<tr>
<td><strong>Other surgical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postoperative bleed</td>
<td>6 (6)</td>
<td>0.01 (0.00-0.04)</td>
<td>5 (0.6-14)</td>
</tr>
<tr>
<td>Gut perforation</td>
<td>3 (3)</td>
<td>0.02 (0.00-0.07)</td>
<td>8 (1.8-12)</td>
</tr>
<tr>
<td>GI occlusion</td>
<td>4 (4)</td>
<td>1.0 (0.1-12)</td>
<td>1.9 (1.4-13)</td>
</tr>
<tr>
<td>GI bleed</td>
<td>3 (3)</td>
<td>9 (1.3-12)</td>
<td>20 (13-26)</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>45 (48)</td>
<td>0.05 (0.01-11)</td>
<td>3 (0.4-28)</td>
</tr>
<tr>
<td><strong>Infections</strong></td>
<td>45 (48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV disease*</td>
<td>20 (21)</td>
<td>0.1 (0.02-2)</td>
<td>3 (1.0-18)</td>
</tr>
<tr>
<td>EBV disease*</td>
<td>8 (9)</td>
<td>0.3 (0.1-2)</td>
<td>3 (1.3-20)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>8 (9)</td>
<td>1.8 (0.02-19)</td>
<td>7 (0.6-36)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>7 (7)</td>
<td>0.3 (0.03-16)</td>
<td>2 (0.5-29)</td>
</tr>
<tr>
<td>Abcess</td>
<td>6 (6)</td>
<td>0.06 (0.03-1.9)</td>
<td>5 (2-14)</td>
</tr>
<tr>
<td><strong>Bone health</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>12 (13)</td>
<td>1.3 (0.3-17)</td>
<td>15 (3-18)</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>2 (2)</td>
<td>4.0-5</td>
<td>10.0-16</td>
</tr>
<tr>
<td>Avascular necrosis</td>
<td>2 (2)</td>
<td>2.0-13</td>
<td>16-17</td>
</tr>
<tr>
<td><strong>Neurological</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td>3 (3)</td>
<td>0.02 (0.01-0.1)</td>
<td>17 (5-18)</td>
</tr>
<tr>
<td>Learning disabilities</td>
<td>4 (4)</td>
<td>6 (3-18)</td>
<td>14 (11-18)</td>
</tr>
<tr>
<td>PTLD</td>
<td>4 (4)</td>
<td>1.8 (0.2-13)</td>
<td>9.1 (3.3-14)</td>
</tr>
<tr>
<td>Depression/anxiety</td>
<td>8 (9)</td>
<td>14 (4-18)</td>
<td>15 (11-20)</td>
</tr>
<tr>
<td>Growth hormones</td>
<td>11 (12)</td>
<td>4 (1.3-15)</td>
<td>10 (6-16)</td>
</tr>
</tbody>
</table>

HAT = hepatic artery thrombosis, GI = gastrointestinal, CMV = cytomegalovirus, EBV = Ebstein-Barr virus, PTLD = post-transplant lymphoproliferative disorder
* = CMV and EBV disease include viremia and elevated liver enzymes with or without symptoms, not mere seroconversion.
Follow-up time from LT to complication and age presented as median (range) in years.
Table includes all individual complications. Most patients had several, and eight (9%) had none.
Approximately half of patients (48%) had acute rejections. Most (31 patients, 71%) only had one acute rejection episode, nine patients had two episodes, and four patients 3-4 episodes each. All late acute rejections (≥ 3 years after LT) were diagnosed in patients aged 19-28. Only two cases of steroid-resistant rejections occurred, and they were treated with ATG.

Half of patients (48%) suffered from different types of infections, with CMV disease causing most of the burden. Of the eight patients with EBV disease post-LT, four developed PTLD, but all are in remission 3-22 years after diagnosis. Only 20 patients had experienced both rejections and infections.

Osteoporosis was diagnosed from lumbar spine bone mineral density measurements in 12 patients. Only two of these patients, however, had received LT for acute liver failure, while most had suffered for years of chronic liver diseases also affecting bone metabolism. All three cases of epilepsy were diagnosed within a month of LT, and were possibly caused by encephalopathy. Eight patients (9%) had received psychiatric treatment for depression or anxiety, similar to the general population of Finnish adolescents.

At LT, the mean height z-score was -1.1 ± 2.0, and 35 patients (36%) had height z-scores below -2.0. Eleven patients received growth hormones after LT. For 68 patients with more than three years of follow-up after LT, the mean height z-score was -0.8 ± 1.2, and only eight of these patients (12%) had height z-scores below -2.0 (p = 0.0005 for difference in percentage of children with short stature at and after LT).

The rates of different cardiovascular risk factors of 66 living patients are summarized in Table 10. Risk factors tended to accumulate especially in those individuals who were overweight or obese (Figure 9).

**Table 10. Cardiovascular risk factors of 66 living LT recipients.**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overweight (BMI 25.0-29.9 kg/m²)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Obese (BMI &gt; 30.0 kg/m²)</td>
<td>9 (14)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>11 (17)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>11 (17)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 9. Number of cardiovascular risk factors according to body-mass-index. Risk factors include BMI > 25 kg/m², hypertension, dyslipidemia, and insulin resistance.

Current immunosuppression

After LT, all patients were started on triple immunosuppression including CsA, AZA, and MP. Side effects or immunological instability, however, often lead to treatment changes. At the end of follow-up, only eleven LT recipients (17% of survivors) continue with the first combination of immunosuppressants. Details of current immunosuppression are summarized in Tables 11 and 12. Only four LT recipients are on monotherapy, two on CsA and two on tacrolimus.

Table 11. Immunosuppressants used by the 66 liver transplant recipients at the end of follow-up.

<table>
<thead>
<tr>
<th>Immunosuppressant</th>
<th>Users, n (%)</th>
<th>Dose (mg/day)</th>
<th>Dose (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNI</td>
<td>64 (97)</td>
<td>125 (100-165)</td>
<td>2.9 (2.0-3.6)</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>41 (62)</td>
<td>2500 (2000-5250)</td>
<td>80 (50-120)</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>23 (35)</td>
<td>1080 (1000-1260)</td>
<td>19 (11-22)</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>54 (82)</td>
<td>1.0 (1.0-1.5)</td>
<td>0.03 (0.02-0.06)</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>37 (56)</td>
<td>37.5 (25.0-65.6)</td>
<td>1.1 (1.0-1.3)</td>
</tr>
<tr>
<td>Mycophenolate</td>
<td>17 (26)</td>
<td>1080 (1000-1260)</td>
<td>19 (11-22)</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>36 (55)</td>
<td>1.0 (1.0-1.5)</td>
<td>0.03 (0.02-0.06)</td>
</tr>
</tbody>
</table>
Table 12. Most common combinations of immunosuppressant at the end of follow-up.

