Stellingen

behorende bij het proefschrift

Late Concentration-Controlled Calcineurin Inhibitor Withdrawal with Mycophenolate Mofetil in Renal Transplant Recipients

1. Late AUC-gecontroleerde onttrekking van een calcineurineremmer van een combinatie van onderhoudsimmuunsuppressie met prednison, een calcineurineremmer en mycofenolaat mofetil kan de lange termijn uitkomsten van stabiele niertransplantatiepatiënten met een standaard immunologisch risicoprofiel verbeteren (dit proefschrift).

2. Therapeutische drug monitoring van mycofenolaat mofetil gedurende en na (late) onttrekking van een calcineurineremmer van onderhoudstherapie met prednison, mycofenolaat mofetil en een calcineurineremmer kan onderdosering voorkomen en het risico op acute rejectie verminderen (dit proefschrift).

3. Onttrekking van een calcineurineremmer verlaagt de gemiddelde 24-uurs bloeddruk bij niertransplantatiepatiënten (dit proefschrift).

4. Achteruitgang van de diastolische linker ventrikelfunctie kan met het onttrekken van een calcineurineremmer van een triple regime met prednison, mycofenolaat mofetil en een calcineurineremmer bij niertransplantatiepatiënten worden voorkomen (dit proefschrift).

5. De verschillende immunosuppressiva hebben zowel voor- als nadelen. De ideale onderhoudstherapie na niertransplantatie is die combinatie van immunosuppressiva, die het beste past bij de specifieke kenmerken van de patiënt.

6. Het concept van de draagbare kunstnier is veelbelovend, maar de beste draagbare nier blijft nog steeds een transplantatienier.

7. Omdat zelfmanagement kan bijdragen aan een betere kwaliteit van leven, meer ziekte inzicht en betere therapietrouw, dient naar een toename van het aantal thuisdialyse patiënten in Nederland gestreefd te worden.

8. Gezamenlijke besluitvorming (shared decision making) moet worden bevorderd in de spreekkamer, maar de patiënt dient wel de keuzevrijheid te houden om de arts te laten beslissen.

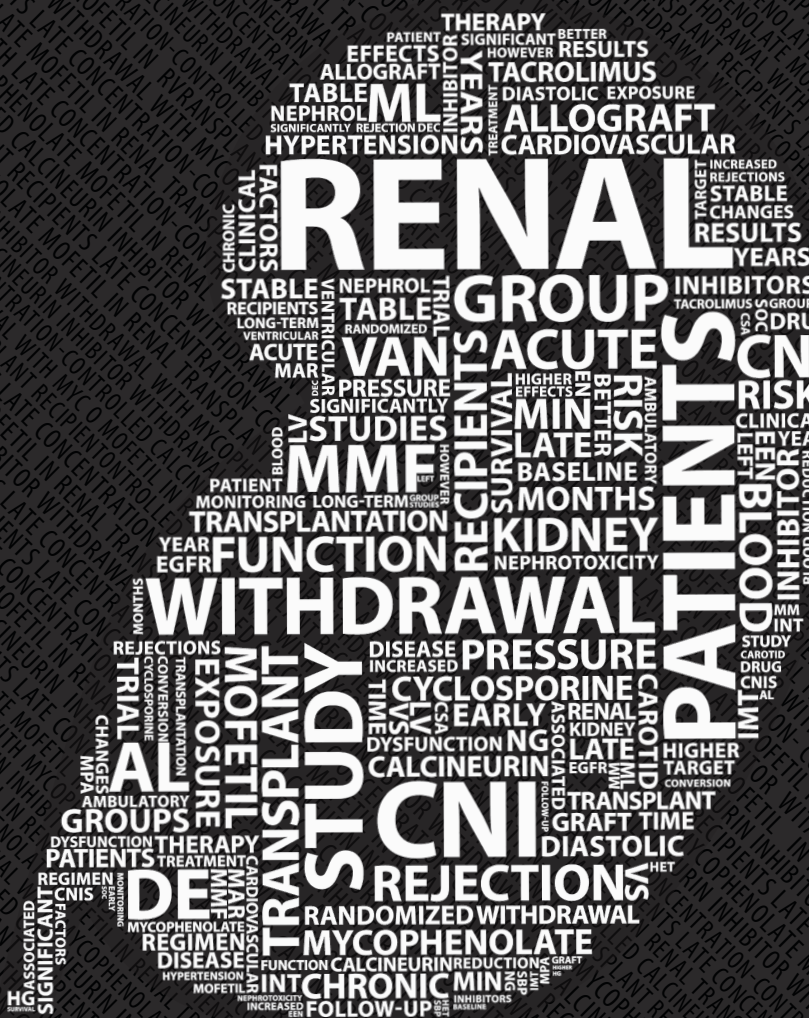
9. Promoveren getuigt van ambitie, discipline en doorzettingsvermogen en een zekere mate van masochisme.

10. Te weten dat je onwetend bent, is het begin van alle wijsheid (Marion Zimmer Bradley, The Mists of Avalon, 1983).

Jacqueline S. Mourer
21 januari 2014

Late concentration-controlled calcineurin inhibitor withdrawal with mycophenolate mofetil in renal transplant recipients

JACQUELINE S. MOURER



Late Concentration-Controlled Calcineurin Inhibitor Withdrawal with Mycophenolate Mofetil in Renal Transplant Recipients

Jacqueline S. Mourer

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Late Concentration-Controlled Calcineurin Inhibitor Withdrawal with Mycophenolate Mofetil in Renal Transplant Recipients

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CONTENTS

CHAPTER 1

Introduction

INTRODUCTION

Kidney transplantation is the optimal therapy for patients with end-stage renal disease (ESRD). Recipients of a renal transplant have a better quality of life, due to less restrictions and morbidity from chronic dialysis treatment, and improved survival compared to patients on dialysis while on the waiting list [1-2]. The reduction of acute rejection rates by the introduction of immunosuppressive drugs, in particular calcineurin inhibitors (CNIs, cyclosporine (CsA) and tacrolimus) and mycophenolate mofetil (MMF), and progress in surgical care have improved early graft function and 1-year graft survival over the past decades [3-7]. Epidemiological studies, however, have demonstrated that the enhanced short-term survival has not translated into a concomitant improvement in long-term outcome of first transplants [8-10]. This may be partly explained by the acceptance of higher risk recipients on the waiting list and the declining quality of donor kidneys due to the increase in demand for organs, more extended criteria donors, more non-heart-beating procedures and more unrelated and unmatched living donors [8, 11]. Additionally, long-term graft survival is also affected by events other than the occurrence of acute rejection. Since the number of patients with ESRD is still rising, the shortage of donor kidneys has become worse with growing waiting lists and the attention has shifted to maximising long-term graft survival.

CARDIOVASCULAR MORTALITY

Long-term graft outcome is determined by death with a functioning graft (40-50%) and late kidney graft loss (50-60%) [12-13]. The main cause of mortality is cardiovascular disease, followed by malignancy and infection [14-15]. The mortality due to malignancy has increased as the survival time after transplantation has become longer [14-15]. The incidence of cardiovascular death is reduced in renal transplant recipients compared to patients on dialysis, but is still significantly higher than that observed in the general population [14, 16]. The most common causes of cardiovascular mortality are ischaemic heart disease and cerebrovascular accidents [17]. Congestive heart failure and left ventricular hypertrophy are also more prevalent after renal transplantation [18]. Important risk factors for cardiovascular disease are age, hypertension, dyslipidaemia, (new-onset) diabetes mellitus, obesity and smoking [19-20]. Next to these conventional risk factors, additional risk factors in renal transplant recipients include deteriorating renal function, degree of proteinuria, immunosuppressive drugs, such as corticosteroids and CNIs, treated acute rejection episodes and (opportunistic) viral infections [20-21]. The cardiovascular burden has increased with larger proportions of patients with older age and higher rates of ESRD due to diabetes mellitus, as well as a longer time spent on dialysis [14, 22]. Nevertheless, a

reduction in the cardiovascular death rate has occurred over the past decades, probably as a result of the improved and more aggressive management of cardiovascular disease and cardiovascular risk factors [14, 22]. With more attention paid to the prevention and treatment of cardiovascular disease and its risk factors, it might be possible to prolong patient and graft survival.

CHRONIC TRANSPLANT DYSFUNCTION

Evolution of terminology

Late kidney graft loss is mainly caused by chronic transplant dysfunction (CTD) [23-24]. CTD is clinically characterised by a gradual, but progressive deterioration in renal function starting 3 months post-transplantation, and is often accompanied by the combination of proteinuria and (worsening of) hypertension [24]. The pathogenesis of CTD is multifactorial and is associated with immunological causes, like true chronic rejection, and non-immunological factors, such as donorage, ischaemia/reperfusion injury, hypertension, polyoma virus infection, recurrent glomerulonephritis, CNI toxicity and diabetes mellitus [23, 25]. In the nineties, the term chronic rejection was regarded as misleading and was replaced by the non-specific term chronic allograft nephropathy (CAN) to describe chronic histological changes accompanying deteriorating renal function [26]. It was characterised by interstitial fibrosis, tubular atrophy (IF/TA), glomerulosclerosis and atherosclerosis, but did not discriminate between the specific causes of chronic graft dysfunction [24, 27]. More recently, it was considered to impede an appropriate treatment following an accurate diagnosis. Consequently, the Banff classification of 2005 eliminated CAN as a diagnostic entity [27]. The newer classification distinguishes between chronic changes (IF/TA) with or without specific causes, like chronic T-cell or antibody-mediated rejection, hypertension, CNI toxicity, obstruction or viral infection [27]. If no obvious underlying pathophysiology has been found in an allograft biopsy, the terminology IF/TA with no evidence of any specific aetiology is used [27].

Risk factors

Several studies have examined (longitudinal) protocol biopsies of kidney transplants to determine the natural course and risk factors for CTD [28-32]. The study by Nankivell et al. documented that the large majority of patients (94.2%) with simultaneous kidney-pancreas transplantations had mild tubulointerstitial damage already within the first year post-transplantation [29]. The proportion of patients with severe CAN-IF/TA was minimal at one year after transplantation, but increased over the years (10 years: 58.4%). However, a recent study reported that most patients (with current immunosuppressive regimens) had mild IF/TA both at 1 and 5 years after renal transplantation, whereas only

a minority (17%) developed severe chronic histological changes at 5 years [33]. Earlier tubulointerstitial changes have been associated with pre-existing damage, ischaemia-reperfusion injury, early acute rejection and subclinical rejection [28-29, 32] and are correlated with inferior allograft function and graft survival [34-35]. Chronic tubulointerstitial damage in 2-year protocol biopsies has been associated with older donor age, early acute rejection and CNI toxicity [31]. More recently, attention has shifted to chronic humoral rejection as a major factor in graft loss [36-39].

Calcineurin inhibitor-related nephrotoxicity

CNI-related nephrotoxicity is usually divided into acute and chronic effects. The acute form is characterised by the acute deterioration of renal function over several days. It is caused by afferent arteriolar vasoconstriction leading to a decrease in the glomerular filtration rate (GFR), renal plasma flow and increased renal vascular resistance [40]. CNIs induce an increase in vasoconstrictive factors, like thromboxane and endothelin, and the activation of the renin-angiotensin system in combination with a decrease in vasodilator factors, including prostacyclin, prostaglandin and nitric oxide [41]. Additionally, free radical formation, sympathetic nerve activation and reversible tubular dysfunction are involved in acute CNI-related nephrotoxicity [41].

Chronic nephrotoxicity is related to the long-term use of CNIs and presents with a more gradual decline in renal function, which is progressive over time [42]. Chronic use may induce reversible changes in renal vasculature, but also irreversible damage, such as arteriolar hyalinosis, glomerulosclerosis and (striped) interstitial fibrosis and tubular atrophy [41]. The lesions arise from chronic haemodynamic changes, resulting in ischaemia with the formation of free radicals, an increased expression of transforming growth factor-β and angiotensin II and direct toxic effects on the tubules [41]. The prevalence of chronic CNI nephrotoxicity has been variable in studies with (protocol or for cause) transplant biopsies due to different patient populations and the non-specificity of the histological lesions [42-43]. The study by Nankivell et al. documented that chronic histological changes related to CNIs, were universal in recipients of combined kidney-pancreas transplants ten years post-transplantation [42]. In the recent DeKAF study, however, CNI nephrotoxicity was diagnosed in for cause biopsies in only 30% of patients with deteriorating allograft function with a median post-transplantation time of 7.5 years [43]. Studies in patients treated with CNIs for non-renal solid organ transplants or autoimmune diseases have also indicated chronic kidney dysfunction due to CNI toxicity [44-47].

In addition to nephrotoxicity, CNIs may cause cardiovascular side-effects, such as hypertension, hyperlipidaemia and glucose intolerance, as well as the onset of de novo diabetes mellitus [21].

