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REVIEW

Calcineurin inhibitor minimization protocols in liver transplantation

Stefan A. Farkas,[1] Andreas A. Schnitzbauer,[1] Gabi Kirchner,[2] Aiman Obed,[1] Bernhard Banas[3] and Hans J. Schlitt[1]

1 Department of Surgery, University Hospital Regensburg, Regensburg, Germany
2 Department of Gastroenterology, University Hospital Regensburg, Regensburg, Germany
3 Department of Nephrology, University Hospital Regensburg, Regensburg, Germany

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Correspondence
Stefan A. Farkas MD, PhD, Department of Surgery, University Hospital Regensburg, Franz-Josef-Strauss Allee 11, 93053 Regensburg, Germany. Tel.: +49 941 944 6801; fax: +49 941 944 6802; e-mail: stefan.farkas@klinik.uni-regensburg.de

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Summary

Liver transplant recipients are at increasingly high risk for suffering from impaired renal function and probable need of renal replacement therapy. Extended criteria organs and transplantation of patients with higher model for end-stage liver disease scores further increase this problem. Acute and chronic nephrotoxicity are the trade-off in immunosuppression with potent calcineurin inhibitors (CNIs). As a good renal function is associated with better graft and patient survival, CNI minimization protocols have been developed. Current strategies to overcome CNI toxicity include reduction or withdrawal of CNIs concurrently with switching over to mammalian target of rapamycin inhibitor or mycophenolate mofetil (MMF)-based regimens. This strategy caused an improvement in renal function in a significant number of liver transplantation patients according to several studies. However, total CNI avoidance seems to result in higher rejection rates. To prevent chronic renal dysfunction in patients prone to or with acute renal failure, CNI delay – with induction therapy for bridging – followed by low-dose CNI in combination with MMF are proven strategies without risking higher rejection rates. An individualized, tailor-made immunosuppressive regime, with a special focus on renal function is recommended. This review gave an overview on CNI minimization protocols in liver transplantation also focusing on recently analyzed studies.

Introduction

CNI nephrotoxicity trade-off

Introduction of calcineurin inhibitors (CNIs) for immunosuppression after renal transplantation has been responsible for remarkable improvements in both short-term graft and patient survival [1]. The reduction in early severe acute rejections in particular accounted for this improvement. However, it is becoming increasingly clear that the basis of this immunosuppression, the inhibition of calcineurin, may be linked with nephrotoxicity, hypertension, hyperlipidemia, and new-onset diabetes mellitus, side-effects that may lead to late renal allograft loss [2–4]. Also in liver transplant recipients, CNIs may cause acute and chronic nephrotoxicity [5–7]. It has been shown that exposure to CNIs within the first 6 months after liver transplantation (LTx) represents a risk factor for renal failure (reviewed in Ref. [8]). Other immunosuppressive agents were developed subsequently, including mammalian target of rapamycin (mToR) inhibitors and mycophenolate mofetil (MMF), which are not nephrotoxic *per se*. Current strategies to overcome CNI toxicity, also used in kidney transplantation, include reduction or even stopping administration of cyclosporine (CsA) [9] or tacrolimus (TAC) concurrently with switching over to sirolimus [10–12], everolimus or MMF-based regimens. This strategy has been documented (and there are currently ongoing studies) to achieve an improvement in renal

transplant function or to reduce the deterioration rate in many cases. These measures to deal with CNI toxicity need further documentation as a preserved good renal function seems to not only have an important impact on graft survival but also on patient survival [13].

In addition, the model for end-stage liver disease (MELD) score-based liver allocation procedure selects for transplant candidates with renal dysfunction prior to LTx. Furthermore, transplantation of extended criteria donor organs is associated with increased rates of renal dysfunction after LTx. CNI therapy induces acute and chronic renal dysfunction and other complications. Long-term outcomes, including patient quality of life and survival, are thereby compromised. This is the trade-off in CNIs like CsA and TAC. Reduced-CNI regimens are intensely investigated to reduce nephrotoxic effects, while simultaneously preventing early graft loss. This review gave an overview on CNI minimization protocols in LTx, which are already published or which have been presented recently.

Declining renal function is a common problem in nonrenal transplant patients

Calcineurin inhibitor nephrotoxicity is a common problem in nonrenal transplant recipients [14,15]. In the study by Ojo *et al.* [15], during a median followup of 36 months, chronic renal failure developed in 11 426 patients (16.5%). 29% of these patients required maintenance dialysis or renal transplantation. The 5-year risk of chronic renal failure after transplantation of a nonrenal organ ranges from 7% to 21%, depending on the type of organ transplanted. The occurrence of chronic renal failure among patients with a nonrenal transplant is associated with an increased risk of death by a factor of more than four. In this study, 90% of liver recipients received either CsA or TAC as basic immunosuppression. This was significantly correlated with renal failure after transplantation.

