

Transplant Institute, Sahlgrenska University Hospital
Institute of Clinical Sciences at Sahlgrenska Academy
University of Gothenburg, Göteborg, Sweden

Renal Function after Transplantation of the Liver and Intestine

GUSTAF HERLENIUS

Academic dissertation



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“The natural desire of good men is knowledge”

Leonardo da Vinci



“The Aztecs made such a virtue of cleanliness that their superficial operations were probably more successful than those in Europe until our own century. On the other hand, Aztec thinking was dominated by abstract religion”.

“It was believed that the gods had to be kept alive by continual offerings of blood and hearts from sacrificed people.”

This might, at best, be called the discovery of divine transfusion and transplantation.

From:

Knut Haeger: The Illustrated History of Surgery, page 11

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ABSTRACT

Renal Function after Transplantation of the Liver and Intestine

Gustaf Herlenius

Transplant Institute, Sahlgrenska University Hospital

Institute of Clinical Sciences at Sahlgrenska Academy, University of Gothenburg

Background: Chronic kidney disease (CKD) after liver (LT) or intestinal (IT) transplantation may decrease patient survival. Calcineurin inhibitors (CNI) play a major role in its development.

Aims: Describe long term renal function and risk factors for developing CKD in adults and children after LT and IT. Investigate if CNI discontinuation in adults after LT improves renal function.

Methods: GFR was measured (GFR_m) with either Iohexol or 51-Cr EDTA-clearance in both adults and children at different intervals before and after LT and IT.

Results: After LT in adults (I), GFR_m decreased with 42% after 10 years. Prevalence of CKD increased over time: 12% at 5 years and 29% at 10 years. Eight patients (5%) required renal replacement therapy (RRT). Baseline GFR_m correlated poorly with late renal function. GFR_m at 3 months post-LT correlated well with GFR_m at 5 years and GFR_m below 30 ml/min/1.73m² at 3 months was a risk factor for CKD at 5 years. After IT (II) CKD was almost universal. RRT was required in 20% of the patients. Calculated GFR (MDRD equation) overestimated GFR_m with 30-40%. Children undergoing LT (III) stabilized their renal function after an initial decline. None required RRT. Age above 2 years at LT, hepatic malignancies or metabolic liver diseases as the cause for LT were risk factors for developing CKD. A CNI discontinuation protocol (IV) in 25 adult patients with severe CKD was used with either MMF (n=13) or SRL (n=12). Baseline GFR_m (n=25) was 31±8 ml/min/1.73m². At 3 months GFR_m (n=23) increased to 40±10 ml/min/1.73m² (p=0.0001). There was no significant difference when comparing the MMF and the SRL study arms. Patients (n=8) with baseline GFR_m below 30 ml (CKD stage IV) increased GFR_m at one year with 63% (p=0.003). Patients in the SRL group presented a higher incidence of oral ulcerations and hypertriglyceridemia. Two deaths were reported both probably unrelated to the change in immunosuppression. No biopsy proven rejection episodes occurred.

Conclusion: CKD is a frequent complication after LT and IT. Early renal function may identify patients at risk of developing CKD. CNI discontinuation under the protection of either MMF or SRL was safe and GFR_m increased significantly under the observational period.

Keywords: adult liver transplantation, pediatric liver transplantation, intestinal transplantation, multivisceral transplantation, immunosuppression, calcineurin inhibitors, glomerular filtration rate, renal function, nephrotoxicity, chronic kidney disease, renal replacement therapy, mortality

LIST OF PAPERS

Paper I

Early renal function post-liver transplantation is predictive of progressive chronic kidney disease. Herlenius G, Fistouris J, Olausson M, Felldin M, Bäckman L, Friman S.

Scand J Gastroenterol. 2008 Mar; 43(3):344-9

Paper II

Chronic kidney disease--a common and serious complication after intestinal transplantation. Herlenius G, Fägerlind M, Krantz M, Mölne J, Olausson M, Gäbel M, Friman V, Oltean M, Friman S.

Transplantation. 2008 Jul 15; 86(1):108-13.

Paper III

Stable long term renal function after pediatric liver transplantation.

Herlenius G, Hansson S, Krantz M, Olausson M, Kullberg-Lindh C, Friman S

Pediatric Transplantation. In press

Paper IV

Conversion from calcineurin inhibitor to either MMF or sirolimus improves renal function in liver transplant recipients with chronic kidney disease- results of a prospective randomized trial. Herlenius G, Felldin M, Gustafsson B, Olausson M, Bäckman L, Nordén G, Friman S.

Submitted

ABBREVIATIONS

AE	adverse event
ALF	acute liver failure
AUC	area under the curve
ALD	alcoholic liver disease
AZA	azathioprine
BPAR	biopsy proven acute rejection
C&G	Cockcroft & Gault equation
CCr	calculated creatinine clearance
CIPO	chronic intestinal pseudoobstruction
CKD	chronic kidney disease
CLD	chronic liver disease
CNI	calcineurin inhibitors
CsA	Cyclosporine A
ELTR	European Liver Transplant Registry
ERL	everolimus
ERPF	effective renal plasma flow
ESLD	end stage liver disease
FHF	fulminant hepatic failure
GFR	glomerular filtration rate
GFR _c	calculated GFR
GFR _m	measured GFR
HBV	hepatitis B
HCV	hepatitis C
HD	hemodialysis
HM	hepatic malignancies
HRS	hepatorenal syndrome
IF	intestinal failure
IL-2	interleukin- 2
IS	immunosuppression
IT	intestinal transplantation
KDOQI	Kidney Disease Outcome Quality Initiative
KT	kidney transplantation
LIT	liver and intestinal transplantation
LT	liver transplantation
MDRD	modification of diet in renal disease
MELD	Mayo End Stage Liver Disease
MMF	mycophenolate mofetil
mTOR	mammalian target of rapamycin
MV	multivisceral transplantation
NEPT	neuroendocrine pancreatic tumor
NKF	National Kidney Foundation
NLTR	Nordic Liver Transplant Registry
PN	parenteral nutrition

RRT	renal replacement therapy
SBS	short bowel syndrome
SD	secretory diarrhea
SRL	sirolimus
Tac	Tacrolimus
UNOS	United Network for Organ Sharing
TPN	total parenteral nutrition

CONTENTS

ABSTRACT	5
ABBREVIATIONS.....	7
CONTENTS	9
1. INTRODUCTION.....	11
HISTORICAL ASPECTS OF LIVER AND INTESTINAL TRANSPLANTATION	12
Liver transplantation.....	12
Intestinal transplantation.....	14
CNI EXPOSURE AND RENAL TOXICITY	15
MYCOPHENOLATE MOFETYL AND SIROLIMUS.....	17
PREVALENCE, RISK FACTORS AND IMPACT OF CHRONIC KIDNEY DISEASE AFTER TRANSPLANTATION OF NONRENAL ORGANS.....	17
CHRONIC KIDNEY DISEASE AFTER PEDIATRIC LIVER TRANSPLANTATION.....	22
CHRONIC KIDNEY DISEASE AFTER INTESTINAL TRANSPLANTATION	22
TYPE OF CALCINEURIN INHIBITOR AND RISK FOR DEVELOPING CHRONIC KIDNEY DISEASE	23
ASSESSMENT OF RENAL FUNCTION	23
ASSESSMENT OF RENAL FUNCTION IN CHILDREN AFTER LIVER TRANSPLANTATION.....	24
RENAL SPARING IMMUNOSUPPRESSIVE PROTOCOLS.....	25
CNI AVOIDANCE PROTOCOLS	25
INDUCTION PROTOCOLS.....	26
CNI MINIMIZATION AND DISCONTINUATION PROTOCOLS.....	27
MYCOPHENOLATE MOFETIL AS PROTECTION.....	27
SIROLIMUS OR EVEROLIMUS AS PROTECTION.....	28
2. AIMS OF THE THESIS.....	30
3. PATIENTS & METHODS.....	31
Paper (I): Patients.....	31
Paper (II): Patients	31
Paper (III): Patients	31
Paper (IV): Patients.....	32

Tables 3-6 patient characteristics of patients in Papers I-IV	33
Immunosuppression.....	37
Paper (I): Immunosuppression.....	37
Paper (II): Immunosuppression.....	37
Paper (III): Immunosuppression	37
Paper (IV): Immunosuppression.....	38
GFR measurements.....	39
Paper (I – IV) GFR measurement and Follow-up.....	39
Classification of Chronic Kidney Disease.....	40
Papers (I - IV): classification of CKD	40
Statistical Methods	41
Papers (I, III, IV).....	41
4. RESULTS	42
5. DISCUSSION	46
PREVALENCE OF CKD AND REQUIREMENT OF RENAL REPLACEMENT THERAPY AFTER LIVER TRANSPLANTATION IN ADULTS AND CHILDREN	46
PREVALENCE OF CKD AND THE REQUIREMENT OF RENAL REPLACEMENT THERAPY AFTER INTESTINAL TRANSPLANTATION	48
PATIENTS AT RISK OF DEVELOPING CHRONIC KIDNEY DISEASE AFTER TRANSPLANTATION	49
Liver transplant recipients	49
Intestinal transplant recipients	51
CONSEQUENCES OF CHRONIC KIDNEY DISEASE AFTER TRANSPLANTATION OF A NON RENAL ORGAN.....	52
Liver transplantation.....	52
Intestinal transplantation.....	54
MINIMIZING THE RISK OF CHRONIC KIDNEY DISEASE AFTER TRANSPLANTATION OF A NON RENAL ORGAN	55
Pre transplant setting	55
Preemptive kidney transplantation	56
Post transplant setting.....	57
6. CONCLUDING REMARKS	62
ACKNOWLEDGEMENTS.....	63
REFERENCES	67

Key words:

adult liver transplantation, pediatric liver transplantation, intestinal transplantation, multivisceral transplantation, immunosuppression, calcineurin inhibitors, glomerular filtration rate, renal function, nephrotoxicity, chronic kidney disease, renal replacement therapy, mortality

1. INTRODUCTION

Liver transplantation and transplantation of the intestine have become the treatment of choice for patients with end stage liver disease and for patients experiencing intestinal failure along with the life threatening complications of parenteral nutrition.

The last four decades of advances in surgical technique, patient selection, organ preservation, post transplant intensive care, prevention of opportunistic infections and the discovery of new immunosuppressive drugs and strategies have dramatically improved long- term survival rates. A second positive outcome is the excellent quality of life enjoyed by patients. As a consequence, the focus of care has shifted from the prevention of rejection and surgical complications to the identification and avoidance of factors that may have a negative impact on the long term outcome and survival of these patients.

Long- term immunosuppression with the nephrotoxic calcineurin inhibitors (CNI) cyclosporine A (CsA) and tacrolimus (Tac) significantly increases the risk that a patient will develop chronic kidney disease after transplantation. In the literature there is an increasing body of evidence indicating that the presence of chronic kidney disease after transplantation of a non renal organ has a profoundly negative effect on the long- term survival after transplantation. Therefore, there is currently an urgent need to increase our knowledge about the prevalence of and the risk

factors associated with the development of renal dysfunction and to develop safe and efficient immunosuppressive strategies to prevent, halt or even reverse the progression of calcineurin induced chronic kidney disease.

This study addresses the long- term renal function of adult and pediatric patients who have received hepatic or intestinal allografts. Our aim was to see if it was possible at an early stage to identify patients likely to develop renal dysfunction over the long- term. Also, we implemented a strategy to eliminate the calcineurin inhibitor from the immunosuppressive protocol in adult patients with renal dysfunction after LT to evaluate whether this would improve kidney function while not increasing the risk for acute rejection or severe adverse events.

HISTORICAL ASPECTS OF LIVER AND INTESTINAL TRANSPLANTATION

Many factors have contributed to the success of organ transplantation. Innovations in surgical techniques such as Alexis Carrel's (Carrel 1902) vascular anastomosis and the first successful kidney transplant (KT) by the American Surgeon Joseph Murray (1954) showed the feasibility and potential benefits of organ replacement. In spite of the advances in the surgical field, the monumental hurdle of the immunological barrier still remained. The first firm evidence that transplantation of tissues and organs between individuals that were genetically dissimilar would lead to organ rejection was discovered during World War II by Brazilian- born British zoologist Peter Medawar. Medawar, together with the plastic surgeon Thomas Gibson, was asked by the British government to study why skin grafts applied to the burns of British fighter pilots failed to engraft permanently. They developed a rabbit model to study skin grafting and they were the first to suggest that the immune system was responsible for the destruction of the skin allograft (Gibson 1943; Langnas 2008). Eventually, in 1952, the system of transplant antigens, also known as histocompatibility antigens, was described by a French scientist, Jean Dausset, and his American counterpart, George Snell. The era of clinical and transplant immunosuppression (IS) began with the discovery of antiproliferative agents such as 6-mercaptopurine (1951) by Gertrude Elion and George Hitchins. Its prodrug, azathioprine (AZA), was introduced in 1957. This brought about major advances in clinical KT.

These breakthroughs in the field of immunology in combination with the discovery of drugs with immunosuppressive properties were the main factors that made transplanting organs and tissues possible. Sir Peter Medawar eloquently expressed how the advent of these new drugs would influence the field of clinical transplantation with the words, "immunosuppression is what gives this surgical endeavor its specificity".

Liver transplantation

Discoveries regarding immunosuppression in the early 1960's were crucial in the advancement of liver transplantation. The introduction of steroids in canine models of KT showed that acute rejection could be reversed with large doses of prednisone (Marchioro 1964). The observation that the mean survival time of the canines doubled when they were treated with AZA prior to LT (Starzl 1964) was also of major importance. By the late 1960's the first case where a human

recipient achieved a prolonged survival after an orthotopic liver transplant (OLT) was reported by Dr. Thomas E. Starzl (Starzl 1968). Despite these few initial successes, the 1 year patient survival rate was less than 30% and LT remained an experimental procedure (Ronald W. Busuttil 2005). It was not until the early 1980's, with the introduction of Cyclosporine A (CsA) in England by Sir Roy Calne (Calne 1979; Calne, Rolles et al. 1979) together with the use of anti-lymphocyte preparations and the monoclonal antibody OKT-3 that it was possible to balance the therapeutic benefits and toxic effects of IS. After the introduction of CsA, these experimental procedures evolved to become viable therapeutic options with predictable clinical outcomes. Tacrolimus (Tac), a new macrolide compound isolated from a soil fungus found in Japan (*Streptomyces tsukubaensis*) was found to possess potent IS properties. It was hoped that this agent would further reduce the incidence of rejection and morbidity after LT.

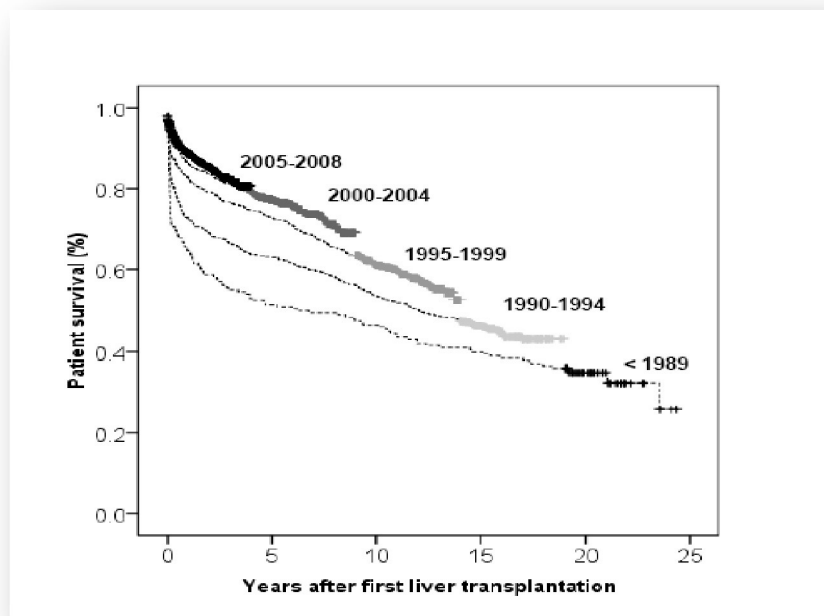


Fig.1 Patient survival from The Nordic Liver Transplant Registry (www.scandiaintransplant.org.)

Most of the initial experience with Tac came from its use in LT. Prospective randomized multicenter trials in Europe (Bechstein, Neuhaus et al. 1996) and in the USA (The U.S. Multicenter FK506 Liver Study Group 1994) found a comparable patient and graft survival between CsA and Tac; however the incidence of acute, steroid resistant and refractory rejection were significantly lower with Tac.