MANAGEMENT STRATEGIES TO IMPROVE LONG-TERM GRAFT SURVIVAL

Strategies to improve long-term graft survival should focus on modifiable factors related to the high cardiovascular mortality and CTD (see Table 1). Non-immune strategies are based on reducing the cardiovascular risk and preserving renal function in renal transplant recipients with chronic renal failure. Additionally, immunological management comprises both preventive and therapeutic interventions aimed at the reduction of nephrotoxicity and cardiovascular side-effects caused by CNIs. This is achieved by the minimisation or elimination of CNIs and conversion to an immunosuppressive drug regimen of corticosteroids combined with an antimetabolite (MMF) and/or a mammalian target of rapamycin (mTOR) inhibitor (sirolimus, everolimus). Attempts to treat CTD are often initiated late after serum creatinine has started to rise and will be less effective, therefore earlier detection is important. Since serum creatinine underestimates decline in graft function, eGFR and proteinuria should be monitored routinely. Additionally, protocol biopsies might be considered to identify patients with subclinical rejection or to diagnose CAN-IF/TA earlier in the course of the disease.

Table 1. Strategies to improve long-term outcome in renal transplantation

Non-immunological management	Immunological management
• Proactive screening for CTD	• CNI minimisation/withdrawal
– Deterioration of allograft function	– MMF-based
– (Protocol) transplant biopsies	– mTOR inhibitor-based
• Hypertension	• CNI avoidance
• Hyperlipidaemia	– Belatacept-based (phase III)
• Diabetes mellitus	
• Smoking	
• Obesity	
• Prevention of incompliance	

CTD, chronic transplant dysfunction; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.

Non-immunological interventions

Treatment of cardiovascular risk factors

Hypertension

The prevalence of hypertension is 50-90% in renal transplant recipients [48-49]. Hypertension, defined as a blood pressure ≥140/90 mm Hg, is an independent risk factor for cardiovascular disease and mortality after renal transplantation [49-50]. Previous studies have

shown that hypertension is an independent predictor of long-term graft survival [49-52]. Hypertension after transplantation is caused by multiple factors, including pre-transplant hypertension, the use of CNIs and corticosteroids, increase in bodyweight, impaired renal function and transplant renal artery stenosis [48]. As in the general population, home blood pressure and ambulatory blood pressure monitoring are superior to office blood pressure measurements in diagnosing hypertension, because of the repeated measurements and absence of the white coat syndrome [53-55].

Clinical practice guidelines for the care of renal transplant recipients recommend a target blood pressure <130/85 mm Hg [56] or <130/80 mm Hg [57], and <125/75 mm Hg for patients with proteinuria [56]. First-line therapy includes lifestyle modifications: weight reduction, exercise and sodium restriction. There is no preferred pharmacological antihypertensive treatment after renal transplantation and no class of antihypertensive drugs is contraindicated [57-58]. Calcium channel blockers are used for the management of hypertension (early) after transplantation, since the vasoconstriction by CNIs is counteracted by their vasodilatory effect [59]. Care should be taken with non-dihydropyridine calcium channel blockers, because of their pharmacokinetic interactions with CNIs and mTOR inhibitors [58]. As inhibitors of the renin-angiotensin system have reno- and cardioprotective effects in the general population and patients with chronic kidney disease [60], the use of angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) may provide benefits in renal transplantation, in particular if proteinuria is present [58]. However, ACEi and ARBs may cause a deterioration of renal function, hyperkalaemia and anaemia [61]. A recent meta-analysis of 60 randomised controlled trials indicated that calcium channel blockers might be favoured over ACEi as antihypertensive treatment in renal transplant recipients, because they improved renal function in contrast with ACEi [62]. Patients with ischaemic heart disease or chronic heart failure may profit from treatment with β-blockers [58]. Diuretic therapy may be necessary to promote the proteinuric effectiveness of ACEi or ARBs, and in case of water retention or hyperkalaemia [48]. In daily practice, most patients require a combination of ≥2 antihypertensive agents to reach target blood pressure.

Hyperlipidaemia

The prevalence of hyperlipidaemia is 50-60% in renal transplant recipients [48]. Immunosuppressive drugs including CNIs, corticosteroids and mTOR inhibitors, but also proteinuria and decreased renal function contribute to high lipid levels [21]. Elevated cholesterol and triglyceride levels are associated with cardiovascular disease after transplantation [19]. In addition, hypercholesterolaemia is an independent predictor of unfavourable graft outcome [63]. In the ALERT study, a randomised controlled trial, lowering of low density lipoprotein (LDL)-cholesterol with 40-80 mg fluvastatin in renal transplant recipients, did not significantly reduce the composite primary endpoint, major adverse cardiac events

(MACE), after a mean follow-up of 5.1 years [64]. Though the secondary endpoint of cardiac death or non-fatal myocardial infarction was reduced by 35% (p=0.005). According to the authors the study was underpowered, but after 2 years extension a significant reduction of 21% in the incidence of MACE was demonstrated (p=0.036) [65]. The treatment with fluvastatin did not result in better graft survival [66]. A systematic review of 16 studies in renal transplant recipients revealed a tendency to reduce cardiovascular events by statins compared to placebo, however no effect on mortality was shown [67]. Despite insufficient data on graft and patient outcome, current practice guidelines recommend target levels of LDL-cholesterol <2.6 mmol/l considering the high cardiovascular risk [57, 68]. Treatment includes lifestyle changes and prescription of a statin. The majority of the statins, except for pravastatin and fluvastatin, are metabolised by the cytochrome P450 isoenzyme CYP3A4, which is inhibited by CNIs [69]. Because of this interaction with CNIs, doses of statins should be reduced to the lowest dose needed to achieve goal levels of LDL-cholesterol to prevent adverse effects such as myopathy. Ezemetibe can be safely administered as monotherapy or as an adjunctive agent to statins to decrease lipid levels [70].

Diabetes mellitus

Pre-transplant diabetes mellitus and new-onset diabetes after transplantation (NODAT) are risk factors for cardiovascular mortality and graft failure [19, 71-73]. The reported incidence of NODAT varies between 4% and 20% during the first year and increases with time post-transplantation [69]. The risk factors for the development of NODAT are patient-related, such as age, a family history of diabetes, ethnicity (African-American and Hispanic) and post-transplant weight gain and also immunosuppressive agents, including corticosteroids, CNIs, and mTOR inhibitors [71, 74]. Corticosteroids cause peripheral insulin resistance, CNIs reduce the secretion of insulin, and mTOR inhibitors affect both insulin secretion and insulin sensitivity [74]. Studies have shown that tacrolimus is more diabetogenic than CsA [71, 75]. First-line treatment of NODAT includes modification of the immunosuppressive regimen and life style changes (diet, weight reduction and exercise) [57, 74]. With screening and early detection, NODAT might even be reversed. The pharmacological management is as for type 2 diabetes mellitus in the general population [69]. According to practice guidelines the glycated haemoglobin level should be targeted at 7.0-7.5% [57].

Immunological interventions

Considering the scope of this thesis only CNI withdrawal or minimisation under MMF-based therapy will be reviewed.

A. CNI withdrawal with azathioprine or MMF

CNI avoidance in renal transplant recipients, receiving a combination of corticosteroids, MMF and induction antibodies, is associated with a very high rejection rate [76]. In one randomised controlled trial (n=54), CsA-free DR-matched patients had a lower GFR (52 vs. 69 ml/min, p=0.029) and an unacceptable high acute rejection rate (70.4% vs. 29.6%, p=0.006) compared to patients with standard CsA-based treatment at 12 months [76]. Consequently, CNI avoidance has never been implemented.

A number of earlier studies have evaluated CsA withdrawal and conversion to a regimen of steroids combined with azathioprine (AZA) [77]. A meta-analysis of ten randomised studies concluded that the incidence of acute rejection was 11% higher after CsA withdrawal than after CsA continuation (p<0.001). However, graft survival was not influenced by withdrawal [77]. Given the beneficial effects of MMF on the incidence of acute rejection during the first 6 months post-transplantation in three large randomised trials [78-80], the attention shifted from conversion to AZA towards MMF. In addition, maintenance therapy with MMF compared with AZA was associated with a decreased risk of declining allograft function in the long-term and late acute rejection up to 65% (p<0.001) [81-82].

A smaller study (n=64), investigating the conversion from CsA to either MMF or AZA at 1 year post-transplantation, found significantly less rejection in the group treated with MMF (11.8% vs. 36.7%, p=0.04) [83]. Subsequently, a number of randomised controlled trials have examined the safety of CNI withdrawal with MMF in stable renal transplant recipients [84-89]. Although early (<1 year post-transplantation) as well as late (≥1 year post-transplantation) CNI elimination resulted in improved renal function in most trials, an increased risk of acute rejection was observed [85-89]. These conclusions were rather disappointing considering the potency of MMF in three large MMF trials [6, 78-80].

Two studies compared early CNI withdrawal (3 months post-transplantation) with MMF withdrawal from a triple-drug regimen with corticosteroids, CsA and MMF [84-85]. One study (n=84) reported better allograft function (71.7 vs. 60.9 ml/min, p=0.012) after CsA withdrawal at 1 year post-transplantation [84]. The incidence of acute rejection was higher (11.3% vs. 5.0%), but this was not statistically significant [84]. The other study by Hazzan et al. (n=108) also demonstrated an improvement in renal function in the CsA withdrawal group (64.7 vs. 56.5 ml/min, p=0.023) at 1 year [85]. However, discontinuation of CsA was associated with more acute rejection episodes (18.5% vs. 5.6%, p=0.045). The patients who developed acute rejection had a significantly higher incidence of subclinical rejection in the transplant biopsy at randomisation and lower systemic exposure to mycophenolic acid (MPA).

The CAESAR trial (n=536) evaluated three treatment arms: low-dose CsA combined with corticosteroids, MMF and daclizumab, CsA withdrawal (low-dose CsA, withdrawal 4-6 months post-transplantation) and standard-dose CsA (no daclizumab) [86]. A higher rate of acute rejection was observed in the CsA withdrawal arm than in the low- and

standard-dose CsA arms (38.0% vs. 25.4%, p=0.027 and 27.5%, p=0.04, respectively) with no benefit in renal function after 12 months. Post-hoc analysis revealed that the rejectors received lower doses of MMF during the study than those who did not have acute rejection [86]. In the CsA withdrawal group, the median area under the concentration-time curve (AUC) of MPA was lower in patients that experienced rejection compared with those without rejection at month 7 [90].

Another study randomised 212 patients to CsA or prednisone withdrawal from a triple-drug regimen with prednisone, CsA and MMF or continuation of triple therapy at 6 months after transplantation [87]. Although significantly more acute and chronic rejection episodes occurred in the CsA withdrawal group (vs. control group: 22.2% vs. 1.4%, p=0.001 and 14.3% vs. 1.4%, p=0.006, respectively), no significant difference in eGFR was reported at 2 years post-transplantation.

In the study by Abramowicz (n=187), late CsA withdrawal from an MMF-containing regimen compared to CsA continuation (12-30 months post-transplantation) resulted in an increase in renal function (67.4 vs. 61.7 ml/min, p=0.05), yet with a significantly higher incidence of acute rejection after a longer follow-up time of 5 years (16% vs. 1%, p=0.0029) [88-89]. Patients with lower doses of prednisone or MMF seemed to have a higher risk of experiencing acute rejection episodes or graft loss suggesting underdosing might have played a role [89].

Since CNI-related nephrotoxicity is considered to be an important contributory factor to progressive graft dysfunction [31, 42], two randomised trials have been conducted to determine whether late CNI withdrawal could be a possible treatment of patients with CAN-IF/TA [91-92]. The Creeping Creatinine Study (n=143) documented better renal function (42.3 vs. 36.6 ml/min, p<0.01) after the elimination of CsA and addition of MMF in CsA-treated patients with declining renal function caused by CAN (>5 years post-transplantation), without the occurrence of acute rejection at 12 months [91]. Another trial in 39 patients with histologically-proven CAN (>6 years post-transplantation) had to be stopped for ethical reasons, because CNI withdrawal from triple-drug therapy led to significantly improved graft function after 6 months [92]. Again there were no acute rejection episodes after CNI withdrawal.

In summary, CNI withdrawal in stable renal transplant recipients may result in better renal function, yet these previous studies underscore the increased risk of acute rejection. The elimination of CNIs in patients with CAN-IF/TA, late after renal transplantation, may stabilise and even improve deteriorating graft function, without the occurrence of acute rejection. Possible risk factors of acute rejection are early withdrawal, subclinical rejection and underdosing of the remaining drugs. Achieving adequate drug exposure to MPA before CNI withdrawal by therapeutic drug monitoring (TDM) may be advantageous to prevent acute rejection.

B. CNI minimisation under MMF-based therapy

There have been several randomised controlled studies investigating de novo CNI minimisation with a regimen of corticosteroids, MMF, and predominantly with antibody induction, as an alternative strategy to avoid the adverse effects of CNIs [86, 93-95]. One trial randomised 313 renal transplant recipients to de novo CsA reduction (target CsA-trough level: 150 ng/ml) and conventional CsA treatment with MMF-containing immunosuppression, and reported similar acute rejection rates and renal function at 6 months post-transplantation [93]. In another study, standard CsA treatment with corticosteroids, AZA and thymoglobulin was compared with either reduced CsA (target trough level: 125-175 ng/ml) or tacrolimus (target trough level: 7-10 ng/ml) with MMF and basiliximab in 240 patients [94]. The eGFR was significantly higher in the groups with reduced dose CNI after 24 months (reduced-dose CsA vs. reduced-dose tacrolimus vs. standard-dose CsA: 59.5 vs. 61.7 vs. 52.0 ml/min/1.73 m^2; p=0.041). The incidence of acute rejection did not differ between the treatment groups. However, a limitation of this study is that the AZA-based immunosuppressive regimen of the control group is not currently considered the optimal conventional treatment.

