Complications following pediatric liver transplantation – a change of paradigm

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List of abbreviations

ACE	angiotensin converting enzyme
ADV	adenovirus
AIH	autoimmune hepatitis
AIHA	autoimmune hemolytic anemia
ALF	acute liver failure
ALT	alanine aminotransferase
ANA	anti-nuclear antibody
AP	alkaline phosphatase
AST	aspartate aminotransferase
AZA	azathioprine
BA	biliary atresia
BC	biliary complication
BDA	biliodigestive anastomosis
BP	blood pressure
CIT	cold ischemia time
CKD	chronic kidney disease
CMV	cytomegalovirus
CNI	calcineurin inhibitor
COD	cause of death
CrCl	creatinine clearance
CSA	cyclosporine A
DCD	donation after cardiac death
DD	deceased donor
DIC	disseminated intravascular coagulation
DM	diabetes mellitus
DNA	desoxyribonucleic acid
EBV	Epstein-Barr virus
ELTR	European liver transplant registry
ERCP	endoscopic retrograde cholangiopancreaticography
EVR	everolimus
GALD	gestational alloimmune liver disease
GFR	glomerular filtration rate
GGT	gamma glutamyl transferase
GI	gastrointestinal
HAT	hepatic artery thrombosis
HCTZ	hydrochlorothiazide
HLA	human leukocyte antigen
HPS	hepatopulmonary syndrome
HTN	hypertension
HUS	hemolytic uremic syndrome
ICU	intensive care unit
IGT	impaired glucose tolerance
IMPDH	inosine monophosphate dehydrogenase
INR	international normalized ratio
IV	intravenous

IS	immunosuppression
ISD	immunosuppressive drug
ITBL	ischemic type biliary lesions
IVC	inferior vena cava
LD	living donor
LDH	lactate dehydrogenase
LKM	liver kidney microsome (antibody)
LRLT	living related liver transplantation
LT	liver transplantation
MAP	mean arterial pressure
MELD	model of end-stage liver disease
MMF	mycophenolate mofetil
MPA	mycophenolic acid
mTOR	mammalian target of rapamycin
NAS	non-anastomotic strictures
NH	neonatal hemochromatosis
NODM	new-onset diabetes mellitus
OLT	orthotopic liver transplantation
OML	oral mucosal lesion
OT	operational tolerance
PCP	pneumocystis carinii pneumonia
PCR	polymerase chain reaction
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
pLT	pediatric liver transplantation
PNF	primary non-function
PTC	percutaneous transhepatic cholangiography
PTCD	percutaneous transhepatic cholangio-drainage
PTH	parathyroid hormone
PTLD	post-transplant lymphoproliferative disease
PVT	portal vein thrombosis
RAS	renin-angiotensin system
ScTx	stem cell transplantation
SIR	sirolimus
SIRS	systemic inflammatory response syndrome
SLT	split-liver transplantation
SMA	smooth muscle antibody
SPSS	statistical package for social sciences
TAC	tacrolimus
TTP	thrombotic thrombocytopenic purpura
UKT	Universitätsklinikum (University Hospital) Tübingen
VC	vascular complication
WD	Wilson’s Disease
WIT	warm ischemia time

1. INTRODUCTION

1.1. Historical overview of pediatric liver transplantation

Liver transplantation (LT) has become a routine treatment for terminal liver failure of both acute and chronic liver disease in children. In 1983, the National Institute of Health Consensus Development Conference declared that liver transplantation was an effective therapy for end-stage liver disease both in adults and children [1]. This declaration was made 20 years after Dr. Thomas Starzl performed the first human liver transplant in 1963 in Denver, Colorado, USA [2]. Improvement of survival in the first decade of pediatric LT (pLT) was hampered by the lack of effective immunosuppressants which led to a high incidence of acute and chronic rejection. Only after the introduction of cyclosporine (CSA) as an immunosuppressant by Sir Roy Calne in 1977, patient outcome after liver transplantation in children improved [3] and has reached a present long-term patient survival rate of more than 90% in experienced pediatric transplant centers [2]. Hence, what started with the pioneering efforts of T.E. Starzl 50 years ago has now become a routinely successful clinical procedure. This achievement was made through refining the surgical techniques, immunosuppression, intensive care, better prevention and control of infection, and earlier re-transplantation when the first graft failed [4, 5]. Over the years, multiple indications for pediatric liver transplants have emerged and will now be discussed in detail before taking a deeper look into further developments in pediatric liver transplantation.

1.2. Indications for liver transplantation in children

1.2.1. Cholestatic disorders

Biliary Atresia (BA) is the most common indication for chronic liver failure in infancy and childhood and accounts for at least 50% of all liver transplants in children. It is a disease of the infant which causes severe damage and

obliteration of the bile duct system leading to profound cholestasis and progressive biliary cirrhosis [6, 7]. For the majority of children, a surgical repair (Kasai procedure) is only temporary. Half of these patients undergo LT within the first 1-2 years of life and 80% during childhood [8]. The fact that BA is the most frequent indication for LT in childhood, explains the very young average age at transplantation. This favors complications such as vascular or biliary strictures due to the very small anatomic structures in technically modified grafts as well as a high likelihood of adverse reactions due to immunosuppression. *Alagille Syndrome* is a rare autosomal dominant disorder caused by a variety of mutations in the *JAG1* gene, which is a ligand in the Notch signaling pathway that controls the development of many organs [9]. Therefore, clinical features vary, but are dominated by liver (chronic cholestasis due to the paucity of interlobular bile ducts) and heart disease (vascular and cardiac anomalies). Further organ systems may be affected. Approximately 50% of patients who present in infancy with clinically evident and progressive liver disease will not survive into adulthood without LT [10]. *Progressive Familial Intrahepatic Cholestasis* (PFIC) is a group of autosomal-recessive inherited disorders of transport proteins. In patients affected by high-impact mutations symptoms of liver disease including cholestasis, pruritus and jaundice are seen in the first year of life. Ursodeoxycholic acid therapy should be initiated in all patients to activate compensatory mechanisms. However, in many PFIC patients with high impact proteins, LT has to be considered before reaching adulthood due to possible fibrosis or end-stage liver disease [11].

1.2.2. Metabolic disorders

Wilson's Disease (WD) is a rare autosomal-recessive disease characterized by the deposition of copper in the brain, liver; cornea, and other organs due to impairment of the biliary route for excretion of dietary copper [12]. The overload of copper inevitably leads to progressive liver and neurological dysfunction. The standard treatment for WD is chelation and zinc. Pediatric liver transplantation is reserved for those patients presenting with acute liver failure or rarely with

chronic failure despite medical therapy [13].

1.2.3. Other indications

Neonatal Hemochromatosis (NH, meanwhile Gestational Alloimmune Liver Disease, GALD) is a rare disease in which liver injury of alloimmune origin and fetal onset is associated with massive extrahepatic iron deposition and liver failure. It presents with signs of terminal liver failure around birth [14]. If the current treatment including exchange transfusions and IV immunoglobulin fails, LT can be lifesaving. *Autoimmune Hepatitis* (AIH) is diagnosed based on the presence of either anti-smooth muscle antibody (SMA) (type1) or anti-liver-kidney microsomal (LKM) antibody (type2). Children with type 2 AIH often tend to be younger, male and more likely to present with acute liver failure. Of children with AIH, 10-20% will eventually need LT [15]. *Acute liver failure* (ALF) in children is defined as a rapid onset hepatic necrosis presenting with or without hepatic encephalopathy and coagulopathy with INR > 2 within 8 weeks of the onset of clinical liver disease. The etiology of 49% of pediatric ALF cases is categorized as “indeterminate”, followed by acetaminophen toxicity (14%), metabolic disease (10%), drug toxicity (5%), and others (11%) [16]. Because there is a chance of spontaneous recovery, 54% of ALF patients will not require LT, approximately 32% of ALF patients require LT and 14% die without LT [16]. *Hepatoblastoma* is the most common pediatric liver tumor that is amenable to LT. Protocol treatment includes chemotherapy to reduce the tumor volume and if a surgical removal is still not possible and no unresectable metastases are found, LT may be offered [17]. The number of transplants due to hepatic tumors is increasing posing a special challenge considering the immunosuppressive therapy due to risk of disease recurrence.

1.3. Surgical transplant techniques

The technique of orthotopic liver transplantation (OLT) has developed since the original technique described by T.E. Starzl. Full-size grafts are still considered

anatomically ideal since the anatomy is preserved, resulting in better outcomes and less surgical complications. However, the shortage of size-matched donor organs for small children stimulated the development of technical innovations to increase the donor pool [2]. Reduced size liver transplantation describes a technique of ex situ liver reduction where a liver is cut to the volume of segments needed and the rest discarded. Usually, either the left lateral segment (segments 2-3) or the full left lobe (segments 2-4) is retained. However, liver reduction has been criticized and has become obsolete due to the loss of potentially transplantable segments. Split-liver transplantation (SLT) also increases the donor pool as it usually simultaneously offers a donor organ for a small (segments 2 and 3, in many cases pediatric) and an adult recipient. Technically, it is a complex procedure that can be performed either in the *ex situ* or *in situ* split technique [18-20]. Unfortunately, the wider application of the split technique is still hindered by the lack of experience and missing universal acceptance in transplant centers. Living related liver transplantation (LRLT) is a further option to increase the donor pool, particularly for infant patients or younger children. Provided that the donor is suitable by anatomic means, contraindications are excluded and perioperative risks are minimized, the donor will most commonly donate the left lateral segment (segments 2-3) or rarely the right (segments 5-8) or left lobe (segments 2-4) of the liver [21]. The surgical risk for the donor is relatively low when the left-lateral segment is transplanted. In addition to alleviate the organ shortage, this option has the advantage that the recipient can be prepared in order to perform the LT in an elective setting. To summarize, the types of grafts used in children include: full-size donor organ following post-mortem donation (51%), reduced liver (21%), living donor liver (20%), and split liver (8%) [22]. It is important to be informed about the type of graft received in order to anticipate technique-specific complications.

1.4. Complications associated with pediatric liver transplantation

Presently, according to data from European centers collected in the European Liver Transplant Registry (ELTR), graft loss in the first year after pLT occurs in

28% of cases and mortality is 15-17%. Early recognition and correction of post-transplant complications improve graft and patient survival. Complications can occur both in the early (during the initial post-transplant hospitalization) or late course after LT (generally after patients have been discharged home, weeks or months to years after transplantation). The majority of complications and deaths occur within the first three months [23, 24]. Primary non-function (PNF) of the graft, with an incidence in pediatrics of < 5% (0-16%) [25], manifests 12-48h after transplantation and includes rising INR (>3), AST or ALT (>5000IU/L), and bilirubin [22]. It results in graft loss, with only one third of children with PNF surviving [26]. Causes include impaired quality of the donor organ, difficulties in organ preservation and retrieval, and technical or immunological complications in the recipient [27]. The only effective treatment is emergent re-transplantation.