<table>
<thead>
<tr>
<th>Medication combination</th>
<th>Users, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsA + AZA + MP</td>
<td>11 (17)</td>
</tr>
<tr>
<td>CsA + AZA</td>
<td>11 (17)</td>
</tr>
<tr>
<td>Tac + AZA + MP</td>
<td>9 (14)</td>
</tr>
<tr>
<td>CsA + MMF</td>
<td>7 (11)</td>
</tr>
<tr>
<td>CsA + MMF + MP</td>
<td>6 (9)</td>
</tr>
</tbody>
</table>

CsA = cyclosporine, AZA = azathioprine, MP = methylprednisolone, Tac = tacrolimus, MMF = mycophenolate.

Liver graft function and histological changes

All surviving patients demonstrated stable graft function as judged by their liver function tests (Table 13). Alterations in just one laboratory value were disregarded.

Table 13. Laboratory data of 66 living LT recipients.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Current reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>31 (18-68)</td>
<td>0-50</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>20 (9-72)</td>
<td>10-40 10-75</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>26 (8-128)</td>
<td>0-50</td>
</tr>
<tr>
<td>Total serum bilirubin (μmol/L)</td>
<td>10 (4-34)</td>
<td>4-20</td>
</tr>
<tr>
<td>Serum prealbumin (mg/L)</td>
<td>202 (102-519)</td>
<td>130-375 200-390</td>
</tr>
<tr>
<td>Factor V (%)</td>
<td>105 % (89-126%)</td>
<td>79-128%</td>
</tr>
<tr>
<td>Serum prothrombin ratio (%)</td>
<td>86 % (74-106%)</td>
<td>70-130%</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.1 (4.2-6.1)</td>
<td>3.8-7 4-6</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.9 (0.6-8.7)</td>
<td>&lt;2.5</td>
</tr>
</tbody>
</table>

HOMA = homeostasis model assessment
Data presented as median (range). For ALT, prealbumin, and plasma glucose, reference values are provided for children under 16 and adults, respectively.

Of 66 surviving LT recipients, 54 (82%) underwent liver biopsy after a median follow-up of 11 (range 3-22) years (study II). The biopsies were considered representative because of their sufficient length (18 mm, range 10-31) and number of portal tracts (14, range 10-44). In 18 patients (33%), biopsies showed near-normal histology with no inflammation, fibrosis, or steatosis. Portal inflammation was present in 14 samples (26%), of which four also demonstrated interface inflammation and two additional lobular inflammation. The inflammatory infiltrates predominantly included lymphocytes (70-100%), followed by plasma cells (5-20%), neutrophils (5-10%), macrophages (5-10%), and eosinophils (5%). Fibrosis was found in 21 biopsies (39%). Fibrosis was mainly mild, as in 16 biopsies changes were Metavir stage 1, three stage 2, and two
stage 3. Macrovesicular steatosis involving 5-30% of hepatocytes was seen in five biopsies, but an additional 18 biopsies demonstrated microvesicular steatosis (altogether 43% of patients). None of the biopsies had visible cholestasis or periportal copper deposits, but 12 biopsies (22%) showed periportal CK7-immunopositivity. Ductular reaction was found in 23 (43%) of biopsies.

**Correlations of histological findings**

Prealbumin levels were higher in patients with near-normal histology than those with histological changes (253 ± 89 mg/l vs. 190 ± 43 mg/l; p=0.007). No significant differences were, however, found between these patient groups in patient or donor age, cold ischemia time, length of follow-up, or incidence of vascular or biliary complications.

Portal inflammation was less frequently found in biopsies of patients still using low-dose MP (14% vs. 47%; p= 0.008). Portal inflammation correlated positively with fibrosis stages (r= 0.350, p= 0.010), but not with liver biochemistry. Anti-nuclear antibodies were present in 14 patients, with titer > 80 in 11 (20%). No correlation was, however, found between anti-nuclear antibodies and portal inflammation. No other autoantibodies were found in any patients, and as none of the patients with autoantibodies had elevated transaminase levels or interface hepatitis, no cases of de novo AIH were diagnosed. Portal inflammation showed no correlations with C4d-immunopositivity, original diagnosis of hepatitis, CMV status, or length of follow-up.

Fibrosis stages correlated negatively with serum prealbumin levels (r = -0.364, p = 0.007) and positively with periportal CK7-immunopositivity (r = 0.546, p < 0.001). The four patients with interface hepatitis also had fibrosis, and all four patients with Metavir stage 2 fibrosis had mild portal inflammatory changes. The two patients with Metavir stage 3 had no signs of inflammation. No correlations were found between fibrosis and surgical complications or length of follow-up.

Both microvesicular and macrovesicular steatosis correlated with the patients’ BMI (r = 0.517, p < 0.001 and r = 0.429, p = 0.001, respectively). Macrovesicular steatosis was also associated with HOMA (r = 0.407, p = 0.021). Higher BMI was found in patients using antihypertensive medication than those without (26.1 ± 6.6 kg/m² vs. 20.5 ± 4.7 kg/m², p=0.004). Hypertensive patients more often had steatosis than normotensive patients (33% vs. 2%, p = 0.001). Steatosis showed no correlation with MP use, inflammation, fibrosis, or donor age.

Periportal CK-7 immunopositivity correlated positively with serum GGT levels (r = 0.495, p < 0.001) and conjugated bilirubin levels (r = 0.402, p = 0.003), but
with no other liver function tests or portal inflammation. Both GGT and conjugated bilirubin levels were higher in patients with CK7-immunopositivity (59 vs. 15 U/L, \( p < 0.001 \) and 5 vs. 2 \( \mu \text{mol/L}, \ p = 0.004 \); respectively). CK7-immunopositivity was present in five of six patients treated for bile strictures. Four patients with bile duct strictures also had ductal reaction. Still half of the biopsies with periportal CK7-immunopositivity were from patients with no clinically diagnosed biliary complications.

The age of the allograft (donor age + follow-up time) was positively associated with serum GGT levels (\( r = 0.472, p < 0.001 \)), conjugated bilirubin levels (\( r = 0.420, p = 0.002 \)), and periportal CK7-immunopositivity (\( r = 0.305, p = 0.027 \)). Allograft age showed no correlation with inflammation, fibrosis, or steatosis. Graft type (whole/reduced), type of biliary anastomosis (end-to-end / Roux-en-Y), cold ischemia time, or the number of rejection episodes showed no significant correlations with histological findings.