In the CAESAR study, no increase in acute rejection risk was found in the group receiving low-dose CsA (target trough level: 50-100 ng/ml) vs. the group with standard-dose CsA at 12 months [86]. The mean eGFR was not statistically different.

The Symphony study evaluated four immunosuppressive regimens in 1645 renal transplant recipients: standard-dose CsA combined with corticosteroids and MMF and low-dose CsA (target trough level as in CAESAR study), low-dose tacrolimus (target trough level: 3-7 ng/ml) and low-dose sirolimus (target trough level: 4-8 ng/ml) combined with corticosteroids, MMF and daclizumab [95]. At 12 months, renal function was better in the low-dose tacrolimus group (65.4 ml/min) than in the other 3 groups (56.7-59.4 ml/min, p<0.001). The low-dose tacrolimus group had a lower rate of biopsy-proven acute rejection (12.3%) than the low-dose CsA (24.0%), low-dose sirolimus (37.2%) or the standard-dose CsA group (25.8%, p<0.001). Post-hoc analysis suggested (lower) MMF dose to be a predictive factor for acute rejection, especially in patients treated with CsA [96]. Disappointingly, in the 3-year follow-up study, the difference in renal function no longer reached statistical significance [97]. Ekberg et al. performed a pooled analysis of three large randomised studies (n=998), the Symphony, the Opticept, and the FDCC study, all of which used a combination regimen of corticosteroids, tacrolimus, MMF and antibody induction [98]. The study indicated, that at reduced doses, tacrolimus was less, but still consistently, nephrotoxic. Furthermore, higher MMF dose was associated with better renal function.

In conclusion, de novo minimisation of CNIs, combined with an MMF-based regimen with antibody induction, is safe with no increase in acute rejection. Low-dose tacrolimus

may improve renal function, however a beneficial effect on long-term graft function has not convincingly been shown, probably because reduced doses are still nephrotoxic.

A number of studies have evaluated early and late CNI reduction with MMF-based maintenance therapy in renal transplant recipients with stable renal function [99-102]. The Opticept trial (n=720) used three dosing regimens (30 days post-transplantation): concentration-controlled or fixed-dose MMF with standard-dose CNI or concentration-controlled MMF with 50% reduced-dose CNI (after 3 months: target trough level CsA: 95-145 ng/ml; tacrolimus: 3-5 ng/ml) [99]. At 12 months, no significant difference in the change in eGFR from baseline or the incidence of acute rejection was observed between the groups. Higher MPA exposure and trough levels were associated with a lower risk of acute rejection in patients receiving tacrolimus [99].

Two randomised controlled trials performed early stepwise CsA reduction managed by monitoring of CsA concentrations at 2 hours post-dose (C_2) in 119 and 250 renal transplant recipients, respectively, at 3 months post-transplantation [100-101]. The lower-C_2 and higher-C_2 groups showed comparable acute rejection rates [101] and renal function at 6 months [100] or 12 months post-transplantation [101]. However, the lower C_2 target ranges of CsA in these studies were not reduced compared to the standard C_2 range currently used (after 4 months: 700-900 ng/ml [100] or 6 months: 600-800 ng/ml [101]).

Pascual et al. conducted a randomised study in 64 stable renal transplant recipients to examine whether the CsA dose could be lowered by 50% more than 12 months after transplantation [102]. The CsA reduction group demonstrated an increase in GFR (from 57.7 to 64.6 ml/min, p=0.01), whereas the control group had stable renal function after 6 months. None of the patients had acute rejection after the reduction of CsA [102].

Two randomised studies investigated whether late CNI minimisation has a beneficial effect on the development of CAN-IF/TA [103-105]. The study by Stoves et al. evaluated MMF/reduced-dose CsA (target trough level CsA: 75-100 ng/ml), switch from CsA to tacrolimus (target trough level: 5-10 ng/ml) and the continuation of standard therapy with CsA in 42 patients with biopsy-proven CAN [103]. Although CsA doses were only reduced by 24% (median trough level: 99 ng/ml), the MMF/reduced CsA group showed a significant increase in renal function after 6 months, when patients with a GFR<20 ml/min were excluded. No episodes of acute rejection occurred during the follow-up. The REFERENCE trial, in 103 CsA-treated patients with deteriorating allograft function, reported that a 50% CsA dose reduction with the introduction of MMF (>6.5 years post-transplantation) improved renal function after 2 years (56.2 vs. 45.1 ml/min, p=0.02), which was sustained after 5 years of follow-up (51.8 vs. 41.3 ml/min, p=0.07), without increasing the risk of acute rejection [104-105].

In conclusion, reducing CNIs with MMF-based immunosuppression may ameliorate renal function and delay the progression of CTD, without the development of acute

rejection. Most studies, investigating CNI reduction from maintenance therapy with MMF in stable renal transplant recipients, did not observe a benefit in allograft function, although there was no increased risk of acute rejection. A limitation of most of these CNI minimisation studies is that the reduction of CNIs was relatively limited or targeted at CNI levels comparable to currently used standard levels. TDM of MPA may allow further reduction of CNIs.

THERAPEUTIC DRUG MONITORING

Although trough levels do not accurately represent systemic drug exposure, pre-dose drug concentrations are used by the majority of transplantation centres to adjust the dose of most immunosuppressive drugs. Twelve-hour AUC monitoring (AUC_{0-12}) has not become routine practice, because of the inconvenience of multiple blood samplings and expenses. Sparse sampling methods with only a few time points correlating with total drug exposure have been developed [106].

Calcineurin inhibitors

The immunosuppressive action of CsA and tacrolimus is based on the inhibition of the phosphatase calcineurin after binding to intracellular cyclophylin and FK-binding protein, respectively [107]. Eventually, calcineurin inhibition results in the reduced production and secretion of cytokines, including interleukin 2. Bioavailability is limited by the transport of intracellular CNIs from enterocytes into the intestinal lumen by the efflux pump P-glycoprotein (PGP) and by metabolisation by the cytochrome P450 isoenzymes CYP3A4 and CYP3A5 in the enterocytes [107-108]. The presence of CYP3A4 and CYP3A5 in the liver mainly account for the systemic clearance of CNIs. The large majority of CNI metabolites (>95%) are excreted in the bile and the remaining <5% in the urine [107-108]. As CNIs have considerable pharmacokinetic variability between and within patients, as well as a narrow therapeutic window, TDM is mandatory [107-108].

Trough levels of CsA correlate poorly with estimates of systemic exposure [109]. Previous studies have demonstrated that the AUC_{0-12} correlates better with acute rejection and nephrotoxicity than CsA pre-dose levels [109-110]. The highest intra- and interpatient variability in CsA exposure occurs during the first 4 hours after oral administration [111]. A shortened AUC_{0-4} correlates well with the AUC_{0-12} and is also a good predictor of renal outcome [109]. Because obtaining multiple samples is considered impractical, especially in an outpatient setting, the C_2 level of CsA has become an alternative parameter reflecting exposure, which provides a better estimation of the AUC_{0-4} than levels at other time points [112]. However, a systematic review investigating C_2 monitoring concluded there is not enough evidence to recommend C_2 monitoring [113]. Estimation of the AUC_{0-12} obvi-

ously becomes more reliable, when more samples are included in the analysis. Therefore, limited sampling strategies using samples at 2 or 3 time points have been developed for CsA [114].

Pharmacokinetic studies of tacrolimus have shown conflicting results concerning the relationship of trough levels and systemic exposure, varying from a poor to a high correlation [115-118]. Reports on the relation between the trough levels of tacrolimus and acute rejection have been inconsistent [119-121], whereas several studies have shown a significant association with nephrotoxicity [98, 120, 122]. Because monitoring of pre-dose levels does not provide a solid reflection of systemic exposure, sparse sampling methods have also been derived for tacrolimus, which more accurately predict the AUC_{0-12} [123-124].

Mycophenolic acid

MMF was introduced as a fixed-dose drug without the need for TDM. The prodrug MMF is hydrolysed to the active immunosuppressive metabolite MPA after oral absorption [125]. MPA is mostly metabolised into the inactive 7-*O*-MPA-glucuronide (MPAG) and, in smaller amounts, into the active MPA-acyl-glucuronide and the inactive 7-*O*-MPA-glucoside [125]. MPAG is excreted in the urine and the bile and is reconverted to MPA after enterohepatic circulation, giving a secondary peak later during the dosing interval [107, 125]. Contrary to tacrolimus, CsA inhibits the enterohepatic recirculation of MPAG by the multidrug resistance-associated protein-2 (MRP-2), which is involved in biliary excretion [126]. MPA is a reversible inosine monophosphate dehydrogenase inhibitor, which inhibits the synthesis of guanine nucleotides resulting in the suppression of lymphocyte proliferation [107, 125]. Adverse effects of MPA include gastrointestinal symptoms, haematological toxicity and infections [125].

Pharmacokinetics of MPA show a large variability between and within patients [107]. A relationship between MPA pharmacokinetic parameters, systemic exposure and pre-dose levels, with acute rejection has been documented in randomised concentration-controlled trials, whereas no correlation with the MMF dose was found [127-128]. Therefore, using TDM might improve MMF-based therapy. Recommendations on MPA-AUC levels early post-transplantation have been derived from these studies [129-130]. The lower threshold of the MPA-AUC of 30 µg·h/ml is based on an increased risk of acute rejection in patients with CsA-based therapy. Above 60 µg·h/ml, the incidence of acute rejection is not reduced any further [130]. Limited sampling strategies during the first 2 to 3 hours have been developed for MPA with either CsA or tacrolimus to estimate AUC_{0-12} [130]. Abbreviated AUCs are superior to trough levels in predicting AUC_{0-12} [131].

TDM of MPA might be helpful to prevent underexposure of MPA and the subsequent occurrence of acute rejection, especially when withdrawing CNIs from MMF-based therapy. There have been no prospective randomised studies investigating CNI withdrawal by individualised MMF dosing. However, a number of CNI withdrawal studies

suggested that underdosing of the remaining immunosuppressive drugs might increase the risk of acute rejection [85-86, 89]. In the study by Hazzan et al., the subgroup experiencing acute rejection episodes had a lower mean MPA-AUC (43 vs. 58 µg·h/ml, p=0.045) [85]. In the pharmacokinetic substudy of the CAESAR study, patients with acute rejection had a lower median MPA-AUC (39.7 µg·h/ml) compared to patients without acute rejection (67.5 µg·h/ml) in the CNI withdrawal group at month 7 [90]. From both studies it can be concluded that the required MPA-AUC might have to be higher (>60 µg·h/ml) with dual therapy with corticosteroids and MMF.

AIM OF THE THESIS

The aim of this thesis was to optimise care for renal transplant recipients in a dedicated outpatient clinic by providing target-driven therapy for modifiable cardiovascular risk factors and to assess the impact of late concentration-controlled CNI withdrawal with MMF on renal allograft function, acute rejection rate and markers of cardiovascular disease.

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CHAPTER 2

Randomized trial comparing late concentration-controlled calcineurin inhibitor or mycophenolate mofetil withdrawal

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ABSTRACT

Background. Early calcineurin inhibitor (CNI) withdrawal with mycophenolate mofetil (MMF) has not become routine practice, due to concerns about excess acute rejection. Therapeutic drug monitoring may be advantageous when the CNI or MMF is withdrawn.
Methods. This prospective, randomized, concentration-controlled withdrawal study enrolled 177 stable renal transplant recipients on maintenance CNI-based immunosuppression, combined with steroids and MMF. After the feasibility phase of the study, patients were randomized to MMF-withdrawal (target area under the concentration-time curve-cyclosporine: 3250 ng·h/mL or tacrolimus: 120 ng·h/mL) or CNI-withdrawal (target area under the concentration-time curve-mycophenolic acid: 75 µg·h/mL).
Results. The estimated glomerular filtration rate (modification of diet in renal disease) remained significantly better after CNI elimination (59.5±2.1 vs. 51.1±2.1 mL/min/1.73 m^2, *P*= 0.006) up to 3 years and resulted in less functional decline, including the subgroup with an estimated glomerular filtration rate less than 50 mL/min/1.73 m^2 at baseline (*P*=0.03). At 6 months, one patient in the MMF-withdrawal group (1.3%) and three in the CNI-withdrawal group (3.8%) experienced acute rejection (*P*=0.62). The defined higher mycophenolic acid exposure was well tolerated.
Conclusion. These data indicate, that with time the large majority of stable renal transplant recipients can safely be reduced to dual therapy with either MMF or CNIs, applying concentration-controlled dosing. CNI-free patients, including those with moderate renal allograft dysfunction, have the benefit of improved renal function, whereas the risk of acute rejection after late withdrawal is low.

INTRODUCTION

Chronic allograft nephropathy (CAN), currently defined by interstitial fibrosis and tubular atrophy (IF/TA) [1], is the most prevalent cause of late graft failure. IF/TA develops early after transplantation and is the consequence of the summated effects of tissue injury, dominated by acute rejection and calcineurin inhibitor (CNI)-related nephrotoxicity [2-3]. Progressive vasculopathy, including arteriolosclerosis, arteriolar hyalinosis, obliteration of peritubular capillaries and glomerular sclerosis become increasingly important over time [4-5].