Risk factors for renal failure after liver transplantation

Different authors [16–26] have shown that severe renal failure after transplantation was associated with a significantly lower survival rate. Independent predictors of permanent renal dysfunction were the presence of serum creatinine >1.2 mg/dl at any time before LTx and a baseline GFR <70 ml/min/1.73 m^2 [25]. Diabetes mellitus, coronary artery disease, primary graft nonfunction, infections and repeated application of blood products added to a significantly higher mortality rate [16–24]. Most of these patients received an immunosuppressive protocol based on CNIs and MMF and steroids or an IL2R blockade. Also, a recent retrospective study [26], which included 1075 patients, showed that onset of chronic renal dysfunction within the first year after LTx was correlated with reduced survival. Chronic renal failure was correlated with calcineurin inhibition with CsA but not with TAC.

Impact of the MELD allocation system on renal failure

The implementation of the MELD score decreased mortality of those awaiting LTx [27–29]. MELD score-based liver allocation procedure selects on the criteria of priority for patients with renal dysfunction prior to LTx. However, the real impact of the MELD allocation system on the risk of chronic renal disease after LTx remains unknown. Machicao *et al.* [30] conducted a single-center cohort study of 174 patients undergoing LTx. Mean value of calculated MELD score in the pre MELD cohort was significantly lower than that in the MELD cohort. However, the incidence and prevalence of chronic renal failure and the need for kidney transplantation or hemodialysis after LTx were comparable between the two cohorts until 2 years after LTx. In multivariate analysis, serum creatinine at the time of LTx was the only variable associated with the development of chronic renal failure. Particularly in the MELD era, CNI toxicity adds significantly to the increased risk for renal impairment and need for renal replacement therapy. It is important to identify the risk factors for permanent renal dysfunction in liver transplant recipients for the high-risk recipients. Also the 'standard' liver transplant recipient today is – especially in countries with organ shortage and long waiting lists – transplanted with a higher MELD score, and thus mostly with marked preoperative renal dysfunction.

Study retrieval method

To analyze CNI minimization protocols, PubMed database was used with the following keywords: Liver transplantation, calcineurin inhibitors, renal dysfunction, tacrolimus, cyclosporine, sirolimus, side-effects, calcineurin inhibitor reduction and/or withdrawal. As a lot of studies of different quality were retrieved by that, the following analysis was restricted to prospective randomized studies, historic controlled case series and some exceptions of special interest. Furthermore, some very recent, unpublished studies were included because of their relevant impact to this field.

CNI delay

One approach to reduce CNI nephrotoxicity is *de novo* immunnosuppression with MMF and delayed introduc-

tion of CNIs. In order to prevent early rejection with CNI delay, a potent induction therapy is needed. Yoshida *et al.* [31] (Table 1) showed in a prospective, randomized study the effectiveness of CNI delay and induction with daclizumab in 148 *de novo* liver recipients. The patients received either MMF + delayed reduced-dose TAC (target trough level 4–8 ng/ml, starting day 4–6), or MMF + normal-dose TAC. There was no significant difference in patient survival or acute rejection. However, the GFR calculated by the Modification of Diet in Renal Disease [32] or the Cockroft–Gault formulas of GFR [33] was significantly better in the delayed, low-dose TAC group than in the standard TAC group 1 week and 6 months after LTx. In conclusion, this is the first prospective randomized study showing that only a short delay of low-dose TAC, in combination with daclizumab and MMF, preserved early renal function after LTx without increasing the risk of acute rejection.

The Vienna group retrospectively evaluated the effect of short-term induction therapy with Thymoglobulin and delayed use of CNI [34] (Table 1). One hundred and twenty-nine patients with initial CNI therapy were compared with a group of 262 patients receiving induction therapy followed by a CNI delay of 3 days. The 1-year data favored the thymoglobulin induction and delayed introduction of CNIs strategy with regards to acute rejection (14.5% vs. 31.8%, $P = 0.0008$) and renal function (creatinine levels: 1.26 vs. 1.37 mg/dl, $P = 0.0.015$; GFR: 81 vs. 72 ml/min., $P = 0.02$).