Biopsy findings led to changes in treatment in 10 patients (19%). In four patients each, immunosuppression was either increased or decreased. One patient demonstrated marked venous congestion and was scheduled to undergo magnetic resonance imaging to find out the cause. One patient was scheduled for more frequent control visits because of a pre-cirrhotic condition. On the other hand, only one subcapsular hematoma was encountered as the sole complication of biopsies, and it resolved after conservative treatment.

**Cholesterol metabolism and FGF**

**Serum lipids and noncholesterol sterols**

Despite similar mean serum concentrations of TC, HDL-C, LDL-C, and triglycerides in LT recipient and control groups, cholesterol synthesis and absorption marker sterols showed significant differences. Overall, the LT recipients’ cholesterol metabolism profile showed increased cholesterol synthesis and decreased absorption in comparison to controls, as depicted by the ratios of synthesis marker lathosterol to absorption markers cholestanol (1.06 ± 1.14 \( \mu \text{g/100 mL} \) vs. 0.61 ± 0.33 \( \mu \text{g/100 mL}, p < 0.001 \) and sitosterol (1.23 ± 0.91 \( \mu \text{g/100 mL} \) vs. 0.68 ± 0.54 \( \mu \text{g/100 mL}, p < 0.001 \); see also study III, Tables 3 and 4). Synthesis marker lathosterol was significantly higher in LT recipients than in controls both as measured serum concentration and as noncholesterol sterol to cholesterol ratio (188 ± 95 \( \mu \text{g/100 mL} \) vs. 148 ± 76 \( \mu \text{g/100 mL}, p = 0.010 \); 129 ± 55 vs. 96 ± 41 \( \times 10^2 \mu \text{g/mg of cholesterol}, p = 0.001 \), respectively, Figure 10). Absorption marker campesterol, on the other hand, was significantly lower in LT
recipients than in controls (serum concentration 326 ± 121 μg/100 mL vs. 481 ± 185 μg/100 mL, p < 0.001). Markers of cholesterol absorption showed strong positive correlations (e.g. serum campesterol and sitosterol: LT recipients r = 0.877, p < 0.001; controls, r = 0.811, p < 0.001, Figure 11), while synthesis and absorption markers were inversely related as a sign of maintained homeostasis of cholesterol metabolism (see also study III, Figure 1). No changes were found in the transportation of noncholesterol sterols, as approximately half of noncholesterol sterols were transported by LDL and 30% by HDL in both LT recipients and controls.

Use of low-dose MP correlated positively with cholesterol absorption and negatively with cholesterol synthesis (MP and serum campesterol: r = 0.386, p = 0.006; MP and serum lathosterol: r = -0.379, p = 0.007). Use of AZA showed a similar association (AZA and lathosterol/cholestanol ratio: r = -0.388, p = 0.006). Age and BMI were associated with increased cholesterol synthesis (age and lathosterol/sitosterol ratio: r = 0.470, p = 0.001; BMI and lathosterol/sitosterol ratio: r = 0.587, p < 0.001). BMI also correlated with higher LDL-C (r = 0.292, p = 0.042) and serum triglycerides (r = 0.380, p = 0.007), as expected. Both fasting and the 2-hour plasma glucose levels of the glucose tolerance test correlated positively with cholesterol synthesis (fasting plasma glucose and serum desmosterol: r = 0.452, p = 0.004; 2-hour plasma glucose and serum desmosterol: r = 0.411, p = 0.022). No correlations were found between gender, original liver disease, donor age, graft type, the levels of liver function tests, or length of follow-up and any of the noncholesterol sterols.

Liver biopsies were available for 47/49 (96%) LT recipients participating in the cholesterol metabolism study. Fibrosis was present in 17 (36%) of the biopsies, and these LT recipients had lower serum TC (3.37 ± 0.66 mmol/L vs. 3.95 ± 0.94 mmol/L, p = 0.035), LDL-C (1.63 ± 0.55 mmol/L vs. 2.17 ± 0.75 mmol/L, p = 0.015), and serum prealbumin levels (173 ± 54 mg/L vs. 218 ± 57 mg/L, p = 0.022) than recipients without liver fibrosis. Microvesicular steatosis was found in 18 (38%) of the biopsies, and LT recipients with steatosis had higher BMI than those without (25.0 ± 7.0 kg/m² vs. 19.2 ± 3.4 kg/m², p = 0.003). Mild portal inflammation (Banff grade 1) with lymphocytes as the predominant cell type was present in 9 (19%) of the biopsies, but showed no correlations with serum lipids. Noncholesterol sterols showed no correlations with any histological changes.
Figure 10. Mean serum lathosterol (marker of cholesterol synthesis) and campesterol (marker of cholesterol absorption) ratios to cholesterol in patients and controls. Both differences between groups are significant at \( p<0.001 \) level.

Figure 11. Correlation of serum sitosterol and campesterol concentrations in patients and controls. Patients = filled circles \((n=49, r=0.877, p < 0.001)\), controls = white circles \((n=93, r=0.811, p < 0.001)\).
**FGF21**

Both measured and log-transformed serum FGF21 levels were significantly higher in LT recipients than controls (250 pg/mL, IQ 110-560 pg/mL vs. 77 pg/mL, IQ 38-180 pg/mL, p < 0.001; 2.4 pg/mL, IQ 2.1-2.8 pg/mL vs. 1.9 pg/mL, IQ 1.6-2.2 pg/mL, p < 0.001; respectively).

In the controls, serum FGF21 levels correlated positively with lathosterol/cholestanol and lathosterol/sitosterol ratios (r = 0.268, p = 0.011; r = 0.258, p = 0.015, respectively). In LT recipients, however, no correlation was found between serum FGF21 levels and cholesterol metabolism. Instead, FGF21 correlated positively with cystatin C levels (r = 0.306, p = 0.034) and negatively with the glomerular filtration rate (r = -0.366, p = 0.043). The difference in log-transformed FGF21 levels between LT recipients and controls remained significant even after excluding the patient quartile (12 patients) with the highest cystatin C levels (2.3 pg/mL, IQ 2.0-2.7 pg/mL vs. 1.9 pg/mL, IQ 1.6-2.2 pg/mL, respectively; p < 0.001).

**Quality of life and sexual health**

At time of HRQoL assessment, median 10 years (range, 2-23) had elapsed since LT.

**HRQoL in children**

HRQoL was similar for the nine LT recipients and 35 age- and gender-matched controls under age 7 years (see study IV, Table 4). LT recipients aged 7-17 (n = 19) only estimated their school functioning to be lower than did controls (domain score 69 ± 16 vs. 81 ± 11, p = 0.004), and their parents strongly agreed (65 ± 21 vs. 81 ± 12, p < 0.001; Figure 12). A third (31%) of these patients was one grade behind in school compared to 5% of controls (p = 0.009). Parents of LT recipients evaluated also physical and social role functioning to be impaired (p = 0.032 and p = 0.008, respectively). Nine patients (47%) in this age group scored within the normal range of controls (mean ± 2 SD) on all HRQoL domains.