Currently, early intervention strategies focus on developing immunosuppressive regimens, that allow CNI minimization and reduce CNI-associated toxicities, while maintaining low acute rejection rates [6-7]. The results of these CNI minimization trials with interleukin-2-receptor blocker prophylaxis and mycophenolate mofetil (MMF), indicate that lower levels of CNI exposure are sufficient in de novo kidney transplant recipients. Minimization or, preferably, elimination of CNIs, appears to be a key decision to potentially modify long-term outcome [8-9]. Early maintenance therapy with steroids and MMF, however, has not become routine practice, mainly due to concerns about acute rejection after CNI discontinuation [10]. Despite the documented salutary effects of MMF on acute rejection [11], when used with CNIs and corticosteroids, several multicenter trials have underscored the increased risk of acute rejection episodes as the major limitation to complete CNI withdrawal [6, 12-13]. It is important to note, however, that the risk of rejection in these studies was higher in patients with lower mycophenolic acid (MPA) exposure. The optimal range of MPA exposure in patients on reduced or no cyclosporine (CsA) was found to be higher than 30-60 µg·h/mL that is usually targeted in combination with CNIs [14]. Therapeutic drug monitoring may be advantageous when CNIs are withdrawn from a triple-drug regimen including MMF [13]. We hypothesized that adequate exposure before late withdrawal of either the CNI or MMF could significantly reduce the risk of acute rejection.

RESULTS

Study population

Of 177 patients entering the study, 158 patients were randomized to either CNI or MMF withdrawal between March 2003 and September 2007. Baseline characteristics were comparable with respect to demographic and transplant-related characteristics, such as donor age, histocompatibility, panel reactive antibodies, acute rejection history, time posttransplant, blood pressure (BP), lipid profile, renal function and proteinuria (Table 1).

Table 1. Baseline characteristics at randomization

		MMF withdrawal (N=79)	CNI withdrawal (N=79)	*P*
Recipient age (yr)		52.7±13.0	52.5±10.8	0.91
Sex (M/F)		0.68	0.71	0.73
Previous rejection (%)		18	18	1.00
PRA>5% (%)		44	45	0.96
HLA mismatch	A locus	1.0±0.6	0.8±0.6	0.12
	B locus	1.0±0.6	1.0±0.6	0.61
	DR locus	0.9±0.7	0.8±0.6	0.71
Time posttransplantation (yr)		3.5±2.6	3.1±2.0	0.26
Donor age (yr)		42.5±14.4	43.3±16.6	0.75
Donor sex (M/F)		0.44	0.51	0.43
Donor sex mismatch (%)		52	40	0.15
Donor type (%LD/DD)		39/61	41/59	0.82
Delayed graft function (%)		34	34	0.96
BMI (kg/m^2)		26.2±4.3	26.6±4.1	0.65
Hemoglobin (mmol/L)		8.4±0.9	8.5±1.0	0.44
MDRD clearance (mL/min/1.73 m^2)		53.9±16.4	58.0±16.1	0.12
Albumin:creatinine ratio (mg/mmol)		2.17 (1.30-5.87)	2.10 (1.00-4.50)	0.49
Cholesterol (mmol/L)		5.3±1.2	5.2±1.0	0.66
LDL-cholesterol (mmol/L)		3.2±1.0	3.1±0.9	0.66
HbA1c (%)		5.8±1.0	5.8±0.9	0.82
Systolic blood pressure (mm Hg)		140.1±16.5	142.2±17.6	0.45
Diastolic blood pressure (mm Hg)		80.2±10.7	81.5±10.2	0.44
Mean arterial pressure (mm Hg)		100.2±11.3	101.7±10.9	0.38
CsA/tacrolimus (%)		75/25	77/23	0.71
MMF/myfortic (%)		96/4	94/6	0.48
CsA dose (mg/d)		208±54	220±61	0.26
AUC CsA (ng·h/mL)		3538±968	3625±782	0.60
Tac dose (mg/d)		5.0±1.3	5.9±2.7	0.18
AUC Tac (ng·h/mL)		158±70	149±65	0.66
MMF dose (mg/d)		1595±498	1737±532	0.09
AUC MPA (µg·h/mL)		42±18	48±21	0.08
Prednison dose (mg/d)		7.0±1.7	7.3±1.8	0.25
Antihypertensive drugs ≥2 (%)		74	72	0.93
Calcium entry blocker (%)		64	62	0.79
ACE inhibitor (%)		42	41	0.82
Angiotensin II receptor blocker (%)		19	10	0.11
Diuretics (%)		34	27	0.28

Data are presented as mean±standard deviation, median and interquartile range or percentages.
MMF, mycophenolate mofetil; CNI, calcineurin inhibitor; M, male; F, female; PRA, panel reactive antibodies; HLA, human leucocyte antigen; LD, living donor; DD, deceased donor; BMI, body mass index; MDRD, modification of diet in renal disease; LDL, low density lipoprotein; HbA1c, glycated hemoglobin; CsA, cyclosporine; Tac, tacrolimus; AUC, area under the concentration-time curve; MPA, mycophenolic acid; ACE, angiotensin converting enzyme.

Immunosuppression

Baseline area under the concentration-time curves (AUCs) of CsA (group B: 3538±968, range: 607-5855 ng·h/mL; group C: 3625±782, range: 1616-5351 ng·h/mL), tacrolimus (Tac) (group B: 158±70, range: 93-400 ng·h/mL; group C: 149±65, range: 41-343 ng·h/mL) and MPA (group B: 42±18, range: 12-94 µg·h/mL; group C: 48±21, range: 18-134 µg·h/mL) were variable (Table 1; see SDC, Figure S1, http://links.lww.com/TP/A647), with underexposure for CsA (<3000 ng·h/mL) in 27% or 20% (*P*=0.36), tacrolimus (<100 ng·h/mL) in 10% or 22% (*P*=0.40) or MPA (<30 µg·h/mL) in 29% and 17% (*P*=0.08) or both drugs 7% or 5% of patients, in group B or C (*P*=0.75). Dose adaptations were guided by a dedicated hospital pharmacist. In the MMF-withdrawal group the mean daily CNI doses were comparable at baseline, week 6 and month 6 (CsA: baseline: 208±54; week 6: 208±46; month 6: 213±47 mg/d; Tac: baseline: 5.0±1.3; week 6: 4.8±1.3; month 6: 5.3±1.8 mg/d). At 3 years the mean CsA-AUC (3524±698, range: 2413-5411 ng·h/mL) was still above the target range in group B, despite dose adjustments after previous study visits, but less variable. The mean Tac-AUC (135±38, range: 62-205 ng·h/mL) and MPA-AUC (75±19, range: 32-121 µg·h/mL)

Table 2. Clinical parameters 3 yr after withdrawal

	MMF withdrawal	CNI withdrawal	*P*
BMI (kg/m^2)	26.9±4.8	26.9±4.6	0.95
Albumin:creatinine ratio (mg/mmol)	1.55 (0.70-4.60)	2.20 (1.00-4.43)	0.29
Cholesterol (mmol/L)	4.8±0.9	4.8±1.1	0.88
LDL-cholesterol (mmol/L)	2.6±0.7	2.7±0.9	0.45
HbA1c (%)	5.8±0.7	5.7±0.7	0.86
Systolic blood pressure (mm Hg)	135.9±18.1	133.4±15.7	0.40
Diastolic blood pressure (mm Hg)	78.3±9.9	79.0±8.4	0.64
Mean arterial pressure (mm Hg)	97.5±10.6	97.1± 9.4	0.85
CsA dose (mg/d)	203±46		
AUC CsA (ng·h/mL)	3524±698		
Tac dose (mg/d)	4.5±1.4		
AUC Tac (ng·h/mL)	135±38		
MMF dose (mg/d)		2142±776	
AUC MPA (µg·h/mL)		75±19	
Antihypertensive drugs ≥2 (%)	77	66	0.27
Calcium entry blocker (%)	63	59	0.62
ACE inhibitor (%)	63	59	0.62
Angiotensin II receptor blocker (%)	23	18	0.44
Diuretics (%)	37	29	0.36

Data are presented as mean±standard deviation, median and interquartile range or percentages.
MMF, mycophenolate mofetil; CNI, calcineurin inhibitor; BMI, body mass index; LDL, low density lipoprotein; HbA1c, glycated hemoglobin; CsA, cyclosporine; Tac, tacrolimus; AUC, area under the concentration-time curve; ACE, angiotensin converting enzyme.

reached the target range with less variability (Table 2; see SDC, Figure S2, http://links.lww.com/TP/A647). The mean daily MMF dose increased from 1737±532 mg/d (baseline) to 2292±598 (week 6) and 2322±682 mg/d (month 6).

Renal function and proteinuria

The estimated glomerular filtration rate (eGFR) was significantly better at 6 weeks (63.1±1.9 vs. 55.2±1.9 mL/min/1.73 m^2, *P*=0.004), 1 year (61.1±1.8 vs. 52.9±1.8 mL/min/1.73 m^2, *P*=0.002) and 3 years after randomization (59.5±2.1 vs. 51.1±2.1 mL/min/1.73 m^2, *P*=0.006) in the CNI-withdrawal group (see SDC, Table S1, http://links.lww.com/TP/A647). The mean difference of eGFR using the modification of diet in renal disease (MDRD) clearance [15] relative to baseline (Fig. 1A) was significantly higher immediately after CNI elimination. Both groups demonstrated a slight decline in MDRD levels over time (see SDC, Table S1, http://links.lww.com/TP/A647), with no statistical difference (*P*=0.91) in slopes during the 3-year follow-up period. In the subpopulation with an initial eGFR less than 50 mL/min/1.73 m^2 (but ≥30 mL/min/1.73 m^2), CNI withdrawal also resulted in significantly better renal function (Fig. 1B). Of note, in this subgroup the rate of eGFR deterioration was significantly less after CNI elimination (slope: +0.008 vs. -0.096 mL/min/1.73 m^2/month, *P*=0.03). During the follow-up median urine albumin/creatinine ratios did not differ between the groups (Table 2).

Acute rejection

At 6 months, one patient in the MMF-withdrawal group (1.3%) and three in the CNI-withdrawal group (3.8%) experienced biopsy-proven acute rejection (BPAR, *P*=0.62) (Table 3). The acute rejection episodes occurred after 161 (group B) and 75, 55 and 84 days (group C) after withdrawal, despite sufficient exposure of the CNI and MMF (see SDC, Table S2, http://links.lww.com/TP/A647 4). None of the patients had an immunological risk profile predictive of acute rejection: none of them were highly immunized or had been treated for rejection before randomization. Only one patient had a high human leucocyte antigen mismatch (group B). One patient in group B (1.3%) and one in group C (1.3%) experienced BPAR late after withdrawal, after 991 and 369 days, respectively. In case of the late rejection in group B, there was suspicion of incompliance and the rejection in group C was most likely induced by underexposure to MPA (not documented) during a diarrhea episode due to cytomegalovirus (CMV) colitis. At 36 months, the incidence of BPAR was not significantly different between both groups (group B: 2.5% vs. group C: 5.1%; *P*=0.68; Table 3).

Chronic transplant dysfunction

During the study, 4 (5.1%) and 5 (6.3%) renal biopsies were performed because of chronic transplant dysfunction (CTD) in group B and C. Transplant glomerulopathy was diag-

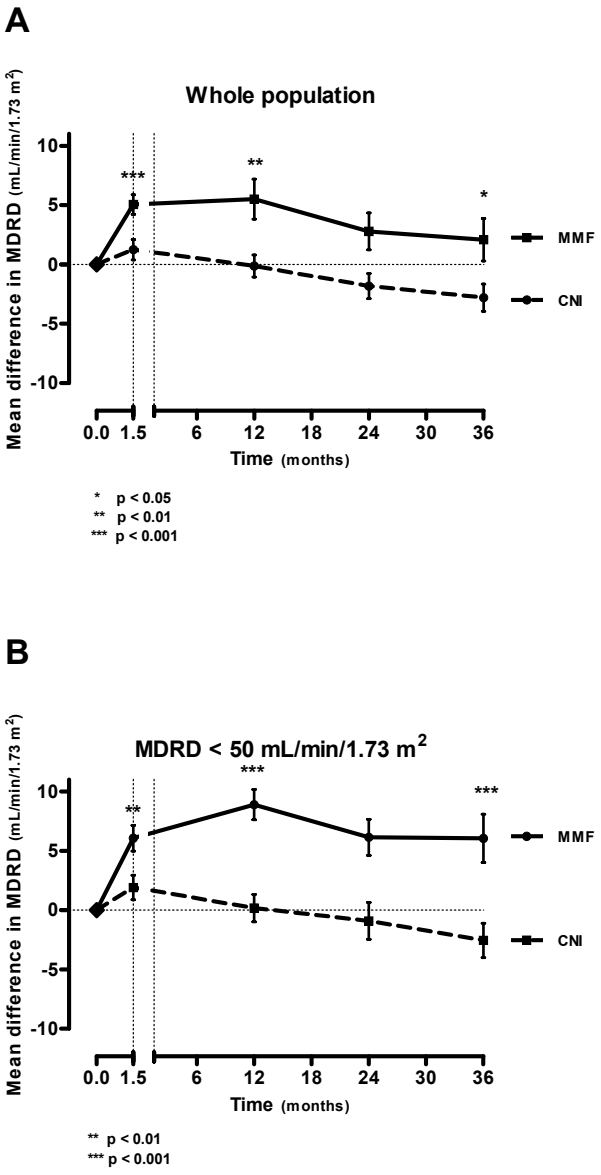

Figure 1. Mean difference in eGFR relative to baseline

Intention-to-treat analysis of renal function (eGFR) estimated using the modification of diet in renal disease (MDRD) formula. Plotted are the mean differences in eGFR relative to baseline with their standard errors of the mean at 6 weeks, 1, 2 and 3 years after either calcineurin inhibitor (CNI)- (solid lines) or mycophenolate mofetil (MMF)-withdrawal (dotted lines).