1.4.1. Vascular and biliary complications

Vascular and bile duct anastomoses of the liver graft can differ in children compared to adults. Arterial reconstruction is mostly performed by end-to-end arterial anastomosis. Due to the small diameter of the vessels in children, anastomoses are difficult to perform and may result in vascular thrombosis or stenosis more frequently. Hepatic artery thrombosis (HAT), as the most feared surgical complication, has a reported incidence of 3% in adults and 7-8% in children [28]. It may present insidiously, with fever, cholangitis or biliary leaks, strictures or abscesses. Early recognition and immediate surgical revascularization may salvage the graft. Hepatic artery stenosis occurs in 5-10% of the cases and can be successfully managed with angioplasty or stenting by an experienced interventional radiologist. Portal vein thrombosis (PVT), rare in adults, occurs in up to 33% of pediatric liver transplant recipients [29]. Early PVT presents with graft dysfunction or gastrointestinal bleeding. Screening with abdominal Doppler ultrasound during the first days after LT is routine to detect vascular thrombosis at a time when intervention is still possible. Following heparinization for the first seven days post pLT, prophylaxis against vascular thrombosis with oral aspirin is standard for the first 2-3 months after LT in the

protocol used at the local Tübingen center as well as in many other centers. Intra-abdominal bleeding, particularly from the cut surface of the liver (in a reduced or split graft), may require relaparotomy to stop the hemorrhage. This complication is often associated with heparine therapy. Rare complications are venous outflow and inferior vena cava obstruction (2-4%, respectively). Construction of the hepatic vein outflow anastomosis can vary. Most commonly in adults, the recipient's retrohepatic part of the inferior vena cava (IVC) is removed along with the liver and replaced by the donor's liver with intact IVC. Alternatively, frequently in children, the “piggyback” technique is used, especially with living related or reduced-size transplantation where it is not possible to remove part of the donor's IVC [30, 31]. Using this technique, the hepatic vein is anastomosed to the donor's preserved IVC end-to-side while creating an enlarged triangular orifice.

Biliary complications (BCs) frequently are sequelae of impaired perfusion and ischemia of the graft before or during harvesting as well as during the postoperative course. Biliary strictures occur as a consequence of ischemia, biliary leaks either are due to ischemia at the anastomosis or due to insufficient surgical closure of bile duct structures. BCs are rather common and occur in 5-30% of children [27] with a varying incidence of 1.6-18% for leaks and 3-23% for strictures, occurring more frequently in technically modified transplants. Biliary anastomotic strictures present with cholangitis or obstructive jaundice, but may also be asymptomatic. Cholangiography and stenting with ERCP (endoscopic retrograde cholagiopancreaticography) or PTC (percutaneous transhepatic cholangiography) may lead to resolution and avoid the need for surgical reconstruction in the majority of cases. If the problem persists, conversion to choledochojejunostomy via a Roux-en-Y jejunal limb may be required [32]. Non-anastomotic biliary strictures (NAS), also referred to as ischemic-type biliary lesions (ITBL), are strictures at any and frequently multiple locations in the biliary system other than the anastomosis. They fortunately are relatively rare since this type of bile duct stricture is regarded as the most troublesome biliary complication. These strictures are often resistant to therapy,

frequently associated with long-term sequelae, and one of the more common indications for re-transplantation [33]. Biliary leaks can occur at various sites in the postoperative period after LT. The majority of postoperative leaks occur at the site of anastomosis, but also at the resection surface of the graft in the case of LRLT or SLT. Patients present with fever or mild graft dysfunction, and if undiagnosed, may progress to biliary peritonitis. HAT or temporary impairment of the arterial perfusion is contributing to 25% of all biliary complications and should be excluded in all cases.

1.4.2. Immunosuppressive therapy

The use of immunosuppressive drugs (ISDs) after LT has been essential from the early beginnings in order to avoid rejection and risk of transplant function impairment. To reach this goal, a spectrum of ISDs is available. Since the first liver transplant, the variety of ISDs has increased and undergone constant change, including the development of new drugs. During the early years of transplantation, ISDs were limited to anti-metabolic agents, such as azathioprine (AZA) and steroids as a global immunosuppressant, often resulting in poor outcomes. Corticosteroids have numerous effects on the immune system including reduction of circulating T-cells as well as inhibition of leukocyte adhesion and inflammatory mediators. The poor side-effect profile of corticosteroids is widely recognized and therefore their eventual withdrawal after serving as a component of the induction therapy has been widely adopted and steroid-free protocols have been introduced. A standard weaning strategy is not universally accepted and some centers do continue low-dose or alternating day treatment to minimize undesired effects. A breakthrough in rejection control came with the introduction of cyclosporine A (CSA), a calcineurin inhibitor (CNI). CNIs activate specific intracellular binding proteins (cyclophiline or FK-506) and block the function of calcineurin, which is a serine/threonine phosphatase activator. This prevents the production of interleukin-2 and thus the activation of T-cells, which are the prime agents of rejection [34]. This targeted therapy led to a marked improvement in post-

transplantation outcome by more than doubling the one year patient survival rate [35]. The next advance was achieved by tacrolimus (TAC), a CNI with higher potency, allowing steroid withdrawal within the first post-transplant year in most patients [36] and excellent control of acute and chronic rejection injury. Besides its efficacy [37], the steroid-sparing effect of TAC is of importance to preserve the growth potential of children [2]. Another advantage of TAC is the lack of cosmetic side-effects associated with CSA which consequently led to a switch to TAC in most patients. CNI levels are measured at their trough and the dose of CNIs is adjusted based on targeted blood levels. In general, TAC has become the predominant CNI and is administered to almost all liver transplant recipients (87%) at the time of hospital discharge. CSA is used in a few patients who are intolerant of TAC, e.g. due to risk of diabetes mellitus development [38]. The acute toxic side effects of CNIs include nephrotoxicity, systemic hypertension (HTN), hyperkalemia, hyperlipidemia, neurotoxicity, gastro-intestinal toxicity and post-transplant diabetes mellitus [27, 34]. Arterial hypertension occurs in 40-80% of liver transplant recipients [39] and often needs to be treated with antihypertensive medication such as an ACE inhibitor (enalapril) or calcium channel blocker (nifedipine) [40]. In addition to a CNI, the current immunosuppressive regimen following LT in children may include the purine antagonist mycophenolate mofetil (MMF) which is added in children with a history of autoimmune hepatitis, previous immunosuppression (IS) or to reduce nephrotoxicity of CNIs. MMF, or rather its active metabolite mycophenolic acid (MPA), is a potent and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), which inhibits the *de novo* synthesis of purines. Since the T- and B-lymphocytes depend on this pathway for their proliferation, MPA has a potent cytostatic effect on lymphocytes and can therefore be used as an ISD [34]. The most important side effects of MMF are gastrointestinal complaints including nausea and diarrhea and hematological changes such as leucopenia, anemia and/or thrombocytopenia. Especially in children, these side effects sometimes require drug discontinuation. The great advantage that MMF has over CNIs is that it does not cause diabetes, HTN, or renal toxicity. Several studies have provided evidence that MMF is even

effective in reversing structural changes in the kidney [41, 42]. Some studies have demonstrated that MMF directly exerts protective effects against inflammation and progression of fibrosis [43]. Biologic agents, such as basiliximab or daclizumab, are monoclonal antibodies inhibiting the T-cell or one of its surface receptors (i.e. IL-2 receptor/ CD 25). Most protocols include these anti-IL2 antibodies for induction with or without steroids. Furthermore, their use is well accepted due to their favorable renal toxicity profile but because of their high cost and requirement of IV administration, they are currently only used for induction therapy or rarely for steroid-resistant rejections [44]. Other ISDs that may be used after transplantation, however off-label in children, are the mTOR inhibitors sirolimus (SIR) or everolimus (EVR). They interact with an intracellular pathway inhibiting the mammalian target of rapamycin (mTOR), which impacts a variety of relevant metabolic functions in humans. They are used in patients with CNI-induced nephrotoxicity because of their renal sparing effect. However, there is currently not enough data to precisely describe the risks and potential benefits of the drugs but several studies for the pediatric population are ongoing. The potential side-effects of mTOR inhibitors may include an increased incidence of altered wound healing and dehiscence, hyperlipidemia, thrombocytopenia, leucopenia and anemia [45].

1.4.3. Rejection and infections

In particular in the early weeks following LT, the balance between adequate immune control and the consequences of over-immunosuppression with an enhanced risk of infection and other toxic effects remain challenging [46]. In clinical practice, it is sometimes difficult to differentiate acute cellular graft rejection from other complications since clinical and chemical findings are often nonspecific. However, in the majority of children, it is associated with increased AST /ALT, bilirubin, alkaline phosphatase and GGT activity, and formally must be confirmed with a liver biopsy [22]. The lowest rate of rejection is seen in children < 6 months of age and the highest in teenagers who show poor compliance with their medications [47]. In stable patients, routine laboratory

evaluation should be obtained every 3-4 months to monitor for biochemical signs of rejection. Acute rejection is treated with increased doses of CNIs or 3-day high dose IV corticosteroids, followed by weaning doses of oral steroids and adaptation of the IS regimen. Induction therapy with IL-2 receptor antibodies can further reduce the rate of acute rejection [48, 49]. In pediatric patients, chronic rejection has been a significant cause of graft loss but the incidence has decreased from 10% to <5% [47]. Chronic rejection may develop after episodes of acute rejection that are not responsive to increased IS and often leads to graft loss [22]. It can occur as early as 6 weeks post-transplant but is usually most common within the first year or during puberty when the incidence of non-compliance increases. The clinical presentation includes jaundice and pruritus. Rescue strategies for chronic rejection include adding additional immunosuppressants to the existing baseline therapy. Non-response to medical management requires re-transplantation.

Infectious complications are important causes of morbidity and mortality especially in the first 3 months post-transplant while immunosuppression is at its highest. Children with chronic liver diseases considered for LT should undergo all available vaccinations before being listed for liver transplantation in order to prevent infection related morbidity after surgery in the state of immunosuppression. Risk factors for infection include CNI and/ or steroid therapy, poor graft function, prolonged intensive care, ventilator dependence, gut perforation, re-transplantation, and the use of anti-lymphocyte antibodies to treat severe rejection [50]. Bacterial infections are common within the first two weeks post-LT. Gram-positive organisms from venous lines remain an important cause of sepsis in the first post-operative week, while gram-negative sepsis is less common with the use of prophylactic antibiotics during and after surgery. As standard medication in the UKT protocol, patients also receive cotrimoxazole for PCP prophylaxis. Risk factors for fungal sepsis include graft dysfunction, HAT, bile leak, bowel perforation, re-intubation, ALF and previous IS (e.g. related to AIH). Most fungal infections are due to candida species but other species may also occur. Fungal infections particularly take place in patients with

complicated post-LT course and are associated with a high mortality. Fluconazole is well tolerated as prophylaxis and therapy, but amphotericin or capsofungin are the main stay of treatment. Herpes simplex, varicella zoster, CMV, EBV and adenovirus are all potential causes of early and late infections, often associated with over-immunosuppression. Young patients, who are more likely to be seronegative for these viruses pre-LT, are susceptible to primary infections post-LT. Rarely, adenovirus infection causes fulminant hepatitis or necrotizing pneumonitis in the early post-LT period with a 45% mortality rate [51]. Seventy percent of children develop primary CMV infection post-LT with a mortality rate of 7% [52]. CMV, as the most prevalent viral infection, is characterized by malaise, fever, leukopenia, thrombocytopenia, arthralgia, and later hepatitis, enteritis, or pneumonitis. Other rare late CMV manifestations include myocarditis, vasculitis, and encephalomyelitis. Valganciclovir is usually given as CMV-prophylaxis for 3 (or 6) months post-transplant depending on risks and observed complications. EBV primary infection is defined as systemic detection of EBV-DNA load and/or EBV seroconversion in recipients who had been EBV-seronegative at the time of transplantation. Associated symptoms may or may not be present. EBV reactivation is defined as detectable EBV-DNA load in patients already EBV-seropositive at the time of engraftment. EBV infection can be categorized as follows: asymptomatic infection, symptomatic infection including either flu-like symptoms (fever, malaise, chills) or infectious mononucleosis (fever, pharyngitis, lymphadenopathy with or without hepatosplenomegaly) and post-transplant lymphoproliferative disease (PTLD) [53]. PTLD describes an abnormal proliferation of EBV-infected B-cells which occurs in the setting of ineffective T-cell function because of pharmacological immunosuppression after organ transplantation. According to different studies, it affects 5-15% of children post-LT [54]. It can present in lymphoid tissue in any organ, although most commonly in the transplanted organ, lymph nodes, gastrointestinal tract, or lungs where it can cause a broad variety of clinical symptoms. Most PTLDs are non-Hodgkin lymphomas and nearly all of them are associated with EBV infection. Diagnosis is made by the histologic evidence of PTLD and EBV association in biopsies of affected organs. Most transplant

centers use EBV DNA levels (PCR) to monitor for viral load indicating acquisition or reactivation of infection. A high and/or persistent EBV load may constitute a valid predictive marker for the later development of EBV-related PTLD [55]. The mainstay of treatment is reduction of immunosuppression, but this therapeutic option is only based on a high EBV load without clinical evidence for PTLD but not on scientific evidence [56]. A promising novel therapeutic option to control PTLD is anti-B-cell antibody therapy (rituximab). Previously a study showed that valganciclovir post LT was contributing to prevention of post LT EBV related complications [57].