Parents of school-aged children estimated physical, social, and school dimensions to be significantly lower for children who were either overweight or obese (p < 0.05 for all), whereas the overweight/obese children themselves reported similar HRQoL as normal weight LT recipients. HRQoL in this age group showed a significant increasing trend with longer follow-up time in the child’s physical (r = 0.503, p = 0.028) and social (r = 0.593, p = 0.009) PedsQL scores.
HRQoL and sexual health in adults

LT recipients (n = 29) who had reached adulthood (age > 18 years) reported physical functioning and general health to be impaired (Figure 13, see also study IV, Table 4). Although LT recipient and control scores for the emotional well-being domain were similar, significant differences were found in individual questions. LT recipients reported getting sick easier than other people (50% answered true or mostly true compared with 13% of controls; p < 0.001) and also expected their health to deteriorate (28% answered true or mostly true compared with 2% of controls; p < 0.001). Still most LT recipients (76%) considered themselves as healthy or almost as healthy as other people, and 64% found their current health status excellent. Seventeen (59%) LT recipients in this age group scored within the normal range of controls (± 2 SD) on all domains.

The LT recipients tended to have lower education levels than controls, as 18% of LT recipients had finished school after 9th grade (compared with 5% of controls) and 7% had studied at university level (compared with 15% of controls), but these differences failed to reach statistical significance.
According to DISF-SR scores, sexual health was similar between LT recipients and controls (see study IV, Table 7). LT recipients had a tendency to be less satisfied with the strength of their orgasms (44% vs. 82%, p = 0.050). Both the orgasm domain and sexual drive/relationship domain correlated positively with general health scores (r = 0.327, p = 0.002; r = 0.254, p = 0.020, respectively). Gender and BMI had no significant effect on DISF-SR scores. Sexually transmitted diseases were rare among LT recipients and controls with self-reported rates of 8% and 7%, respectively. Four LT recipients (of 24 who answered the question, 17%) had hoped for pregnancy and three have become parents.

The mean AUDIT sums were similar for LT recipients and controls (6.1 ± 6.0 and 6.6 ± 4.3, respectively). Smokers scored higher on the AUDIT than did nonsmokers (9.0 ± 6.6 vs. 5.8 ± 4.7, respectively; p = 0.043). Smokers also had higher scores in the sexual drive/relationship domain than did nonsmokers (13 ± 5 vs. 16 ± 5, p = 0.043). AUDIT scores correlated positively with both orgasm (r = 0.313, p = 0.036) and sexual drive domains (r = 0.224, p = 0.039). Smoking and AUDIT scores showed no significant correlations with HRQoL measurements.

Figure 13. Mean SF-36 domain scores for adult patients (n = 29) and controls (n = 62). Differences between groups were significant for domains of physical functioning (p = 0.036) and general health (p < 0.001), marked with asterisks.
HRQoL and complications

Among the LT recipients who participated in the HRQoL study, the mean number of complications per person was 2.3. Six LT recipients (11%) had a clean record of no surgical or medical complications, while five (9%) had five different complications (the maximum in this study). The total number of complications correlated negatively with PedsQL emotional functioning scores ($r = -0.505, p = 0.032$), and the following domains of SF36: emotional wellbeing ($r = -0.424, p = 0.024$), emotional limitations ($r = -0.418, p = 0.027$), vitality ($r = -0.486, p = 0.009$), and general health ($r = -0.405, p = 0.032$). Of individual complications, biliary complications were associated with lower PedsQL scores on the children’s evaluation of physical and social functioning ($p = 0.029$ and $p = 0.046$, respectively) and the parent scores of social role functioning ($p = 0.018$), and osteoporosis was associated with lower parent scores of emotional functioning ($p = 0.024$). For those in the adult group, reoperations were associated with lower SF-36 social functioning scores ($p = 0.020$) and infections were associated with inferior evaluations of general health ($p = 0.034$). Vascular complications, rejection episodes, renal insufficiency, and treatment with growth hormones showed no significant relations with HRQoL measurements.

LT recipients were using on average five (median; range 1-9) different medications including vitamin supplementation. The number of medicines used correlated negatively with the school-aged children's own perceptions of physical functioning ($r = -0.511, p = 0.025$), but their parents' scores were unaffected. No correlation was found between the number of medicines and HRQoL in adults.
DISCUSSION

In this thesis, I have reported the long-term survival and complication rates after pediatric LT in Finland and demonstrated them to be equal to those of larger centers around the world. The results of our cross-sectional studies showed continued low-dose steroid use to be associated with both milder histological changes and a healthier cholesterol metabolism profile than immunosuppression without steroids. Half of LT recipients reported their quality of life to be similar to that of age- and sex-matched controls’, while deteriorated quality of life was associated with complications.

Methodology

The strengths of the general study design include the population-based comprehensive nature of data collection spanning the first twenty years of pediatric LT in Finland, the length of follow-up (median 11 years for those alive), and high participation rates (74-86% in studies II-IV). Validity of studies III and IV is further increased by age- and gender-matched control populations specifically chosen for this purpose. Our greatest limitation is the retrospective data, which allowed for no definitive conclusions on the associations found, and in some cases limited the data available for analyses (e.g. the cumulative doses of MP). Our whole patient population was fairly small, allowing only limited subgroup analyses and no multivariate analyses. In cases where expected results remained nonsignificant, the small population size may have lead to type II statistical errors. For statistical analyses, methods were chosen according to the distribution of each variable. In studies III and IV, all p-values were also adjusted for multiple comparisons to diminish the chances of type I errors.

Graft histology

To assess graft histology, core needle biopsies were examined thoroughly by two experts in the field and the primary researcher (S.K.) until consensus (study II), which increased the validity of histological grading. Core needle biopsies are the gold standard for assessing liver histology. The histological assessment was limited by the facts that biopsies represented a cross-sectional sample and that none of the patients were completely steroid-free. Patients without MP, however, had been off them for median 4 (range 1-18) years, and thus many effects of steroids have had time to dissolve. In addition to conventional stains including hematoxylin-eosin, PAS, and trichrome, the biopsies were also stained for C4d and CK7. Mature hepatocytes are CK7-negative but under persistent
cholestasis they may dedifferentiate to CK7-positive intermediate hepatobiliary cells (207). As concealed cholestasis due to biliary complications may lead to fibrosis (164), we hypothesized that CK7-immunopositivity might also be associated with fibrosis. Our study brought new knowledge into the field of LT histology, as none of the previous studies on histological findings after pediatric LT have used CK7-stains or reported the frequency of liver steatosis.