Bajjoka *et al.* [35] (Table 1) compared retrospectively 118 liver transplant recipients who received Thymoglobulin and delayed initiation of CNI (study group) with 80 liver transplant recipients, who received no antibody and initiation of CNI at day 1 (control group). All patients received MMF and steroids. Delayed CNI initiation with Thymoglobulin was associated with significant improvement in renal function throughout the first year post-transplant: lower serum creatinine (1.4 ± 0.5 vs. 1.7 ± 0.5 mg/dl, $P < 0.001$), a higher eGFR (57.4 ± 20.5 vs. 43.7 ± 14.4 ml/min/1.73 m^2, $P < 0.001$), and less dependence on dialysis (0.8% vs. 13%, $P < 0.001$) in comparison with no antibody and early CNI initiation. There was a trend of a lower incidence of early biopsy-proven acute rejection with Thymoglobulin. Surprisingly, overall infection and cytomegalovirus infection were significantly lower in Thymoglobulin-treated patients.

Recent, unpublished data

A recent study, unpublished until writing of this article, investigating immunosuppression with MMF *de novo* and delayed *and* reduced CNIs is the ReSpECT trial (A. D. Mayer, unpublished data) (Table 1). Daclizumab was given to bridge the CNI-free period in the delayed TAC group. In this, prospective multi-center study, *de novo* liver recipients were randomized to receive either: standard-dose TAC (>10 ng/ml) or 2 g MMF + reduced-dose TAC (≤8 ng/ml) or 2 g MMF + delayed reduced-dose TAC (≤8 ng/ml) to day 5 + daclizumab. All groups received corticosteroids because of center praxis. Four hundred and eighty-five patients were included. The preliminary analysis showed that the difference in calculated GFR (ml/min) from baseline to week 52 was significantly better in the delayed reduced TAC group versus the standard group ($P = 0.007$). In the MMF and reduced TAC arm the calculated GFR was slightly better than the standard regimen, although this did not reach statistical significance. Furthermore, significantly less biopsy proven acute rejections (BPARs) were found in the delayed, reduced TAC group versus the standard group ($P = 0.0054$). The lower rate of BPARs in the MMF and delayed, reduced TAC group compared with the other regimens was probably because of the inclusion of the daclizumab induction therapy in this treatment group.

CNI reduction

Calcineurin inhibitor reduction and introduction of MMF to reduce CNI nephrotoxicity were performed in a prospective, randomized study by Pageaux *et al.* [36] (Table 2). They investigated the effect of MMF introduction, followed by reduction in CNI (CsA) dose, on renal function in patients who received a transplant ≥1 year before enrollment and developed CNI-related chronic renal dysfunction. One study arm received MMF, followed by CNI dose reduction (at least 50%; $n = 29$). The other arm received CNI dose reduction of ≤25% without addition of MMF ($n = 27$). They demonstrated that MMF and low-dose CNI improved renal function at 12 months. No rejection episode was observed with MMF; one rejection episode was observed without MMF. They concluded that the introduction of MMF combined with the reduction of at least 50% of CNI dose allowed the renal function of liver transplant recipients to significantly improve at 1 year, without late acute rejection episodes.

The Hong-Kong group published their results of a case-controlled study on early elimination of steroids and CNI reduction and induction therapy with basiliximab (Table 2) [37]. Thirty-one living donor recipients received 20 mg dosages of basiliximab on day 0 and 4 after transplantation, and maintenance therapy with reduced CNI (TAC) aiming at trough levels between 5 and 10 ng/ml and MMF. The group was compared with 49 patients receiving standard immunosuppression with TAC trough levels between 10 and 15 ng/ml and tapered steroids that were eliminated completely 6 months after transplanta-

Table 1. CNI delay.

Reference	No. patients	Design	Time of IS change	Induction	Immunosuppression/groups	Acute rejection	Survival	Renal function
A. D. Mayer (unpublished data)	485	Prospective randomized multicenter	*De novo*	Yes	A: Standard TAC + IL2 B: MMF + reduced TAC + IL2 C: MMF + reduced delayed TAC + IL2	Significantly lower BPAR in group C: $P = 0.0054$	Not applicable	**Week 52** Significantly better GFR in group C: $P = 0.007$
Yoshida *et al.* [31]	148	Prospective randomized	*De novo*	Yes	A ($n = 72$): MMF + delayed reduced TAC + IL2 B ($n = 76$): MMF + normal dose TAC + IL2	A: 23.2% B: 27.7% $P = 0.68$	**Month 6** A: 86.6% B: 92.9% $P = 0.21$	**1 week after LTx** A: 110.7 ml/min B: 89.6 ml/min $P = 0.019$ **Month 1** A: 86.8 ml/min) B: 70.1 ml/min $P < 0.001$ **Month 6** A: 75.4 ml/min B: 69.5 ml/min $P = 0.038$
Soliman *et al.* [34]	391	Retrospective, comparative with historical group	*De novo*	Yes	A ($n = 129$): CNI B ($n = 262$): delayed CNI + thymoglobulin	A: 31.8% B: 14.5% $P = 0.0008$	**5 years** A: 74.3% B: 70.1% $P = 0.05$	**1 year** A: 72 ml/min B: 81 ml/min $P = 0.02$

Table 2. CNI reduction.