Since long-term maintenance IS increases the risk of infectious and malignant complications as well as other side-effects, one of the most important management strategies is to find ways of reducing IS toxicity while sustaining sufficient immunological protection of the graft. The surgical procedure itself and perioperative management remains challenging but has become a routine procedure even in small children or patients severely affected, e.g. in acute liver failure. However, with significantly improved short-term prognosis, issues regarding long-term safety of IS treatment and quality of life come into focus.

1.5. Objectives of this thesis

This thesis aims to answer the following questions:

1. **Has the spectrum and frequency of complications following liver transplantation in children changed in the current era compared to previous periods, specifically: has there been a change in frequency and impact of ISD related complications?**

2. **What are the consequences of the current spectrum of post-transplant complications for patient management?**

2. METHODS

From February 2005 to October 2011, a total of 52 pediatric patients underwent OLT at the University Hospital in Tübingen. Their data was collected by retrospective chart review including medical records, operative reports, anaesthesia protocols, ICU medical records, and microbiological and pathological reports.

2.1. Donor and recipient data

The donor parameters include age, sex, and cause of death (COD).
The recipient parameters include age, sex, ethnicity, diagnosis, and blood group. Further data was collected at different time points:

Pre-transplant screening:
- Weight, height, blood pressure, mean arterial pressure (MAP)
- Physical exam findings and clinical findings /review of systems (edema, cerebral bleeding, cyanosis, hepatomegaly, splenomegaly, skin alterations, encephalopathy, jaundice, ascites, fatigue, variceal bleeding, renal failure)
- Laboratory findings:
    - Electrolytes (sodium, potassium)
    - Osteoporosis parameters (calcium/ phosphate/ PTH), INR
    - Renal function (creatinine, creatinine clearance, cystatin c, urea)
    - Liver function (total/ direct bilirubin, albumin, AST, ALT, GGT, AP)
    - Blood count (thrombocytes, haemoglobin, leukocytes, lymphocytes, neutrophils, eosinophils), cholesterol, glucose, pH, bicarbonate, uric acid, LDH
- Auto antibodies (ANA, SMA, LKM): screening for autoimmune hepatitis
- PCR for EBV, CMV, ADV: pre-transplant viral status

Preoperative admission for transplantation/ Transplant procedure:
- Physical exam findings, blood pressure, MAP, basic laboratory findings
- Transplant procedure: MELD (model of end-stage liver disease)/ PELD

(pediatric end-stage liver disease), graft type, type of biliary/ vascular anastomoses, length of ICU stay, warm ischemia time (WIT), cold ischemia time (CIT), immunosuppressive therapy and other medication, histopathology

One, three and six months post-transplant:
At 1, 3 and 6 months the following data was obtained:
Physical exam findings, blood pressure, MAP, laboratory findings, auto antibodies (ANA, SMA and LKM), PCR (EBV, CMV and ADV), current medications, complications and possible therapeutic interventions

Yearly follow-up:
Each yearly follow-up includes a documentation of the patient's physical exam findings, blood pressure, laboratory findings and immunological as well as viral diagnostics.

2.2. Statistical methods

Clinical, biochemical and outcome parameters were entered in a database. Using SPSS version 20.0, cohorts with early and delayed referral were compared in the univariate analysis using Fisher´s Exact Test for categorical and 1-way ANOVA for continuous variables unless mentioned otherwise. Correlations were analyzed using the Spearman coefficient with testing two-tailed significance. Candidate variables at the univariate level were selected for multivariate regression if $p<0.05$. Multivariate logistic regression analysis was then used to estimate the relative risk and 95% confidence intervals. Differences were considered significant for p-values < 0.05.

3. RESULTS

3.1. Study population

3.1.1. Patient characteristics

The 52 children who underwent liver transplantation between 2005 and 2011 at the University Hospital Tübingen (UKT) were included in this retrospective single center analysis. Two patients were excluded from the study because of an additional history of stem cell transplantation (ScTx), one of them died in the context of transplant failure after ADV infection. The characteristics of the remaining 50 children are listed in table 1.

Table 1: Characteristics of the study population at the UKT 2005-2011

Characteristics	**Value**
Total number of transplants	52
Excluded (ScTx)	2
Included	50
Follow-up (range of years)	1-7 yr (median: 2 yr)
Gender	31 boys, 19 girls
Age at OLT	21 days-19.3 yr (median: 21months)
Height at OLT	52-179 cm (median: 79.3 cm)
Weight at OLT	3.6-79 kg (median: 9.9 kg)
less than 7 kg	32% (n=16)
Ethnicity	Europe: 82% (n=41)
	Near/ Middle East: 14% (n=7)
	Far East: 4% (n=2)
Blood group	A: 48% (n=24)
	0: 40% (n=20)
	B: 10% (n=5)
	AB: 2% (n=1)

3.1.2. Indications for liver transplantation

Primary indications for LT at our centre include both cholestatic disorders, e.g. biliary atresia, metabolic disorders, e.g. Wilson's disease, conditions leading to cirrhosis, e.g. autoimmune hepatitis, acute liver failure and tumors such as hepatoblastomas. For further details see table 2.

Table 2: Indications for liver transplantation at the UKT 2005-2011

Diagnosis	**Value**	**%**
Biliary atresia (BA)	26	52
Acute liver failure (ALF)	5	10
Alagille syndrome	3	6
Progressive familial intrahepatic cholestasis (PFIC)	2	4
Hepatoblastoma	2	4
Autoimmune hepatitis (AIH)	2	4
Idiopathic cirrhosis	2	4
Gestational alloimmune liver disease (GALD)	2	4
Non-cirrhotic portal hypertension	2	4
Caroli-syndrome	1	2
Crigler-Najjar syndrome	1	2
Wilson's disease (WD)	1	2
Liver cirrhosis	1	2

3.1.3. Transplant procedure

Mean PELD score at transplant was 15.5 (range of PELD 0-41, median: 16.0), valid for all children less than 12 years. Mean MELD score at transplant was 14.3 (range of MELD 10-25, median: 14.0), valid for all children older than 12 years, relevant for organ allocation in the North-American UNOS system. The time spent on the ICU ranged from 3 to 75 days (median: 6 d). Cold ischemia time (CIT) ranged from 30min - 19h 22min (median: 6.5 h), CIT is declining for the more recent transplantations. CIT in LDLT ranged from 30min - 3h 35min (mean: 63.5min), CIT in SLT ranged from 2h 3min - 16h 27min (mean: 10h 28min), and CIT in full-size LT ranged from 3h 26min - 19h 22min (mean: 9h 3min). Warm ischemia time (WIT) was between 17- 70 min (median: 38 min).

Different surgical transplant techniques were used for transplantation (see figure 1). Sixty-four percent (n=32) of liver grafts were from deceased donors (DD), including full-size grafts and split grafts from which segments 2-3 (left-lateral lobe) were transplanted in 11 patients (22%) and segments 1, 4-8 (right lobe) in 7 patients (14%). Liver transplantation from living donors (LD) was performed with the left-lateral lobe in 30% (n=15) and the left lobe (segments 2-4) in 3 patients (6%).

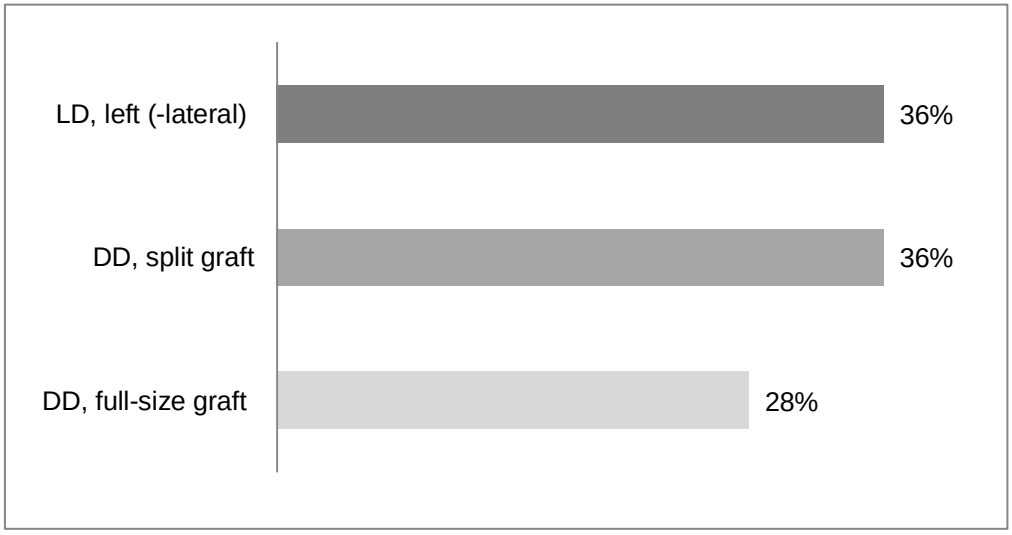

Figure 1: Graft types at the UKT from 2005-2011; LD: living donor, DD: deceased donor

The hepatic artery anastomosis was mostly performed end-to-end (86%, n=43) whereas the portal vein anastomosis was end-to-end in all patients (98%, documentation in 1 case inconclusive). To anastomose the hepatic vein, the so called “piggy-back” technique was used in 52% (n=26), in 42% (n=21) of patients the inferior vena cava was replaced by donor cava and in the rest of the patients the method was not specified. The biliary anastomosis was either end-to-end (choledocho-choledochostomy) in 16 patients (32%) or in the form of a biliodigestive anastomosis (BDA) in 58% (n=29) of patients.

3.1.4. Donor data

The donor age including all post-mortem and living donors ranged from 0-55 years (median: 25 years), including 35 female and 15 male donors. Their CMV IgG Status was positive in 25 donors, negative in 23 donors and unknown in two donors (see table 3).

Table 3: Donor data from deceased and living donors at the UKT 2005-2011

Characteristic	**DD (n=32)**	**LD (n=18)**
Age, years	0-55 (median: 17)	19-50 (median: 33)
Sex, male/female	13/19	2/16
CMV status, pos/neg	18/13 (1 unknown)	7/10 (1 unknown)
Body weight, kg	4-95 (median: 55)	

The deceased donors include 14 full size transplants and 18 split transplants. COD in DD was: 3 anoxia, 1 cerebrovascular accident, 5 cerebral edema, 2 intracranial bleeding, 3 meningitis, 8 subarachnoidal hemorrhage, 6 traumatic brain injury, 1 suicide, 2 trauma, 1 unknown. The living donors include 14 mothers, 1 father, 1 step father, 1 adoptive mother, 1 grandmother.

3.2. Patient and graft survival in different transplant periods

In a literature review, articles describing pLT in different time periods were reviewed and the respective patient and graft survival data are listed in table 4. The data was compared to the results at the UKT from 2005-2011 where both patient and graft survival rates were 100%. No patient with isolated LT died or had to receive a re-transplant.