**Cholesterol metabolism and FGF21**

We used measurements of serum cholesterol precursor sterols and plant sterols to assess cholesterol metabolism in LT recipients and controls matched for age and gender (study III). For estimating cholesterol synthesis, also deuterium incorporation and mass isotopomer distribution analysis techniques are available (27) but they were inconvenient for our study since different methods are necessary to measure cholesterol absorption. Exact measurement of cholesterol absorption, on the other hand, requires 72-hour collection of feces and would thus have significantly affected participation rates. We used several instead of one serum marker for both cholesterol synthesis and absorption as recommended (26). Information on the patients’ and controls’ diet was not collected, although it may influence the serum noncholesterol sterol levels (208). Other confounding factors were, however, taken into account, including the circadian rhythm of cholesterol metabolism, metabolic diseases, and drugs affecting cholesterol metabolism (27,131). In addition to the noncholesterol sterols, we measured serum FGF21 levels for the first time in LT patients, as FGF21 has been proposed to be a predictor of cardiovascular risk and serum levels have been elevated in patients with liver injury and NAFLD (139,209).

**Quality of life**

LT recipients and healthy controls matched for age, gender, and place of residence filled validated HRQoL questionnaires (study IV). HRQoL was measured with two different generic tools to allow for the wide age range of both LT recipients and their respective controls. We specifically wanted to compare HRQoL to that of healthy controls since, despite major surgery and continued immunosuppression, LT aims at being a key to normal life. Our study includes the self-reports of all LT recipients ≥ 7 years, because the difference between self- and proxy-reported HRQoL is a common finding. All responses were collected by an investigator uninvolved in clinical care (S.K.) to assure truthful responses. Our study is made unique by several factors, including the combination of both surgical and medical complications with HRQoL assessment and by the fact that almost half of the participating LT recipients had already transitioned from
pediatric to adult care. In many HRQoL reports from other centers, patient material is highly selective, with children not maintaining regular follow-up with their LT centers and those not fluent in English or Spanish excluded, and participation rates may be as low as 42% (177,178). Instead, we included all LT recipients from a 20-year period and were able to reach an excellent participation rate (86%) and highly representative sample of LT recipients. We also directly enquired about sexual functioning as well as alcohol and tobacco from those survivors of pediatric LT, who had already gone through transition. These studies in young adults are still very rare in the LT population.

Survival and complications

Our patient survival rates of 84% (95% CI 77-91%) at one year, 78% (70-86%) at three years, and 71% (60-79%) at ten years and graft survival rates of 76% (95% CI 68-83%) at one year, 69% (60-77) at three years, and 62% (53-72%) at ten years are similar to larger centers, although this comparison is complicated by the fact that 95% confidence intervals are rarely reported (92,107,112).

Our surgical complication rates are also similar to those reported from other centers, as summarized in Table 14. The most significant difference to reported complication rates is our complete lack of post-LT diabetes and our very low rate of dyslipidemia. Neither difference can be explained by genetic differences between study populations, as both conditions are common in the general population of Finns.
Table 14. Comparison of complication rates after pediatric liver transplantation in Finland and in the literature.

<table>
<thead>
<tr>
<th>Complication</th>
<th>Frequency in Finland (%)</th>
<th>Frequency in literature (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic artery thrombosis</td>
<td>11</td>
<td>7-8</td>
</tr>
<tr>
<td>Portal vein thrombosis</td>
<td>3</td>
<td>2-11</td>
</tr>
<tr>
<td>Bile complications</td>
<td>12</td>
<td>15-24</td>
</tr>
<tr>
<td>Gut perforation</td>
<td>3</td>
<td>6-10</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>45</td>
<td>50-70</td>
</tr>
<tr>
<td>Infections*</td>
<td>45</td>
<td>38</td>
</tr>
<tr>
<td>PTLD</td>
<td>4</td>
<td>5-10</td>
</tr>
<tr>
<td>Hypertension</td>
<td>17</td>
<td>18-28</td>
</tr>
<tr>
<td>Dyslipidemia**</td>
<td>8</td>
<td>26-30</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>9-13</td>
</tr>
<tr>
<td>Obesity</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Height &lt; -2 SD</td>
<td>11</td>
<td>13-24</td>
</tr>
</tbody>
</table>

PTLD = post-transplant lymphoproliferative disorder, SD= standard deviations.
Complication rates in Finland are reported for all patients (n = 99), except rates of hypertension, dyslipidemia, diabetes, obesity, and growth retardation are reported for those alive at end of follow-up (n = 66).

* = The frequency of infections in Finland includes all infections, while the frequency from literature only includes severe infections of the first post-operative month.
** = The frequency of dyslipidemia in Finland includes any change in serum cholesterol (total, HDL, or LDL) or triglyceride levels, while the frequency from literature only includes triglyceridemia.

Literature from references 20, 63-64, 82, 92-94, 104-105, 110-112, 114, 117-119, 125-127, 140-144, 147-150.

The North American Studies of Pediatric Liver Transplantation (SPLIT) research group has defined the ideal 10-year survivor of pediatric LT as one fulfilling the following criteria: no retransplantation, no chronic rejection, no PTLD, no renal dysfunction (calculated GFR ≥ 90 mL/min/1.73 m²), growth > -2 SD for the healthy population, no diabetes, no antihypertensive agents, no antiseizure medication, no prednisone, and normal serum levels of ALT, bilirubin, albumin, and GGT (94). In a highly selective population of 167 LT recipients with more than 10 years follow-up, only 32% of patients fulfilled these criteria according to patients’ self-reported complication rates (94). Leaving out steroid use which is part of our treatment protocol, 42 of our 66 survivors have been followed for more than 10 years after LT, and 19 (45%) of them fit the composite profile of the ideal survivor after meticulous review of patient records.

**Survival after liver malignancies**

Among the 12 patients transplanted for HB and HCC at our center, survival was similar to the overall population of LT recipients. We found no correlation...
between PRETEXT staging and survival, which may be due to difficulties in distinguishing between stage III (compression) and stage IV (ingrowth) (58).

AFP levels and responses to chemotherapy expectedly predicted survival (210). In retrospect, one of the two patients who died of tumor recurrence was already resistant to chemotherapy prior to LT and thus this tumor was too advanced to be cured even by LT. Some centers also recommend routine post-LT chemotherapy to improve survival (210,211). Our results suggest, however, that excellent survival rates are achievable without additional exposure to adverse effects of routine chemotherapy. Chemotherapy post-LT may still be valuable for patients with vascular tumor invasion or a large proportion of viable tumor in the explanted liver.