The addition of Tac to the immunosuppressive armamentarium has been a major step forward in the field of liver transplantation. Currently more than 4000 patients have received liver transplants in the Nordic countries. According to the Nordic Liver Transplant Registry (NLTR-www.scandiaintransplant.org) the one and five-year patient survival after LT are 88% and 78% respectively (2001-2008, n= 1863). The corresponding one and five year patient survival rates from the European Liver Transplant Register (ELTR) and, The United Network for Organ Sharing (UNOS) in the United States appear in Table 1.

Patient survival				
	1 year	5 years	n	Date accessed
ELTR	82 %	71 %	74 534	December 2008
UNOS	88 %	74 %	98 000	November 2009

Table 1. Patient 1 and 5-year survival rates from the liver transplantation registries in Europe (**ELTR**), and the USA (**UNOS**).

www.ELTR.org & www.UNOS.com

Today, thanks to the advances in surgical technique, anesthesia, and intensive care, modern IS, and prophylaxis against opportunistic infections, liver transplantation has evolved into a well established therapeutic option available for those patients with end stage liver disease (ESLD) for whom there is no other treatment available.

Intestinal transplantation

Transplantation of the intestine (IT) either isolated, or in combination with other organs such as the liver (LIT) or liver, pancreas and stomach as a multivisceral (MV) graft, represents one of today's paramount challenges in clinical transplantation. The evolution in this particular field of transplantation mirrors to a great extent the experience with LT in the sense that improvement in patient and graft survival has been coupled with the discovery of more efficient and safer IS drugs. Tac has played a fundamental role in this sense.

Prior to the introduction of Tac, the initial experience with IT was, to say the very least, discouraging. Initial reports from Europe and the USA showed a more than a 90% graft loss due to rejection as well as a forbiddingly high patient mortality rate due to infections and post

transplant lymphoproliferative disease (PTLD) (Okumura and Mester 1992; Reyes, Bueno et al. 1998). These discouraging results led to a moratorium for the few active programs on both continents. When Tac was introduced in 1989, IT transplantation began to evolve into what it is today, a widely performed and successful procedure offering the patient good quality of life and in close to 80% of the cases independence from parenteral nutrition (PN) and intravenous fluids. (Sudan, Iverson et al. 2000; Abu-Elmagd K 2002; Grant, Abu-Elmagd et al. 2005; Langnas 2008).

Current results from the Intestinal Transplantation Registry show that close to 2000 patients worldwide have received intestinal allografts. The one and five-year patient survival rates are close to 80% and 60%. Centers of excellence demonstrate 1 and 5 year patient survival rates of 95% and 77% respectively, their ongoing investigations focus on lowering long-term causes of graft loss such as chronic rejection (Mazariegos, Squires et al. 2009). These results are now comparable to LT results and superior to long-term lung transplantation survival rates. (www.intestinaltransplant.org and international symposium of intestinal transplantation, September 2009, Bologna, Italy).

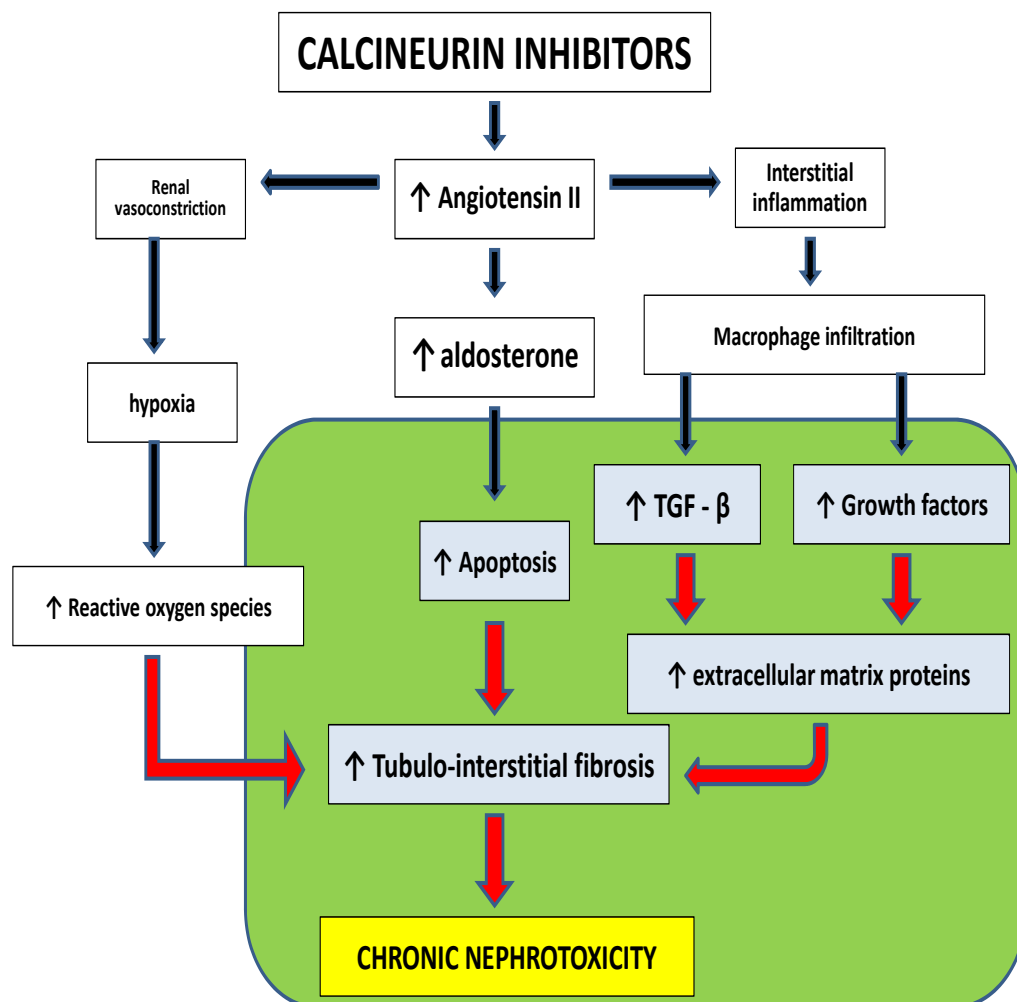
CNI EXPOSURE AND RENAL TOXICITY.

Renal dysfunction after transplantation of a non renal organ is multifactorial. The deranged homeostasis inherent in end stage liver disease in combination with drug toxicity sets the stage for a progressive and, in many cases, unrelenting deterioration of renal function.

The patient with established liver cirrhosis and portal hypertension presents a splanchnic vasodilatation manifested as a relative hypovolemia and renal hypoperfusion. This renal insult may be further accentuated by other events such as sepsis, gastrointestinal hemorrhage, sepsis secondary to spontaneous bacterial peritonitis, and the use of nephrotoxic drugs and contrast dyes (Gines and Schrier 2009). In addition, physiological events induced by the transplant procedure itself may aggravate the ensuing renal dysfunction. Hemorrhage and the ischemia reperfusion injury activate polymorph neutrophils which creates on the surface of the endothelium a prothrombotic and proinflammatory environment. Along with the cascade involving depletion of nitrous oxide and oxidative stress, there is extensive cellular damage and a profound imbalance in both systemic and renal circulatory regulatory mechanisms. These series of events may result in a pronounced systemic inflammatory response that can lead to renal and multiorgan failure (Charlton, Wall et al. 2009). The nephrotoxicity of the CNI's is enhanced in a milieu like this.

Although biochemically distinct, CsA and Tac have similar action mechanisms as well as a similar pattern of nephrotoxicity. Acute CNI nephrotoxicity is dose dependent and the clinical manifestation most commonly seen is a functional decrease in renal blood flow and GFR mediated primarily by an afferent arteriolar vasoconstriction (Yokoyama, Hayakawa et al. 1995). Toxicity usually resolves within 24-48 Hrs after dose reduction. Chronic nephrotoxicity associated with prolonged CNI exposure has been associated with the development of glomerular afferent arteriolar hyalinosis, tubular atrophy and interstitial fibrosis (Myers, Ross et al. 1984; Laine, Krogerus et al. 1994; Campistol JM 2000). Vasoconstriction at the arteriolar level is primarily induced by a CNI mediated increase in the production of endothelin-I and

angiotensin II, enhanced norepinephrine release from sympathetic nerve terminals (Moss, Powell et al. 1985) and an imbalance in the homeostasis in the prostaglandin-thromboxane cascade (Campistol JM 2000). Additionally, matrix accumulation is observed as a consequence of a CNI mediated increase in angiotensin resulting in an upregulation of profibrotic molecules such as transforming growth factor- β and endothelin-1 (Textor, Burnett et al. 1995; Johnson DW 1999). Matrix accumulation may be further enhanced by an inhibition of matrix degradation by a deficient metalloproteinase activity (Johnson DW 1999; Esposito, Fornoni et al. 2000). These mechanisms have also been identified in kidneys after renal transplantation and in animal models of renal ischemia (Bicknell, Williams et al. 2000; Jain, Bicknell et al. 2000). Ultimately, these multiple pathways result in progressive interstitial fibrosis, glomerular injury and tubular atrophy leading to progressive proteinuria (Myers, Ross et al. 1984). The presence of proteinuria in itself may further enhance the vicious circle for the development and progression of interstitial fibrosis (Nangaku, Pippin et al. 1999; Wang, LaPage et al. 2000).



Adapted from Charlton et al Liver transplantation, vol 15. No 11, S-11 Nov 2009.

MYCOPHENOLATE MOFETYL AND SIROLIMUS

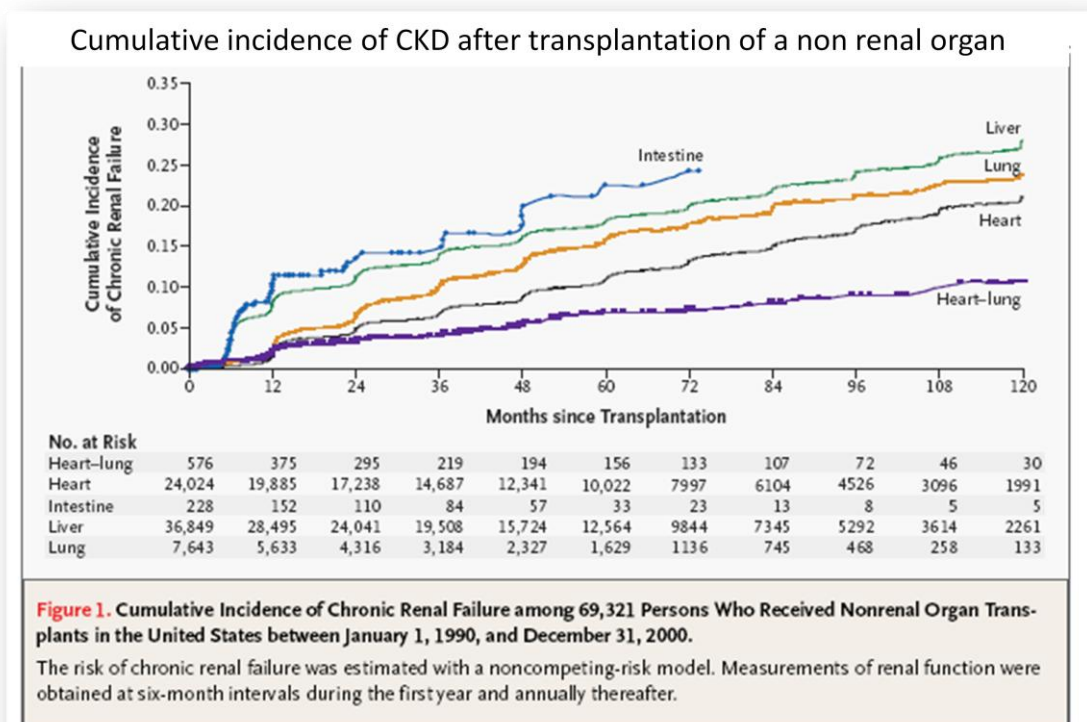
There are other immunosuppressive drugs that have been discovered and which represent a substantial progress in maintenance immunosuppression after organ transplantation. One of these drugs is mycophenolate mofetil (MMF) a semisynthetic ester prodrug of mycophenolic acid (MPA), which is a reversible inhibitor of the inosine-monophosphate-dehydrogenase required for the de novo synthesis of guanine nucleosides. The central immunological effect of MPA resides in the inhibition in the production of variety of cytokines, glycosilation of adhesion molecules in leukocytes and the proliferation of arterial smooth muscle cells (Lipsky 1996). MPA has also been seen in vitro to exert antiviral and antifibrotic effects (Klupp, Bechstein et al. 1997; Bahra, Neumann et al. 2005). The benefits of this drug were initially observed in KT and heart transplants. Due to its lack of nephrotoxicity the drug was subsequently adopted in a variety of protocols for liver, pancreas and intestinal transplantation. MMF is usually used as a component of a multidrug regimen and recently after LT in patients with CNI induced CKD aiming to either reduce or discontinue CNI (Barkmann, Nashan et al. 2000; Schlitt, Barkmann et al. 2001; Orlando, Baiocchi et al. 2007; Farkas 2008) .

Other drugs with interesting properties and the potential of offering the possibility to reduce or eliminate the CNI's, are the mammalian target of rapamycin (mTOR) inhibitors. These drugs are represented by the macrocyclic lactone rapamycin or sirolimus (SRL) and its more polar derivative everolimus (ERL). SRL and ERL exhibit a similar mode of action but have a different pharmacokinetic behavior. SRL inhibits the response to interleukin 2 (IL-2) and thereby blocks T- and B-lymphocyte activation by binding to FK-binding protein-12 in a manner similar to Tac. However, unlike the Tac-FKBP-12 complex which inhibits calcineurin, the sirolimus FKBP-12 complex binds directly to the mTOR also called FKBP-rapamycin associated protein kinase (FRAP), and it acts as a key regulator of cell proliferation, growth and apoptosis. FRAP kinase plays a crucial role in the progression of the cell cycle from G1 to S phase (Formica, Lorber et al. 2004; Marti and Frey 2005). SRL has been shown to prevent acute rejection episodes and to decrease steroid or CNI exposure. In many studies it has been shown to be effective as a substitute for or in combination with CNI's. SRL and ERL are devoid of nephrotoxic properties when exposed to the healthy quiescent kidney or when not used in combination with CNI's. There is however, a certain amount of evidence that these drugs may enhance CNI nephrotoxicity (Marti and Frey 2005). The same antiproliferative properties that confer their immunosuppressive properties may interfere with mechanisms of for repair of tissue injury and may therefore exacerbate pre-existing renal damage. Nevertheless, several studies have shown that despite these properties, SRL may be a helpful adjuvant drug in the pursuit of CNI-sparing or discontinuation regimens (Fairbanks, Eustace et al. 2003; Farkas 2008; Flechner 2008; De Simone P 2009)

PREVALENCE, RISK FACTORS AND IMPACT OF CHRONIC KIDNEY DISEASE AFTER TRANSPLANTATION OF NONRENAL ORGANS

The chronic use of CNI after transplantation of non renal organs has been strongly linked to the development of chronic kidney disease (CKD) (Dagöo T 1997; Fisher, Nightingale et al. 1998; Cohen A.J 2002; Davis, Gonwa et al. 2002; Gonwa 2003; Moreno, Cuervas-Mons et al. 2003;

Morard 2006; Aberg, Koivusalo et al. 2007). CKD complicates medical management as it results in increased morbidity, mortality and costs (Brown, Lombardero et al. 1996; Cohen A.J 2002). There are currently several reports on the impact of CKD on long-term patient survival. A large population based-cohort study (n=69 321) performed in the USA to investigate the incidence of severe CKD (defined as a calculated GFR of 29 ml/min/1.73m² or less) after transplantation of a non renal organ (heart, lung, liver and intestine) revealed that the cumulative incidence of CKD was 16.5% after a median follow-up of 36 months (Ojo, Held et al. 2003). Furthermore, the authors found that 29% of these patients required renal replacement therapy (RRT) either as hemodialysis or KT. Additionally, the five-year risk of chronic renal failure varied according to the type of organ transplanted - from 6.9 % among recipients of heart-lung transplants to 21.3 % among recipients of intestine transplants. Recipients of liver allografts were reported to have a cumulative incidence of CKD close to 20% second only to the recipients of intestinal transplants. One of the main findings of this study was that the presence of CKD increased the risk of death by a factor of four.



Ojo AO, Held PJ, Port FK, et al. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; 349 (10): 931.