Table 4: Patient and graft survival from 1985 to 2010

Period	Studies	5 yr patient survival	5 yr graft survival
1985-1990	Otte et al. [18]	73,6%	80,5%
	Esquivel et al. [58]	69,2%	73,0%
	Lloyd-Still et al. [59]	64,0%	
1990-1995	Drews et al. [60]	78,0%	68,0%
	Busuttil et al. [23]	77,0%	75,0%
	Yamanaka et al. [61]	75,0%	63,0%
1995-2000	Miller et al. [62]	80,9%	78,0%
	Deshpande et al. [63]	88,1%	86,1%
	Bourdeaux et al. [64]	88,5%	83,0%
2000-2005	Wallot et al. [65]	91,4%	87,3%
	Gridelli et al. [66]	91,0%	80,0%
	Oliveros et al. [67]	96,0%	89,0%
2005-2010	**UKT**	**100,0%**	**100,0%**

The data shows a constant improvement in both patient and graft survival over the past 25 years. The respective mean patient and graft survival for every 5 year period is depicted in figure 2.

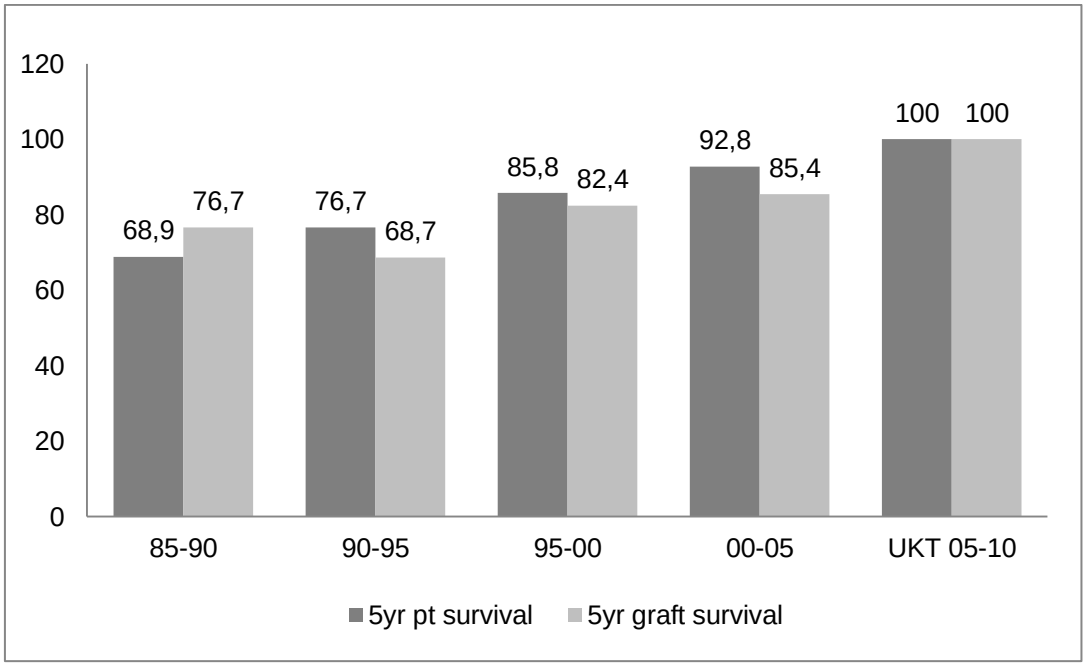

Figure 2: Patient and graft survival from 1985-2010

3.3. Vascular and biliary complications

3.3.1. Results at the UKT from 2005-2011

A total of 136 complications were analyzed. In the following, the categories of complications will be discussed divided up by vascular complications, biliary complications, rejection and infections as well as complications associated with calcineurin inhibitor immunosuppression.

Vascular complications (VCs) at the UKT from 2005-2011 were found in a total of 9 patients (18%). See table 5.

The following vascular complications at the UKT required interventional treatment: The HAT was treated by relaparotomy and surgical revision. The PVT (affection of segments 2 and 3 only) which led to portal HTN required treatment in the form of a thrombectomy. The venous stenoses were treated by balloon angioplastic dilatation.

Table 5: Vascular complications at the UKT from 2005-2011			
Complication		**Value**	**%**
Thrombosis	Hepatic artery thrombosis (HAT)	1	2
	Portal vein thrombosis (PVT)	1	2
Stenosis	Hepatic artery stenosis	1	2
	Portal vein stenosis	2	4
	Inferior vena cava stenosis	2	4
	Hepatic vein stenosis	2	4

Relaparotomy

A total of 15 patients (30%) required a relaparotomy due to the following causes: post-operative bleeding occurred in 10 patients (20%), abscesses in 2 patients, suspected or actual hypoperfusion in 2 patients and HAT in 1 patient. At the UKT, a direct abdominal closure could be performed in all but one case in which closure was performed as a two-step procedure.

Biliary complications (BCs) at the UKT from 2005-2011 occurred in 36% of the patients (n=18). See table 6.

Table 6: Biliary complications at the UKT from 2005-2011		
Complication	**Value**	**%**
Biliary anastomotic strictures	14	28
Biliary leaks	2	4
Ischemic type biliary lesions (ITBL)	2	4

The BCs at the UKT mostly required surgical intervention due to recurrent episodes of cholangitis. The interventional treatment included surgical revision with a new BDA in 10 patients presenting with biliary strictures and biliary leaks.

Three patients required ERCP: in the first patient balloon angioplasty was performed, the second received a stent placement and in the third, flushing due to sludge was done. As a change in management, percutaneous transhepatic cholangio-drainage (PTCD) was increasingly used as a therapeutic option in 6 cases, requiring re-intervention in 2 cases, leading to resolving stricture and stable biliary drainage in 4 cases.

In terms of BCs, it is common to differentiate between early complications and late complications. This differentiation was also used for the complications seen at the UKT from 2005-2011 resulting in the following:
Early complications (occurring during the initial post-transplant hospitalization) were observed in 7 patients (3 biliary strictures, 2 ITBL, 2 biliary leaks).
Late complications (occurring after patients have been discharged home) were observed in 11patients (11 biliary strictures).

3.3.2. Potential predictors of biliary complications

The data of the 50 patients was analyzed for potential predictors of biliary stenoses after pediatric liver transplantation. Figures 3-5 show that pre-transplant parameters have a significant influence on the occurrence of biliary stenoses including alkaline phosphatase, bilirubin and sodium. Patients with lower pre-transplant levels of alkaline phosphatase ($p < 0.005$), direct bilirubin ($p < 0.038$), and sodium ($p < 0.012$) seem to experience biliary stenoses more frequently. This indicates a potential relevance of recipient-specific laboratory parameters on the frequency of biliary complications.

In our study group no significant differences in the number of biliary stenosis were found for CIT, WIT, PELD, MELD, donor age, weight of the recipient.

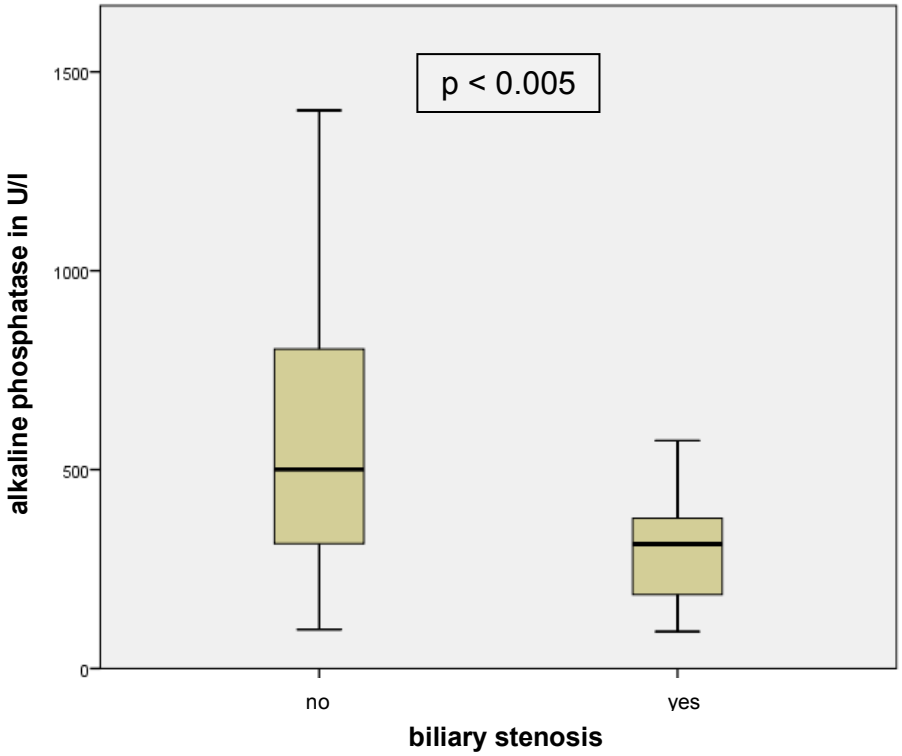

Figure 3: Pre-transplant alkaline phosphatase

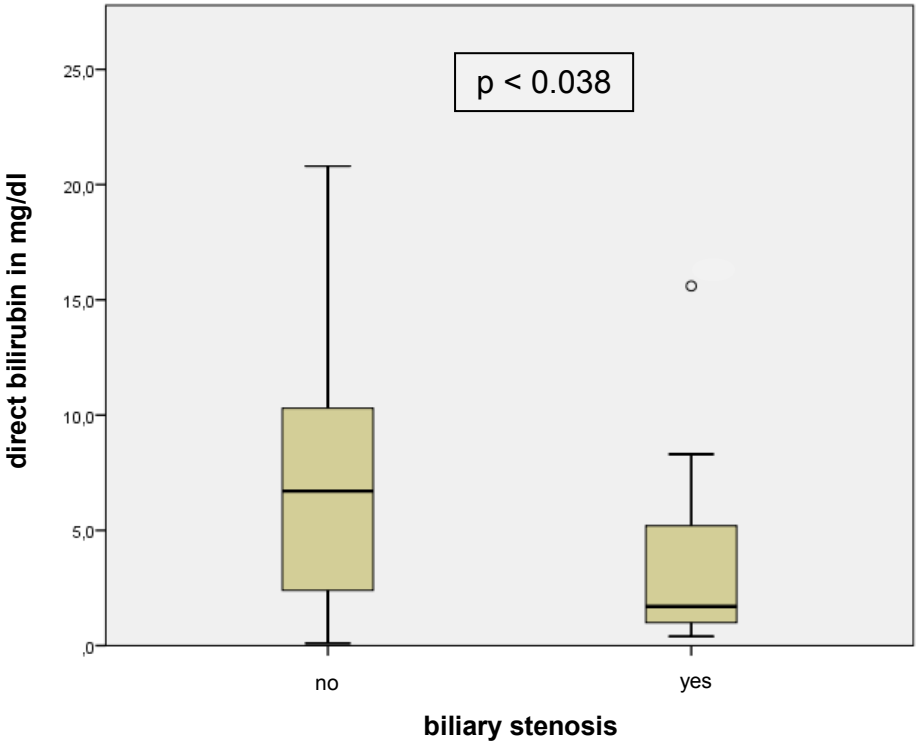

Figure 4: Pre-transplant direct bilirubin

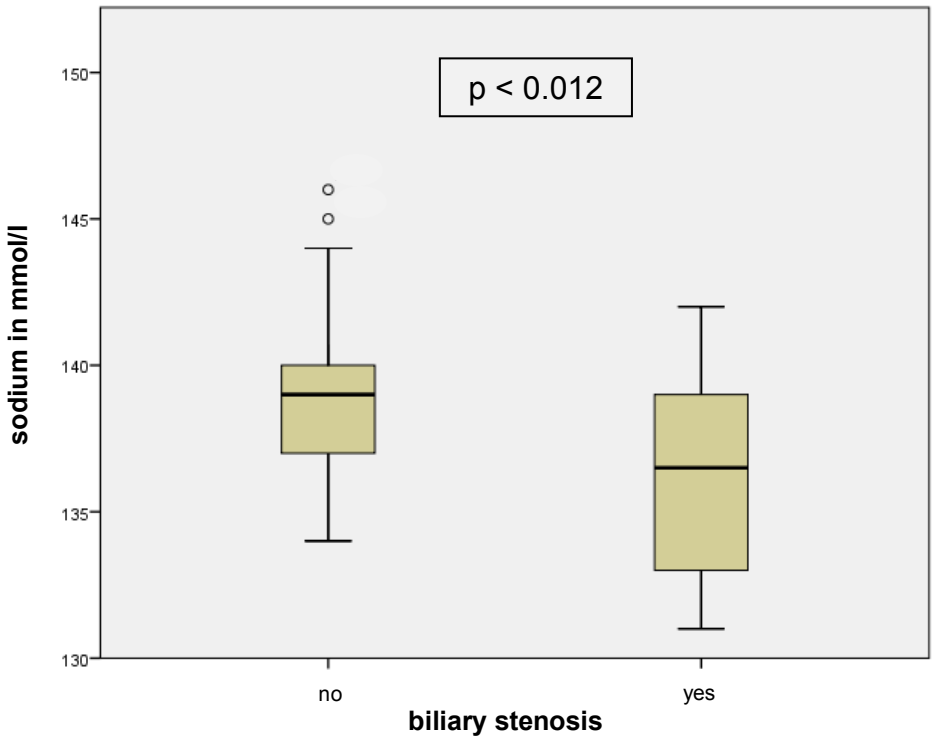

Figure 5: Pre-transplant sodium

The influence of the pre-transplant sodium was also shown to be significantly associated with the number of biliary stenosis in a stepwise multivariate analysis ($p < 0.007$). In this analysis, sodium was the only independent parameter that was associated with the occurrence of biliary stenoses.