Currently the guidelines for surgical treatment of HB recommend LT as primary treatment of PRETEXT stage IV cases and emphasize avoidance of heroic liver resections (54,212). Also in our study, survival was better among the patients who underwent primary LT than those who received a “rescue” transplant. The same may also be true for HCC, as liver regeneration after partial hepatectomy may induce both growth and malignant transformation of occult tumors and subsequently tumor recurrence (213). All cases of HCC at our center were found because of tumor-induced symptoms, and tumors were subsequently at an advanced state at diagnosis. None of our patients with HCC fulfilled the Milan criteria for LT (solitary lesion ≤ 5 cm or maximum of three tumors with none > 3 cm), and only one of our patients fit even the more liberal University of California, San Francisco (single tumor < 6.5 cm or maximum of three tumors with none > 4.5 cm and cumulative tumor size < 8 cm) or 5/5 criteria (maximum of five tumors with the largest being 5 cm). Still only one of five patients transplanted for a large HCC (tumor diameters 7.5-12 cm after chemotherapy) died of tumor recurrence. These results also support the suspicion of HCC being a different disease in children and adults (57), and LT should be considered in children with large HCC confined to the liver.

**Immunosuppression**

Although biopsy proven acute rejection rates decrease with use of tacrolimus, patient and graft survival are similar in patients receiving tacrolimus or CsA (63,116,214). Also rates of fibrosis are similar in patients receiving either CNI (163). Histological findings of biopsies are, however, not the only measure to be taken into account when deciding on a child’s immunosuppressive medication.

Regarding immunosuppression, children are very different from adults. Children need higher initial and maintenance doses of immunosuppressive medi-
cations than adults, because of differences in drug-binding affinity of plasma proteins, distribution volumes, and drug metabolism (116,215,216). More intense immunosuppression and more frequent primary infections with e.g. EBV in part explain the higher incidence of PTLD found in children. Also adverse effects of immunosuppression may differ from adults, and the balance between efficiency and toxicities remains problematic. The choice of CNIs is further complicated by the fact that LT recipients with acute rejections have an overall lower cancer risk after LT than patients without rejections (9). Furthermore, infections cause significantly more deaths in children than do rejections (119). As several factors (including e.g. growth, infections, PTLD, and renal function) need to be considered, the best approach may be lenient individualization of immunosuppression. Although many centers are fascinated by the newest immunosuppressive agents, evidence supporting their cost-effectiveness is scarce, with the exception of basiliximab (214). Our results suggest that a more conservative immunosuppressive regime with slight individual modification depending on adverse effects may offer excellent treatment results.

Liver histology

Protocol liver biopsies of symptom-free long-term survivors of pediatric LT showed inflammation in 26%, fibrosis in 39%, microvesicular steatosis in 43%, and periportal CK7-immunopositivity in 22%. The fibrosis stage correlated with C7-immunopositivity and inflammation. Immunosuppression including low-dose MP seemed to protect against inflammatory and fibrotic changes in the liver graft, while MP use showed no correlation with insulin resistance or liver graft steatosis. The age of the allograft correlated with serum GGT and conjugated bilirubin levels as well as periportal CK7-immunopositivity. Half of the participating LT recipients (29 persons, 54%) have already transitioned to adult health care, a time period potentially hazardous to both the LT recipient and liver graft (191,217), which makes our results seem even more promising.

Both inflammation and fibrosis rates were less frequent in our study population than in several recent reports (13,14,163). Despite many confounding factors between studies from different centers, one presenting explanation is our immunosuppressive regime including prolonged use of low-dose steroids. Steroid avoidance is based on its potential adverse effects which are dose dependent and may have been overestimated in patients on alternate-day low-dose medication (12,218). This may be especially true at our center, as Finnish LT recipients use extremely low steroid doses (≤ 0.1 mg/kg/day). Our immunosuppressive protocol of long-term triple medication (CsA, AZA, and low-dose MP) may also protect patients from de novo AIH, which may actually be a part in the continuum of immune-mediated injury (115,219). Ekong et al reported de novo AIH in 22% of pediatric LT recipients (163), but no cases fulfilling the
diagnostic criteria were found in our study. Especially in children, chronic inflammatory changes often lead to fibrosis (13,165). Since the rate and grade of inflammation among our LT recipients were low, so were also the rates and grades of fibrosis.

Besides inflammation, fibrosis may result from subtle cholestasis due to biliary complications and disturbed bile secretion (164). Although none of our patients had histological cholestasis, periportal copper deposits, or significantly elevated serum bilirubin levels, twelve biopsies (22%) showed periportal CK7-immunopositivity and ductular reaction was present in 23 biopsies (43%). Half of the CK7-immunopositive biopsies were from patients without clinically diagnosed biliary complications. These changes may be caused by partial bile duct obstruction and possibly exacerbated by hypoxia (21). Ductular reaction may also be caused by mild persistent cholestasis and the associated relative bile acid overload. These reactive changes in turn induce mild inflammation (207,220), thus binding inflammatory and fibrotic changes even closer together.

Increasing donor age influences both outcomes and graft function after LT (64,112,221). Actual graft age (donor age + follow-up time) has to our best knowledge never been correlated with clinical markers or liver biochemistry. Although the oldest grafts in our study were 72 years old, allograft age correlated with higher serum GGT and conjugated bilirubin levels as well as periportal CK7-immunopositivity. This should be no surprise because despite their marvellous regeneration potential, also hepatocytes undergo telomere shortening (222), and thus extracting a second lifetime out of a donor liver may prove inconceivable. Children listed for LT should therefore be entitled to the livers of the youngest donors.

Steatosis is a well-recognized risk in adults after LT (168,169), but has gone unmentioned in all previous reports on histological findings after pediatric LT. As obesity is a growing global problem and reported rates among LT recipients are also fairly high (141,143), we find it highly unlikely that no steatosis would be present elsewhere. Our patients demonstrated a clear correlation between BMI, insulin resistance, antihypertensive medication use, and steatosis. We found no cases of steatohepatitis, as none of the biopsies showed hepatocyte ballooning. Non-alcoholic fatty liver disease was mild, since in the most severe case 30% of hepatocytes were affected by macrovesicular steatosis.