Reference	No. patients	Design	Time of IS change	Induction	Immunosuppression/groups	Acute rejection	Survival	Renal function
Pageaux *et al.* [36]	56	Prospective randomized	Within 3 months after inclusion, patients at least 1 year after LTx	No	A (n = 29): MMF + CNI reduction 50% B (n = 27): MMF + CNI reduction 25%	No BPAR observed	**1 year after inclusion** A: 96% B: 100%	**1 year after inclusion** A: 51.7 ml/min B: 44.8 ml/min P = 0.04
Liu *et al.* [37]	80	Case-controlled study	*De novo*	Yes	A: reduced CNI + MMF + IL2 B: Tac + steroids	**6 months** A: 6% B: 27% P = 0.038	No information provided	No information provided
Tzakis *et al.* [38]	90	Retrospective, comparative with historical group	*De novo*	Yes	A: alemtuzumab and low-dose TAC B: Tac + steroids	**1 year** A: 46% B: 55% P = 0.12	**1 year** A: 95% B: 92% P = 0.88	**1 year** $P < 0.05$: renal function P = 0.004: incidence of nephrotoxicity favoring group A
Herrero *et al.* [39]	11	Uncontrolled case study	1 year after LTx	No	MMF + CNI reduction or withdrawal	**63 weeks** 2 patients	**63 weeks** 100%	**63 weeks** Creatinine-clearance 38.16–47.01 ml/min P = 0.005
Chan *et al.* [40]	14	Uncontrolled case study	1 year after LTx	No	A: azathioprine + CNI discontinuation B: azathioprine + CNI reduction	**24 months** No deaths	**24 months** No acute rejections	**24 months** A: 2.42–1.72 mg/dl P = 0.0004 B: 2.08–1.85 mg/dl P = 0.069

tion. Notably, 94% of the patients had chronic hepatitis B infection. Results showed a lower incidence of hepatitis B infection or hepatocellular carcinoma breakthrough in the induction therapy group. The onset of diabetes was lower as was the incidence of acute rejections.

The Miami group revealed a positive effect of induction with alemtuzumab and low-dose TAC ($n = 40$) compared with standard TAC therapy without induction ($n = 50$) [38] (Table 2). The incidence of acute rejection was significantly lower during the first 2 months after LTx ($P = 0.002$) and slightly lower overall in the study group (average TAC trough level <6.5 ng/ml) versus the control group at 12 months (46% vs. 55%, $P = 0.12$). The mean creatinine levels were significantly lower in the reduced TAC group ($P < 0.05$) as well as incidence of nephrotoxicity ($P = 0.004$, conversion from TAC to non-CNIs).

A recent retrospective study of the Berlin group [26] (Table 2), which included 1075 patients, showed that onset of chronic renal dysfunction within the first year after LTx was correlated with reduced survival. The CNI CsA, not TAC, was an independent risk factor for the occurrence of chronic renal failure. Moreover, the authors demonstrated that in patients with advanced chronic renal failure dose, reduction of CNIs and the addition of MMF did not alter creatinine serum levels compared with CNI treatment alone.

Herrero *et al.* [39] (Table 2) described a small number of patients with impaired kidney function, where MMF was added and CsA was tapered. Only those patients, who were completely free of CsA during the observation period, had reduced serum creatinine and urea levels as well as an increased creatinine clearance (CrCl).

Chan *et al.* [40] described CsA discontinuation in 14 patients because of nephrotoxicity (serum creatinine levels of >1.5 mg/dl) and maintenance with azathioprine. In the patients in whom CsA was discontinued, the mean serum creatinine level decreased from 2.42 ± 0.48 to 1.72 ± 0.39 mg/dl ($P = .00004$). In another group of patients where CsA was only reduced, the mean serum creatinine level did not decrease significantly.

CNI withdrawal

Several CNI-sparing and -avoiding regimens were investigated and established in patients with chronic renal dysfunction after LTx. In 2001, Schlitt *et al.* [41] (Table 3) reported a beneficial effect of CNI withdrawal and replacement by MMF in patients developing renal dysfunction after LTx in a prospective randomized trial. In 28 patients with renal impairment, CNI was replaced with MMF in a stepwise pattern in half the group (study patients); the other half (controls) stayed on CNI immunosuppression. At the end of the study, mean (SD) serum creatinine had fallen by 44.4 (48.7) μmol/l in study patients compared with 3.1 (14.3) μmol/l in controls; a mean difference of 41.3 μmol/l. Moreover, systolic and diastolic blood pressure as well as serum uric acid decreased significantly in the study group but not in the control group. However, three reversible episodes of acute graft rejection occurred in study patients during MMF monotherapy, whereas none occurred in the control group.