3.3.3. Vascular and biliary complications in different transplant periods

To compare the number of VCs and BCs from this study with different transplant eras, a literature review of data from different time periods was done. Comparing the results, a notable decrease in the number of VCs can be found from 1985 until the present day whereas the number of BCs increased over the years. See table 7 and figure 6.

Table 7: Vascular and biliary complications from 1985-2010				
Period	**Studies VC**	**total VC**	**Studies BC**	**total BC**
1985-1990	Wozney et al. [68]	68,0%	Bhatnagar et al. [69]	10,0%
	Lerut et al. [70]	10,0%	Letourneau et al. [71]	38,0%
	Lallier et al. [72]	21,0%	Lallier et al. [73]	25,0%
1990-1995	Bell et al. [74]	16,0%	Patkowski et al. [75]	18,7%
	Yamanaka et al. [61]	15,0%	Egawa et al. [76]	16,3%
	Bilik et al. [25]	25,0%	Heffron et al. [77]	13,6%
1995-2000	Mali et al. [78]	19,7%	Miller et al. [62]	18,0%
	Eid et al. [79]	14,0%	Gridelli et al. [66]	27,0%
	Sieders et al. [80]	21,0%	Bourdeaux et al. [64]	26,0%
2000-2005	Broniszczak et al. [81]	16,8%	Oliveros et al. [67]	29,9%
	Shirouzu et al. [82]	15,4%	Anderson et al. [83]	26,0%
	Yilmaz et al. [84]	24,7%	Kling et al. [85]	33,0%
2005-2010	**UKT**	**18,0%**	**UKT**	**36,0%**

Figure 6 displays the data from table 7 in a graph:

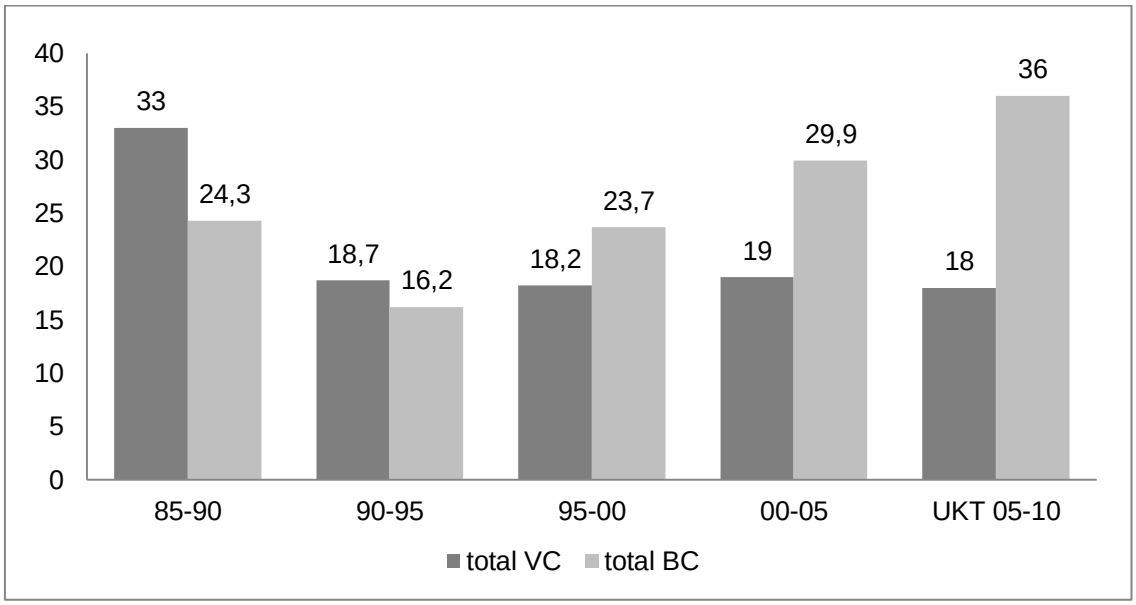

Figure 6: Vascular and biliary complications from 1985-2010

To specify the different BCs reported in the studies, the number of biliary leaks from 1985-2005 ranged from 4%-20% vs. 4% at the UKT from 2005-2010. The number of biliary strictures is reported to range from 5-34% [86] vs. 36% at the UKT.

The VCs in the era from 1985-2005 are further differentiated in the studies as well: HAT ranged from 4.9-42% vs. 2 % at the UKT from 2005-2010, PVT and portal vein stenosis ranged from 1.5-16.7% vs. 6% at the UKT, hepatic artery stenosis ranged from 1.2-11% vs. 2 % at the UKT, hepatic vein stenosis and thrombosis ranged from 1.2-6% vs. 4% at the UKT and IVC stenosis and thrombosis ranged from 1-3% vs. 4% at the UKT.

3.4. Rejection and infections

To compare the number of rejections and infections, a separate literature review was done for both number of rejections and infections in different transplant eras of the past. Table 8 and figure 7 display the significant decline in the number of rejections over the past years. The number of rejections in the UKT program includes acute rejection in 3 patients (6%) and chronic rejection in 1 patient (2%) which was due to non-compliance.

Table 8: Number of rejections after pLT from 1990-2010

Period	Studies	Rejection
1990-1995	Drews et al. [60]	68,0%
	Dunn et al. [87]	57,0%
1995-2000	Shepherd et al. [88]	46,0%
	Martin et al. [89]	60,3%
2000-2005	Sanada et al. [90]	46,6%
	Indolfi et al. [91]	32,3%
2005-2010	**UKT**	**8,0%**

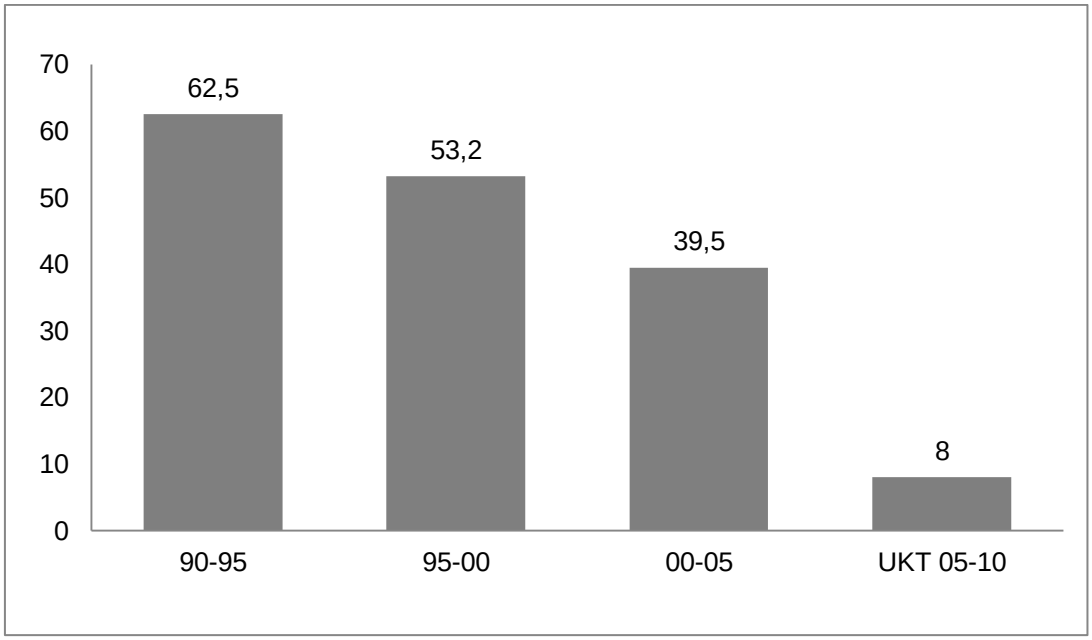

Figure 7: Number of rejections from 1990-2010

The frequency of LT associated infections has also changed in 2005-2010 compared to previous periods. The result of the literature review is displayed in table 9 and graph 8 including the total number of patients with post-transplant infections, the number of bacterial infections as well as CMV and EBV (including primary infections and clinically relevant re-activations).

Table 9: Number of infections after pLT from 1985-2010

Period	Total	Bacterial	CMV	EBV
1985-1995	54,0% [92]	62,0% [60]	33,0% [60]	63,0% [93]
	72,0% [94]	68,0% [94]	41,0% [95]	31,4% [96]
1995-2005	52,0% [88]	51,9% [97]	19,8% [98]	50,6% [99]
	60,0% [100]		18,0% [91]	56,0% [101]
05-10 UKT	**46,0%**	**16,0%**	**12,0%**	**34,0%**

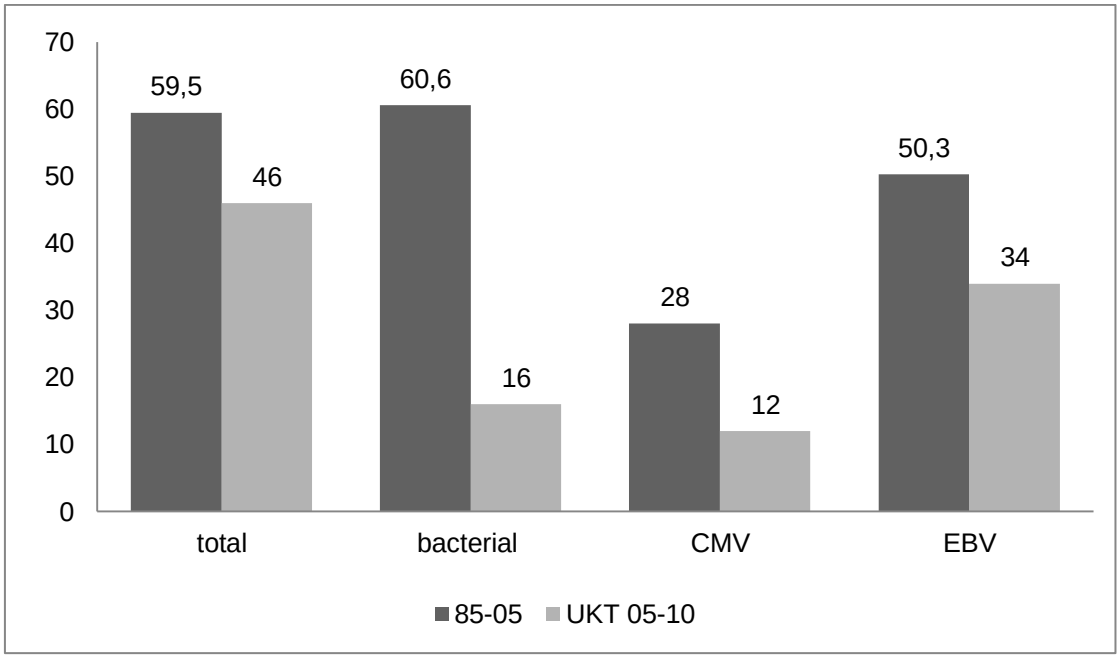

Figure 8: Infections after pLT in 1985-2005 compared to UKT 2005-2010

Infectious complications are less frequent now compared to past periods. Especially bacterial infections are significantly less common than previously reported. The bacterial infections seen at the UKT in 2005-2010 include mostly pneumonia (including two cases with pneumocystis carinii) and sepsis.