We encountered only one minor complication (2%), while treatment changes were made in ten patients (19%). Histological changes showed very limited associations with biochemical markers of liver function and injury. Although patients may remain symptom-free for years while their liver slowly grows more fibrotic, biopsies are both useful and justifiable to plan treatment and make changes appropriately. The updated follow-up protocol of our center accordingly includes liver biopsies at five year intervals.
Cholesterol metabolism and cardiovascular disease risk

Despite similar serum and lipoprotein cholesterol and triglyceride levels, LT recipients showed increased markers of cholesterol synthesis and decreased markers of cholesterol absorption in comparison to controls. Cholesterol homeostasis was still maintained also in LT recipients. Immunosuppression including AZA and low-dose MP was associated with lower markers of cholesterol synthesis and higher markers of cholesterol absorption suggesting a favorable effect on cholesterol metabolism. Also FGF21 levels were higher in LT recipients than in controls. In controls, serum FGF21 correlated positively with cholesterol synthesis and negatively with cholesterol absorption, but in LT recipients this association was lacking.

The balance of cholesterol metabolism in LT recipients had clearly shifted toward increased cholesterol synthesis and decreased intestinal absorption. Still the equilibrium between cholesterol synthesis and absorption was maintained, although sometimes high cholesterol absorption leads to loss of this homeostasis (223). A cholesterol metabolism profile of high synthesis and low absorption is associated with obesity, insulin resistance, and NAFLD (26,132,224). In children and adolescents, such a profile predicted the presence of cardiovascular risk factors at middle age (25). These previous findings combined with our results suggest that our LT recipients are at increased risk of acquiring the metabolic syndrome despite having an overall lipid profile similar to age- and gender-matched controls.

Low-dose MP and AZA seemed to have beneficial effects on the patients’ cholesterol metabolism by associating with lower synthesis and higher absorption and more closely resembling the cholesterol metabolism profile of the control group. Diem et al concluded that complete steroid withdrawal is recommendable, although reported serum cholesterol levels showed a positive effect already after a switch from daily steroids to alternate-day dosing (29). Low-dose steroids have a positive effect on kidney graft survival (225), and their immunosuppressive effects present already in very low doses (0.1 mg/kg/day at our center). Thus low-dose MP use allowing for minimal CNI doses may be associated with a similar protective effect also after LT.

Serum FGF21 levels vary greatly in healthy populations (226), and also among our controls levels varied 90-fold. Variability was even greater among LT recipients (340-fold). Although FGF21 has correlated positively with BMI and liver fat accumulation (136,209), in our study FGF21 showed no correlation with BMI, serum lipids, or biopsy-proven steatosis. Serum FGF21 levels were instead associated with impaired kidney function similar to a study of Chinese kidney disease patients (138). Serum FGF21 levels also showed a negative correlation with progression of liver fibrosis in the group of LT recipients with the best
kidney function. We are bound to agree with Gälman et al (227) that instead of being a simple metabolic regulator, FGF21 may also reflect ongoing adaptive or protective processes, as demonstrated by the higher FGF21 levels found in LT recipients with no histological liver fibrosis. The novel association we found between serum FGF21 levels and cholesterol synthesis among the controls, however, suggests that FGF21 and cholesterol metabolism are still connected in healthy subjects. This interconnection may be mediated by liver X receptors which function as sensors of whole body cholesterol, suppress human FGF21 promoter activity and play a role in the activation of stellate cells thus contributing to the process of liver fibrosis (228). We speculate that the increase in serum FGF21 may be a complex phenomenon, with several potential causes, including impaired kidney function, liver steatosis, or impaired glucose intolerance. All of these conditions are, however, associated with an increased risk for dyslipidemia and cardiovascular diseases. The changes in cholesterol metabolism and FGF21 levels as well as the accumulation of cardiovascular disease risk factors in LT recipients should therefore be taken seriously by clinicians caring for young LT recipients in order to prevent later morbidity and mortality.

Although immunosuppressive medication is associated with the increased cardiovascular disease risk of LT recipients, several other factors also play a role. The return to normal daily life and eating habits together with normalization of the hypermetabolic state of end-stage liver disease may contribute to weight gain and insulin resistance (194). Patient education on healthy eating and the importance of physical exercise are therefore vital for long-term disease-free survival.

Quality of life

HRQoL was similar in LT recipients and controls under seven years. In school-aged children, the HRQoL school domain was significantly impaired in comparison to controls, whereas among the LT recipients who had reached adulthood, physical functioning and general health yielded reduced scores. Still all HRQoL domains reached scores within the normal range of controls in 54% of LT recipients aged over seven. The total number of complications correlated negatively with emotional HRQoL domains and the general health score of adults. Sexual functioning was similar to healthy peers according to DISF-SR scores, although LT recipients seemed to be less frequently satisfied with the strength of their orgasms.

Previous studies have reported HRQoL of LT recipients to be lower than that of healthy controls or even similar to children receiving cancer therapy (15,16,177). Still half of the LT recipients in our study scored within the normal range defined by the control group on all HRQoL domains, and 68% of adult survivors
considered their current health status excellent. We are inclined to raise the question of whether LT recipients actually represent two distinct groups: those with decreased HRQoL due to complications and those with normal HRQoL. Variance should not be overlooked, as some LT recipients thrive as well as or even better than their healthy peers, thus reaching the original LT goal.

The total number of surgical and medical complications correlated negatively with emotional HRQoL and general health. Biliary complications were independently associated with inferior HRQoL even years after LT. This may be due to visible jaundice, prolonged or repeated treatment episodes which enhance the stigma of illness. Although vascular complications are severe problems, they mostly present within the immediate post-LT period and, after successful treatment, leave no long-term health impairment. Most LT recipients with renal insufficiency also had normal HRQoL scores, which is understandable as none required hemodialysis known to reduce HRQoL (229). The awareness of progressing renal insufficiency may still contribute to the effect multiple complications have on the emotional HRQoL domains. It may also be one contributing factor to the LT recipients’ expectations of poorer health in the future. Obesity was only associated with inferior HRQoL scores of proxy-reports and adults.

Risk-taking often polarizes in youths and young adults (186,187), which we also confirmed in the correlations between smoking, alcohol consumption, and sexual activity. Although the adult survivors in our study actually consumed alcohol less frequently than controls, they still raised due concern in health care providers. The LT recipients in our study tended to have lower education levels than controls, which is in line with a recent French study (230). Specific cognitive impairments (e.g. visuospatial) after pediatric LT may affect both achievement level and education (231). Åberg et al showed employment status to correlate with HRQoL in Finnish adults after LT (232), but the adult population in our study may be too young to show this effect. Rates of chronic pain were similar between LT recipients and controls. HRQoL improved significantly with longer follow-up, which may be due to the decreasing frequency of life-threatening operations and infections and consequently increasing confidence about the future.