(A) Whole population (B) patients with an eGFR at baseline <50 mL/min/1.73m^2.

Table 3. Safety 3 yr after withdrawal

	MMF withdrawal (N=79)	**CNI withdrawal (N=79)**	***P***
Patient survival 3 yr	92.4	94.9	0.75
Graft survival censored for death 3 yr	98.7	98.7	1.00
BPAR:			
6 mo	1.3	3.8	0.62
1 yr	1.3	5.1	0.37
3 yr	2.5	5.1	0.68
Therapy failure:	15.2	19.0	0.53
Graft loss	1.3	1.3	1.00
Patient death	7.6	5.1	0.75
Acute rejection	2.5	5.1	0.68
Switch to:	8.9	13.9	0.32
CS/CNI/MMF	2.5	6.3	0.44
CS/MMF	6.3	0	0.06
CS/CNI	0	6.3	0.06
CS/mTOR inhibitor	0	1.3	1.00
Informed consent withdrawn	5.1	2.5	0.68
Lost to follow-up	2.5	2.5	1.00
Renal biopsies*	7.6	11.4	0.42
Histological diagnosis:			
BPAR	2.5	5.1	0.68
Chronic changes:			
Transplant glomerulopathy	0	2.5	0.50
IF/TA	5.1	5.1	1.00
Arteriolar hyalinosis	3.8	5.1	1.00
Global glomerulosclerosis	2.5	2.5	1.00
Recurrent disease: IgA nephropathy	0	2.5	0.50
Adverse events :			
Diarrhea	0	3.8	0.25
Infections:			
None	68.3	57.6	0.23
Single	15.0	13.6	0.82
≥2	16.7	28.8	0.11
Anemia Hb<7 mmol/L	11.4	22.8	0.06
Malignancy**	7.6	5.1	0.75
New-onset diabetes mellitus	6.5	5.2	1.00

Data are presented as percentages. *with histological diagnosis, **excluding basal or squamous cell carcinoma of the skin.

BPAR, biopsy-proven acute rejection; CS, corticosteroids; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; IF/TA, interstitial fibrosis and tubular atrophy; IgA, immunoglobulin A; Hb, hemoglobin.

nosed in two patients in the CNI-withdrawal group (2.5%). Four biopsies in both groups (5.1%) demonstrated chronic changes specific for CAN-IF/TA (Table 3). Two of these four patients in the CNI-withdrawal group had been switched to CNI-based therapy; one because of an episode of rejection and another due to leucopenia. Two biopsies in the CNI-withdrawal group (2.5%) demonstrated recurrence of IgA nephropathy.

Patient and graft survival

The 3-year patient survival was 92.4% in the MMF-withdrawal group and 94.9% in the CNI-withdrawal group (P=0.75) (Table 3). The 3-year graft survival censored for death was 98.7%, both in the MMF-withdrawal and CNI-withdrawal group (P=1.00). Causes of graft loss were CAN-IF/TA (group B) and chronic rejection (group C).

Treatment failure

Treatment failure defined by graft loss, patient death, acute rejection or change of immunosuppressive therapy was not different between the groups (Table 3). The main reason for reinstituting triple therapy was acute rejection in both regimens. Patients were switched to the other treatment arm because of adverse effects of the remaining drug: in case of the MMF-withdrawal group: CAN-IF/TA (1), gout (1), neuropathy (1), gingival overgrowth (1) and co-administration of verapamil (1); in case of the CNI-withdrawal group: diarrhea (2), recurrent infections (1), pancreatitis (1) and leucopenia, later definitely attributed to allopurinol (1).

Blood pressure and lipid profile

Systolic and diastolic BP decreased in both groups. At the end of the study, there were no significant differences in systolic and diastolic BP between the groups. There was a trend to use more antihypertensive drugs in the MMF-withdrawal group (Table 2). Cholesterol and low density lipoprotein-cholesterol fell during the study, with no differences between the groups.

Adverse events

The defined MPA exposure was well tolerated; diarrhea occurred in only three patients in the CNI-withdrawal group (3.8%) (Table 3). In retrospective, these patients already had diarrhea at inclusion, but had not mentioned this. Consequently, two of them were switched to dual therapy with steroids and a CNI. The other patient developed CMV-colitis and was switched to triple therapy because of acute rejection. Anemia (hemoglobin <7.0 mmol/L) was found more frequently in the CNI-withdrawal group (22.8% vs. 11.4%, P=0.06). One patient developed persistent leukocytopenia that resolved after cessation of allopurinol. There was no difference in the occurrence of single or recurrent infections, new onset diabetes mellitus or malignancies between the groups (Table 3).

DISCUSSION

This study used therapeutic drug monitoring to adjust the dose of MMF or the CNI to the target levels in stable renal transplant patients before withdrawing one of the drugs from a triple-therapy regimen. Only 1 of 79 patients (1.3%) in the MMF-withdrawal group and 3 of 79 (3.8%) in the CNI-withdrawal group experienced acute rejection during the first 6 months after withdrawal. During the follow-up, patients were dosed based on infrequent AUC monitoring as it was a relatively low-risk population. Higher MPA exposure was well tolerated and late withdrawal of CNIs led to an immediate and significant improvement in renal function, remarkably also in patients with an eGFR less than 50 mL/min/1.73 m^2 (but still ≥30 mL/min/1.73 m^2). These findings are in line with previous studies regarding CNI withdrawal in patients with progressive deterioration of renal allograft function [16-17]. The CONVERT trial showed that late CNI conversion to sirolimus resulted in better renal function, only for those with a baseline eGFR more than 40 mL/min [18]. Our results extend these observations to maintenance therapy with MMF and patients with an eGFR more than or equal to 30 mL/min/1.73 m^2.

The sustained increase in renal function directly after CNI withdrawal most likely resulted from reversal of renal vasoconstriction. CNIs cause acute reversible and chronic irreversible nephrotoxicity. Acute nephrotoxicity is induced by afferent arteriolar vasoconstriction, which is followed by a reduction in renal blood flow and GFR [19-20]. Repeated daily episodes of renal vasoconstriction and ischemia might result in chronic structural changes of preglomerular vasculature, glomeruli and tubulointerstitium. Chronic CsA administration in a renal transplant rat model induced segmental sclerosis in glomeruli [21]. Additionally, CNIs induced upregulation of transforming growth factor-β, which may vary widely between individuals and on the renal molecular level [22-24]. Although CsA and tacrolimus are structurally different compounds, they have similar nephrotoxic potential at the clinical, histological and molecular level [24-25]. The catalytic subunit of calcineurin consists of multiple isoforms and the α-isoform has a predominant role in kidney development [26]. Nephrotoxic changes associated with CNIs may be the result of chronic inhibition of α-activity in tubular epithelial cells. Mice lacking the α-isoform of calcineurin have impaired renal function and histological changes and matrix expansion with increased fibronectin and transforming growth factor-β comparable with CNI-induced nephrotoxicity in humans [27]. Despite dose adaptation, the actual CNI exposure remained higher than the predefined targets. This may also play a role in the observed renal function over time.

This prospective controlled trial demonstrates that, in contrast with early withdrawal [6, 13, 28-29], late concentration-controlled CNI withdrawal was successful in more than 95% of patients, with a low risk of acute rejection. In the CAESAR study, CsA tapering between month 4 and 6, followed by withdrawal, resulted in 50% increase in acute rejection [6].

Posthoc analysis, however, revealed that the risk of rejection was highest in patients with low MPA exposure, and that the optimal range of MPA exposure in patients on reduced or no CsA was close to 60 µg·h/mL [6]. This observation compares well with a study that evaluated acute rejection after withdrawal of CsA or MMF three months posttransplantation [30]. Low MPA exposure was shown to be an independent risk factor for acute rejection. In the Opticept trial, a maintenance regimen with concentration-controlled dosing of MMF combined with low dose CNI was not inferior with respect to the occurrence of acute rejection, indicating the utility of concentration-controlled exposure to MMF with CNI withdrawal [31]. It appears therefore critically important, that dosing and target concentrations of the remaining immunosuppressant are chosen such, that adequate overall immunosuppression is maintained during CNI minimization or withdrawal.

Of note, none of the patients could have been predicted to have rejection based on their immunological risk profile. This underlines the importance of frequent examination of renal function during the first months after withdrawal. The 5-year follow-up study by Abramowicz reported that acute rejection also occurred long-term after CNI withdrawal [13]. During the follow-up of our study, only one patient in each group experienced a rejection episode, after the first year. One occurred as a consequence of CMV-colitis resulting in underexposure to MPA, the other one was related to incompliance with the prescribed CNI. This suggests that active AUC-monitoring prevented underexposure, and the occurrence of acute rejection at later time points. Physicians and patients should however be aware of the risk of underimmunosuppression in case of an intercurrent illness, especially with dual therapy.

Despite the proof of principle, the current study has several limitations. This is a single-center study in a selected group of predominantly Caucasian kidney transplant recipients (78.5%) and consequently the results may not be directly extrapolated to other populations. Second, although the rejection rate in both withdrawal groups was low, the current study was not designed or powered to show a difference in this outcome. Outside the small feasibility phase, there was no control group continuing on triple immunosuppression. However, next to excellent prevention of acute rejection, it is also unlikely that graft function would have improved in a CNI- and MMF-based control group. Only 25% of patients in the MMF-withdrawal group continued with tacrolimus, whereas nowadays tacrolimus has become the most frequently prescribed CNI after renal transplantation. However, CsA and tacrolimus share similar nephrotoxic potential, and therefore our findings may be generalized to currently used regimens with tacrolimus and MMF. Finally, no protocol biopsies were obtained at the time of randomization or during follow-up to identify patients with subclinical rejection and/or CAN-IF/TA. According to the study protocol, a renal biopsy was performed when serum creatinine increased more than 15%. Because serum creatinine is a relatively insensitive marker, the rate of (subclinical) rejection or CAN-IF/TA may have been underestimated.

In summary, late concentration-controlled CNI or MMF withdrawal can be safely implemented in the large majority of stable renal transplant recipients. To prevent insufficient immunosuppression, MMF and CNI doses can be adjusted based on exposure measurements. Maintaining low toxicity and adequate levels of the remaining immunosuppressant, CNI elimination may lead to improved graft and patient outcomes, especially in the increasingly prevalent proportions of recipients with extended criteria kidney transplants and gradual loss of renal function.

MATERIALS AND METHODS

Patients

Recipients of a first or second deceased or living donor kidney transplant, more than or equal to 6 months posttransplantation and on a CNI-based regimen (Cyclosporine, Novartis, Basel, Switzerland or Tacrolimus, FK506, Astellas Pharma, München, Germany), with corticosteroids and MMF (Cellcept, Hoffman-La Roche, Basel, Switzerland) were considered for enrollment. To be included, subjects must have had less than or equal to 2 rejection episodes after transplantation, no acute rejection during the 6 months before enrollment, and stable renal function with an MDRD clearance more than or equal to 30 mL/min/1.73 m^2 for at least 3 months. Exclusion criteria were chronic diarrhea or gastrointestinal disorders that interfere with the absorption of oral medication; active peptic ulcer disease; malignancy, except successfully treated nonmetastatic basal or squamous cell carcinoma of the skin; pregnancy or lactation; panel reactive antibodies more than 60% at the time of transplantation; current or historic treatment with unlicensed, investigational drugs or other prohibited medication; and (active) systemic infection (including CMV) requiring therapy at study entry.