In 2007, Orlando *et al.* [42] (Table 3) evaluated in 42 patients with CNI chronic toxicity whether conversion to MMF monotherapy could be as effective as the CNI-combination standard scheme. CNIs were tapered by 25% of the initial dose every month until complete withdrawal if possible and replaced by increasing doses of MMF. MMF was introduced with 0.5 g and increased to 1.5 g. Only 25% of the patients needed 1.5 g MMF. Thus, CNIs could be completely withdrawn in 41 of 42 patients. The mean length of MMF monotherapy was 27.3 ± 6.4 months. Renal function improved in 31/36 (89%) cases. In particular, creatinine dropped from 1.8 ± 0.4 to 1.56 ± 0.4 mg/dl. GFR increased from 47.8 ± 10.4 to 57.6 ± 17 ml/min ($P < 0.05$ for creatinine and GFR versus baseline). Blood levels of cholesterol and triglycerides decreased in 13 out of 17 (76%) and 15 out of 17 (89%) patients respectively. Arterial hypertension improved in four of five (80%) cases. A total of eight patients showed clinically an acute rejection episode, which was resolved by escalation of MMF to a daily dose of 2 g.

The evolving literature on mTOR inhibitors shows mixed results concerning conversion from CNIs to mTOR inhibitors because of renal preservation [43]. In a randomized controlled trial, a significant, if modest, improvement in the change of GFR was shown 1 year from the time of conversion [44] (Table 3). But there was no significant difference in absolute GFR. Shenoy *et al.* [45] randomized 40 patients with renal dysfunction (24-h CrCl 40–80 ml/min) to be withdrawn from CNI and receive SRL or to continue CNI (control arm) (Table 3). The mean time of conversion was 4.4 years after LTx. Improvement in 24-h CrCl was seen in the SRL arm at 3 months (75 SRL vs. 56 ml/min control, $P = 0.012$), whereas at 12 months there was only a trend toward improvement in the SRL arm (72 SRL vs. 58 ml/min control, $P = 0.09$). Two patients, one in each study arm, developed steroid-sensitive rejection. Side-effects of SRL were limited. Campell *et al.* [23] showed that patients with preserved renal function after LTx, remained stable concerning renal function within 1 year after being switched to sirolimus (Table 3). Fairbanks *et al.* [46] (Table 3) found a 27% increase in eGFR after switching 21 patients from CNI to sirolimus because of renal

Table 3. CNI withdrawal.

Reference	No. patients	Design	Time of IS change	Induction	Immunosuppression/groups	Acute rejection	Survival	Renal function
Schlitt *et al.* [41]	28	Prospective, randomized, controlled	At least 6 months after LTx	Yes	A: MMF (switched from CNI based regimen) B: CNI based regimen	A: $n = 3$ B: $n = 0$	100%	**Creatinine day 0:** A: 168·1 μmol/l B: 139·1 μmol/l **Creatinine at 6 months** A: 123·7 μmol/l B: 136·0 μmol/l $P = 0{\cdot}002$
Orlando *et al.* [42]	42	Case series	At least 1 year after LTx	Not applicable	MMF-monotherapy, CNI withdrawal within 4 months	8 steroid sensitive cases	100%	**Baseline** Creatinine: 1.8 mg/dl and GFR: 47.8 ml/min $P < 0.005$ **12 months** Creatinine: 1.56 mg/dl and GFR: 57.6 ml/min $P < 0.005$
Watson *et al.* [44]	30	Prospective randomized, controlled	At least 11 months after LTx	Not applicable	A: CNI based B: Sirolimus-based	A: 0 B: 2	A: 1 death B: 0 deaths	**Change in GFR baseline to month 3** A: +0.6 ml/min B: +6.8 ml/min $P = 0.024$ Absolute GFR between the study groups at 3 months favoring group B: $P = 0.02$, but not at 12 months $P = 0.07$
Shenoy *et al.* [45]	40	Prospective randomized, controlled	At least 6 months after LTx	Not applicable	A: Sirolimus-based B: CNI based	A: 5% B: 5%	A: 5% B: 5%	**Baseline creatinine clearance (CrCl):** A: 64 ml/min B: 60 ml/min **12 month CrCl:** A: l72 ml/min B: 58 ml/min $P = 0.09$
DuBay *et al.* [48]		Case–control retrospective	At least 90 days after LTx	Not applicable	A: Sirolimus-based B: CNI-based	A: 5% B: 4%	A: 12% B: 14%	**CrCl at baseline:** A: 37.0 ml/min B: 37.3 ml/min **CrCl at 12 months:** A: 37 ml/min B: 35 ml/min
Chang *et al.* [49]	14	Uncontrolled case series	*De novo*	No	SIR (or early switch from) (CNI) + MMF + steroids	6 acute rejections	0	Creatinine 2.2 mg/dl baseline 1.2 mg/dl 3 months