Recommendations on e.g. prevention of infections can affect HRQoL depending on how strictly the LT recipients’ family follows the given guidelines. Especially during adolescence, the pure knowledge that tattoos, tanning, smoking, and travel to the most exotic, primitive corners of the world are associated with higher disease risk than for “normal” people, can increase anxiety and feelings of isolation. The question remains, how to provide all this education on risk factors and lifestyle restricting guidelines without increasing the stigma of illness or anxiety levels, or decreasing the patients’ subjective perception of HRQoL? HRQoL is associated with the level of self-esteem (152), which should
thus be boosted by both health-care providers and family members. Parents of adolescent LT recipients have reported higher stress levels than adults of healthy teens (177), and this may also affect the adolescents’ self-perceived level of functioning and self image of “sick or healthy”. Besides counseling the youth during transition, their parents should be offered more psychological support as well to allow their child to grow more independent and responsible.

All nonadherent deceased LT recipients in our study (4 of 33, 12%) fell into the age group of 16-23 years, which is the age of transition from pediatric to adult services. Our center has hosted a transition-clinic from the beginning of 2005, which all patients attend after they turn 13. A list of topics open for discussion is available to both the LT recipient and doctor, and besides vaccinations, travel, and tattoos, it also includes several issues related to sexual health and fertility. Still 61% of LT recipients over 18 years reported having received insufficient information on the effect of LT and immunosuppression on fertility. Some of the LT recipients transferred to adult services already before the clinic and the delicate subject may also have been addressed inadequately or precociously, or repetition after transition may have been deficient. In France, LT recipients without physical problems felt greatly embarrassed by their scars (230). Scars were also an issue for 30% of American adolescents after LT (152). Self-esteem and physical appearance may have influenced also the difference we found between LT recipients’ and controls’ satisfaction with orgasms. Physicians caring for adolescents and young adults should never overlook the weight of these issues, if they want to influence patient adherence. A well-established, functioning transition clinic with involvement also from adult health care is essential in assuring the good results of LT are carried on to the future of these patients.

**Future considerations**

Although tissue engineering is a field of growing interest, actual clinical applications, such as therapeutic liver repopulation by transplanted hepatocytes or growing a functioning liver outside the human body, are still just a dream (233). Thus improvement of the current treatment protocols after LT continues to be vital for the patients' wellbeing.

Children with deteriorated health at LT have worse outcomes (63), and thus children with chronic hepatic conditions should be listed early enough. Early listing would also allow for better donor selection, as younger donor age is associated with improved short-term liver graft survival rates (92). Donor age may prove especially important decades later as the LT recipient and liver graft reach late adulthood and the true effects of hepatocyte ageing are seen (222). LT should, however, never be attempted too early (234). New algorithms and
criteria for patient listing better simulating multidisciplinary evaluation may still bring forth new patient groups who benefit significantly from LT. The last word on LT criteria in children suffering from hepatic malignancies has also not been said (55,235).

The subtly spreading fibrosis found in liver grafts of long-term survivors of pediatric LT remains a mystery. So far, only some connections with preceding events have been recognized, but the mechanisms relevant in the development of fibrotic changes after LT are unknown. Chronic liver damage leads to activation of hepatic stellate cells, which then up regulate expression of tissue inhibitors of metalloproteinases. This in turn leads to the inhibition of matrix metalloproteinases and subsequent accumulation of matrix proteins in the extracellular matrix (236,237). Earlier evidence suggests that different matrix metalloproteinases may be involved in acute rejections diagnosed in the immediate post-LT period (238), but no studies have so far been conducted in long-term survivors of LT.

For a successful transition of care, both pediatric and adult staff need to familiarize themselves with developmental aspects of adolescence and communication with youth (239). Poor adherence of the caring team to the young patient weakens the adherence of the young to their treatment. This includes meeting too many different doctors and the focus of care being too disease-oriented (188). Surprisingly, no prospective studies are available for transition of care after solid organ transplantation. In very few reports have the voices of the transitioning youths been heard. We have already begun a project aimed at bringing forth the opinions of the youths in transition and expect to report our results in a few years. Thus we hope to improve the transition process in a truly patient-centered direction.
CONCLUSIONS

Liver transplantation is the only remedial treatment and a life-saving procedure for children suffering from end-stage liver disease. Since children have a long life expectancy, even subtle changes in liver histology and cholesterol metabolism may have significant effects in the long run. Accumulated complications may also decrease the LT recipients’ perception of their quality of life.

Based on the results of this study, the following conclusions are drawn:

1. Survival and complication rates after pediatric liver transplantation at a relatively small center can reach levels similar to those of larger centers. Survival rates after liver transplantation for hepatoblastoma and hepatocellular carcinoma may also be similar to those of other indications despite original tumor size. Excellent treatment results for unresectable hepatoblastoma and hepatocellular carcinoma are achievable without posttransplant chemotherapy.

2. Due to the low incidence of biopsy-associated complications, frequent treatment changes after liver biopsies, and limited correlations of histological changes with liver biochemistry, protocol biopsies are both useful and justifiable in the long-term follow-up of pediatric liver transplant recipients.

3. Cholesterol synthesis was increased and absorption decreased in LT recipients compared with controls. This unfavorable cholesterol metabolism profile may predispose to increased cardiovascular disease risk in later life. Also the regulation of FGF21 in LT recipients was disturbed.

4. Indefinitely continued use of low-dose steroids and associated lower doses of calcineurin inhibitors seemed to have a beneficial effect on both long-term liver graft histology and the cholesterol metabolism profile of LT recipients. Adult height was affected more by the growth retardation caused by end-stage liver disease than the very low doses of steroids.

5. In a majority of LT recipients, long-term quality of life can be similar to healthy controls. Specific targets for improvement include psychosocial adjustment of both the child and family members and consideration of school issues, as these will have long-term effects on the overall life of LT recipients. Sensitive subjects such as sexual health should also be addressed more often.

6. After the first posttransplant year, patient and graft survival is excellent until adolescence and the time of transition of care. The quality and value of a systematic transition program involving both pediatric and adult health care providers should be highlighted.
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REFERENCES


(27) van der Wulp MY, Verkade HJ, Groen AK. Regulation of cholesterol homeostasis. Mol Cell Endocrinol 2012. Epub ahead of print


(103) Aberg F, Jula A, Hockerstedt K, Isoniemi H. Cardiovascular risk profile of patients with acute liver failure after liver transplantation when compared with the general population. Transplantation 2010;89:61-68.


(136) Dushay J, Chui PC, Gopalakrishnan GS, Varela-Rey M, Crawley M, Fisher FM, et al. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 2010;139:456-463.

(137) Chavez AO, Molina-Carrion M, Abdul-Ghani MA, Folli F, DeFronzo RA, Tripathy D. Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. Diabetes Care 2009;32:1542-1546.


REFERENCES


(176) Varni JW, Seid M, Kurtin PS. PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations. Med Care 2001;39:800-812.


REFERENCES


REFERENCES