Study design

Prospective, single-center, randomized open controlled study in stable kidney transplant recipients on a triple-drug regimen (Fig. 2). The trial was performed in conformity with the Declaration of Helsinki, Good Clinical Practice guidelines. Informed consent was obtained from all patients. The study was approved by the Leiden University Medical Center ethics committee (ISRCTN81895822). The first (feasibility) phase randomized 58 patients (1:1:1) to MMF or CNI withdrawal or continuation of their current regimen (CsA-C_2 target 700 ng/mL). No acute rejection episodes occurred and the relatively high MMF doses were well tolerated after withdrawal. Encouraged by these results, in the second (extension) phase of the study another 119 patients were randomized (1:1) to the MMF-withdrawal or CNI-withdrawal group. All patients were stratified for the occurrence of previous acute rejection and prior cardiovascular events, defined as

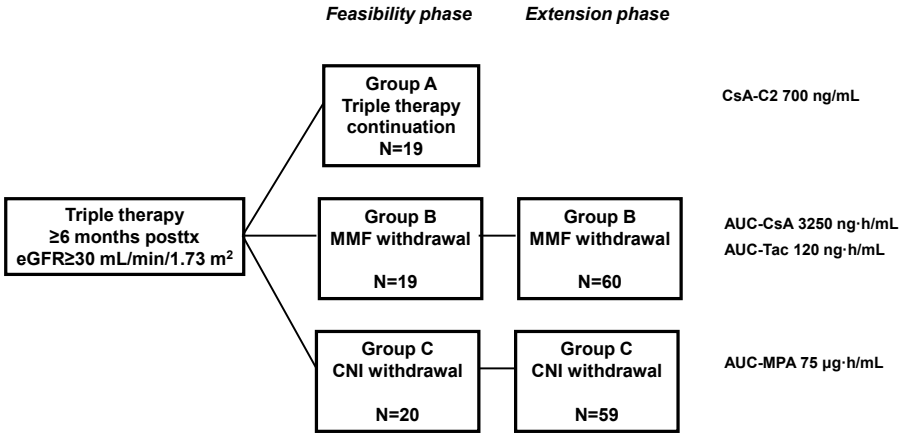

Figure 2. Study design
Posttx, posttransplantation; eGFR, estimated glomerular filtration rate; MMF, mycophenolate mofetil; CNI, calcineurin inhibitor; CsA, cyclosporine; AUC, area under the concentration-time curve; Tac, tacrolimus; MPA, mycophenolic acid.

myocardial infarction or a percutaneous coronary intervention. At enrollment, systemic exposure (CNI and MPA) was assessed and patients were randomized using envelopes. The computer-generated randomization list and envelopes were provided by an independent trial pharmacist. Study visits were scheduled after 6 weeks and 3 monthly during the first year, and subsequently at 2 and 3 years. Follow-up of key parameters such as renal function, BP, serum lipids, hematologic parameters, malignancies, patient and graft survival were continued in each patient for 3 years to assess long-term outcome.

Treatment Plan

In eligible patients, systemic drug exposure (CsA or Tac, MPA) was measured by an AUC_{0-12} before randomization to one of the study arms. We used a population-based two-compartmental pharmacokinetic model combined with Bayesian estimation and limited sampling as previously described [32-33]. The limited sampling strategy for the CNIs included a trough level and additional levels 2 and 3 hours postdose. For MPA, blood samples were collected before and 1, 2 and 3 hours postdose. Systemic exposure was assessed at baseline, week 6 (2 weeks after withdrawal), month 6, year 1, year 2 and year 3. Doses were adjusted to reach the defined targets guided by an independent trial pharmacist. In case of unexpected large differences with previous results or, if required in relation to the patients clinical condition, more frequent assessments were initiated.

Group A continued their current treatment regimen aiming at C_2-levels of 700 ng/mL (range: 600-800 ng/mL). In group B (MMF-withdrawal) CsA was dosed to reach the defined target AUC_{0-12} of 3250 ng·h/mL (range: 3000-3500 ng·h/mL) and tacrolimus of 120 ng·h/mL (range: 100-140 ng·h/mL) before withdrawal. The targets and limited

strategy have been described previously [25]. In group C (CNI-withdrawal) the MMF dose was adjusted to reach the defined MPA-AUC_{0-12} target of 75 µg·h/mL (range: 60-90 µg·h/mL) before withdrawal. The CNI or MMF dose was reduced 50% every two weeks and withdrawn after week 4. At the time of CNI withdrawal, the daily dose was temporarily increased to 20 mg/day for 1 week, followed by 15 mg/day the next week and than back to their maintenance dose. In stable renal transplant recipients on tacrolimus we maintained prednisolone at 5 mg/day, and 7.5 to 10 mg/day in all other patients.

Study end-points

Primary read-out was the change in creatinine clearance (eGFR estimated using the MDRD formula) after 3 years. Secondary endpoints included the number and severity of acute rejection episodes, CTD, graft loss, patient death, adverse events, BP and serum lipid profile. CTD was defined by the need to perform a renal biopsy in case of more than 15% deterioration of renal function. BP was measured in sitting position after 5 minutes rest using a digital BP monitor. Patients received concomitant medication according to defined standards for the treatment of hypertension (systolic BP<140 mm Hg and diastolic BP<90 mm Hg). Type, number and dosage of antihypertensive drug(s) were recorded during the study period. A target low density lipoprotein-cholesterol less than 2.6 mmol/L was defined for the whole study population. Any infection that required hospital admission was documented including site of infection and microorganism.

Statistical Methods and Power

The analyses were performed according to the intention-to-treat principle. A sample size of 63 evaluable patients per group was calculated to have 80% power to detect an increase of creatinine clearance of 10 mL/min, assuming a standard deviation of 20 mL/min and α (2-z)=0.05. The power of the actual sample size is 88%. For comparison between two categories of interval variables, Student *t* test (parametric) or Mann-Whitney test (nonparametric) was used. Nominal variables were compared by a chi-square test or Fisher exact test. Results are given as mean±standard deviation for interval variables and numbers and percentages for nominal variables. A linear mixed model was used to analyze the longitudinal data of renal function in the withdrawal groups. Briefly, the model assumed that the MDRD clearance level at baseline of each patient might change after withdrawal and then follow a linear trend. The model had two parameters: the change of MDRD clearance after withdrawal with respect to baseline and the slope of the subsequent trend of the MDRD clearance. The parameters were assumed to be random and might differ between patients. The null hypotheses were tested that there were no differences in the two parameters between both groups. The analyses were performed using SPSS software (version 16.0).

Acknowledgements

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SUPPLEMENTAL DIGITAL CONTENT

Table S1. MDRD clearance over time according to linear mixed modelling

		MMF withdrawal	CNI withdrawal	*P*
Whole population		**N=79**	**N=79**	
MDRD clearance (mL/min/1.73 m^2)	baseline	53.9±1.8	58.0±1.8	0.12
	6 weeks	55.2±1.9	63.1±1.9	0.004
	1 year	52.9±1.8	61.1±1.8	0.002
	3 years	51.1±2.1	59.5±2.1	0.006
Slope (mL/min/1.73 m^2/month)		-0.096	-0.088	0.91
<50 mL/min/1.73 m^2		**N=33**	**N=30**	
MDRD clearance (mL/min/1.73 m^2)	baseline	39.0±1.3	42.2±1.4	0.10
	6 weeks	40.9±1.7	48.3±1.8	0.004
	1 year	38.5±1.7	48.0±1.8	<0.001
	3 years	36.7±2.0	48.5±2.1	<0.001
Slope (mL/min/1.73 m^2/month)		-0.096	+0.008	0.03
≥50 mL/min/1.73 m^2		**N=46**	**N=49**	
MDRD clearance (mL/min/1.73 m^2)	baseline	64.7±1.7	67.7±1.7	0.21
	6 weeks	65.4±1.9	72.1±1.8	0.01
	1 year	63.2±1.9	69.1±1.9	0.03
	3 years	61.7±2.6	66.4±2.5	0.20
Slope (mL/min/1.73 m^2/month)		-0.084	-0.148	0.48

Data are presented as model-predicted values and standard errors of the whole population and of subpopulations with a modification of diet in renal disease (MDRD) clearance < and ≥50 mL/min/1.73 m^2.
MMF, mycophenolate mofetil; CNI , calcineurin inhibitor.

Table S2. Rejection at 6 months

	MMF withdrawal N=79	CNI withdrawal N=79
Number (%)	1 (1.3%)	3 (3.8%)
Sex (M/F)	M	M
Age (yr)	54	59; 23; 55
HLA mismatch	1-2-2	1-1-0; 0-0-0; 1-1-0
PRA (%)	4	8; 2; 0
Prior rejection	no	no
Previous transplantations	no	no
Systemic drug exposure at baseline (CNI ng·h/mL, MPA µg·h/mL)	CsA: 4142; MPA: 56	Tac: 127; MPA: 37 CsA: 2936; MPA: 55 CsA: 3664; MPA: 34
Systemic drug exposure at time of rejection (CNI ng·h/mL, MPA µg·h/mL)	CsA: 2832	MPA: 60; MPA: 68; MPA: 61
Time posttransplantation (yrs)	1.0	2.5; 1.5; 1.5
Time after withdrawal (d)	161	75; 55; 84
Intercurrent illness	no	no

MMF, mycophenolate mofetil; CNI, calcineurin inhibitor; M, male; F, female; HLA, human leucocyte antigen; PRA, panel reactive antibodies; MPA, mycophenolic acid; CsA, cyclosporine; Tac, tacrolimus.

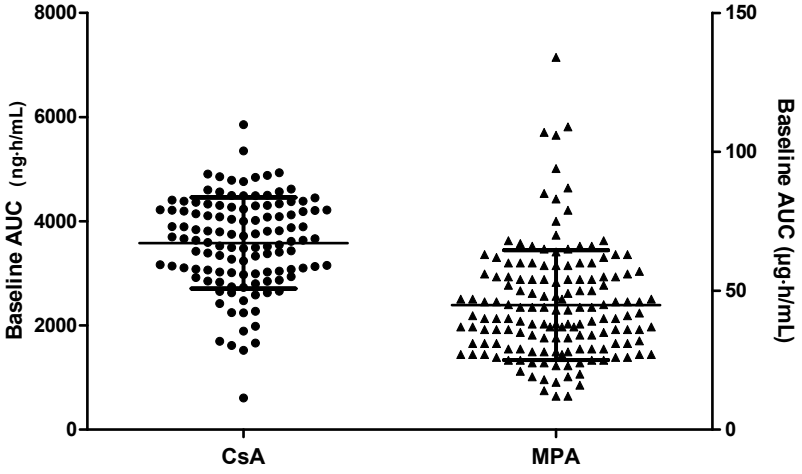

Figure S1. Baseline exposure to CsA and MPA

Baseline exposure as measured by area under the concentration-time curve (AUC) of cyclosporine (CsA) and mycophenolic acid (MPA) of all included patients. Mean±standard deviation are shown.

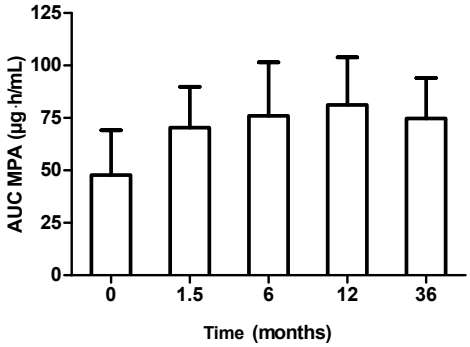

Figure S2. Exposure to MPA

Exposure as measured by area under the concentration-time curve (AUC) of mycophenolic acid (MPA) in calcineurin inhibitor (CNI)-withdrawal group. Mean and standard deviation at baseline and 6 weeks, 6 months, 1 and 3 years after withdrawal are shown.

CHAPTER 3

Late calcineurin inhibitor withdrawal prevents progressive left ventricular diastolic dysfunction in renal transplant recipients

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ABSTRACT

Background. Calcineurin inhibitor (CNI)-based therapy is associated with adverse cardiovascular effects. We examined the effects of late CNI or mycophenolate mofetil (MMF) withdrawal on echocardiographic parameters.

Methods. This study was conducted as a substudy of a randomized trial in stable renal transplant recipients on a triple CNI-based regimen with prednisone and MMF that evaluated late concentration-controlled withdrawal of the CNI or MMF on renal function. A total of 108 patients (age, 52.3±11.5 years, 67% male, at a median of 2.0 years after transplantation (interquartile range 1.3-3.3 years), estimated glomerular filtration rate, 57±16 mL/min/1.73 m^2, 66% on cyclosporine and 34% on tacrolimus) entered the cardiovascular substudy examining echocardiographic parameters at baseline and 2 years after randomization. In all patients traditional cardiovascular risk factors were treated according to predefined targets.

Results. Late CNI withdrawal prevented progressive development of left ventricular (LV) diastolic dysfunction, as assessed by markers of LV diastolic function (mitral deceleration time and mitral annular e' velocity). Conversely, in the MMF-withdrawal group, the left atrial (LA) volume index (an indicator of chronic LV diastolic dysfunction) was significantly increased at 2 years (from 24.1±6.7 to 27.0±7.0 mL/m^2, $P<0.05$). In addition, CNI withdrawal resulted in a higher proportion of patients achieving the predefined blood pressure targets (<130/85 mm Hg: 41.5% vs. 12.7%, $P=0.001$) at 2 years while requiring less antihypertensive drugs. Changes in LA volume index were significantly associated with treatment arm ($P=0.03$), and changes in systolic ($P=0.005$) and diastolic blood pressure ($P=0.005$).

Conclusions. Late CNI withdrawal, from a triple-drug regimen in stable renal transplant recipients, prevented progressive deterioration of LV diastolic function and facilitated better blood pressure control.

INTRODUCTION

The introduction of newer immunosuppressants, and the calcineurin inhibitors (CNIs) in particular, has significantly reduced acute rejection rates, but the recent epidemiological data continue to indicate that long-term graft survival has not improved accordingly [1]. Chronic allograft dysfunction, currently defined by interstitial fibrosis and tubular atrophy, remains the dominant cause of late graft loss and CNI-induced, acute and/or chronic, nephrotoxicity is considered an important contributor to the progression of chronic allograft dysfunction [2]. Moreover, CNI-based therapy has unfavourable cardiovascular side effects such as hypertension, dyslipidemia and new-onset diabetes [3]. To date, cardiovascular disease (CVD) remains the leading cause of morbidity and mortality after renal transplantation.