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Other reports of CKD after liver transplantation in the adult population have demonstrated a high frequency of renal dysfunction (Gonwa, Klintmalm et al. 1995; Dagöo T 1997; Lafayette, Pare et al. 1997; Gonwa, Mai et al. 2001; Cohen, Stegall et al. 2002; Nair, Verma et al. 2002; Gonwa 2003; Pawarode, Fine et al. 2003; Burra, Senzolo et al. 2009; Charlton, Wall et al.

2009). However, there is a great variability among these reports most likely due to the different definitions of CKD used.

Cohen et al. from the US defined CKD as a measured GFR of less than 40 ml/min/1.73m²CKD and reported a prevalence of 27% at 5 years (Cohen 2002). A European multicenter study using calculated GFR and defining CKD as a GFR below 60 ml/min/1.73 m² found a prevalence of 35% at 5 years (Burra, Senzolo et al. 2009). Another European group has reported a prevalence of mild to moderate CKD defined as a serum creatinine between 125-250 mmol/L at 5 years after LT of 78 % (Fisher, Nightingale et al. 1998).

The high variability of the prevalence of CKD can be explained by methodological differences in assessing renal function as well as differences in definition of CKD or acute kidney injury (Barri, Sanchez et al. 2009). However, an additional factor that may reflect a true difference in the population under scrutiny can be geographical and endemic differences for example of hepatitis C infection which ultimately may influence the prevalence of CKD (Burra et al., Ojo, Åberg, Manrique 2008).

Possible risk factors that may contribute to the development of CKD after transplantation of a non renal organ have been explored in many studies. Well recognized risk factors such as diabetes mellitus, atherosclerotic vascular disease and arterial hypertension have been described as well as other transplantation specific variables such as type and duration of CNI treatment, the indication for LT, age, baseline renal function, post transplant acute kidney injury and gender. A summary of some of the most important studies exploring these and other potential risk factors for developing CKD after LT is presented in Table 2.

In the previously mentioned study by Ojo et al. (Ojo, Held et al. 2003) a series of general risk factors for CKD were identified which were significant irrespective of the organ transplanted. These risk factors were age, gender, pretransplantation GFR, diabetes mellitus and hepatitis C infection. For the LT recipients in particular, all of the aforementioned risk factors were significant. CsA –based IS was also significant, as well as the era of transplantation. CsA-based IS was associated with an increased relative risk of CKD (RR: 1.25).

Lafayette and coworkers in an analysis of 115 LT patients found that the presence of renal dysfunction (defined as elevation of serum creatinine >1.0 mg/dl) prior to transplantation was the strongest predictor of patient survival and post transplant renal function. None of the other pre or perioperative factors were predictive of death or renal dysfunction. Half of the patients had renal dysfunction defined as a serum creatinine above 1.5 mg/dl after LT (Lafayette, Pare et al. 1997).

In a retrospective study from Birmingham, UK, by Fisher et al., which was based on 883 LT patients, a high prevalence of mild (above 125 µmol/L) to moderate (above 250 µmol/L) CKD at 5 years (78%) was reported. Elevated serum creatinine levels at 3 months and 1 year post LT were risk factors for developing severe CKD. The authors could also show that elevated CsA levels at 1 month and a high cumulative dose at 5 years were risk factors for the development of late-onset CKD (Fisher, Nightingale et al. 1998).

Table 2. Studies of risk factors for development of CKD

Author	Study	Method	Predictive variable (s)	Endpoint(s)	Prevalence CKD	n
Ojo et al.	Population based cohort study retrospective	GFRc	Type of organ transplant Pre transplant GFR Post transplant acute renal failure DM, AHT, race, gender, Csa, HCV Era of transplantation	Severe CKD GFR < 29 ml/min/1.73m ²	18% (5 years)	36 849
Campbell et al.	Retrospective	S-creatinine	Duration of renal dysfunction pre LT, cause of liver disease, DM, bilirubin, INR	6 & 12 month S-creatinine	50%	69
Morard et al..	Retrospective	GFRc	CNI levels (Csa & Tac) at 1 and 5 years	< 50 ml/min at 5 years	11% (5 years)	134
Gonwa et al.	Retrospective	GFRm	Presence of HRS pre LT	RRT post LT, long term function (3 years)	10% (HRS) 0.8% (no HRS)	300
Lafayette et al.	Retrospective	S-creatinine	↑creatinine (>1.0 mg/dl)	Acute renal failure	23%	115
Fisher et al.	Retrospective	S-creatinine	↑ creatinine 3 & 6 months post LT Age, CMV infection, re LT Csa levels	S-creat. >125 mmol/L S-creat >250 mmol/L	>125mmol/L:78% >250mmol/L: 4%	883
Cohen et al.	Retrospective	GFRm	Correlation of GFRm at 1 year post LT with GFRm at 3 years (r: 0.72)	CKD < 40ml/min/1.73m ²	27% (5 years)	353
Burra et al.	Pro & retrospective	GFRc	HCV (+), gender, baseline creatinine & GFR→CKD at 1 yr 1 yr GFR→GFR at 5 yrs	GFR<60 ml/min/1.73m ² at 1 & 5 years	1 year:35% 5 year: 36%	396
Åberg et al.	Retrospective	GFRc	Acute liver failure (ALF) Chronic liver disease (CLD) Liver tumor (LT)	CKD<60 ml/min/1.73m ²	ALL:16% (10 yrs) CLD 46% (5 yrs) ALF:26% (5yrs) RRT: n=7	396
Barri et al.	Retrospective	S-creatinine & GFRm	Acute Kidney Injury (3 definitions)	GFRm < 30 ml/min/1.73m ² at 1 and 2 years post LT	19% (1 year) 20% (2 years)	1050

Cohen et al. from the Mayo clinic in Rochester reported on 191 patients followed with iothalamate clearance as a measure of renal function. Renal dysfunction defined as a GFR below 40 ml/min/1.73m² was present in 27% of the patients at 5 years after LT.

The cumulative incidence for the requirement of RRT in the whole group was 10% at 10 years. They could also demonstrate a significant correlation with GFR_m at one year and late renal function. Baseline GFR correlated poorly with GFR at 3 years (r: 0.3). In summary, this important study demonstrated that pretransplantation renal impairment did not reliably predict long-term renal failure; there was no difference between the two commonly used CNI's with respect to nephrotoxicity and late onset renal failure could be predicted by renal function at one year (Cohen, Stegall et al. 2002).

A recent European multicenter study reported by Burra et al. investigated the prevalence of CKD after LT. CKD stage III of the NKF classification at one and five years were the major endpoints of the study. A sub analysis was done to investigate the possible influence of hepatitis C infection (HCV) on the prevalence of CKD. Prevalence of CKD was 35% and 36% at one and five years respectively (n=406 and n=203). Median GFR was significantly lower in the HCV (+) patients at both time points. Multivariate analysis identified baseline serum creatinine, gender and HCV (+) as significant predictors of GFR at 1 year, however, hepatitis C positivity was the only significant risk factor irrespective of gender. One year GFR was predictive of GFR at 5 years.

One of the few studies that has specifically addressed the underlying liver disease as a risk factor for development of CKD is one by Åberg et al. from Helsinki, Finland. Patients (n=396) were divided into cohorts according to underlying disease; chronic liver disease (CLD, 70%), acute liver failure (ALF, 23%) and hepatic malignancies (HM, 7%). GFR_c (C&G) was utilized and renal dysfunction was classified according to the NKF/KDOQI guidelines. GFR decreased significantly compared to baseline in all cohorts during the first year post LT. During this first year the incidence of severe CKD (stage 4) decreased only in the group with acute liver failure. The authors speculated as to whether this effect was secondary to a hepatorenal component, thus implying reversibility, in the acute liver failure group. In the group with ALF 65% of the patients achieved a substantial improvement with a GFR above 60 mls/min/1.73m² at 1 year after transplantation. The corresponding improvement in the group with chronic liver disease was only 27%. The tumor group showed a progressive decline in GFR over time. Notably the incidence of CKD at 5 years (9.7%) was lower than the one reported by Ojo at five years post LT. The authors discussed as to whether this discrepancy could possibly have been explained by comparing results between a national registry and outcomes from a single center study and the high proportion of patients with ALF in the Finnish study. Another possibility which might have contributed to the difference in cumulative incidence of CKD between these studies could have been the lower prevalence of hepatitis C in the series from Finland. Hepatitis C infection is a risk factor for development of CKD as shown by the aforementioned studies by Ojo et al. and Burra et al. (Burra, Senzolo et al. 2009).

CHRONIC KIDNEY DISEASE AFTER PEDIATRIC LIVER TRANSPLANTATION

There is a growing body of knowledge about late renal function after LT in children (McDiarmid 1996; Berg, Ericzon et al. 2001; Fine 2005; Wiesmayr, Jungraithmayr et al. 2005; Herzog, Martin et al. 2006; Bishop, Burniston et al. 2009). Unfortunately, analogous to the adult experience, the true incidence and prevalence of CKD in the pediatric population is obscured by the lack of uniformity in the methods used to assess renal function as well as the definition and classification of renal dysfunction. This makes it difficult and cumbersome to compare the results of many of these clinical studies.

The prevalence of CKD reported in the literature varies from 0% (Wiesmayr, Jungraithmayr et al. 2005) to almost 80% (Mention, Lahoche-Manucci et al. 2005). Interestingly, irrespective of the methodology, there seems to be a similar pattern in the evolution of renal function in children after LT in many of these reports. Berg et al. report a significant decline in GFR during the first year after LT but a stable renal function thereafter (Berg, Ericzon et al. 2001). In 12 children with a longer follow-up of 6 years, both GFR and effective renal plasma flow (ERPF) seemed to stabilize, a finding concordant with that of Laine et al. (Laine, Krogerus et al. 1994) and Mc Diarmid et al. (McDiarmid, Ettenger et al. 1990). Berg et al. additionally showed that only their subgroup of 13 children with metabolic disease had a significant fall in GFR and ERPF over time, emphasizing the possibility of preexisting renal impairment, and potentially of an ongoing renal dysfunction.

In conclusion, much of the evidence in the current literature suggests that renal function after liver transplantation in children remains stable in the long - term, and in particular those children of less than 2 years of age at the time of transplantation, where kidney development and growth is ongoing. This might be the explanation to why the decline seen in the renal function of the adult LT population is not seen to the same extent in the pediatric LT recipients.

CHRONIC KIDNEY DISEASE AFTER INTESTINAL TRANSPLANTATION

Information regarding renal dysfunction after intestinal transplantation is scarce. Historically the recipients of intestinal allografts have been subjected to high serum levels of Tac, often in the range of 15-20 ng/ml (Farmer, Shaked et al. 1996; Reyes, Bueno et al. 1998; Tzakis, Kato et al. 2005) which theoretically should increase the risk of developing CKD. Tzakis and coworkers reported a progressive decline in renal function in 24 adults with a calculated creatinine clearance (CCr) that decreased approximately 40% from baseline after a 2 year follow-up (Ueno T. 2006). The same group also investigated renal outcome in a pediatric population (n=36) after IT (Ueno T. 2006). They found an initial decline of renal function that gradually improved during the first year post IT. After the first year, mean GFRc remained stable, a trend which differed considerably from the adults. The authors discuss the possibility that renal maturation in the pediatric population may offset the decrease in renal function after transplantation. With regards to the impact of renal function on the long term outcome after IT, Watson and co workers (Watson, Venick et al. 2008) performed a retrospective analysis of patients undergoing IT from 1991 to 2006 (n= 62). They used a GFRc to evaluate renal

function. Renal dysfunction was observed in 16% of the patients post-IT. The most frequent predictors of post-IT renal dysfunction were a preoperative GFRc less than 75% of normal, pre-IT location of the patient in the intensive care unit, and high-dose Tac immunosuppressive therapy. A GFRc less than 75% of normal at weeks 1, 4 and after a year was predictive of poor patient survival ($P < 0.05$). They conclude that given the strong correlation of renal disease with poor outcome, preserving renal function is an important factor for the improvement of long-term outcomes in IT recipients. Ojo et al. found that the cumulative incidence of CKD after transplantation of a non renal organ was the highest for recipients of intestinal allografts (14% at 3 years and 21% at 5 years). A separate model for analysis of potential risk factors for developing CKD after IT was not performed due to the relatively scarce number of patients.

TYPE OF CALCINEURIN INHIBITOR AND RISK FOR DEVELOPING CHRONIC KIDNEY DISEASE

Results of the few clinical studies comparing renal outcome between CsA and Tac after LT have been varied and non conclusive. Platz et al. (Platz, Mueller et al. 1994) found a similar incidence of severe CKD in LT patients receiving either CsA (n=60) or Tac (n=61). Early postoperative renal insufficiency was observed to a similar extent in patients treated with CsA (38.3%) and Tac (42.6%). Late renal insufficiency appeared in 23.3% of CsA- and in 29.4% of Tac-treated patients. These results demonstrate a similar outcome in terms of both early and late nephrotoxicity and have been confirmed by others (Fisher, Nightingale et al. 1998). In contrast, other studies have demonstrated superior renal function after LT on a Tac-based protocol (McDiarmid, Colonna et al. 1993; Berg, Ericzon et al. 2001). Ojo and associates have also reported that the relative risk of developing CKD was higher for patients on CsA compared to Tac (RR 1.25; $p < 0.001$) (Ojo, Held et al. 2003). There are some studies assessing the fibrogenic potential of Tac and CsA in renal transplants that suggest that Tac may exert a less fibrogenic influence on transplant glomeruli than CsA (Bicknell, Williams et al. 2000). Moreover animal experimental models of renal ischemia suggest that Tac possesses lower fibrogenic potential than CsA (Jain, Bicknell et al. 2000).

ASSESSMENT OF RENAL FUNCTION

Knowledge of the methodology involved in the assessment of renal function is essential when evaluating the current body of published data concerning CKD after the transplantation of a non renal organ.

To date, the vast majority of the studies focusing on this subject rely on serum creatinine measurements or on calculated creatinine clearance (CCr) as a surrogate marker for GFR.

These calculations of GFR are based on a series of equations incorporating serum creatinine along with a diversity of other variables such as age, gender, body weight and length. The difficulty with using serum creatinine as one of the factors for calculating GFR is that it is an endogenous body substance. Creatinine is produced at an almost constant rate from muscle derived phosphocreatine. It is an insensitive marker of early decrease in GFR, because as renal function deteriorates, there is a coupled increase in tubular creatinine secretion (Heilman 2005; Goldsmith 2007). This has been confirmed in studies of heart transplant recipients with advanced renal dysfunction secondary to chronic CsA induced nephropathy (Tomlanovich, Golbetz et al. 1986). The authors demonstrated that ^{99m}Tc-DTPA and inulin were unrestricted by the glomerular capillary wall and behaved as true filtration markers, while creatinine was progressively hypersecreted by renal tubules as the nephropathy progressed. The ensuing enhancement of creatinine clearance over GFR therefore blunted the expected rise in serum creatinine levels. This is a serious limitation since a rise in serum creatinine is not detected until 50% or more of the GFR is lost (McDiarmid 1996). Creatinine plasma levels can also be artefactually elevated or decreased in a series of different circumstances such as state of hydration, use of drugs such as steroids and diuretics, liver disease, dietary protein intake, age, race and body habitus (Arieff and Chidsey 1974; Gaston 2005; Goldsmith 2007). Another pitfall in the use of creatinine based formulas is that creatinine is measured by two different techniques (Jaffe reaction and the enzymatic method) in the clinical laboratory which will yield different results in particular with the Jaffe reaction in the presence of hyperbilirubinemia (Goldsmith 2007). Lastly, many of these formulas have not been validated in patients with end stage liver disease or post LT or IT (Gonwa, Jennings et al. 2004).

A Canadian prospective study of de novo LT patients (Cantarovich, Yoshida et al. 2006) correlated GFR measured with ^{99m}Tc-DTPA with the Cockcroft & Gault (C&G), Nankivell, 1/serum creatinine (1/SCr) and modified diet in renal disease (MDRD) formulas. Measurements and calculations were performed at baseline and at one and three months after LT. The most important finding of this study was that all formulas correlated poorly with measured GFR; 1/SCr (r^2 : 0.11 and 0.2), C&G (r^2 : 0.31 and 0.35), MDRD (r^2 : 0.27 and 0.35) and Nankivell (r^2 : 0.11 and 0.2).

Another study by Gonwa et al. (Gonwa, Jennings et al. 2004) has confirmed the poor correlation of commonly used prediction equations in a large cohort of adult LT patients (n=1447). Neither pretransplant nor three – month estimates of measured GFR demonstrated good predictive value of the equations in LT recipients. Only 66% percent of estimates were within 30% of the measured GFR and in accordance with the aforementioned study by Cantarovich et al. the best correlation was found with the 6 variable-MDRD formula (r : 0.7 and r^2 : 0.49).