With regard to viral infections, the results of this study show a decrease in the total number of CMV and EBV infections. CMV infection today was seen in less than half of the patients compared to data from 1985-2005. Hence, the prophylactic regimen of valganciclovir for 3 (or 6) months post-transplant may be effective in reducing the number of CMV complications. A correlation to the Donor CMV-status could be observed: out of the 6 patients in this study cohort who developed a clinically relevant CMV infection, 5 donors were CMV positive. Out of these 5 patients, 4 had been CMV negative before LT. EBV primary infection (positive PCR) occurred in 17 patients (34%). The spectrum of EBV-related morbidity ranged from asymptomatic and symptomatic infections to EBV-associated PTLD which occurred in 2 patients (4%). Both PTLDs were treated with a reduced immunosuppressive regimen and rituximab (anti CD 20).

To summarize, the spectrum of complications with regard to the surgical procedure and infectious causes has changed. The number of biliary complications has increased whereas the number of infections and vascular problems has decreased. Some complications (e.g. primaray non-function) were not observed at all in analysis of the recent cohort.

3.5. Complications associated with CNI immunosuppression

A differentiation of post pLT complications does not allow a strict division between the events occurring in the operative and post-operative phase both related to the surgical procedure and multidisciplinary acute peri- and post-operative management. However, a separate analysis of CNI-related morbidity is important due to the fact that these complications alone require significant efforts in management resources. These complications are summarized in table 10.

Table 10: CNI-associated complications at the UKT from 2005-2011

Complication	n	%
Hypertension	32	64
Renal insufficiency	14	28
Hyperkalemia	5	10
Hematological alterations	5	10
GI symptoms	5	10
Neuropsychiatric disorders	3	6
Diabetes / impaired glucose tolerance	3	6
Failure to thrive	2	4
Chronic oral mucosal lesion	2	4
Food allergy	1	2
Celiac disease	1	2

Arterial HTN and renal insufficiency both play a major role in the post-transplant care today since they are common and require antihypertensive medications. Hyperkalemia as well as acidosis are likely associated with renal insufficiency. The observed hematological complications show a spectrum of disorders including: 2 patients with anemia, 1 pancytopenia, 1 autoimmune hemolytic anemia (AIHA), 1 myelosuppression. GI-symptoms include both diarrhea and recurrent episodes of vomiting in 3 patients each, one being affected by both symptoms. Neuropsychiatric complications included both seizures in 2 patients and 1 patient with psychosis. Causality with CNI is likely due to synchronicity of CNI treatment and neurologic symptoms, the lack of further identifiable other etiology and the control of symptoms following modifications of CNI treatment. Diabetes/ impaired glucose tolerance (IGT) is also known as a common side-effect of this immunosuppression and seen in 3 CNI-treated patients. Failure to thrive, oral mucosal lesions (OMLs), food allergy and new-onset celiac disease are non-classifiable complications that may be associated with pre-existing conditions or ISDs.

3.6. Dominance of CNI-associated complications

Table 10 shows that 73 (54%) out of a total of 136 complications occurred as direct side-effects of calcineurin inhibitor immunosuppression. This dominance of CNI-associated side-effects over surgical and infectious complications expresses a change of paradigm in the spectrum of complications following pLT. If infectious complications and rejections, which in a broader sense are also related to CNIs, are also included in the group of CNI-associated complications, the dominance is even more evident with 94 (69%) out of 136 total complications. This shift towards CNI-associated complications is also depicted in figure 9 (non-CNI- associated complications include vascular and biliary complications and complications that required a relaparotomy).

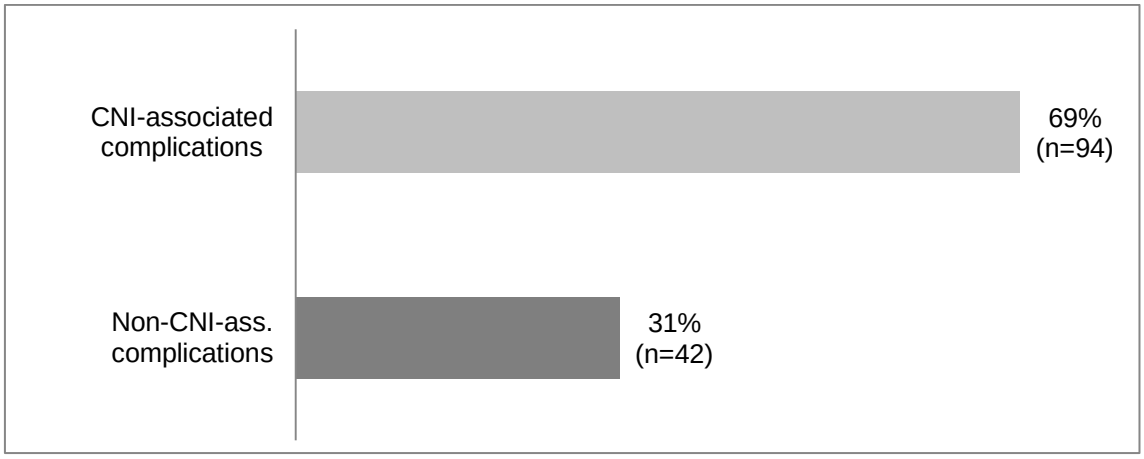

Figure 9: Dominance of CNI-associated complications

3.7. CNI therapy and alternatives

The immunosuppressive regimens that were administered at the UKT in the immediate post-OP period after LT, as well as 1, 3, 6 months and one year later are shown in table 11. The IS regimens more than one year after LT are not listed because of a small number of follow- up patients.

Table 11: Immunosuppressive regimen at the UKT until 1 year after pLT

	CNI	MMF	mTOR inhibitor
perioperative	100% 49 TAC, 2 CSA	14% (n=7)	-
1 month	98% 48 TAC, 2 CSA	28% (n=14)	-
3 months	98% 47 TAC, 2 CSA	28% (n=14)	-
6 months	97% 42 TAC, 4 CSA	23% (n=11)	4% (n=2)
1 year	86% 34 TAC, 4 CSA	27% (n12)	11% (n=5)

To decrease CNI-associated side effects two strategies were applied: firstly, the reduction of CNI levels and addition of a further IS (e.g. MMF) and secondly, once intolerable side-effects were identified, a CNI-free regimen was established. One option to replace CNIs is the use of mTOR-inhibitors such as everolimus or sirolimus. Out of the 50 patients in our study population, 13 (26%) received a secondary modification of their immunosuppressive medications to a CNI-free regimen (see table 12). The switch to CNI-free immunosuppression was done 5 months to 10 years following LT.

Table 12: Change to CNI-free immunosuppression to reverse adverse effects

Adverse effects	No.	CNI-free regimen	Outcome
Nephrotoxicity, Hyperkalemia	2	EVR/ MMF	2 resolved
	2	EVR	1 converted to LD steroids/ MMF, 1 resolved
	1	EVR/ LD steroids	resolved
severe food allergy, eczema	1	EVR	improved
AIHA	1	EVR/ LD steroids	elevated transaminases, azathioprine added
Pancytopenia	1	SIR	improved
Chronic OMLs	2	1 MMF, 1 SIR	1 improved
Persistent high EBV load	1	EVR/ MMF	resolved
PTLD	1	MMF / LD steroids	rejection, cyclosporine added

As indicated in table 12, there were various adverse effects (e.g. renal or haematological problems) that made the switch from a CNI-based therapy to a CNI-free regimen necessary. The CNI-related adverse events were resolved in 9 of the 13 patients and the new IS regimen was tolerated well. Side effects of secondary non-CNI treatment are listed in table 13.

Table 13: Complications of CNI-free regimen and their management		
Complication	**Action taken**	**Number**
Rejection (biopsy proven)	cyclosporine introduction	1
Transiently elevated transaminases	adaptation of EVR levels in 1 case, azathioprine in 1 case	2
Edema of lower extremities, proteinuria	switch EVR – SIR	2
Relaparotomy indicated	replacement of mTOR-inhibitors	1

The effect of changing IS-regimen to CNI-free immunosuppression is shown in the improvement of the GFR both pre and post conversion in 4 patients:

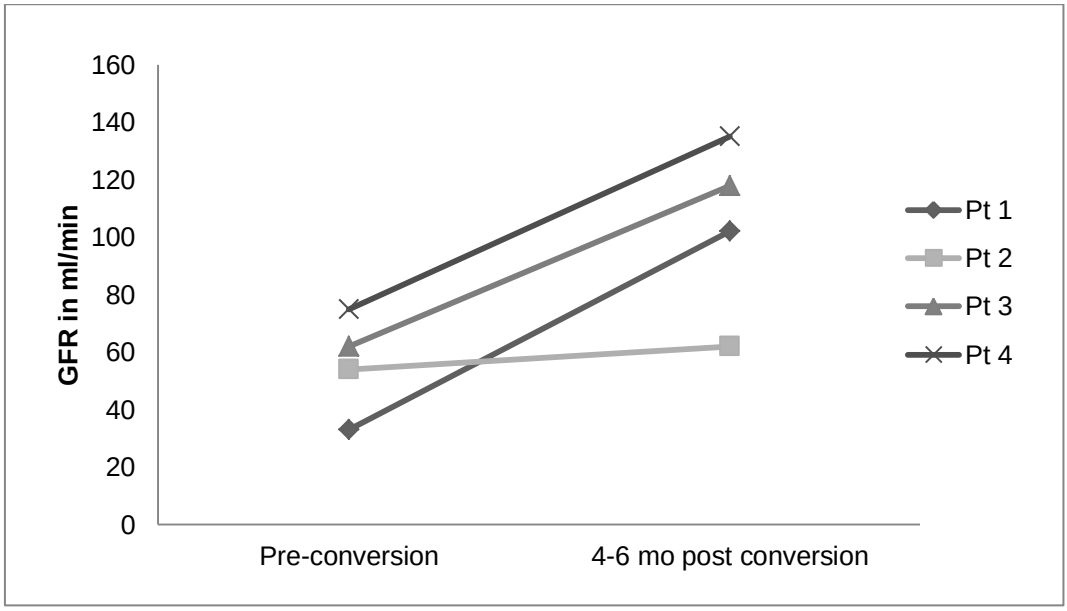

Figure 10: GFR improvement in 4 patients pre- and post-conversion; GFR: glomerular filtration rate

4. DISCUSSION

This is the first retrospective study comparing the spectrum of complications of a single pLT program to data published in previous periods. The primary objective was to analyze whether there was a change in the type and frequency of complications after pLT, with emphasis on the relation of CNI-related complications versus complications related to the surgical procedure, and to discuss the consequences of the current spectrum of post-transplant complications for patient management. We were able to show that, associated with improvement in patient and graft survival, the spectrum of complications after pLT has changed significantly during the periods studied. Compared to previous studies, a significant shift from complications related to the surgical transplantation procedure towards CNI-associated complications was observed in our study cohort. This dominance of CNI-associated complications points to a need to reconsider the strategies in current post LT management.