Previous studies have shown that patients with end-stage renal disease (ESRD) already have a high cardiovascular risk burden at the initiation of dialysis with left ventricular hypertrophy (LVH) as the most commonly observed cardiac abnormality [4-5]. LVH is an important determinant of sudden cardiac death and heart failure both in the general population and in dialysis patients [4, 6]. Additionally, left atrial (LA) dilatation has also been shown to be an independent predictor of all-cause mortality in ESRD patients, beyond that of left ventricular (LV) mass and systolic function [7-8]. Favourable structural cardiac changes, such as LV mass regression and improvement of LV function, that may alter the long-term cardiovascular risk, have been described after renal transplantation [9-12].

Currently, there are conflicting data regarding the effects of calcineurin on the myocardium. A recent study in cardiac transplant recipients reported that replacement of the CNI with sirolimus resulted in LV mass regression, with an improvement in diastolic function [13]. In experimental animals, however, calcineurin activation appears to be a key player in mediating the development of LVH, and calcineurin inhibition could prevent LVH without affecting LV systolic function [12, 14-15]. Moreover, genetic inhibition of calcineurin has failed to result in LVH development in a mouse model but, instead, resulted in diastolic dysfunction [16].

In a recently reported randomized trial in stable renal transplant recipients, we assessed the impact of late concentration-controlled CNI or mycophenolate mofetil (MMF) withdrawal on renal function after 3 years [17]. CNI elimination resulted in better renal function, whereas the risk of acute rejection was low in both withdrawal groups. We report the results of a cardiovascular substudy that evaluated the impact of CNI or MMF withdrawal on echocardiographic parameters 2 years after randomization.

RESULTS

Patients

A total of 119 patients entered the cardiovascular substudy between December 2005 and September 2007 (see Figure , SDC, http://links.lww.com/TP/A689). A total of 11 patients were not available to complete the 2-year follow-up visit. In the MMF-based group: 1 patient died, 1 returned to dialysis, 1 developed a posttransplantation proliferative disorder, 1 did not show for the 2-year visit and 2 withdrew informed consent owing to the perceived intensity of outpatient visits. In the CNI-based group, 3 patients died, 1 developed a Merkel cell tumor and 1 withdrew consent.

The recipient-, donor- and transplant procedure-related factors of the 108 patients, who completed the 2-year follow-up visit, are summarized in Table 1. There were no significant differences between the randomized groups with respect to the type of transplant, time posttransplantation, immunological or cardiovascular risk profile. At baseline, there were also no differences in systolic blood pressure (SBP), diastolic blood pressure (DBP) or proportions of patients with the target blood pressure (BP) of lower than 130/85 mm Hg.

Table 1. Baseline characteristics after randomization

Variable		CNI-based n=55	MMF-based n=53	*P*
Recipient:				
Age, yr		51.0±12.8	53.6±9.9	0.26
Gender, % male		63.6	69.8	0.50
PRA>5% , %		38.2	39.2	0.91
Body mass index, kg/m^2		25.5±3.5	27.0±4.1	0.11
Diabetes mellitus, %		14.5	20.8	0.40
Transplant:				
Donor age, yr		42.0±14.2	45.2±15.9	0.29
Donor gender, % male		47.3	46.2	0.91
HLA mismatch	A-locus	1.0±0.6	0.9±0.6	0.26
	B-locus	1.0±0.6	1.0±0.7	0.99
	DR-locus	0.9±0.6	0.9±0.7	0.63
Time posttransplantation, yr		1.9 (1.3-3.4)	2.2 (1.2-3.2)	0.93
Delayed graft function, %		32.7	35.8	0.73
Previous rejection, %		7.3	7.5	1.00
Clinical parameters:				
Mean arterial pressure, mm Hg		99±12	102±11	0.18
SBP, mm Hg		138±15	144±18	0.09
DBP, mm Hg		80±11	82±11	0.39

Table 1. Baseline characteristics after randomization (continued)

Variable	CNI-based n=55	MMF-based n=53	*P*
BP<130/85 mm Hg, %	21.8	18.9	0.70
eGFR, mL/min/1.73 m^2	56±16	57±16	0.63
Albumin: creatinine ratio, mg/mmol	2.3 (1.6-5.7)	2.3 (1.4-5.6)	0.73
Total cholesterol, mmol/L	5.4±1.3	5.2±0.9	0.30
LDL-cholesterol, mmol/L	3.3±1.0	3.0±0.8	0.14
HbA1c , %	5.8±1.0	5.7±0.9	0.78
Immunosuppression:			
Prednisone dose, mg/day	7.0±1.7	7.3±1.9	0.36
CsA:Tac, %	64:36	68:32	0.64
CsA dose, mg/day	212±56	225±54	0.17
Trough level CsA, ng/mL	122±51	120±41	0.81
AUC CsA, ng·h/mL	3599±962	3687±811	0.79
Tac dose, mg/day	5.0±1.3	6.0±2.8	0.48
Trough level Tac, ng/mL	9.6±5.9	8.9±4.0	0.94
AUC Tac, ng·h/mL	158±70	146±67	0.71
MMF dose, mg/day	1517±523	1712±602	0.09
AUC MPA, µg·h/mL	43±19	50±24	0.12
Concomitant medication:			
Antihypertensive drugs ≥2, %	72.7	67.9	0.57
Calcium channel blocker, %	69.1	60.4	0.34
ACE-i, %	45.5	49.1	0.71
Angiotensin II receptor blocker, %	16.4	3.8	0.05
Beta blocker, %	69.1	58.5	0.25
Statin, %	54.5	67.9	0.15
Ezetimibe, %	0	3.8	0.24
Insulin, %	9.1	13.2	0.55
Oral antidiabetics, %	7.3	5.7	1.00

Data presented as mean±SD, median and interquartile range or percentages .
CsA; cyclosporine; eGFR, eGFR based on Modification of Diet in Renal Disease equation; HLA, human leucocyte antigen; PRA, panel reactive antibodies; Tac, tacrolimus.

Clinical follow-up

In the first 6 months after randomization, 2 patients in the MMF-based (3.8%) and 1 patient in the CNI-based group (1.8%) experienced an acute rejection episode (*P*=0.49). Two (3.8%) patients in the MMF-based and 3 (5.5%) in the CNI-based group had a cardiac event (*P*=1.00). In the MMF-based group, 1 patient had atrial fibrillation and 1 had endocarditis; in the CNI-based group, 1 patient had supraventricular tachycardia and 2 had myocardial infarction. During follow-up, the immunosuppressive regimen was changed

Table 2. Clinical parameters and (concomitant) medication at 2 years

Variable	CNI-based n=55	MMF-based n=53	*P*
Clinical parameters:			
Body mass index, kg/m^2	26.8±3.5	27.3±4.3	0.52
Mean arterial pressure, mm Hg	103±10	97±10	0.002
SBP, mm Hg	141±14	135±18	0.06
DBP, mm Hg	84±10	77±9	0.001
BP<130/85 mm Hg, %	12.7	41.5	0.001
eGFR, mL/min/1.73 m^2	54±17	59±17	0.11
Albumin:creatinine ratio, mg/mmol	1.1 (0.65-3.70)	1.5 (0.85-4.15)	0.38
Total cholesterol, mmol/L	4.6±0.9	4.4±0.7	0.10
LDL-cholesterol, mmol/L	2.6±0.7	2.4±0.6	0.15
HbA1c, %	5.8±0.8	5.8±0.8	0.95
Diabetes mellitus, %	22.6	17.0	0.47
Immunosuppression:			
CsA dose, mg/day	192±46		
Trough level CsA, ng/mL	114±35		
AUC CsA, ng·h/mL	3341±529		
Tac dose, mg/day	5.0±1.3		
Trough level Tac, ng/mL	8.9±2.4		
AUC Tac, ng·h/mL	145±27		
MMF dose, mg/day		2240±792	
AUC MPA, µg·h/mL		75±20	
Switch to, %	3.6	11.3	0.16
CS+CsA+MMF	1.8	1.9	1.00
CS+Tac+MMF	0	5.7	0.12
CS+Tac	0	3.8	0.24
CS+MMF	1.8	0	1.00
Concomitant medication:			
Antihypertensive drugs ≥2, %	81.8	67.9	0.06
Calcium channel blocker, %	61.8	56.6	0.59
ACE-i, %	72.7	69.8	0.74
Angiotensin receptor blocker, %	16.4	7.5	0.24
Beta blocker, %	58.2	49.1	0.35
Statin, %	80.0	84.9	0.50
Ezetimibe, %	14.5	9.6	0.56
Insulin, %	9.1	13.2	0.55
Oral antidiabetics, %	12.7	9.4	0.76

Data presented as mean±SD, median and interquartile range or percentages.
CsA, cyclosporine; CS, corticosteroids; eGFR, estimated GFR based on Modification of Diet in Renal Disease equation; Tac, tacrolimus

in 6 (11.3%) patients of the MMF-based and 2 (3.6%) patients of the CNI-based group (*P*=0.16). Triple therapy was reinstituted in 4 patients in the MMF-based group and 1 patient in the CNI-based group. Two patients in the MMF-based and 1 patient in the CNI-based group were switched to the other treatment arm.

The clinical characteristics at 2 years are summarized in Table 2. At 2 years, patients in the CNI-based group had a higher mean arterial pressure (*P*=0.002), SBP (*P*=0.06) and DBP (*P*=0.001). More patients in the MMF-based group achieved the predefined target BP of lower than 130/85 mm Hg (41.5% vs.12.7%, *P*=0.001). In addition, compared with that at baseline, a higher proportion of patients reached the target BP in the MMF-based group (baseline, 18.9%; 2 years, 41.5%; *P*=0.01). In the CNI-based group, less patients achieved the target BP (baseline, 21.8%; 2 years, 12.7%; *P*=0.36) and there was a trend to use more antihypertensive drugs (≥2 drugs; *P*=0.06). Overall, the type of antihypertensive drugs used did not differ between the groups. Despite the increased use of angiotensin converting enzyme inhibitors (ACE-i) or angiotensin receptor blockers in both groups, the estimated glomerular filtration rate (eGFR) remained better in the MMF-based group (59±17 vs. 54±17 mL/min/1.73 m^2, *P*=0.11, Table 2). Six weeks after CNI withdrawal, the Δ-eGFR was 4.5±1.0 mL/min/1.73 m^2 in the MMF-based group versus 0.8±1.0 mL/min/1.73 m^2 in CNI-based patients (*P*=0.012). The Δ-eGFR between week 6 after randomization and year 2 was not significantly different between the groups (*P*=0.42). There was no difference in the degree of proteinuria, as assessed by repeated urine albumin/creatinine ratios. A significant decrease in lipid levels was observed (*P*<0.001) in both groups, but there were no significant differences between the groups. There was also no significant difference in the proportions of patients with diabetes mellitus or in the glycated hemoglobin (HbA1c) levels.

Echocardiography

There were no significant differences in echocardiographic variables between the groups at baseline (Table 3). None of the patients had moderate or severe valvular disease. After 2 years of follow-up, there were no significant changes in LV end-diastolic volume (LVEDV) index (from 60.6±11.7 to 60.0±11.4 mL/m^2, *P*=0.47) and LV end-systolic volume (LVESV) index (from 21.9±6.2 to 21.2±6.0 mL/m^2, *P*=0.15) in the overall population. In addition, no changes in LV ejection fraction (LVEF) were observed (from 64.1±6.0% to 64.8±6.0%, *P*=0.24). Similarly, the LV mass index showed no change after 2 years (from 95.1±26.5 to 97.1±25.9 g/m^2, *P*=0.28) in the whole population.

For LV diastolic function for the entire group, mitral deceleration time was significantly increased compared with that at baseline (from 196.4±50.4 to 207.8±51.7 ms, *P*=0.013). Similarly, the LA volume index increased from 23.2±6.4 to 25.2±7.1 mL/m^2 (*P*<0.001) at 2 years.