ASSESSMENT OF RENAL FUNCTION IN CHILDREN AFTER LIVER TRANSPLANTATION

In the pediatric population renal function is highly variable depending upon age. As an example, a plasma creatinine concentration of 88.4 $\mu\text{mol/L}$, represents a normal renal function in an adolescent but more than a 50% loss of renal function in a 5-year-old child (Atiyeh, Dabbagh et al. 1996). In the pediatric liver transplant population, GFRc with the Schwartz

formula has been shown to correlate poorly with GFR_m (Berg, Ericzon et al. 2001). Current recommendations favor measuring GFR with either Iohexol or 51-Cr EDTA-clearance (Heilman 2005).

Samyn et al. from King's College in the UK, investigated the correlation of cystatin C with GFR_m in 62 children after LT (Samyn, Cheeseman et al. 2005). They found that cystatin C levels correlated well with 51-Cr EDTA measurements and was much more reliable than creatinine and GFR_c calculated with the Schwartz equation. In their experience a cystatin C cutoff level of 1.06 mg/L had a high sensitivity (91%) and specificity (81%) for predicting a GFR_m level of less than 80 ml/min/1.73m². Their recommendation is to use this method as a screening tool and to subsequently perform a GFR_m if cystatin C levels suggest a GFR of less than 80 ml/min/1.73m².

A recent study incorporating cystatin C along with a number of clinical parameters such as body weight and length to create a new GFR-predictive formula has been reported by American investigators in a multicenter study. The study group included 349 children from the Chronic Kidney Disease in Children cohort. The formula that generated the most accurate GFR estimate included height, serum creatinine, cystatin C, blood urea nitrogen, and gender. With this new formula the authors found that the correlation between estimated and measured GFR was 0.88. 88% of estimated GFRs were within 30% of GFR_m and 46% within 10% of GFR_m (Abraham, Schwartz et al. 2009). However, this new formula still needs to be validated in the liver transplant population.

RENAL SPARING IMMUNOSUPPRESSIVE PROTOCOLS

During the early period after LT a viable strategy has been to implement induction protocols with non nephrotoxic drugs such as anti-IL2 receptors or ATG to delay or minimize the use of CNI. In long- term survivors, other non-nephrotoxic IS drugs such as MMF and the mTOR inhibitors (SRL and ERL) have been introduced as part of two accepted strategies to mitigate the progression of posttransplantation kidney disease by limiting CNI exposure. This is done by using either one of the drugs as a complement to the CNI-based protocol to be able to safely reduce the CNI levels (CNI minimization) or to completely discontinue the administration of the CNI and replace it with either MMF, SRL, or ERL (CNI discontinuation) (Farkas 2008; Flechner, Kobashigawa et al. 2008; Charlton, Wall et al. 2009).

CNI AVOIDANCE PROTOCOLS

In a review by the Scientific Registry of Transplant Recipients, which analyzed the United Network of Organ Sharing database, the use of CNI's was reported in 97% of patients discharged from the hospital after LT in the United States in 2002. Reports of CNI avoidance are scarce and some of the reports reveal an increased risk of ductopenic rejection, graft loss and even death (Sandborn, Hay et al. 1994; Flechner, Kobashigawa et al. 2008).

INDUCTION PROTOCOLS

Since early renal function after LT has been shown to correlate with the development of CKD (Cohen, Stegall et al. 2002; Velidedeoglu, Bloom et al. 2004) there should theoretically be an advantage by delaying or minimizing the use of CNI's in the early period after LT.

In an effort to study this possibility, Sellers et al. reported on a retrospective, nonrandomized study comparing 209 adult liver transplants with daclizumab induction to 115 transplants with no induction. Patient and graft survival were similar in both groups. Acute rejection within the first 6 months was less frequent in the induction group (25.4% vs. 39.1%, $P = 0.01$). Sustained renal improvement in recipients with pretransplant renal dysfunction (serum creatinine >1.5 mg/dl) was seen in the induction group, while the cohort without induction presented a steadily deteriorating renal function (Sellers, McGuire et al. 2004).

Yoshida et al. report in a US multicenter trial the results of a protocol with Daclizumab induction with delayed (day 4-6) low-dose (4-8 ng/ml) Tac started after LT vs the standard Tac protocol. The study arm included 72 patients. MMF and steroid tapering were used in both study arms. GFR follow-up was performed with GFRc (MDRD and C&G). The study arm had a significantly higher GFRc at 1 month (87 vs 70 ml/min/1.73 m²) and 6 months (75 vs 70 ml/min/1.73 m²) post LT when the MDRD equation was used to calculate GFR. There was no difference in the rate of rejection episodes or mortality between the study groups (Yoshida, Marotta et al. 2005).

An important study in this field is a European multicenter study with 517 (included in full analysis set) adult de novo LT patients with serum creatinine of 200 μ mol/L or less at study entry. This was a prospective, randomized, open-label trial investigating the effect of lower levels and delayed introduction of tacrolimus on renal function. The primary end point was the change from baseline GFR (C&G) at week 52. Renal function was also assessed by a GFRc with an abbreviated MDRD equation. Secondary endpoints were requirement of RRT, biopsy proven acute rejection episodes, graft loss and patient survival among others. Patients were followed for one year even if prematurely discontinuing study treatment. The patients were randomized to one of three study arms: standard dose Tac with steroids, reduced dose Tac, MMF and steroids, and finally, delayed (day 5) introduction of reduced dose Tac with induction treatment with daclizumab (within 12 hrs post LT and day 7). The study demonstrated that the patients in the study arm with delayed introduction of reduced dose Tac under the protection of MMF and daclizumab showed less impairment of renal function compared to the standard Tac and steroids protocol without an increased frequency of acute rejection episodes, graft loss or mortality. Although these results are promising in the short term the authors recognize that it remains to be shown if this will translate into a reduced risk for late CKD, rate of RRT or mortality (Neuberger, Mamelok et al. 2009).

CNI MINIMIZATION AND DISCONTINUATION PROTOCOLS

MYCOPHENOLATE MOFETIL AS PROTECTION

The use of MMF with CNI reduction or elimination has shown that it is possible to achieve significant improvement in renal function without compromising safety or efficiency.

Pageaux et al. in a European prospective, multicenter, randomized study of 56 LT recipients (27 study patients and 29 controls) with CNI-related chronic renal dysfunction found that a 50% reduction of the CNI dose under MMF protection allowed the renal function to improve significantly after 1 year of follow-up. No rejection episodes or severe adverse events were reported (Pageaux, Rostaing et al. 2006).

In contrast to this study several earlier efforts with MMF as a renal sparing agent have had varying results and have in some cases been hampered by side effects of the drug and an increased risk of acute rejection. Herrero et al. in a prospective study described 11 patients with CNI nephrotoxicity (serum creatinine >1.5 mg/dl) that received MMF with the intent to reduce CNI administration. Complete CNI discontinuation was achieved in 7 patients while more than a 50% reduction of the CNI was accomplished in 10/11. Patients were followed up for a mean of 63 +/- 5 weeks after the switch of IS. Renal function was assessed with GFR calculated with the C&G equation. In the seven patients that achieved CNI discontinuation there was a mild but statistically significant decline from baseline from 2.2 to 1.9 mg/dl (P = 0.05). Creatinine clearance increased from 38 to 42 ml/min (P = 0.005). Mild anemia was reported in six patients and two patients presented acute rejection episodes that responded to steroids and switch to Tac therapy (Herrero, Quiroga et al. 1999).

Barkmann and co workers from Germany analyzed in a prospective non randomized study the course of 22 liver transplant patients with renal dysfunction in whom MMF was introduced and CNI were either markedly reduced or completely withdrawn. The focus of this analysis was on the safety and tolerability of introducing MMF treatment (final dose 1.5-3 g / day) in liver transplant recipients as well as on the efficacy of the protocol with regard to reversal of renal dysfunction. Renal dysfunction was defined as a serum creatinine of 125 µmol/L or higher. In the first 18 study patients, 2 g/day of MMF was introduced abruptly, and cyclosporine or Tac doses were tapered over a 2-week period. In the remaining four patients, MMF was introduced stepwise over a 4-week period. In the study group, improvement in renal function could be observed in 14 of the 22 patients (64%) at 3 months and in 14 of 19 (74%) patients followed for 1 year. Two patients developed graft dysfunction during the study period. In one case CsA was reintroduced because of suspected acute rejection. The second patient required hepatic retransplantation; histological examination of the first graft at showed neither typical signs of acute or chronic rejection nor of hepatitis C or findings that could be attributed to the change in IS. The authors concluded that switching to MMF improved serum creatinine in the majority of the patients. The number of side effects were limiting for treatment in 20% of the cases (Barkmann, Nashan et al. 2000).

The same German group headed by Schlitt and associates studied the possibility of CNI discontinuation with MMF substitution in 14 patients with CNI induced nephrotoxicity (defined as serum creatinine above 125 µmol/L). They found a significant improvement in serum

creatinine over a 6 month observational period while in the control group (n=14) that remained on its prior low CNI protocol there was no change in this variable. Improvements in serum uric acid and blood pressure were also seen in the study cohort. However, three reversible episodes of biopsy proven acute rejection episodes were found in the study group while there were none in the control group. In conclusion an improvement in renal function was seen after CNI discontinuation but at the cost of an increased risk of rejection (Schlitt, Barkmann et al. 2001).

The authors of these three initial studies with MMF described the risk of acute rejection episodes related to the modifications in IS. To further illustrate this potentially serious problem Pham, P. T. and co workers recently reviewed the literature (Pham, Pham et al. 2009) in this field and found in eleven studies with MMF as renal-sparing agent a frequency of acute rejection ranging from 8% to 29%. The authors concluded that large scale randomized controls are needed to truly elucidate the risk-benefit ratio of these strategies (Pham, Pham et al. 2009).

SIROLIMUS OR EVEROLIMUS AS PROTECTION

Other authors have suggested that the use of SRL or ERL in a similar fashion to what has been described for MMF. Multiple studies have suggested a beneficial effect upon renal function using the drug either alone or in combination with a low dose CNI-protocol.

Yang et al. from Beijing, China, reported on 16 patients with CNI nephrotoxicity that were switched to SRL as their primary IS drug at a median of 8.5 months after LT. The authors found a significant improvement after CNI discontinuation, one late rejection episode and good compliance to the protocol. Median follow-up was 2.4 years (Yang 2008).

Nair and co workers from the USA found a moderate improvement in renal function after CNI discontinuation and replacement with SRL in 16 patients (Nair, Eason et al. 2003).

Dubay et al. in Canada in a retrospective, case control study, compared SRL conversion against a well matched CNI reduction arm in 57 patients. All but 5 of the SRL patients were completely withdrawn from CNI therapy. The authors found that conversion to SRL did not offer any significant improvement when compared to the CNI reduction arm. There was not either a difference in the rate to progression to RRT, rejection or death. Patients exposed to calcineurin inhibitors for more than 5 years or those with an initial (calculated) creatinine clearance of less than 30 mL/minute who were converted to sirolimus did worse than control patients maintained on low-dose calcineurin inhibitors. The prevalence of side effects were significantly higher among the patients receiving SRL. The authors concluded that with no statistical advantage and a higher rate of complications, SRL cannot be recommended for the purpose of preventing progression of CNI-induced nephrotoxicity (DuBay, Smith et al. 2008).

Since many of these studies have been small retrospective and single-center reports, the evidence thus far with regards to renal preservation can be labeled as suggestive but not conclusive (Cotterell, Fisher et al. 2002; Fairbanks, Eustace et al. 2003; Sanchez, Martin et al. 2005; Maheshwari, Torbenson et al. 2006; Jensen, Wiseman et al. 2008).

There are however two studies that in spite of being limited in size, are methodologically sound, randomized, and controlled which deserve to be commented upon separately.

Watson et al. from Cambridge, UK prospectively assigned 27 patients at least 6 months after LT to either conventional CNI-based IS (n=14) or to a switch to SRL-based IS (n=13). The final end point was the difference in GFR from baseline to one year after inclusion with GFR measured with Chromium EDTA. Most of the improvement in GFR was seen at 3 months post conversion. There was a significant, if modest improvement in GFR at 3 months but not at 12 months in the SRL arm. Patients who remained on CNI based IS experienced no change in renal function. Two patients had acute rejection episodes both related to low levels of SRL (Watson, Gimson et al. 2007).

De Simone et al. studied the effect upon renal function of using everolimus (ERL) a SRL analog. In a 6-month randomized controlled study, patients (n=72) either substituted the CNI with ERL or received ERL with low dose CNI. The control group (n=73) continued to receive the standard CNI protocol (Tac, or CsA with or without MMF, AZA or steroids). Inclusion criteria included a calculated creatinine clearance (CCr) in the range of 20-60 ml/min. Patients in the CNI discontinuation arm had ERL target levels of 6-12 ng/ml while the patients in the low CNI arm had target levels of 3-8 ng/ml. The primary end point was a change in CCr from baseline to 6 months. The study was powered (80%) to detect a difference of 8 +/- 16 ml/min at a significance level of 0.05. This primary end point of the study was not achieved. Among patients that continued in the ERL protocol (by 6 months CNI discontinuation was achieved in 80% of the patients) the mean increase in CCr was 2.1 and 3.8 ml at 3 and 6 months respectively, versus 2.4 and 3.5 in controls. Adverse events were more frequent in the ERL arm. Two patients (one in each study arm) had biopsy proven acute rejection episodes. This study was hampered by a high frequency of protocol violations in the control arm where 25% CNI dose reductions allowed by the protocol were exceeded in 77% of the patients thus making it difficult to interpret the difference in CCr (De Simone, Metselaar et al. 2009).

2. AIMS OF THE THESIS

Renal impairment suffered by the recipients of liver and intestinal allografts has become increasingly described in the literature. There is strong evidence that CKD after transplantation of these organs represents a significant risk to overall morbidity and mortality.

The purpose of this thesis was to investigate and describe the long-term renal function in adults and children after transplantation of the liver and intestine.

The specific aims were:

- Describe the prevalence of chronic kidney disease and the need for renal replacement therapy after transplantation of the liver and intestine in adult and pediatric patients.
- Identify risk factors for developing severe chronic kidney disease after liver transplantation.
- Evaluate if early measurements of GFR after liver transplantation can identify patients at risk of developing chronic kidney disease later on after liver transplantation.
- Investigate if the discontinuation of calcineurin inhibitors results in an improvement in renal function in adult patients with chronic kidney disease after liver transplantation.

3. PATIENTS & METHODS

Paper (I): Patients

During the period of November 1988 and November 2001, a total of 468 adult patients received 522 LT at our institution. Of these 468 patients, 125 died within the first 5 years after LT and were not included in this study. Pediatric patients were also excluded (n=34). The study cohort consisted of 152 adult patients with at least a five year-follow-up of renal function after LT with a GFR measured with either Chromium EDTA or iohexol clearance. Furthermore, 121 patients with incomplete GFR measurements but with enough data to calculate GFR according to the MDRD equation served as a control group with respect to demographics and baseline GFR. Characteristics of the two cohorts are presented in Table 3.

Based on pretransplant GFR_m, patients were classified into groups above or below 70 ml/min/1.73m². Patients were also classified according to post transplant GFR measurements at 3 months and 1 year (above or below 30 and 40 ml/min/1.73m²) and age (above or below 50 years) as well as calcineurin inhibitor (CNI) levels at 1 year post LT (cyclosporine A >150 ng/ml and tacrolimus >10 ng/ml). The cutoff values of 30 and 40 ml/min/1.73 m² at 3 months and one year were based on the NKF/KDOQI as well as other publications in this field (Cohen, Stegall et al. 2002).

Paper (II): Patients

Thirteen patients received intestinal grafts at our institution since October, 1998. Eleven of these patients (10 adults and 1 child) received multivisceral (MV) grafts including stomach, pancreaticoduodenal complex, liver and small intestine. Of the MV recipients 2 adults received a kidney allograft en bloque with the MV graft due to low baseline GFR_m (41 and 42 ml/min/1.73m² respectively). One adult patient received a combined liver and small intestine graft and lastly one child received an isolated small intestinal graft. Of these 13 patients six died. Three of these patients died within 6 months of the transplantation and were excluded from the analysis. The remaining 10 patients with a survival of at least 6 months constitute the study cohort. The characteristics of these 10 patients are presented in Table 4

Paper (III): Patients

During the period May 1992 to February 2006, 37 children received liver allografts at Queen Silvia's Pediatric Hospital in Göteborg, Sweden. One patient died early post transplantation due to multiorgan failure and poor initial liver function. Of the remaining 36 children (study group) baseline GFR measurements were available in 31. The causes for liver disease appear in Table 5. GFR measurements were performed prior to LT (n=31) and yearly thereafter. A total of 28

children had a complete GFRm follow-up at 5 years and 13 children at 10 years. Patients were divided into two groups according to age at the time of LT. The first group consisted of children younger than two years of age while the second group consisted of children older than two. The rationale for this age-based dichotomy is based on the findings that children reach adult values for mean GFR at approximately 2 years of age. For further analysis, patients were also divided into separate cohorts according to type of underlying liver disease.