impairment. Only one acute rejection occurred. In a retrospective study by Nair *et al.* [47], a switch to sirolimus monotherapy did not improve renal insufficiency in 43% of the patients.

Recently, DuBay *et al.* [48] presented a study, where 75 LTx patients were switched to sirolimus because of CNI-related chronic kidney dysfunction at a minimum of 90 days and a median of 45 months. Sirolimus was maintained for a median duration of 18 months. Calculated CrCl was stabilized but did not improve. This group was compared with a well-matched CNI reduction control arm. No difference in creatinine levels were found after 1 year or rates in progression to renal replacement therapy [48] (Table 3). Moreover, the overall prevalence of side-effects was significantly higher in the sirolimus group compared with the control group. This was the case especially in patients with already severe renal dysfunction (CrCl < 30 ml/min). Chang *et al.* [49] reported their experience with sirolimus in patients for whom CNIs were contra-indicated (Table 3). In this study, 14 patients received *de novo* or early switch from CNIs immunosuppressive regimen to sirolimus (5–10 mg loading dose, followed by 1–4 mg/day). Creatinine levels improved from 2.2 ± 1.1 mg/dl at the initiation of sirolimus to 1.2 ± 0.6 mg/dl at 3 months. Six of the 14 patients experienced acute rejection [49].

Recent, unpublished data

J. A. Thompson (unpublished data) from the University of Minnesota reported (Table 3) about long-term efficacy CNI withdrawal following LTx. The retrospective study includes 100 patients started on CNIs and then taken off primarily because of impaired renal function, and then either put on MMF + steroids or sirolimus. Mean follow-up time was 28.4 months after CNI discontinuation (MMF: 30 months, sirolimus: 27 months). Mean time to CNI discontinuation after LTx was 47.8 months (MMF: 67 months, sirolimus: 20 months). After CNI discontinuation, there was one episode of BPAR in the MMF group that finally led to patient's death. Six BPAR were observed in the sirolimus group (four mild, two moderate). They found an improved creatinine (creatinine decrease ≥ 20%) in 46% of the patients changed to MMF and in 32% changed to sirolimus. A worsening of the creatinine (creatinine increase ≥ 20%) was found in 13% of the patients receiving MMF and 21% of the patients receiving sirolimus. Although this abstract provides preliminary, retrospective data, it is so far the only series with the longest follow up after CNI withdrawal and replacement by MMF or sirolimus.

The Spare-the-Nephron Liver study is a recently analyzed, prospective, multi-center study of liver recipients which has been conducted in similarity to the 'Spare the Nephron' renal study. In the Spare-the-Nephron renal study, 28% of the patients receiving MMF + sirolimus improved renal function compared with 8% and 4% in the MMF + CNI group after 12 months (T. C. Pearson, unpublished data). In the Spare-the-Nephron Liver study pre randomization, all liver patients received MMF + CsA or MMF + TAC. Antibody induction and/or corticosteroids were administered according to individual center practice. Some 1–6 months after transplantation, the patients were randomized into three arms. The first arm received MMF + sirolimus, the second arm MMF + CsA and the third arm MMF + TAC. The 12-month analysis was presented on the ATC 2008 (A. Sebastian *et al.*, unpublished data). GFR was calculated [32] at month 12. Mean time from transplant to randomization was 54 days in the MMF + sirolimus and 52 days in the MMF + CNIs group. Mean GFR increase, from baseline to month 12, was 22% in the MMF/SRL group and 5% in the MMF/CNI group. Significantly, more severe rejections (BPAR Grade ≥ II) were found in the sirolimus containing group as compared with the CNI group (15% vs. 4%). However, 31% of patients in the MMF + sirolimus (vs. 15% in the MMF + CNI group) were withdrawn for agonistic side-effects of MMF- and SRL-like bone marrow suppression, anemia and hyperlipidemia. However, the definite 24-month analysis has to be expected.