Several contributors to this change in the spectrum of complications can be identified. Underreporting in previous (early) studies may play a role. Even though hypertension and nephrotoxicity, the most prevalent side effects of CNIs, were also described in earlier transplant periods [102, 103], they were not the primary focus. Two decades ago, the focus in pediatric liver transplantation was on improvement of the surgical procedure, e.g. through expanding the donor pool by introduction of living-donation or the splitting procedure, while improving survival of both patients and grafts with a limited spectrum of ISDs. Therefore, in this context, CNI-associated undesired effects had potentially been less in focus compared to other risks of high impact such as transplant failure and severe infections which were responsible for many deaths in the previous periods of pLT. In comparison, in our study no deaths from serious bacterial, fungal, or viral infections were reported. The high incidence in earlier studies may be associated with more severe degrees of liver disease at the time point of presentation and less experience in the operative and perioperative management. Compared to previous periods the broadened experience in use of technically modified and living donation grafts in patients at risk may have

also had a positive influence on patient and graft survival. In addition, the availability of novel antiviral drugs has changed post-operative treatment. CMV or EBV naïve infants who receive CMV or EBV positive grafts are particularly vulnerable to infections with these viruses. The use of the CMV antiviral prophylaxis valganciclovir has reduced CMV-associated risk even though studies continue to report CMV disease rates as high as 10-14% [104, 105] which is similar to our study.

In this study, we reviewed the outcome of 50 primary liver graft recipients in a 1-7 year follow-up period. Survival rates for this selected group were excellent, being 100% for both grafts and patients with isolated liver grafts. This compares favorably with recently published data from the European liver transplant registry that showed 1-year patient survival rates of 85% and graft survival rates of 75% [106]. These single center results may indicate that liver transplantation has reached the threshold of a new era where survival of the patient is considered the likely outcome and attention will now be focused more on long-term patient and graft survival. Different studies have been published which also describe a patient survival rate of 100% but lack a similar high graft survival rate. Broering et al. [107] reports a series of 132 consecutive pediatric liver transplants with an actual 6-months patient survival rate of 100%. Three children died after hospital discharge (98% actual patient survival rate). The high patient survival rate was not sustained by a high graft survival: 3-month and actual graft survival rates were 92% and 86%, respectively, leading to a total number of 16 re-transplants (12%). The three main reasons for graft loss were PNF (n=5), arterial thrombosis (n=4) and chronic rejection (n=5). Borenstein et al [108] published patient and graft survival rates of 100% after 13 living donor liver transplants in children. The limitation of this study is the very small size of the study cohort. Between 1993 and 2002, the group from Brussels performed 235 primary liver transplants with a 5-year recipient survival rate of 85% in the cadaveric group (n=135) and 92% in the LRLT group (n=100) [109]. The group from Kings College in London published an actual 6-month patient survival rate of 96.2% after 80 consecutive pediatric liver transplants

[63]. The different authors identified factors that contributed to their good outcomes. Broering et al. recognized that the year of transplantation turned out to be the strongest significant factor in predicting patient survival, underlining the learning curve in their center. Further contributing factors included careful selection of good quality grafts, adequate choice and performance of the splitting technique, the technique of graft implantation and standardized post-transplant care, including close follow-up of the hepatic flow [107].

Compared to previous reports on pLT we were able to show a constant decline in the total number of surgical complications, especially in the number of vascular problems. However, biliary complications remain a major source of morbidity after LT, leading to repeated interventions and adaptations of therapeutic regimens. The reasons may be the frequent use of technically modified grafts and vulnerability to ischemia-reperfusion damage. Manifestations of bile duct damage are seen at the anastomotic region or at multiple locations of the donor biliary system (ITBL). As shown in the literature review, the incidence of BCs did not vary much since the first LT, with an overall reported incidence still between 10 and 35% [110]. Potential contributors to a persistent number of BCs are discussed by various authors. Verdonk et al. observed an increase of biliary anastomotic strictures after 1995 [32], possibly related to an increased use of organs with extended donor criteria. According to Seehofer et al. major risk factors include old donor age, partial liver grafts and prolonged ischemia time. These risk factors could be partially confirmed in our analysis regarding cold ischemia time and old donor age. Even though our center did not confirm partial liver grafts as a significant risk factor, technically modified grafts are frequently discussed with regard to the increase in BCs. Darius et al. [110] also hypothesized that the use of alternative techniques (reduced-size, split and living donation) in pLT contributed to an increase in the incidence of BCs. However, they came to the conclusion that graft type was not found as an independent risk factor for the development of BCs. According to their analysis only HAT and acute rejection were significant risk factors for anastomotic BCs. An analysis of the SPLIT database demonstrates a 24-month

BC rate of 17% for whole organ recipients, 28% for split recipients, 25% for reduced size, and 40% for live donor [111]. While the use of living donor and technical variants of deceased donor grafts does increase the BC rate according to studies from the SPLIT database, it is important to remember that these technically modified grafts contributed to significantly reducing the shortage of size-matched organs for children on the LT waiting list. Even though the different authors agree that BCs following pLT remain an important and challenging problem, many factors that increase the risk for BCs like donor age or steatosis may be difficult to address with regard to the shortage of suitable donors. However, it is generally accepted that the best prerequisite for low biliary morbidity is adequate perfusion with active bleeding at the biliary ends, preservation of periductal tissue and avoidance of vascular injury to the bile duct vessels, e.g. by extensive cautery [112]. Concepts on how to manage biliary complications vary. The majority of centers use ERC plus balloon dilatation with [113] or without [114] stent placement for the treatment of anastomotic strictures in patients with preserved biliary anatomy. Since about 75% of pediatric LT are performed with a bilioenteric anastomosis [111], most biliary complications in this patient group are managed by percutaneous intervention +/- stent placement. Darius et al., despite its more invasive nature, prefer primary surgical intervention which in their experience provides better results in terms of quality of life than repeated radiological interventions.

Due to improvement of post-LT survival data, CNI-associated adverse effects and complications have moved into focus as the treatment post LT is aiming at undisturbed development and minimizing therapy associated morbidity. The impact of CNI-related side-effects is variable: they may be serious and result in mortality, e.g. through PTLD, their impact may be moderate but significantly impair quality of life, e.g. food allergies, or they may frequently affect extrahepatic organ function, e.g. renal dysfunction and arterial hypertension. Hypertension and nephrotoxicity as the most prevalent side effects of CNIs are associated with significant morbidity and mortality in both children and adults [115-117]. In pediatric LT recipients, it has been shown that hypertension is

predictive of long-term renal insufficiency [118]. This result corresponds with our findings at the UKT since all children developing renal insufficiency have a history of preceding hypertension except for one. As such, a repeatedly elevated blood pressure or decreased GFR requires treatment to prevent long-term consequences. In fact, treating patients for hypertension even if they do not meet the conventional criteria (>95th percentile) for treatment is an approach used by a growing number of centers to prevent renal comorbidities [119-123] since patients who develop post-LT chronic renal failure due to CNIs have a more than four-fold higher mortality than those who do not [124, 125]. A recent longitudinal study by Anastaze Stelle et al. of 24 pediatric liver transplant recipients on TAC also analyzed renal function and hypertension [126]. They found that the lower the TAC level, the better the renal function (Creatinine Clearance, CrCl). This indicates that the CNI-dose plays an important role which at the same time implies an approach to prevent renal damage. The dose dependency was also confirmed in other studies which showed that long-term low-dose CNI therapy was not associated with deterioration of renal function in children [127, 128]. Further investigations examined the number of patients requiring antihypertensive therapy: at three months post-OLT, 65.2% of patients needed antihypertensive therapy while only 33% were receiving antihypertensives 36 months post-OLT [126]. Among these patients the ones with a combined therapy including nifedipine and enalapril had better renal function than patients on one drug alone, although the observed effect on CrCl did not reach statistical significance [40]. The mechanism of TAC-related hypertension is still incompletely elucidated, but it appears that TAC creates an imbalance of vasoconstrictors and vasodilators contributing to renal and systemic vasoconstriction [129-132]. In general, there is an acute and a chronic CNI-induced nephropathy. The acute form of CNI toxicity may be reversed when CNI administration is reduced or withdrawn. In contrast, the chronic form of CNI-induced nephrotoxicity is characterized not only by renal vasoconstriction but also by the development of structural damage, including arteriolopathy and tubulointerstitial fibrosis, which are irreversible and need to be avoided due to the potential to cause end-stage renal disease [133]. To conclude, identifying

and treating hypertension early on may prevent long term morbidity and improve prognosis and quality of life since more than 90% of patients receive an immunosuppressive regimen based on CNIs after LT [134].

At our center 100% of patients received a CNI (48 TAC only, 1 CSA only, 1 TAC and CSA consecutively) in the period following LT. After 1, 3 and 6 months, 98% of the IS was CNI based. One year after LT, only 86% still received a CNI based therapy whereas the rest of the patients were switched to a CNI-free regimen due to CNI-related adverse effects. Hence, immunosuppressive strategies are needed to reduce the incidence of CNI-associated complications. There are different options as well as new approaches in order to reduce the number of CNI-related side-effects: One strategy is the individual minimization and weaning off IS. Present research efforts are focusing on approaches aiming at operational tolerance (OT) which is defined as stable graft function for 1 year without IS. There have been several published reports of the successful weaning of 11-38% of liver transplant recipients from IS [135]. A study at the Kyoto University Hospital reported that 88 of 581 pediatric living donor liver recipients (15%) were operationally tolerant [136, 137]. Feng et al. [138] performed a pilot study including 20 highly selected pediatric recipients of parental living donor liver transplants who were discontinued from their immunosuppressive therapy by a stepwise reduction over a minimum of 36 weeks. As a result, 60% of the patients (n=12) remained off IS for at least 1 year with both normal graft function and stable allograft histology and were termed “operationally tolerant”. Many of the 12 operationally tolerant children experienced serious adverse events after weaning such as biliary obstruction, cholangitis or portal vein stenosis. The 8 “non-tolerant” patients had to be treated with an increased or a re-initiation of immunosuppression therapy mostly due to acute or indeterminate rejection. Severe or steroid refractory acute rejection, chronic rejection, graft loss, or deaths were not observed. The challenge now is to find markers for operational tolerance and thus wean patients from CNI using these markers as guidance and safety parameters in the process. Waki et al. [139] studied the role of anti-human leukocyte antigen

(HLA) antibodies after pediatric living-donor liver transplantation, trying to investigate whether the presence of HLA antibodies impeded the development of OT. They concluded that post-transplantation HLA antibodies were associated with the inability to reach OT. A prospective study with more patients is necessary to confirm the predictive value of HLA antibodies for OT. Another novel approach of tolerance induction is the use of mesenchymal stem cells (MSCs). MSCs inhibit the immune response in vitro, and thus are promising candidate cells to promote acceptance of transplanted organs in vivo. Popp et al. [140] demonstrated that donor-derived MSCs induce long-term allograft acceptance in a rat heart transplantation model when concurrently treated with a short course of low-dose MMF. They concluded that MSCs constitute a promising tool for the induction of graft tolerance but further investigation in clinical trials is needed. Considering the results from the above mentioned studies, CNI minimization in the form of complete withdrawal may be an option to limit or to improve CNI toxicity, provided that CNI exposure has not already caused irreversible damage. However, the balance between efficacy and the risk of rejection needs to be carefully weighed [141-145].