Paper (IV): Patients

25 adult liver-transplant patients with stable liver graft function and renal dysfunction were recruited and randomized to either one of 2 protocols with CNI discontinuation. The renal inclusion criterion was a GFRm in the range of 15 to 45 ml/min/1.73m² measured on at least two successive occasions at least 3 months apart. The indications for LT are shown in Table 6. Nine female and 16 male patients were included. The median age at the time of inclusion was 59 years (range 25-66) and the median years after LT was 4.4 (range 1-13) years. Three patients did not complete the one year follow-up. One patient in the MMF arm with a previously known thrombosis of the portal vein and portal hypertension died 21 weeks after inclusion due to gastrointestinal bleeding from esophageal varices. A second patient was withdrawn from the study 20 weeks after inclusion due to progressive proteinuria and severe edema, SRL was discontinued and Tacrolimus was reinitiated. This patient underwent successful renal transplantation two and a half years later. Another patient on SRL died 6 months after inclusion in the study of sepsis and multiorgan failure secondary to a hepatic artery thrombosis. Twenty three of the patients were on Tacrolimus based and two were on Cyclosporine A based immunosuppression at the time of inclusion in the trial.

Randomization The study was approved by the local ethical review board and the Medical Products Agency in Uppsala, Sweden. All patients gave informed consent prior to inclusion in the study. Patients were then assigned to one of the study arms according to a randomization list supplied by an external pharmacy in Stockholm.

Patient Stratification Patients with a complete one year follow-up (n=22) were divided into two groups according to baseline GFR at the time of inclusion in the study. The first group consisted of those patients (n=14) with a GFRm of 30 ml/min/1.73m² or more, while the second group (n=8) included those patients with a GFRm inferior to 30 ml/min/1.73m². The level of GFRm was chosen by taking into consideration the classification of CKD by the National Kidney Foundation –Kidney Disease Outcome Initiative corresponding to stage IV CKD or severe CKD. For further analysis, patients were also divided into separate cohorts according to age at the time of randomization (older or younger than 60 years of age) or if more or less than 2 years had passed since LT as a surrogate marker for CNI exposure.

Tables 3-6 patient characteristics of patients in Papers I-IV

Table 3. Patient characteristics Paper (I) study and control group		
<i>Variable</i>	<i>Study cohort (GFRm)</i>	<i>Control cohort (GFRc)</i>
<i>GFR method</i>	Cr EDTA/iohexol	MDRD
<i>n</i>	152	121
<i>Mean age (SD)</i>	49(12)	49 (11)
<i>Gender F/M</i>	74/78	53/68
<i>Cholestatic</i>	56%	50%
<i>ALD</i>	11%	16%
<i>HBV</i>	6%	7%
<i>HCV</i>	10%	7%
<i>Other</i>	17%	21%
<i>FHF</i>	5%	6%
<i>% RRT</i>	5%	6.5%
<i>% Loss GFR*</i>	36%	34%
<i>Baseline MDRD</i>	78 ml/min/1.73 m ²	76 ml/min/1.73 m ²

<p><i>% Loss GFR*= loss of GFR in percent at 5 years</i></p> <p>AHD: alcoholic liver disease FHF: fulminant hepatic failure HBV: hepatitis B HCV: hepatitis C GFRc: calculated GFR GFRm: measured GFR MDRD: Modification of diet in renal disease</p>

Table 4. Patient characteristics Paper (II)

<i>Demographics, diagnosis and type of intestinal graft</i>				
<i>No.</i>	<i>Sex</i>	<i>Age</i>	<i>Diagnosis</i>	<i>Type of graft</i>
1	F	4	SD/ESLD	MV
2	M	44	NEPT	MV
3	F	56	SBS/ESLD	LIT
4	F	58	NEPT	MV
5	F	40	SBS/ESLD	MV+K
6	F	67	CIPO	MV
7	F	44	NEPT	MV
8	F	37	SBS/ESLD	MV+K
9	F	24	CIPO	MV
10	F	4	HD	I

MV= multivisceral, **MV+K**= multivisceral and kidney transplant
LIT=liver and intestine, **I**= isolated intestine, **SD**= secretory diarrhea,
ESLD end stage liver disease, **NEPT**= neuroendocrine pancreatic
tumor, **SBS**= short bowel syndrome, **CIPO**=chronic intestinal
pseudoobstruction, **HD**= Hirschsprung's disease

Table 5. Patient characteristics Paper (III)	
Diagnosis (n=36)	
	n
BILIARY ATRESIA	14
CHOLESTATIC LIVER DISEASE	8
Chronic autoimmune hepatitis	1
Criggler-Najjar	1
Alagille's Syndrome	1
Cryptogenic cirrhosis	2
Progressive familial intrahepatic cholestasis	1
Primary sclerosing cholangitis	2
MALIGNANT LIVER DISEASE	6
Hepatoblastoma	5
Hepatocellular carcinoma	1
METABOLIC LIVER DISEASE	3
Hereditary tyrosinemia	1
Glycogenosis type IB	1
α -1 antitrypsin deficiency	1
OTHER DISEASES	5
Acute liver failure	2
Budd-Chiari's disease	1
Portal vein thrombosis	1
Hepatic hemangioma	1

Table 6. Diagnosis patients Paper (IV)

<i>DIAGNOSIS</i>	<i>(n)</i>
Alcoholic cirrhosis	8
Alcoholic cirrhosis + HCV	2
Primary sclerosing cholangitis	2
Cryptogenic cirrhosis	2
Chronic autoimmune hepatitis	2
HBV associated liver cirrhosis	2
HCV associated liver cirrhosis	2
Primary Biliary Cirrhosis	3
Other	2

HBV: hepatitis B virus

HCV: hepatitis C virus

Immunosuppression

Paper (I): Immunosuppression

During the period 1988 to 1995 immunosuppression consisted of antithymoglobulin (ATG) induction and CsA was initiated within 24-48 hours after surgery at 10 mg/kg divided into two doses to maintain trough levels of 200-250 ng/ml during the first three months after transplantation. CsA levels of 50-100 ng/mls were maintained after 1 year post transplantation. Since 1996 our standard protocol has consisted of steroids and Tac with 0.1 mg/kg divided into two doses during the immediate postoperative phase to maintain trough levels between 10-15 ng/mls during the first three months post transplantation. Tac dosage is thereafter reduced to achieve trough levels between 5-10 ng/ml after three months post LT. In the whole cohort calcineurin maintenance immunosuppression consisted of CsA in 56% (n=88) of the patients while the remaining 44% (n=64) were maintained on Tac.

Paper (II): Immunosuppression

In this Paper two main immunosuppressive protocols have been used since the initiation of our intestinal transplant program in 1998. Tacrolimus has been the mainstay of therapy in both protocols. From 1998-2003, Tac in combination with steroids and the IL-2 receptor antagonist daclizumab were used. Twelve hour trough levels for Tac in this protocol was 15-20 ng/ml throughout the first 3 months post-transplantation and thereafter tapered to 10 ng/ml. After 2003 antithymocyte globulin induction with Tac monotherapy in a steroid free protocol has been used as described by the Pittsburgh group. Target levels of Tac were 10 ng/ml during the first six months after transplantation and thereafter tapered to about 5 ng/ml by the end of the first year post-transplant. Rejection was treated with a steroid bolus and thereafter the steroids were quickly tapered. Steroid resistant rejection episodes were treated with OKT-3.

Paper (III): Immunosuppression

In Paper (III) during the period 1992 to 1997 the basal immunosuppressive protocol consisted of CsA, steroids, and azathioprine. Tac was introduced to our unit in 1997. Until the change in 1997, CsA was given orally to maintain target 12-hour trough levels of 250-300 ng/ml during the first three months and 200-250 ng/ml from three to six months after transplantation. Levels were thereafter decreased gradually to 150-200 ng/ml at 1 year after transplantation. All of the children that were initially maintained on CsA (n=9) were later switched to Tac. Tac was given initially to the remaining 27 children as an initial oral bolus dose of 0.15 mg/kg twice daily. Doses were adjusted to obtain plasma levels of 10-15 ng/ml for the first six months and 5-10 ng/ml for the following six months. Beyond the first year after LT, Tac levels were maintained close to 5 ng/ml and were adjusted according to trends in serum creatinine and GFRm levels. Six children also received ATG induction therapy during the first week post transplantation.

CHEMOTHERAPY: All children with hepatoblastoma (n=5) were treated with doxorubicin and cisplatinum prior to transplantation and 2 patients received additional cycles of chemotherapy after transplantation. The child with hepatocellular carcinoma received chemotherapy consisting of etoposide, cyclophosphamide and thalidomide five years after LT due to the presence of lung metastases.

Paper (IV): Immunosuppression

MYCOPHENOLATE MOFETIL

In the MMF arm (n=13) the CNI dose was gradually reduced over a 4 week period while the MMF dose was increased gradually every week from 250 mg bid to a target total dose of 1000mg bid. All patients achieved the target MMF dose and had discontinued the CNI by week 4 after inclusion. Dose adjustments were performed according to the presence of side effects of the drug.

SIROLIMUS

In the SRL arm (n=12) CNI discontinuation was performed the day the patient was included in the study. The patients then received a single bolus dose of 10 mg of SRL followed by three consecutive daily doses of 8 mg. The first trough level was obtained at day 7 +/- 2 and the administered dose was adjusted thereafter to achieve the target trough level of 10 ng/ml. The interval with which SRL trough levels were measured depended upon the stability of previous measurements. Extra measurements were also performed 48-72 hours after dose adjustment.

CORTICOSTEROIDS

In both arms Prednisolone was administered at 10 mg daily with a subsequent taper to 5 mg by 6 months which was maintained until completion of the study.

GFR measurements

Paper (I – IV) GFR measurement and Follow-up

GFR measurements were performed with chromium-51 EDTA clearances or with iohexol clearances at the Departments of Clinical Physiology at Sahlgrenska University Hospital (adults) or at Queen Silvia's Pediatric Hospital according to well-established study protocols (Brochner-Mortensen, Giese et al. 1969; Brochner-Mortensen 1972). The results of these methods have not been reported separately in any of the Papers since they can be considered equivalent and have been shown have highly significant correlation with the renal clearance of inulin over a wide range of GFR values (Gaspari, Perico et al. 1995; Granerus 2000; Aurell, Frennby et al. 2002). The given dose of 51-Chromium EDTA was adjusted to age, body weight and renal function. The number of venous blood samples taken, time interval in between blood samples and the total time of clearance measurement was also adjusted according to renal function of the patient determined either by earlier measurements, serum creatinine levels or calculated GFR. In the adults GFR measurements were performed with a one-compartment model to evaluate the area under the curve (AUC) with 3-4 blood samples taken after injection of the tracer.

In Paper (I) a baseline measurement was done at the time of listing for LT, at 3 months post transplantation, and thereafter at 1, 3, 5 and 10 years. Basically the same protocol was used for the patients in Paper (II). However, after the 1-year control GFR measurements were performed on a yearly basis. Several of the patients also had several additional measurements performed outside of the established protocol if there was clinical or laboratory evidence of rapidly progressing deterioration of the renal function.

In the pediatric patients studied in Paper (III) a standard clearance technique was used where Chromium EDTA is injected at an age adjusted dose and blood samples are taken at 2 and 4 hours post injection. The GFR was calculated from the slope and intercept of the blood clearance curve. GFR measurements were performed at the time for listing for LT (baseline) at 3-6 months post LT and yearly thereafter. In some cases additional measurements were performed if there was evidence of deterioration of the renal function.

In Paper (IV) patients underwent routine GFR measurements according to the adult follow-up protocol at our institution. The results of these routine measurements dictated whether a patient was eligible for the CNI discontinuation study (main inclusion criterion was GFR_m 15- 45 ml/min/1.73m²). A baseline measurement was performed at the time of inclusion to the study, at 3 months after inclusion and at termination of the study at 1 year.

Classification of Chronic Kidney Disease

Papers (I - IV): classification of CKD

In all Papers the classification proposed by the Kidney Disease Outcome Quality Initiative (KDOQI) of the National Kidney Foundation was used. The main end point in Papers (I) and (III) was GFR below 30 ml/min/1.73m² corresponding to stage IV (severe kidney disease) or need for RRT. In the remaining Papers, (II&IV) the classification was not used as an endpoint for factorial analysis but rather to describe the populations

CLASSIFICATION OF CHRONIC KIDNEY DISEASE

National Kidney Foundation / Kidney Disease Outcome Quality Initiative
(NKF/KDOQI)

Stage	Description	ml/min/1.73m²
I	kidney disease with markers of kidney damage	normal or increased GFR
II	mild chronic kidney disease	60-90
III	moderate chronic kidney disease	30-59
IV	severe chronic kidney disease	15-29
V	kidney failure	need for renal replacement therapy

(1)

1. HOGG RJ, FURTH S, LEMLEY KV, et al. National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease in children and adolescents: evaluation, classification, and stratification. *Pediatrics* 2003; **111**: 1416-1421.

Statistical Methods

Papers (I, III, IV)

In Papers (I, III and IV) results that were normally distributed were reported as mean values \pm 1 SD. Values not following a normal distribution in Paper (IV) were reported as median and range. The same was done in Paper (II) due to the limited data sample.

In Paper (I) the longitudinal follow-up of GFR_m in the whole cohort was studied with a repeated measures two-way ANOVA to test if there were any significant changes in the mean GFR_m over time. Correlations of baseline GFR_m, GFR_m at 3 months and 1 year with GFR_m at 5 years after LT were calculated with Pearson's correlation coefficient. The coefficient of determination was reported to explain how much of the percentage variation of the dependent variable (GFR_m at 5 years) could be explained by the independent variables. To analyze the relationship between categorical variables the Fisher's exact test was used.

Variables considered to be potential risk factors for the development of CKD stage 4-5 or need for RRT (age above 50 years at LT, diagnosis, baseline GFR_m, GFR_m at 3 months and 1 year, CNI levels at 1 year, length of stay in ICU) were initially analyzed in a univariate model. Those variables found to be significant were then incorporated and analyzed in a multivariate logistic regression model.

In Paper (III) analysis of mean GFR_m in cohorts grouped according to age and diagnoses, an ANOVA or Student's two-sample T-test was used. For reasons of clarity, the mean GFR_m was reported with the standard error of the mean (SEM) in all figures except in Figure 1.

For analysis of differences in the incidence of adverse effects, blood pressure and infectious episodes between the two study cohorts in Paper (IV), Fisher's exact test was used.

P-values of less than 0.05 were considered statistically significant in all analysis performed.

For all Papers data was analyzed using SAS version 8.2 (SAS Institute Inc. Cary, NC., USA) and SPSS versions 12.0 to 17.0 statistical software (SPSS; Chicago, Ill., USA).

4. RESULTS

In the following section we discuss the major findings from the four Papers (I-IV).

The results from each Paper are summarized in the section below

Paper I: *Early renal function post-liver transplantation is predictive of progressive chronic kidney disease.*

- After adult LT there was a progressive decline in renal function coupled with an increasing prevalence of CKD stage 4-5 which affected almost one third of the patients ten years after liver transplantation.
- Early renal dysfunction post LT ($\text{GFR}_m < 30 \text{ ml/min/1.73m}^2$) was a significant independent risk factor for the development of CKD stage 4-5 long after LT.
- GFR_m levels at 3 months and 1 year post LT correlated strongly with GFR_m at 5 years post LT.
- CsA levels ($>150 \text{ ng/ml}$) and Tac levels ($>10 \text{ ng/ml}$) at one year after transplantation were independent risk factors for the development of renal failure or the need for RRT after liver transplantation.

Paper II: *Chronic kidney disease--a common and serious complication after intestinal transplantation.*

- CKD is a common and serious complication after intestinal transplantation affecting 90% percent of the patients in our series.
- The need for RRT was seen in 20% of the patients after intestinal transplantation.
- Preemptive renal transplantation by adding a kidney allograft en bloque with the MV graft was surgically safe and may have contributed to a delay in the initiation of RRT in two adults with MV allografts.
- The pediatric recipients in our series seem to have had the capacity to preserve and even recover renal function after intestinal transplantation.
- Adult patients with intestinal failure and complete dependency on PN or TPN seem to be at a particular risk of developing poor renal function due to poor baseline renal function when compared to the group of patients with NEPT.
- The strategy of CNI discontinuation and replacement with sirolimus in four adults with rapidly progressive CKD and proteinuria (n=2) was safe and relatively well tolerated with regards to side effects. There was not a significant improvement of the renal function after CNI conversion.
- Calculated GFR with the MDRD equation consistently overestimated true GFR with approximately 30-40%.