Another recent trial is the RESCUE study investigating whether everolimus allows for CNI reduction or discontinuation and may improve renal function in liver transplant recipients. In a multicenter, open-label, randomized, two-arm, 6-month study (+12-month extension), liver transplant patients with CNI-related renal impairment (60 ml/min ≤ CrCl ≥ 20 ml/min) continued on a standard CNI-based immunosuppressive regimen or were switched to a reduced-dose/discontinued everolimus-based immunosuppressive regimen (target 3–8 ng/ml). The mean change in CrCl (everolimus 1.0 ± 10.3 ml/min vs. controls 2.3 ± 7.8 ml/min; $P = 0.046$) at 6 months did not differ significantly (F. Nevens, unpublished data).

Discussion

CNI delay

Those studies, wherein CNI regimen were changed *after* occurrence of renal dysfunction, raised the question whether CNI delay after transplantation would be beneficial for preserving renal function. MMF *de novo* was the immunosuppression of choice in most of these studies, often combined with induction therapy using an anti IL2 receptor antibody. The first prospective randomized trial with delayed TAC by Yoshida *et al.* [31], revealed a significantly better GFR in patients at 6 months after transplantation as compared with those who received TAC

from day 1 after LTx. This is somehow surprising, as the TAC delay was only for 4–6 days after LTx. But also studies by Soliman *et al.* [34] and Bajjoka *et al.* [35] in which CNI delay lasted only 3 days (in combination with induction therapy) showed a significant increase in kidney function compared with CNIs from day 1.

These data point out that kidney function is at particular risk very early after LTx with a relatively long-lasting effect, especially if kidney function was already impaired. This is also confirmed by the ReSpeCT Study (A. D. Mayer, unpublished data). Concerning rejection, no significant increase was found with delayed CNI therapy. This therapeutic regimen – with delayed CNIs, MMF and induction along with corticosteroids – corresponds with the regimen we installed at our center at the University of Regensburg/Germany after implementation of the MELD allocation system in the Eurotransplant area. Moreover, patients needing renal replacement therapy did not receive CNIs before recovery of renal function. Although the number of patients needing renal replacement therapy before and after transplantation was the same in both groups (which is in accordance with the study by Machicao *et al.* [30]), the duration of renal replacement therapy after transplantation was shorter with delayed CNI therapy. A prospective study (PATRON trial) will follow (A. A. Schnitzbauer *et al.,* unpublished data).

CNI reduction and withdrawal strategies

The initial studies with CNI minimization were performed in patients that had already developed CNI nephropathy [36,41,50]. The follow up was up to 1 year in smaller patient numbers. These studies showed for the first time that introduction of MMF combined with the reduction of at least 50% of CNI dose allowed the renal function of liver transplant recipients to significantly improve at 1 year. Now longer term results are coming up, however still retrospective. In the abstract by J. A. Thompson (unpublished data) CNIs were taken off after 3 years. In 58 patients during 2 years of observation, one biopsy-proven rejection occurred, which led to the death of the patient at re-transplantation. In 46% of the patients, creatinine improved significantly. These data support that CNI withdrawal is mostly safe and improves renal function in a large fraction of the patients. Nevertheless, rejections on MMF monotherapy remained a problem for some patients. They can occur surprisingly late after LTx, as shown in these studies.

Another important point has to be considered in these studies. Only half of the patients showed a significant improvement of renal function after withdrawal of CNIs. This means that either they were taken off too late – when CNI nephropathy was already irreversible – or CNIs did not contribute to nephropathy in these patients. Thus, it should be distinguished between CNI-nephropathy and nephropathy caused by other reasons like diabetes, vasculitis or glomerulonephritis. Renal biopsy is a reliable tool to diagnose calcineurin-inhibitor-induced nephrotoxicity. In chronic toxicity, the medial hyaline deposits beaded in afferent arterioles are found and the interstitium displays striped fibrosis and tubular atrophy [51–54]. Therefore, it should be considered imperative to perform a renal biopsy before CNI withdrawal or if CNI withdrawal did not improve renal function.