A second strategy is the use of alternative ISDs with improved toxicity profiles. After a reduction or even withdrawal of CNI some months after LT, adequate immunosuppression can be maintained by adding MMF or mTOR inhibitors [145-149]. It has been shown that regimen change led to improved kidney function without resulting in a higher rejection rate. However, not all patients seem to profit from this strategy, possibly because irreversible kidney damage has already taken place [150]. Few studies have evaluated the long-term use of MMF in liver transplanted children with renal dysfunction. Aw et al. [151] studied the efficacy of MMF as renal rescue in 14 pediatric liver transplant recipients with CNI-related nephrotoxicity. Side effects of MMF included acute rejection in three children, leukopenia in two and backache in one. They found that MMF allowed the recovery of renal function from CNI-related nephrotoxicity in more than 70% of pediatric liver transplant recipients with renal impairment. Tannuri et al. [152] evaluated 191 liver transplanted children out of which 11 patients

developed renal dysfunction secondary to prolonged use of CSA or TAC. All of these were switched to a MMF immunosuppressant protocol with reduced doses of CNIs. As a result, 82% showed improvement of renal function parameters in comparison with the pretreatment values. No episodes of acute or chronic rejection or increases in infection rates during the period were detected. Evans et al. [153] report that in 92% of children with renal dysfunction after LT, MMF treatment provided safe and effective immunosuppression and allowed CSA or TAC to be discontinued or reduced, leading to improved renal function. At our center, 6 patients were switched to MMF due to CNI-related side-effects. All of them improved under MMF except for one.

The number of studies investigating mTOR inhibitor-based immunosuppression using either sirolimus (SIR) or everolimus (EVR) in children is still limited. The few published prospective randomized-controlled studies are limited by small patient cohorts and short follow-up periods [154-156]. Hocker et al. [157] compared SIR-based immunosuppression and CNI minimization in pediatric renal transplant recipients with chronic CNI nephrotoxicity. They concluded that in pediatric transplant recipients with declining kidney function due to CNI-induced nephrotoxicity, a switch to SIR-based therapy or CNI minimization were associated with a comparable improvement of GFR after 12 months of observation. The main adverse event under SIR therapy was transient hyperlipidemia in 70% of patients. No patient in either group experienced an acute rejection episode. Gibelli et al. reported on their experience with SIR in pediatric liver transplant recipients at a single-center. Out of 318 liver transplanted children, 13 were converted to SIR therapy because of TAC-related side effects. The indications were PTLD in 11 patients, nephrotoxicity and de novo AIH in one patient each. After conversion, no episodes of acute rejection were observed, PTLD recurred in one patient and one child developed hyperlipidemia. They concluded that conversion from TAC to SIR in selected pediatric liver transplant recipients was safe and especially children with PTLD may benefit from IS with SIR after LT. In our study, 11 patients were switched to an mTOR-inhibitor based regimen due to CNI-associated side-effects with an overall positive result and few mTOR-related side-effects.

A third strategy to reduce IS toxicity is the combination of CNI with adjunctive drugs. One example is the combination of CNIs and hydrochlorothiazide with a possible protective effect on both hypertension and renal function. Hoorn et al. [158] confirmed the hypothesis that CNIs induce hypertension by stimulating the renal sodium chloride co-transporter (NCC). They showed that TAC did not affect blood pressure in NCC-knockout mice, whereas the hypertensive response to TAC was exaggerated in mice overexpressing NCC. Therefore, they suggested reversing TAC-induced hypertension by adding an NCC-blocking drug like hydrochlorothiazide. The use of these inexpensive and well-tolerated thiazide diuretics was also started in the most recent patients of the study group at the UKT with the attempt to prevent the complications of CNI treatment. So far, this approach may prove to be effective in treating CNI-induced hypertension and hyperkalemia, but exact results need to be further evaluated. A different experimental approach in a rat model is described by Hoskova et al. [159] who discovered that TAC-induced hypertension and nephrotoxicity were attenuated by dual inhibition of the renin-angiotensin system (RAS). Their experiments led to the result that TAC-induced arterial hypertension in rats was prevented by dual RAS inhibition, using perindopril and losartan, as well as by a combination of amlodipine and metoprolol. However, significant nephroprotection (reduced albuminuria) was only observed in animals on dual RAS inhibition. In addition, histological analysis revealed that RAS inhibition noticeably diminished glomerulosclerosis and tubulo-interstitial injury which further supports the notion that RAS inhibitors display efficient renoprotective properties during CNI treatment.

Further research is needed to develop immunosuppressive strategies with improved toxicity profiles. Recent progress in the development of biologicals, i.e. antibodies and fusion proteins, allow precise targeting of the immune system, preventing the non-immune side effects encountered with current protocols. In particular, targeting of the two most important co-stimulation pathways critical for T-cell activation, i.e. B7/CD28 and CD40/CD40L, has

provided excellent results in many experimental models of organ transplantation. This has led to the clinical development of belatacept, a cytotoxic T-lymphocyte-associated antigen 4 immunoglobulin (CTLA4-Ig) fusion protein, which has proved to be efficient in preventing acute rejection in kidney transplant recipients [160]. Its use is associated with improved renal function and a better metabolic profile than CNIs. However, a trial analyzing efficacy in liver transplanted patients had to be stopped due to an increased prevalence of intracranial bleeding.

In summary, the establishment of novel operative techniques, the development of more effective immunosuppressants, an improved overall patient management and timing of transplantation as well as the decrease in the number of infections and the introduction of CMV prophylaxis and other additional medication may have contributed to a significantly improved patient and graft survival with a consecutive shift in focus on quality of life issues and adverse effects of the immunosuppressive therapy.

4.1. Limitations and benefits of this study

Our study has several limitations. Due to the retrospective nature of the study, a limited number of patient values are missing. Furthermore, a follow-up range from 1-7 years with a median of only 2 years is insufficient to completely estimate the long-term implications of CNI exposure on kidney function and other long-term side-effects. Lastly, the results of our single center study group, limited by its number of patients (50), cannot be easily transferred to other patient groups.

This study does, however, contribute to the increasing number of publications highlighting the changes in management of pLT in the last periods and the challenges in the administration of CNIs. It focuses on the discussion of relevant consequences for patient management and long term outcomes. One important contribution of this study is to demonstrate a change in the spectrum of post-

pLT complications in comparison with previous reports. Another important contribution is to highlight different approaches of CNI-free immunosuppression avoiding CNI- associated toxicity and thus allowing adequate and undisturbed child development and improved quality of life.

5. SUMMARY

Until recently, the postoperative course of pLT was complicated most frequently by surgical complications such as arterial or portal vein thrombosis and severe infections, leading to both graft and patient loss. The objective of this study is to demonstrate a significant shift in pLT-related complications to a dominance of CNI-associated side effects. Therefore, a retrospective chart review of 50 children (31 boys, 19 girls) was performed who underwent pLT between 2005 and 2011. Most common indications were biliary atresia in 52% and acute liver failure in 10%. Age at pLT ranged from 21 days-19years (median: 21 months), body weight was 3.6-79 kg (median 9.9 kg). Immunosuppressive regimen was based on tacrolimus (TAC) and tapered prednisolone. Full-size post-mortem grafts were transplanted in 28%, split grafts (left-lateral, left lobe or right lobe) and living- donation grafts in 36% each. Range of PELD was 0-41 (median: 16). Patient and graft survival rates improved over the last decades having reached 100% at the UKT from 2005-2011. The number of VCs with currently 18% (including HAT in only 1 patient) as well as the rate of rejections and infections decreased over the last years. BCs remain a constant problem occurring in 18 patients (36%), of which 28% were biliary anastomotic strictures. A literature review was done to show the development in the spectrum and number of complications in previous periods of pLT. In our study, 94 (69%) out of 136 complications were associated with CNI-treatment of which hypertension (64%) and nephrotoxicity (28%) were most prevalent. Other CNI-related side-effects included hematological or neuropsychiatric disorders, GI-symptoms, diabetes, food allergy or failure to thrive which at times required a discontinuation from CNIs and the introduction of alternative therapeutic regimens. This indicates that future management of patients after pLT needs to increasingly focus on strategies to reduce CNI-toxicity including the individual minimization and weaning, the use of alternative ISDs with beneficial toxicity profiles (MMF, mTOR inhibitors) or the combination of CNI with adjunctive drugs (e.g. HCTZ) to ensure adequate development and improved quality of life.

6. ZUSAMMENFASSUNG

Der postoperative Verlauf nach LT im Kindesalter wurde bis vor kurzem vorwiegend durch chirurgische Komplikationen wie z.B. Thrombosen oder Infektionen bestimmt, die zu Patienten- und Transplantatverlust führten. Ziel dieser Studie ist es eine signifikante Veränderung der post-LT Komplikationen mit zunehmender Dominanz CNI-assoziierter Nebenwirkungen aufzuzeigen. Dazu wurden die Daten der 50 Kinder (31 Jungen, 19 Mädchen), die von 2005 bis 2011 eine LT am UKT erhielten, retrospektiv analysiert. Die häufigsten Indikationen waren GA in 52% und ALV in 10%. Das Alter bei LT reichte von 21 Tagen bis 19 Jahren (Median: 21 Monate), das Körpergewicht lag zwischen 3,6-79 kg. Die Immunsuppression bestand aus Tacrolimus und Prednisolon. Ganze Organe von post mortem Spendern wurden in 28% transplantiert, Teil-Transplantate bzw. Lebendspenden in jeweils 36%. Sowohl Patienten- als auch Transplantatüberleben haben sich in den letzten Jahrzehnten deutlich verbessert mit aktuell jeweils 100% im Zeitraum von 2005-2011 am UKT. Dies wurde erreicht durch einen Rückgang in der Anzahl von Gefäßkomplikationen (18%) sowie Infektionen und Abstoßungsreaktionen. Komplikationen der Gallenwege bleiben ein anhaltendes Problem in 36% der Patienten. In einer Literaturrecherche wurden das Spektrum und die Anzahl an Komplikationen nach LT in der Vergangenheit analysiert. Von den beobachteten 136 Komplikationen waren 94 (69%) mit der CNI-Therapie assoziiert, wobei Bluthochdruck (64%) und Nierenschädigung (28%) am häufigsten waren. Als weitere CNI-assoziierte Komplikationen wurden z.B. neuropsychiatrische oder hämatologische Störungen, Diabetes oder Nahrungsmittelallergien beobachtet. Daher wurde z.T. eine Umstellung der Immunsuppression auf eine CNI-freie Therapie nötig. Diese Ergebnisse zeigen, dass die Patientenbehandlung nach LT künftig das Ziel haben muss CNI-Komplikationen zu verhindern. Möglich wäre z.B. der Gebrauch alternativer Immunsuppressiva wie mTOR-Inhibitoren oder die Ergänzung der CNI-Therapie durch andere Medikamente (z.B.HCTZ). Nur so kann eine adäquate Entwicklung und Lebensqualität von LT-Patienten gewährleistet werden.

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10. Erklärung zum Eigenanteil

Hiermit erkläre ich, dass ich die der Medizinischen Fakultät der Universität Tübingen zur Promotion eingereichte Arbeit mit dem Titel:

„Complications following pediatric liver transplantation
– a change of paradigm“

selbstständig und ohne unzulässige Hilfe Dritter oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus fremden Quellen wörtlich oder inhaltlich übernommenen Gedanken sind als solche kenntlich gemacht. Die Daten sowie die Literaturrecherche, welche zu den Ergebnissen der vorliegenden Analyse führten, habe ich selbstständig erfasst und ausgewertet. Das Manuskript sowie die darin enthaltenen Tabellen und Abbildungen wurden von mir erstellt.

Bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskriptes habe ich Unterstützung von Dr. med. Ekkehard Sturm erhalten. Er hat die Arbeit betreut, war an der Entwicklung der Fragestellung und Konzeption der Arbeit beteiligt, hat inhaltliche und formale Hilfestellung gegeben und das Manuskript korrigiert. Herr Prof. Handgretinger hat die Arbeit als Doktorvater mitbetreut.

Die vorgelegte Dissertation wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Tübingen, 02. Februar 2015 Mirjam Böckle

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