Paper III: *Stable long term renal function after pediatric liver transplantation*

- A significant number of children experience mild to moderate kidney disease early after LT
- The decrease in the GFRm was most apparent during the six months immediately following transplantation. However, renal function seemed to stabilize after that point and no significant decline was found in the long-term follow-up.
- None of the children required either dialysis or renal transplantation.
- Children younger than two years of age at transplantation had a stable renal function both early on and well after liver transplantation while the older children lost a greater percent of their renal function in the early period after transplantation.
- In particular, children with hepatic malignancies and inborn errors of metabolism seemed to be the most vulnerable to the renal insult which accompanies liver transplantation and calcineurin based immunosuppression.

Paper IV: *Conversion from calcineurin inhibitor to either MMF or sirolimus improves renal function in liver transplant recipients with chronic kidney disease- results of a prospective randomized trial*

- CNI discontinuation and replacement with either MMF or SRL resulted in a significant improvement in renal function
- After CNI discontinuation the renal function improved significantly even in those patients with a severe CKD (< 30 ml/min/1.73m²).
- The protocol was effective with no biopsy proven acute rejection episodes in either of the study arms.
- There was no significant difference in the frequency of severe adverse events between the study arms. Oral ulcerations and persistent hypertriglyceridemia was more common in the SRL group. Severe proteinuria was seen in one patient in the SRL arm.

5. DISCUSSION

In summary, these Papers we have described that CKD affects almost one third of the adult patients at ten years after liver transplantation and that CKD is almost universal in those patients that have received intestinal allografts. Pediatric recipients of liver allografts develop a significant renal dysfunction early after transplantation, but in contrast to their adult counterparts, renal function recovers and stabilizes in the long term. We have also described in the adult liver transplant population that early measurements of GFR correlate strongly with GFR measurements at five years, and that low GFR levels at 3 months post transplantation can help to identify those patients at risk of developing late CKD. Furthermore, we confirmed that a CNI discontinuation protocol under the protection of either MMF or SRL in patients with severe CKD after liver transplantation was safe and resulted in a significant improvement in GFRm during the one year observational period of the study.

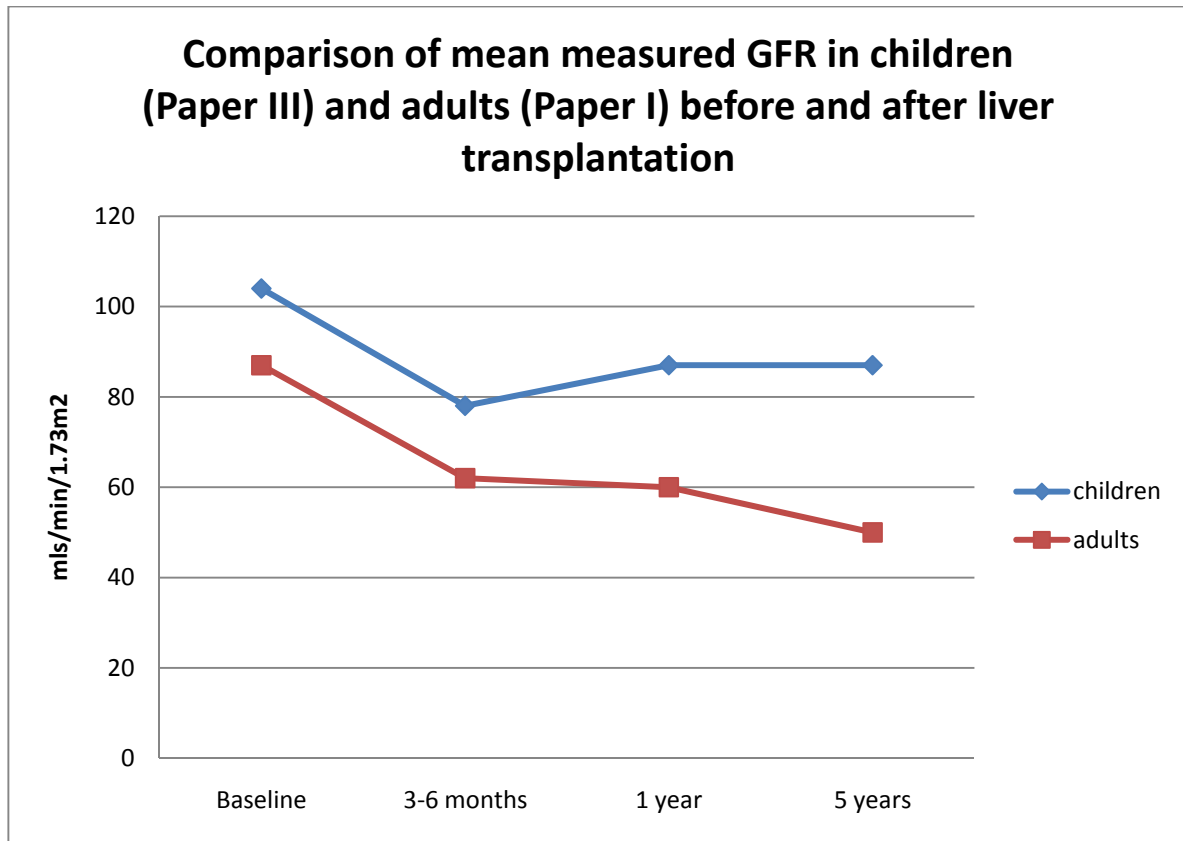
In the following section we will discuss these findings in relation to the current literature

PREVALENCE OF CKD AND REQUIREMENT OF RENAL REPLACEMENT THERAPY AFTER LIVER TRANSPLANTATION IN ADULTS AND CHILDREN

The overall prevalence of CKD and the need for RRT is in our experience with the adult LT recipients (Paper I) in agreement with other important publications in the field. In the adult cohort of patients we found an early and significant decrease in GFRm (29%) during the first 3 months post LT. Thereafter renal function stabilized but, a continuing decreasing trend in GFRm over time was evident. At 5 years after LT 12% of the 152 patients followed had developed CKD stage IV-V and at 10 years the corresponding prevalence had increased to 29% in the 41 patients with long-term follow-up patients. These results are similar to the 18% cumulative incidence of CKD (at 5 years) and close to 30% at 10 years, reported by one of the most important publications in this field (Ojo, Held et al. 2003). Other clinical studies report similar results (Cohen, Stegall et al. 2002; Pawarode, Fine et al. 2003). With regards to the incidence of need for RRT after LT Ojo et al. described that 4.7% (n=3297) of the 69 321 patients followed required RRT. In our 152 patients, 5.2% (n=8) developed renal failure and required RRT at a median of 8 years after LT.

In the pediatric population (Paper III) it is interesting to note that, in analogy with their adult counterparts, a significant decrease in GFRm occurs early on after LT (Figure 6). At 6 months after LT more than 2/3 of the children had CKD stage II (57%) or stage III (17%). However, in the long-term, we found two fundamental differences with the adult population; first, none of the children progressed to a CKD stage IV and in the children with a sustained decrease in

renal function, CKD improved from stage III to stage II by the end of the observational period. Secondly, none of the children required RRT. With regards to the stability of long-term renal function in children after LT, prevalence of CKD, and the need for RRT our findings are consistent with those of other authors (McDiarmid 1996; Berg, Ericzon et al. 2001; Bishop, Burniston et al. 2009).



Even if the collective data on the pediatric population seems to indicate that there is stability and even a certain degree of reversibility in the renal impairment there are some concerns that need to be addressed. Although the pediatric kidney is resilient, and children start off with a greater nephron mass than their adult counterparts do, after 20 or more years of CNI exposure the probability that they will have a significant degree of renal impairment is high. This implies that a substantial amount of these children may very well develop renal failure in early adulthood. Until we are safely able to minimize or altogether avoid CNI exposure, long-term renal impairment with the ensuing threat of renal failure will cast a shadow over the long-term prognosis of pediatric transplantation.

PREVALENCE OF CKD AND THE REQUIREMENT OF RENAL REPLACEMENT THERAPY AFTER INTESTINAL TRANSPLANTATION

The findings regarding long-term renal function after intestinal transplantation are worrisome. In spite of impressive results with induction therapies and tolerogenic protocols that have allowed a substantial reduction in the utilized serum trough levels of Tac there is, in the few reports that exist in the world literature, convincing evidence that renal impairment will pose a serious threat to these patients' long-term results.

Even though our series of patients that have received an intestinal graft is rather small (Paper III), this was to a certain degree compensated for through the superior accuracy attained by measuring GFR with iohexol or ⁵¹-Chromium EDTA. In the few series currently published (Ojo, Held et al. 2003; Tzakis, Kato et al. 2005; Ueno T. 2006; Ueno T. 2006; Watson, Venick et al. 2008) serum creatinine derived formulas have been used to describe the evolution of renal function post transplantation. Serum creatinine is a poor marker for renal function in malnourished patients with muscular wasting, hallmark clinical findings of the patient with intestinal failure. Additionally, none of the creatinine derived formulas have been validated in patients with intestinal failure and even less in patients who have undergone an intestinal transplantation.

In Paper II we compared GFR_m with GFR calculated with the MDRD equation and found that calculating GFR with this equation results in an overestimation of GFR by 30-40%. This implies that there is an increased risk of underestimating the severity of renal dysfunction when using a calculated GFR to assess renal function in these patients. The true prevalence of CKD after IT remains elusive. There are few clinical reports and direct comparisons of results are difficult due to substantial differences in populations, types of transplant procedures and methods of renal assessment.

The UCLA group (Watson, Venick et al. 2008) found that in general that adults had significantly worse postoperative renal function over time than did children, a finding that is comparable to the adult and pediatric reports from the University of Miami (Ueno T. 2006; Ueno T. 2006) and our own, albeit limited, experience with only two pediatric recipients for comparison. Notwithstanding, the difference between the adult and pediatric recipients recipients was striking in our series. One of the children recovered 100% of her renal function while the second child (with unilateral renal agenesis) lost only 20% from baseline at 6 months after transplantation. In the adult patients the results were quite different. GFR_m at 3 months post transplantation decreased to 50% of the baseline value. At 1 year, median GFR in the adult patients was reduced by 72% (n=5) and two patients developed renal failure within the first year and required hemodialysis, one of these patients later received a kidney transplant. The UCLA group defined renal dysfunction as a GFR_c below 75% of normal GFR and found that

by this definition 15% of the children had renal dysfunction after transplantation. In their series none of the recipients required RRT post IT. However one must bear in mind that two thirds (45/68) of their patients were children who received isolated intestinal grafts and had a mild renal dysfunction at baseline. The adult patients in their series shared these same features.

The experience from the University of Miami parallels our adult experience of a significant loss in GFR post IT. At 2 years after transplantation mean GFR_c decreased to 44% of mean baseline values ($p=0.0001$). Interestingly, mean baseline GFR_c for their cohort ($n=24$) was 114 ml/min/1.73m², in other words substantially higher than the mean baseline GFR_m in our series. Unfortunately, there is no data concerning requirement for RRT in the Miami series. The pediatric experience from Miami ($n=44$) with a GFR_c calculated with the Schwartz equation revealed a baseline mean GFR of 138ml/min/1.73m² which decreased to 80% of the baseline value at 18-24 months post IT, which was a smaller decrease in GFR in the children than in their adult counterparts (Ueno T. 2006).

PATIENTS AT RISK OF DEVELOPING CHRONIC KIDNEY DISEASE AFTER TRANSPLANTATION

The etiology of renal impairment is multifactorial. Renal dysfunction may already exist prior to the LT as is the case in patients with ESLD and HRS, patients with hepatitis C infection, polycystic kidney and liver disease, diabetes or hypertension. Patients with intestinal failure and dependence on parenteral nutrition and intravenous fluids are also at a high risk of developing severe renal dysfunction (Buchman, Moukarzel et al. 1993). Both pre and post transplant risk factors for the development of CKD after transplantation of non renal organs have been described in the literature and will be discussed in this section.

Liver transplant recipients

In general, those risk factors known to predispose to renal insufficiency include varying degrees of elevated pretransplant serum creatinine (Gonwa, Klintmalm et al. 1995; Gonwa 2003; Barri, Sanchez et al. 2009; Charlton, Wall et al. 2009) as well as hepatitis C infection, diabetes mellitus and hypertension and age (Ojo, Held et al. 2003). In particular hepatitis C infection has been linked with histopathological changes that may have functional implications such as membranous glomerulonephritis, mixed essential cryoglobulinemia, and membranoproliferative glomerulonephritis (Morales, Campistol et al. 1997; Morales, Pascual-Capdevila et al. 1997)

Our main goal was to try to identify those patients at risk for developing CKD stage IV-V early after LT in the adult population by using GFR_m data retrieved both before and after LT (Paper I). We found that baseline GFR measurements could only explain one third of the variability of the GFR measured at 5 years post LT ($r^2: 0.27$). However, measured GFR at 3 months post LT correlated just as well with those measurements performed at 1 year post LT; $r^2: 0.46$ and 0.52

respectively, with renal function at 5 years after LT ($P < 0.001$). To further categorize this information, a stratification according to the level of GFR_m (above and below 30 ml/min/1.73m²) was performed at 3 months post LT to investigate its potential as a risk factor for CKD stage IV-V at 5 years. In a multivariate analysis GFR levels below 30 ml/min/1.73m² at 3 months post transplant were significantly associated with the development of CKD Stage 4-5 at 5 years post-LT ($P = 0.03$).

Our findings are in accordance with other publications in this field (Cohen, Stegall et al. 2002; Neuberger 2002; Pawarode, Fine et al. 2003). These authors also found a strong correlation between early renal function after LT and the development of severe CKD post LT. Pawarode et al. could among other predictive factors demonstrate that a GFR of 30 ml/min/1.73m² or less at 3 months predicted severe renal dysfunction. Cohen's findings with GFR_m, which in particular makes their series interesting, were similar to ours with a strong correlation of GFR at 1 year and GFR at 5 years. Furthermore, they found that patients with a GFR of < 40 ml/min/1.73m² at one year were at risk of developing severe CKD late after LT. Our findings are also in agreement with these studies with regards to the poor correlation of measured GFR prior to LT with renal function late after LT. A possible explanation for this poor correlation could be the presence of renal dysfunction secondary to HRS, a situation that is reversible after successful LT. From this perspective another study by Wadei et al investigating renal biopsy findings in patients with ESLD demonstrated that clinical criteria commonly used to determine kidney function and to assess chronicity of the renal disease did not relate to histological evidence of fixed renal damage and did not identify potentially reversible renal failure. This lack of agreement might also explain why pre liver transplant GFR does not consistently predict post liver transplant renal function (Wadei, Geiger et al. 2008).

The etiology of renal dysfunction after LT is multifactorial; therefore our study does not attempt to exactly determine its causes. However some previously described risk factors such as hepatitis C infection were interesting due to its increasing prevalence in the transplant population in general and its association with renal dysfunction after LT (Burra, Senzolo et al. 2009). Contrary to the results of the multicentric European center study and the study by Ojo et al., we were not able to show an association of HCV infection and increased prevalence of CKD. This may perhaps reflect the relatively low proportion of patients with HCV infection in our study cohort (10%) compared to the US and continental Europe in the aforementioned series where the frequency of HCV infected patients was 21%-37%. Our population and results in this sense are very similar to those reported by Cohen (HCV: 15%) (Cohen, Stegall et al. 2002) and Åberg et al. (HCV: 3%) (Åberg, Koivusalo et al. 2007) from Finland.

In the literature there is conflicting information in relation to the effect of the cumulative dose and serum levels of CNI's on renal function. Fisher et al, from Birmingham UK found in a multivariate analysis that CsA levels at 1 month and cumulative doses of CsA were significantly associated with the development of late CKD after LT ($p = 0.007$ and 0.01). Morard et al. also found this (Morard 2006) while others have failed to verify this relationship (Bach, Feutren et al. 1990; Feutren, Friend et al. 1990). When analyzing CNI levels at 1 year post LT in our series in a multivariate analysis as a risk factor for development of CKD stage IV we found no significance. However, when potential risk factors for the requirement of RRT

were sought Tac levels over 10 ng/ml and CsA over 150 ng/ml at 1 year post LT were significant predictors (P=0.015).