A further step to avoid CNI nephropathy is replacement of CNIs by mTOR inhibitors like sirolimus. However, the evolving literature on mTOR inhibitors shows mixed results concerning conversion from CNIs to mTOR inhibitors because of renal preservation [43]. Watson *et al.* [44] found a significant, if modest, improvement in the change of GFR at 1 year from the time of conversion, but no significant difference in absolute GFR. Shenoy *et al.* showed in a late conversion trial (mean time of conversion 4.4 years) an improvement in CrCl at 3 months but not at 12 months. Fairbanks *et al.* [46] found an increase in eGFR after switching from CNI to sirolimus in 25% of the patients. Nair *et al.* [47] found no improvement in renal insufficiency in 43% of the patients after switching to SRL. The recent study by DuBay *et al.* [48] showed a stabilization of CrCl but no improvement after switching to SRL. These results are in contrast to the above-mentioned studies. Differences in study methodology may partly explain these conflicting results. DuBay *et al.* used a well-matched CNI reduction arm, which was not done in other studies. Ultimately, a multicenter, randomized controlled trial is necessary to clarify the role of sirolimus in ameliorating CNI- induced renal toxicity after LTx. Furthermore, in the study by DuBay *et al.* [48], especially patients with severe renal dysfunction had more side-effects because of SRL. Regarding the lack of effect of a switch to sirolimus, in particular for patients with already severe renal dysfunction, it has to be noted that CNI nephropathy is probably irreversible if detected too late. Thus, a switch too late only offers probably more side-effects than benefits for the patients. Furthermore, it has to be noted that a switch to sirolimus can induce proteinuria in patients after heart or kidney transplantation [12,55,56].

In the very recent 'Spare the Nephron liver study' (A. Sebastian *et al.*, unpublished data) (preliminary analysis), immunosuppression was started with MMF + CNIs. CNIs were replaced by sirolimus in the study group 54 days (mean) post-transplant. After 12 months, renal function improved significantly compared with the CNI-containing regimen; however, severe rejections were more frequent in the sirolimus-containing group. Furthermore,

31% of the patients did not tolerate MMF + sirolimus because of severe side-effects, compared with 15% in the MMF + CNI group. Similar side-effects of MMF and sirolimus, like bone marrow suppression, anemia and hyperlipidemia, are a disadvantage of this combination. Also, reduced compliance because of gastrointestinal side-effects of MMF may add to higher rejection risks. This is supported by a recent abstract (H. Sollinger, unpublished data) where compliance and rejection were investigated retrospectively in 1700 kidney transplant recipients receiving either CellCept or Myfortic. Drug discontinuation and dose reduction occurred more often in the CellCept group; this was paralleled by a higher risk of rejection.

Taken together, total avoidance of CNIs is not without risk. This regime – or at least CNI delay – should be considered for patients with impaired renal function at the time of transplantation. However, only patients with CNI nephropathy benefit from CNI withdrawal concerning renal function. A total avoidance of CNIs from the beginning of immunosuppression (not only a delay) would be desirable concerning nephropathy. However, an immunosuppressive regimen based only on MMF and steroids for all patients appears too risky. This supports the idea of an individualized, tailor-made immunosuppressive regime, where renal function has to play a very important role in the decision making. As shown by the studies by Yoshida *et al.* [31], Soliman *et al.* [34] and the recent, however preliminary results from the ReSpECT study (A. D. Mayer, unpublished data) CNI delay seems to be a reasonable approach – in combination with induction therapy – to prevent patients at risk for renal failure from the need for renal replacement therapy.

On the other hand, induction therapy with long-lasting effects like alemtuzumab is not without risk [57–60]. Thus, it should be considered to use only moderate induction therapy in severely sick patients with higher MELD scores.

In the future, new diagnostic tools (e.g. metabolic profiling) should be developed to identify patients at risk of renal failure earlier, before probably irreversible structural damages occur. Different studies showed that immunosuppressant-induced changes of metabolite patterns in urine were associated with a combination of changes in glomerular filtration, changes in secretion/absorption by tubulus cells, and changes in kidney cell metabolism [61,62]. A combination of these biomarkers including urine metabolites could probably provide this information in the future.

Until then, especially patients with higher MELD scores have to be regarded at risk for renal dysfunction after LTx. For those patients, CNI delay is recommended. To quantify and detect renal dysfunction better, CrCl and quantitative measurement of proteinuria should be documented before LTx and during follow up. Patients developing a CNI nephropathy during maintenance immunosuppression thus can be detected early. Then a kidney biopsy should be performed and taken into account to prove CNI nephropathy.

Conclusion

Preserved good renal function does improve graft survival and overall patient survival after LTx. Therefore, CNI minimization protocols have been developed. Current strategies to overcome CNI toxicity include reduction or withdrawal of CNIs along with switching to mTOR inhibitor- or MMF-based regimens. These strategies have been documented in several recent and ongoing trials to achieve an improvement in renal function in a large proportion of the LTx patients. However, total CNI avoidance seems to result in higher rejection rates. Current CNI avoidance regimens with MMF and mTOR inhibitors aiming at improvement in renal function, avoidance in early graft loss and reduction of side-effects have to be further refined and investigated. CNI delay and reduction in patients prone to renal failure are established strategies to preserve renal function. An individualized, tailor-made immunosuppressive regime, where renal function plays a central role in the decision making is recommended. However, concerning a general approach to CNI minimization, more prospective, randomized studies have to be performed.

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