In the pediatric population, the risk factors for development of renal impairment that have been described by earlier publications are mainly for children with inborn errors of metabolism and antihypertensive therapy (Berg, Ericzon et al. 2001). Other authors have described that the loss of GFR post LT is larger in those children older than two years of age at the time of transplantation compared to those that were younger than two. However, there is a long term recovery and stable GFR over time and no children required RRT (Bishop, Burniston et al. 2009). We have seen a similar trend (Paper III) in the GFR measurements according to age above and below two years. Those children under the age of two years lost 13% of the GFRm compared to baseline while those older than two years lost 31% (P<0.05). This difference disappears over time. We did however see that those children with malignant liver disease and metabolic liver disease had the largest loss in percentage of GFRm from baseline; 32% and 35% respectively. This is in agreement with what Berg et al. described in their cohort of children that underwent LT for inborn errors of metabolism as well as other authors (Herzog, Martin et al. 2006). In summary, children over the age of two years at the time of LT, and children with hepatic malignancies and inborn errors of metabolism seem to be in particular vulnerable to the renal insult which accompanies LT and calcineurin based immunosuppression.

Intestinal transplant recipients

Candidates awaiting ITx have unique risk factors for developing renal dysfunction including TPN dependence (Buchman, Moukarzel et al. 1993), exposure to nephrotoxic medications, fluid and electrolyte alterations, recurrent septic episodes and, dehydration. Additionally, in the most severe stages of intestinal failure there might be the presence of an impending hepatic failure secondary to intestinal failure associated liver disease (IFALD). In the most severe forms of IFALD there exists severe bridging fibrosis of the liver or manifest cirrhosis accompanied by portal hypertension and HRS. In these desperate cases the patient will have failure of three major organ systems; gastrointestinal tract, liver and kidneys prior to the transplant.

In Paper II, baseline GFR was 67 (22–114) mL/min/1.73 m² for the whole cohort (n=10). The patients with intestinal failure (n=7) had severely impaired renal function prior to transplantation with a median GFRm of 42 ml/min/1.73m² (22-73 ml/min/1.73m²). The presence of renal dysfunction prior to IT was also described by the UCLA group where at the time of transplant GFRc was 83% of normal ie a mild renal dysfunction. The Miami group also found mild renal dysfunction in adults prior to IT (Ueno T. 2006). This finding differs markedly with our experience. One plausible explanation for this discrepancy could be methodological, where the calculation of GFR used by Watson et al. and Ueno et al. will overestimate GFR by approximately 30-40%, thus downstaging the degree of renal dysfunction. Other factors of importance related to the population studied by Watson et al. is that their series was made up of exclusively patients that received isolated intestinal allografts

implying that they had normal or near to normal liver function; whereas in our series three patients had ESLD with HRS and one of the children presented with renal agenesis. Furthermore they excluded from their series those patients with a combined intestinal and kidney transplants while two of our adult recipients of MV grafts had kidneys included in the graft due to severe renal impairment. Lastly, patients with permanent TPN dependence will often present with significant renal dysfunction (Buchman, Moukarzel et al. 1993). The median time of TPN duration in the UCLA series was 17 months while all of our patients with intestinal failure had at least five years of TPN dependence prior to evaluation.

In contrast, the patients presenting with neuroendocrine pancreatic tumors (n=3 adults) in our series had a near normal renal function with a median GFRm of 94 ml/min/1.73m² (93-114). The patients with NEPT are therefore, in this context, a completely different set of patients without most of the mentioned renal risk associated with intestinal failure and parenteral nutrition. Therefore, one would expect that this would be reflected in the long term renal function post transplantation. Unfortunately, as we have seen in our series, these patients despite near to normal baseline renal function will develop severe CKD and need for RRT. In the patients with NEPT the median loss in percentage of GFR from baseline to a median of two years (0.5-2.5 years) follow-up was 84% (68-90%) and one patient required RRT at 6 months post MV. This clearly illustrates the complexity and hazard of these procedures where the renal stressors are probably a combination of extensive surgical trauma, liberal use of vasopressors in the face of hemodynamic instability, CNI, other nephrotoxic drugs, and sepsis among other factors. Furthermore, in the stable phase after a successful transplantation these patients may face other clinical situations that may aggravate an already compromised renal function such as high stomal output with dehydration, inadequate fluid intake and opportunistic polyoma virus infections which may affect renal function.

In summary, when facing the prospect of an IT or MV, all candidates should be considered to be at a potential risk of developing significant renal dysfunction after transplantation irrespective of age, baseline renal function, diagnosis or the type of procedure.

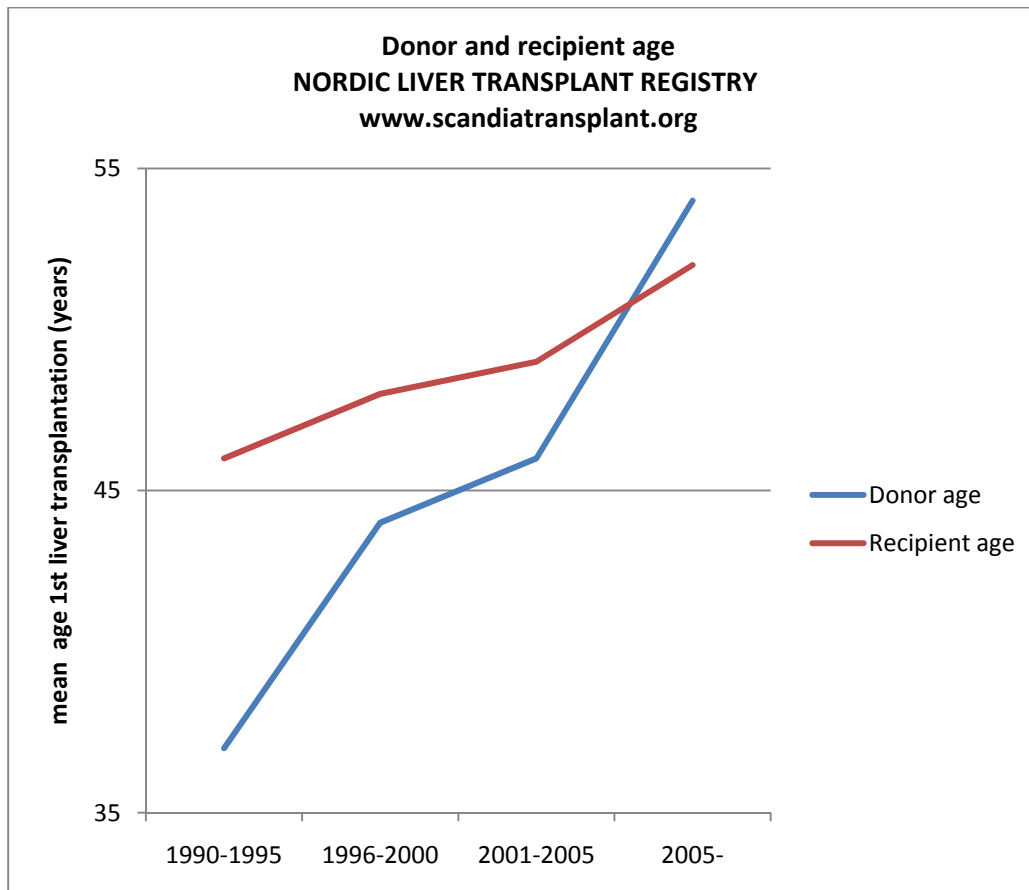
CONSEQUENCES OF CHRONIC KIDNEY DISEASE AFTER TRANSPLANTATION OF A NON RENAL ORGAN

Liver transplantation

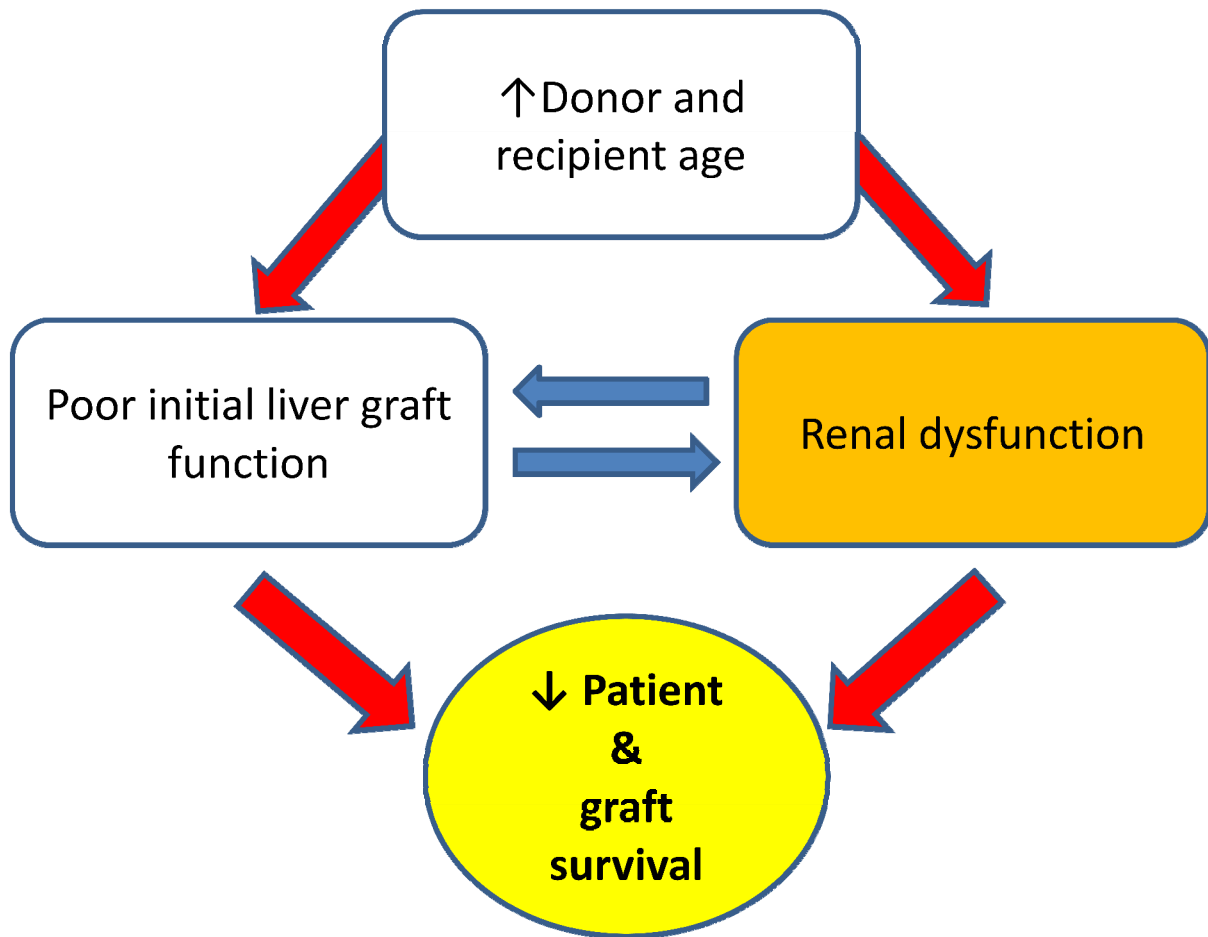
It is evident in the large number of studies that have been previously discussed that the presence of CKD is a frequent complication that has a profound impact on long term outcome.

Given the trend toward increasing longevity in the overall population of recipients of non renal transplants, the population exposed to the risk of CNI renal toxicity will also gradually increase. This will undoubtedly further increase the risk of chronic renal failure and the need for long-term renal-replacement therapy. But, perhaps most importantly, the presence of CKD

after transplantation of a non renal organ has an impact on long term survival. Furthermore, there is a clear tendency toward transplanting older patients with liver allografts from older donors. There is also a strong negative impact of increased donor age on liver transplant outcomes (Feng, Goodrich et al. 2006; Merion, Goodrich et al. 2006).



These trends in patient and donor selection criteria may in the future prove to be detrimental taking in consideration the risk increment for CKD posed by increasing recipient age. The additional risk of poor initial liver graft function or non- function associated with increasing donor age, may further jeopardize patient survival (Feng, Goodrich et al. 2006; Merion, Goodrich et al. 2006) as well as increase the risk of renal impairment in long term survivors after LT. This vicious circle may be further enhanced by the fact that acute renal injury post LT may also compromise liver allograft survival (Barri, Sanchez et al. 2009).



Intestinal transplantation

Studies that address long term survival after IT in the context of renal impairment with measured GFR are virtually nonexistent at this time. However, the Paper by Watson et al. from UCLA provides important insights. The authors describe risk factors for developing renal dysfunction post IT in 62 patients and correlate the percentage of normal GFR_c for age and gender to investigate if renal dysfunction was predictive of post IT survival. They found that overall patient survival was 78% at 1 year and 56% at 5 years. A GFR_c less than 75% of normal at day 7 (log-rank test, $P < 0.05$, hazard ratio HR 1.5), day 28 ($P < 0.05$, HR 1.2), and at 1 year ($P < 0.04$, HR 6.0) was predictive of worse patient survival. It is evident from these data that the risk inflicted by renal dysfunction on survival increases both rapidly and significantly over time. At one year post IT those patients with less than 75% of expected GFR had a six fold higher mortality than the cohort with an expected GFR above 75%. In contrast those with an expected GFR above 75% were all alive after 5 years (Watson, Venick et al. 2008).

Lastly, short term survival after IT has improved substantially while long term survival according to the international registry has failed to improve to the same extent (Dr. David Grant, www.intestinaltransplant.org and international symposium of intestinal transplantation. September 2009, Bologna, Italy). This obscure and relentless late mortality has mainly been attributed to late acute rejection episodes as well as an increasing incidence of what has been termed chronic allograft enteropathy, an entity which might be part of the spectrum of chronic rejection. However, the most common causes of death after intestinal transplantation still remain related to the immunosuppressive load. What role the presence of CKD may play in this setting still remains to be investigated.

Global data of more than 10 years of CNI exposure in adults are indeed concerning. It appears that at some point in time renal impairment in adults appears to become irreversible. The question of when this happens, if it is predictable and if it is at all avoidable remains largely unanswered. Given this continuing development, this trend may ultimately lead to an increase in the caseload of patients that will require resources for treatment of end stage renal disease and ultimately RRT such as hemodialysis or kidney transplantation. Apart from the previously mentioned risk for hampering long term survival after LT, this may result in significant fiscal consequences for health care services (Washburn, Meo et al. 2009) and in the long term even a negative impact on the already burdened waiting list dynamics of renal allografts for transplantation.

MINIMIZING THE RISK OF CHRONIC KIDNEY DISEASE AFTER TRANSPLANTATION OF A NON RENAL ORGAN

Pre transplant setting

Maintaining an adequate renal function in the pre transplant setting is not only important to prevent renal dysfunction post transplantation but also because renal dysfunction is an important predictor of death in patients with decompensated cirrhosis as evidenced by the MELD score (Gines and Schrier 2009). As a consequence, the mission of preserving renal function begins already with the cirrhotic patient or the patient with intestinal failure in the phase prior to LT.

Identifying, preventing and treating causes of renal dysfunction is a difficult challenge in which the experienced nephrologist plays a pivotal role together with the hepatologists, gastroenterologists, intensivists and surgeons caring for these patients. The medical team's concerted success in eliminating or alleviating the detrimental factors affecting renal function will have a profound effect on the patient survival both before and after the transplant procedure.

One of the cornerstones of an adequate medical management prior to transplantation is the monitoring of renal function. Monitoring should be extended into the post transplant period in

order to evaluate adjustments in the dosage of the CNI and other nephrotoxic drugs such as aminoglycosides. This monitoring should preferentially be performed with measured GFR in accordance with a recent consensus report by the International Liver Transplantation Society Expert Panel on Renal Insufficiency in Liver Transplantation (Charlton, Wall et al. 2009). Other important parameters to evaluate prior to transplantation and to follow post transplantation are urine analysis, and urine protein creatinine ratios. In the presence of proteinuria an immunological assay to rule out autoimmune causes of renal involvement should be performed as well as an anatomical evaluation of the kidneys with ultrasound, computed tomography or magnetic resonance depending upon the clinical setting. If a diagnosis cannot be established with these modalities a renal biopsy should be considered.

Preemptive kidney transplantation

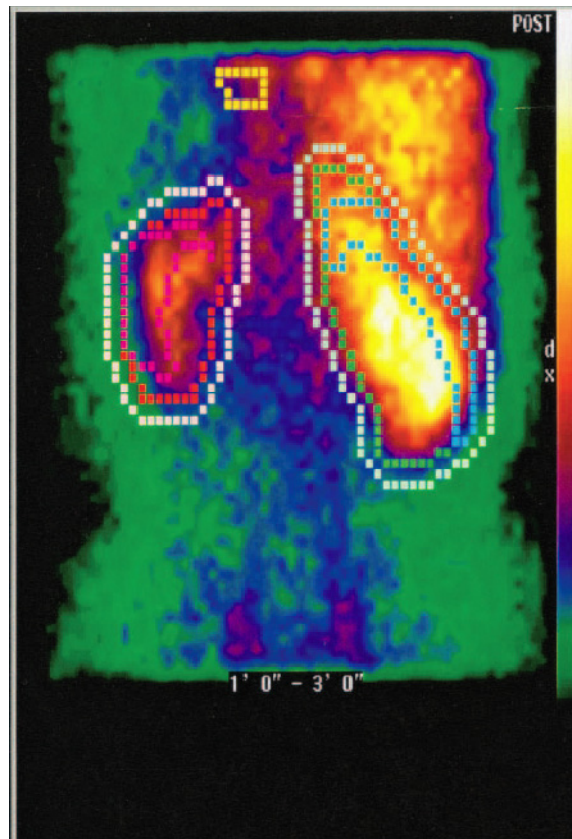
Assessing renal function in the presence of advanced liver disease is a difficult challenge. The importance of this evaluation resides in whether or not the renal injury is reversible after LT or IT. The etiology of the renal disease may offer some guidance since certain conditions such as diabetic nephropathy, polycystic kidney disease and chronic glomerulonephritis will have are of a more permanent character and can be expected to progress over time particularly in combination with CNI-based IS. On the other hand, patients with a HRS have better post transplant renal outcomes due to the reversibility of this condition as liver function improves. In this particular situation a combined liver and kidney transplant is not indicated. In addition to a meticulous pretransplant assessment, guidance by nephrological expertise should provide the necessary information for the decision of whether or not a liver or intestinal transplantation should be combined with a kidney graft.

Guidelines for when a patient should receive a kidney allograft simultaneously with a hepatic graft have varied and are center specific (Davis, Gonwa et al. 2002; Tanriover, Mejia et al. 2008). However, in the recent guidelines by the First ILTS Expert Panel Consensus Conference on Renal Insufficiency in Liver Transplantation certain criteria for simultaneous liver- kidney transplantation were presented (Charlton, Wall et al. 2009); in summary, those patients with end stage renal disease requiring RRT prior to LT are considered candidates. In addition, patients with CKD not on dialysis but with a documented GFR of less than 30 ml/min/1.73m² (either calculated by the MDRD 6 equation or measured) and significant proteinuria. The third group amenable to a combined approach would be the group of patients with sustained acute renal failure and dialysis twice weekly with more than 6 weeks duration. Lastly, patients with a primary non reversible renal disease such as metabolic or genetic diseases such as hyperoxaluria and polycystic kidney disease would also qualify for a combined liver-kidney transplant.

In the field of intestinal transplantation there are no current guidelines in this respect. In existing clinical reports and from the international registry it is difficult to extract how frequently this procedure has been performed and what the long term outcome has been.

In our particular program, decision making was based on our experience in the field of LT where we anticipated a loss of at least 30% of GFR from baseline within the first year post transplantation. Since two of our MV candidates had severe baseline renal impairment we found it convenient to include a kidney allograft “en- bloc” with the MV graft. One of the

recipients died after one year with marginal renal function. The second patient is still alive almost 6 years post transplantation with marginal renal function and in preparation for HD and evaluation for a kidney transplant.



Posterior view renal scintigram performed 5 months after combined multivisceral and kidney transplantation. The kidney graft is located on the right and its image is superimposed with that of the native right kidney.
(Herlenius, Fagerlind et al. 2008)

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Post transplant setting

As has been seen in the studies described previously, and also from our own experience, early renal events seem to dictate long term outcomes (Paper I). Furthermore, it is during this early period post transplantation when the renal function undergoes the steepest decline (Paper I, II, III). One may therefore reason that it is during this time frame when the kidneys are most vulnerable to the combination of pre transplantation pathology, surgical trauma, drugs, and the cascade of immunological events that accompany the ischemia and reperfusion stages of organ

transplantation. With this in mind, it may well be that it is during this early stage where we have a “window of opportunity” to lessen the nephrotoxic effects of the CNI’s and to have a positive influence on long term renal function.

Early post transplant setting

In those patients in whom we a priori know there is a high risk of developing CKD, strategies that allow us to maintain low levels of CNI’s during the early phase post transplantation are attractive. Lessons can be learned from the field of kidney transplantation where some of these renal protection strategies have been implemented. One of the most important studies in this field is a European multicenter randomized controlled study in which 1645 renal transplant recipients were enrolled (Ekberg, Tedesco-Silva et al. 2007). The authors compared low-dose Tac (3-7 ng/ml), low dose SRL (4-8 ng/ml) and low dose CsA (50-100 ng/ml) in combination with daclizumab, MMF and corticosteroids and a control arm with standard dose CsA (150-250 ng/ml) with MMF and corticosteroids. The cohorts were followed for one year. At the conclusion of the study, the group with low dose Tac had the lowest rate of acute rejection (12%) compared to the low dose CsA group (24%), low dose SRL group (37%), and standard dose CsA (26%). Patients in the low dose Tac group also had the highest mean GFRc in comparison with the other treatment groups.

The combination of low dose and delayed introduction of Tac in 517 de novo LT recipients has been explored by Neuberger et al. as mentioned previously (Neuberger, Mamelok et al. 2009). In summary, this important study demonstrated that the patients in the study arm with delayed introduction of reduced dose-Tac under the protection of MMF and daclizumab showed less impairment of renal function compared to the standard Tac and steroids protocol without an increased frequency of acute rejection episodes, graft loss or mortality.

The evidence from these two large clinical studies suggests that delaying and decreasing the dose of the CNI is a safe and efficient strategy to reduce exposure to CNI in the early post transplant phase after KT and LT. Further studies are needed to clarify whether this strategy will render an enduring improvement on the prevalence of CKD and patient survival after LT.

Late post transplant setting

The presence of preexisting renal abnormalities in patients coming to LT may increase the risk for development of renal dysfunction late after liver or intestinal transplantation. Besides the glomerular changes associated with HCV infection, diabetes and arterial hypertension there are other entities associated per se with advanced liver disease. These preexisting lesions have been labeled as hepatic glomerulosclerosis (Axelsen, Crawford et al. 1995) and are characterized by mesangial expansion, capillary wall thickening, immunoglobulin deposition (IgA, IgG and IgM) among others (Axelsen, Crawford et al. 1995; Wadei, Geiger et al. 2008). To date, knowledge of whether these glomerular abnormalities persist after transplantation and how restitution of liver function will affect them is lacking. Of the ten clinical Papers studying long term renal function after LT that were reviewed in the discussion section of this thesis (Table 2), only the study by Åberg et al. reported biopsy findings after LT in four patients with ESRD after LT. Of these patients, three had a histologically confirmed CNI toxicity and the remaining patient had a pre existing IgA nephropathy (Aberg, Koivusalo et al. 2007). In our experience (Paper II) we found histological confirmation of CNI toxicity in the only case that underwent a

biopsy; a female patient with NEPT that received a MV graft. She required hemodialysis 6 months after her transplant.

In spite of the compelling evidence in the literature implicating CNI in the development of CKD after transplantation of renal (Nankivell, Borrows et al. 2003) and non renal organs, one cannot cast aside the possibility of other influences which may contribute to the threat posed against renal functional integrity. Currently, there is an increasing trend to include renal histology in the evaluation of LT candidates with impaired renal function, especially in the situation where a combined liver and kidney transplant is being considered (Tanriover, Mejia et al. 2008; Wadei, Geiger et al. 2008). In the field of renal transplantation a similar and novel approach based on histological findings and cluster analysis based upon Banff scores in patients with new-onset late graft dysfunction has been able to distinguish subgroups with different outcomes (Matas, Leduc et al. 2009). Although the method described by Matas et al. specifically focuses on renal allograft outcomes, the inclusion of a more specific characterization of histological renal changes seems to render important prognostic information, an attractive concept that perhaps could be extrapolated and implemented into the field of liver transplantation to a greater extent.

In the long term survivors after LT and IT we are confronted with a renal pathology of a different nature. Late after transplantation of a non renal organ, the typical findings of CNI renal toxicity; glomerulosclerosis and interstitial fibrosis, are features with a limited degree of reversibility (Nankivell, Borrows et al. 2003; Bahirwani and Reddy 2009). At this stage, the degree of renal involvement will depend upon the presence or not of pretransplantation renal pathology, degree and duration to CNI exposure, the clinical evolution after transplantation, additional medical strategies unrelated to IS therapy undertaken to preserve renal function, and even the diagnosis for which LT was performed. In short, the conditions for altering the physiopathology of CNI induced nephrotoxicity are different than in the early post transplantation phase. However, the improvement in renal function seen in many of the previously discussed reports of CNI reduction or elimination protocols including our own experience (Paper IV) suggests that some of these features may have a certain degree of reversibility.

The results of renal sparing immunosuppressive protocols in long term survivors after LT are as we have discussed previously, varying. Questions remain about to which degree the progression of CKD can be either prevented or even reversed by altering the way the CNI are administered. There is a clear recognition that the CNI are a key element in the development of CKD thereby laying the foundation for the strategies of CNI reduction or elimination under the protection of SRL, ERL or MMF. These strategies have been reviewed in the introductory part of this thesis as well as in recent Papers by Pham et al. (Pham, Pham et al. 2009) and the International Liver Transplantation Society Expert Panel on Renal Insufficiency in Liver Transplantation (Charlton, Wall et al. 2009).

Since calcineurin nephrotoxicity is cumulative (Nankivell, Borrows et al. 2003) we opted for a CNI elimination protocol (Paper IV). The target group for the study was patients with moderate to severe CKD (15-45 ml/min/1.73m²) with a stable liver allograft function. Patients were randomized to one of two study arms, each one with CNI discontinuation and either MMF or SRL as adjuvant IS. The conviction of the unfavorable effects of the CNI on renal function

has influenced the daily medical practice at our institution where the tendency of the attending physicians is to maintain the lowest effective level of CNI as possible to avoid nephrotoxicity. We anticipated that this practice would pose a problem if seeking to obtain a control group in which the CNI levels were not, according to protocol, to be further reduced even in the face of deterioration of renal function. Other groups have encountered similar problems in their study design rendering results that were difficult to interpret due to protocol violations in close to 80% of the patients (De Simone, Metselaar et al. 2009). Consequently, when attending a patient with renal dysfunction, it has become an accepted practice at our institution to minimize or discontinue CNI under the protection of either MMF or SRL at the discretion of the attending physician. We therefore considered it an ethical dilemma to leave patients with renal dysfunction on standard low dose CNI protocol solely to obtain a control group for this clinical trial. Mean Tac levels at the time of inclusion were 5.4 +/- 1.7 ng/ml in 23 patients and the two patients on CsA, had serum trough levels of 70 and 90 ng/ml respectively at inclusion. Therefore, we reasoned that CNI level reduction was maximized and further reductions could imply an increased immunological risk. Furthermore, since there is no alternative proven therapeutic method for CKD due to CNI nephrotoxicity, we felt that with thorough information to the patient and their informed consent after approval of the local Ethics Committee as well as of the Medical Products Agency in Sweden, our study would follow the principles outlined in the Declaration of Helsinki (WMA 2007) <http://www.wma.net/e/policy/pdf/17c.pdf>.

Our main objective was to evaluate the improvement in renal function after CNI discontinuation during a 12- month observational period. Secondary aims were to assess efficacy and safety of conversion from a CNI based immunosuppression to either SRL or MMF based maintenance therapy. Renal function was followed with measured GFR (GFR_m) using Chromium EDTA clearance at baseline, 3 months and one year. A total of 25 patients were included, MMF (n=13) and SRL (n=12). One patient in the SRL arm was retired from the study due to progressive proteinuria. Two patient deaths occurred that were unrelated to the change in IS. Twenty two patients were followed for one year. In accordance with the already discussed experiences of other groups, we also found a significant (p=0.0005) overall improvement in renal function (35%) after discontinuation of CNI- maintenance IS. We found it remarkable that largest improvement in GFR_m was in the group of patients with the most advanced renal impairment (GFR_m below 30 ml/min/1.73m²) in which GFR_m improved with 63% after CNI retirement. This raises the question of which is the lowest GFR_m threshold for which CNI discontinuation will be effective? This is an important question that perhaps can be answered in a future multicenter study with a patient population stratified according to baseline GFR_m at inclusion. The protocol was effective with no BPAR episodes in any of the study arms. There were no differences between the groups with regards to AE except for the presence of oral ulcerations and mean elevated serum triglycerides, side effects that are well described with the use of SRL. In this context it is important to note that the majority of the side effects in the SRL arm occurred early after the switch in IS and probably reflect trough levels that were initially higher than those aimed for in the protocol. It is possible that if these levels would have been within the therapeutic range the outcome would have been different. One concern is the development of progressive proteinuria. In one patient in the SRL arm this resulted in a premature retirement from the study. In total four patients presented with proteinuria at study inclusion (2 in each study arm) which progressed during the study despite preventive measures such as initiation of ACE- inhibitors.

Emerging data have shown potential nephrotoxicity from SRL (Marti and Frey 2005; Jensen, Wiseman et al. 2008). One of the few studies of patients with native kidney disease (n=11) demonstrated acute kidney failure after SRL administration in 6 of these patients. Although no biopsies were performed in this study, which is a drawback, the timing of the onset of acute renal failure within 6 weeks after starting SRL and the recovery of renal function after stopping the medication is consistent with the hypothesis that the use of SRL had an acute detrimental effect on renal function (Fervenza, Fitzpatrick et al. 2004).

In addition, Dittrich et al. described in four patients with chronic renal allograft dysfunction who developed nephrotic range proteinuria after conversion from a CNI-regimen to SRL. Complete resolution of proteinuria occurred in all cases after reintroduction of CNI (Dittrich, Schmaldienst et al. 2004). Lastly, the results from the previously discussed report by Dubay et al. in 57 LT recipients with CNI related CKD showed a higher rate of complications in the patients in the SRL arm and their final recommendation, based on their experience, was that SRL conversion cannot be recommended for the purpose of ameliorating CNI induced nephrotoxicity (DuBay, Smith et al. 2008).

In conclusion, reports of CNI conversion to SRL- based regimens often emanate from small uncontrolled single-center studies with only a short term follow-up of renal function. Results have been varied and the evidence still remains inconclusive (Jensen, Wiseman et al. 2008). Further large scale multicenter randomized controlled trials will be necessary to provide the answer to if SRL eventually will have a role in minimizing CNI-induced renal toxicity after LT. The benefits of reducing long-term cardiovascular, metabolic, and oncologic risks as side-effects of CNI may justify these attempts.

Based on the prevailing literature, there exists a strong physiologic rationale for minimizing or replacing CNI with non nephrotoxic drugs such as MMF and SRL in the face of CNI- induced CKD. In spite of the results from the previously mentioned publications suggesting there are concerns regarding the efficacy of MMF and the potential nephrotoxic properties of SRL, these protocols have in experienced centers been shown to be safe and to offer the potential to improve renal function. However, these strategies should be implemented under close observance by an experienced transplant team and a nephrologist working in unison. Ultimately, the choice of which immunosuppressive drug to use for CNI replacement should be dictated by the individual characteristics of the patient.

6. CONCLUDING REMARKS

In this thesis we found :

- A high prevalence of CKD after transplantation of the liver or intestine in adults and children although the latter seem to have a better function in the long term.
- The natural history of CKD varies according to the patient population studied as well as the type of transplant procedure performed.
- Early renal function post transplantation correlates with late function which may permit a timely identification of those patients at risk of developing renal dysfunction.
- Discontinuation of CNI in adult patients with severe CKD after LT was safe and rendered a significant improvement in renal function during the observational period of the study.

The importance of these findings resides in the fact that renal dysfunction affects overall results and long term patient survival after transplantation. Future studies aimed at describing the underlying mechanisms and the natural history of CNI induced nephrotoxicity after transplantation are needed. The knowledge acquired may offer a better understanding of how the CNI or other alternative drugs should be used in order to minimize renal dysfunction and to reliably predict renal outcomes. Until this knowledge is available, further large-scale, randomized controlled trials with the aim to evaluate the true benefit of CNI discontinuation should be undertaken. These studies should include well defined measures of renal outcome and an adequate period of follow-up. In addition, it would be justified to study how the increasing prevalence of CKD in an aging transplant population affects health care costs and kidney allocation resources as the demand for dialysis and kidney allografts for these patients increase.

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