Table 3 summarizes the changes in echocardiographic parameters using linear mixed models and after adjustment by randomization arm, MMF-based versus CNI-based therapy. Compared with baseline, there were no differences between the groups in

Table 3. Echocardiographic parameters at baseline (randomization) and after 2 years of follow-up

Variable	CNI-based (n=55)		MMF-based (n=53)		*P* (between group)
	Baseline	2 yr	Baseline	2 yr	
LVED diameter, cm	4.6±0.5	4.6±0.7	4.7±0.6	4.7±0.4	0.95
LVES diameter, cm	2.8±0.4	2.8±0.5	2.8±0.5	2.9±0.5	0.07
LV septum thickness, cm	1.1±0.2	1.1±0.2	1.1±0.2	1.1±0.2*	0.07
LV posterior wall thickness, cm	1.0±0.2	1.1±0.6	1.0±0.2	1.0±0.2	0.53
LVEDV index, mL/m^2	61.5±12.0	60.8±11.8	59.8±11.4	59.2±10.9	0.46
LVESV index, mL/m^2	22.3±6.4	21.2±6.1	21.5±5.9	21.2±5.9	0.16
LVEF, %	64.0±6.1	65.2±6.2	64.3±5.7	64.4±5.9	0.24
LV mass index, g/m^2	97.6±26.3	99.6±26.3	92.5±26.7	94.5±25.5	0.27
Mitral E:A ratio	1.04±0.36	1.00±0.38	0.91±0.22	1.00±0.42	0.52
E-wave deceleration time, ms	195.5±56.8	217.8±57.5*	197.5±43.0	197.0±42.6	0.013
Mitral annular e', cm/s	6.2±2.1	6.1±2.1	6.0±1.8	6.6±2.0*	0.16
Mitral E:e' ratio	13.3±6.5	13.6±7.6	11.9±5.4	12.2±6.3	0.51
Myocardial performance index	0.34±0.07	0.36±0.08*	0.36±0.08	0.34±0.09	0.41
LA volume index, mL/m^2	24.1±6.7	27.0±7.0*	22.3±6.0	23.2±6.7	<0.001

Data presented as mean±SD. *P<0.05 versus baseline.
Analysis performed with linear mixed model, introducing randomization (CNI- or MMF-based therapy) as covariate.
E:A, E-wave: A-wave.

changes in LVEDV, LVESV indices and LVEF after 2 years (Table 3). In particular, LV mass index did not show any significant change over time in the overall population or in 1 of the randomized groups (Table 3).

The assessment of LV diastolic function, however, demonstrated a significantly larger and more rapid prolongation of E-wave deceleration time (from 195.5±56.8 to 217.8±57.5 ms, P<0.05) in the CNI-based group, whereas the E-wave deceleration time remained unchanged (from 197.5±43.0 to 197.0±42.6 ms) in the MMF-based group (Fig. 1A). The association between changes in E-wave deceleration time and randomization arm remained significant after correcting for changes in eGFR, SBP and DBP, and body mass index over time (P=0.025; Fig. 1A). Using linear mixed models, the changes in E-wave deceleration time were not associated with changes in eGFR (P=0.17), SBP (P=0.53), DBP (P=0.55) or body mass index (P=0.21).

Mitral annular e' velocity demonstrated a significant improvement at 2 years in the MMF-based group (from 6.0±1.8 to 6.6±2.0 cm/s, P<0.05), but remained unchanged in the CNI-based group (from 6.2±2.1 to 6.1±2.1 cm/s, P=0.53, Table 3).

LA volume index was subsequently employed to assess the effects of chronic LV diastolic dysfunction. There was a significantly larger and more rapid increase in LA volume index (from 24.1±6.7 to 27.0±7.0 mL/m^2, P<0.001, Fig. 1B) in the CNI-based group at follow-up. In contrast, no change in LA volume index was observed in the MMF-based group

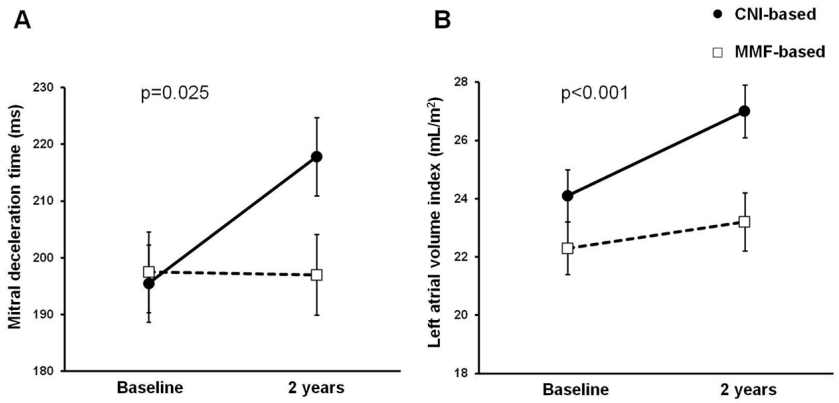

Figure 1. Changes in mitral deceleration time and LA volume index between baseline and 2 years after randomization, after MMF withdrawal (n=55) or CNI withdrawal (n=53) from triple maintenance therapy in stable renal transplant recipients. Linear mixed models were used to analyze the differences over time, correcting for changes in eGFR, SBP and DBP, and body mass index. Error bars denote the standard errors of the mean.

(from 22.3±6.0 to 23.2±6.7 mL/m^2, *P*=0.15, Table 3). The LA volume index increased by 12% in patients who continued the CNI at 2 years. Conversely, the LA volume index increased only marginally by 4% in patients who had their CNI withdrawn (mean change, 2.9±5.0 vs. 0.9±4.4 mL/m^2, *P*=0.03). The larger and more rapid increase in LA volume index observed in the CNI-based group remained significant after correcting for changes in eGFR, SBP and DBP, and body mass index (*P*<0.001, Fig. 1B). Using linear mixed models, the changes in LA volume index were significantly associated with treatment group (*P*=0.03) and changes in SBP (*P*=0.005) and DBP (*P*=0.005). However, changes in eGFR (*P*=0.19) and body mass index (*P*=0.70) over time did not significantly influence the observed changes in LA volume index.

The interobserver and intraobserver reproducibility of the echocardiographic measurements are outlined in Table 4. Overall, intraobserver and interobserver reproducibility were good for the different echocardiographic measurements, with very small biases and narrow limits of agreement.

Table 4. Intraobserver and interobserver reproducibility for echocardiographic measurements of LV and LA dimensions and LV diastolic function

	Intraobserver	**Interobserver**
LVED diameter, cm	-0.12±0.70	0.04±0.84
LVES diameter, cm	0.03±0.58	-0.11±0.82
LV septum thickness, cm	0.02±0.32	-0.08±0.36
LV posterior wall thickness, cm	-0.01±0.34	-0.12±0.26
E-wave deceleration time, ms	-2.4±26	-5.2±30
LA volume, mL	-0.4±10	-1.4±12

DISCUSSION

This is the first study to indicate that late CNI withdrawal from a triple-drug regimen in stable renal transplant recipients could prevent progressive development of LV diastolic dysfunction, as determined by mitral deceleration time and mitral annular e' velocity. Furthermore, LA volume index, an indicator of the chronicity and severity of LV diastolic dysfunction [18], continued to increase in patients who had MMF withdrawn and remained on CNI therapy during the 2-year follow-up period. Additionally, this randomized study demonstrated that MMF-based maintenance therapy facilitated better BP control.

This study was conducted as a cardiovascular substudy of a prospective randomized trial, which demonstrated that late concentration-controlled CNI withdrawal resulted in better renal function compared with MMF withdrawal, with a low risk of acute rejection after 3 years [17]. Although most randomized studies have shown an improvement in renal function, (early) CNI withdrawal has been associated with an increased risk of acute rejection [19-22]. The risk of rejection in these studies was, however, higher in the patients with lower mycophenolic acid (MPA) exposure [19, 22-23]. The results of the main study indicated that area under the concentration-over-time curve (AUC) monitoring of the remaining drug prevents underdosing while reducing the risk of acute rejection after late CNI withdrawal.

This study highlighted that, despite employing strict predefined BP targets and treatment protocol, BP was still insufficiently controlled in a significant proportion of patients at 2 years. This finding is in agreement with previous cohort studies [24-25], which documented that 46% of renal recipients may have SBP of 140 mm Hg or higher at 1 year posttransplantation [24]. This is particularly accentuated in patients who continued on CNI-based therapy, because they had significantly higher BP compared with patients with MMF-based maintenance therapy. CNIs have multiple mechanisms by which they may promote arterial hypertension [26-27]. First, CNIs promote afferent arteriolar vasoconstriction via the sympathetic nervous system and upregulation of the renin-angiotensin-aldosterone system [28]. Furthermore, they reduce the secretion of vasodilator cytokines while mediating an increase in vasoconstrictor cytokines. Accordingly, the prevalence of posttransplant hypertension was reported to be significantly increased (50-60%) after the introduction of CNIs in the 1980s [29].

Poor BP control has been associated with inferior allograft function and long-term outcomes in renal transplant recipients, and conscious efforts should be made to optimize BP control in this population [25, 30]. The present study showed that CNI withdrawal facilitated better BP control.

Arterial hypertension is a well-known risk factor for LVH and heart failure in patients with renal failure including transplant recipients [31]. In the present study, however, no

significant change in LV mass was observed at 2 years between the 2 groups, despite the significantly higher BP observed in the patients who remained on CNI-based therapy. A few explanations may support this observation. First, on the basis of the following criteria–LV mass index greater than 134 g/m^2 (men) and greater than 110 g/m^2 (women) –most of our patients did not have LVH at the time of randomization [32-33]. In fact, only 22.2% (8/36) of our female and 11.1% (8/72) of our male patients had LVH criteria at baseline. Therefore, it is not surprising that no significant interval change in LV mass was observed in our population, in whom LVH was only present in a minority before randomization. Second, there may have been a role for the strict clinical treatment protocol we introduced to titrate antihypertensive medications according to predefined BP targets. Furthermore, the use of ACE-i or angiotensin receptor blockers was high in the present study, with most of the patients (70.4%) using 2 or more antihypertensive drugs already before randomization. Because the renin-angiotensin-aldosterone system is activated in CNI-treated patients [34], antagonizing it with an ACE-i or angiotensin receptor blocker may suppress the renin-angiotensin-aldosterone system-mediated cell growth and perhaps LVH development. Finally, there are conflicting data regarding the effect of calcineurin on cardiac hypertrophy [13-16, 35]. In mice models, the activation of calcineurin has been shown to play a role in mediating the development of LVH. Its inhibition, either by CNI administration or the genetic inhibition of calcineurin, could prevent LVH [14-16], although other investigators were not able to show similar observation [35]. Therefore, the present study also does not support the hypothesis that CNI withdrawal could result in LV mass regression.

Nonetheless, the present study documented that CNI withdrawal from a triple-drug regimen in stable renal transplant recipients, prevented progressive development of LV diastolic dysfunction. Furthermore, significant increases in LA volumes at 2 years were observed in patients who continued their CNI, also after adjusting for changes in eGFR, BP and body mass index. These changes were significantly influenced by changes in SBP and DBP. In contrast, the patients allocated to the MMF-based group did not show changes in LA volume index. This group of patients reached the target BP (<130/85 mm Hg) in a significantly higher proportion than the patients in the CNI-continuation group. This seems to suggest that CNI withdrawal may stabilize LV diastolic dysfunction in patients with chronic renal disease, probably by also influencing BP control.

LV diastolic dysfunction develops early in most cardiac diseases and may be responsible for up to one third of patients with heart failure in the general population despite preserved LV systolic function [36]. In ESRD patients, at least 50-60% have LV diastolic dysfunction [37]. Impaired LV diastolic function and elevation in LV filling pressures are associated with high mortality in ESRD patients [38]. In addition, chronically elevated LV filling pressures will result in LA enlargement in the absence of significant valvular or primary myocardial diseases in patients with sinus rhythm [18]. In this regard, LA enlarge-

ment has been shown to be an independent predictor of all-cause mortality in ESRD patients, beyond that of LV mass and systolic function [7-8].

Nevertheless, whether the favorable diastolic parameters observed in this study are reflective of the consequence of the indirect effects of better renal function or better BP control or the direct effect of CNI withdrawal, or all of these, remains to be ascertained. So far, CNI-based therapy has been associated with LVH development and myocardial fibrosis and contributes to diastolic abnormalities of the cardiac allograft in heart transplant patients [39]. Moreover, a recent study has indicated that LV diastolic dysfunction progressed after renal transplantation, despite LVH regression and improvement in LV systolic function, and suggested that cyclosporine treatment may be the cause of worsening LV diastolic function [11]. Thus, it seems conceivable that by arresting LV diastolic dysfunction with CNI withdrawal (as indicated by the present study), a more favorable cardiovascular risk profile can be expected in renal transplant recipients.

In summary, the present study provides evidence that late CNI withdrawal with MMF prevents progressive development of LV diastolic dysfunction, an important parameter of cardiovascular morbidity and mortality in renal transplant recipients [7-8, 38]. In addition, the MMF-based regimen allows better BP control compared with CNI-based maintenance therapy. Several limitations should be acknowledged. First, the present study is a single-center study in a selected group of renal transplant recipients, with stable renal allograft function at a median of 2 years after transplantation and with a relatively low immunological risk profile. Therefore, the results may not be directly extrapolated to other populations. Second, this study was not designed to discriminate between the adverse effects of CNIs on BP and renal function or the myocardium itself. Finally, the assessment of myocardial fibrosis content with current imaging techniques would have strengthened the results of the present study. However, this goal was beyond the scope of the present study.

MATERIALS AND METHODS

Study design and patients

The main study was a prospective, open-label, randomized-controlled single-center trial that enrolled a total of 177 renal transplant recipients and evaluated renal function after 3 years [17]. Stable patients on CNI-based maintenance therapy with MMF and steroids, at least 6 months after transplantation with a stable eGFR of 30 mL/min/1.73 m^2 or greater, were randomized to CNI or MMF withdrawal. All patients were randomized with stratification for previous acute rejection and prior cardiovascular events (including myocardial infarction or percutaneous coronary interventions). Patients were dosed using a population-based pharmacokinetic model, limited sampling and Bayesian estima-

tion [40-41]. The predefined targets for the AUC_{0-12} were for mycophenolic acid AUC of 75 µg·h/mL (range, 60-90 µg·hr/mL) for the MMF-based therapy group, and cyclosporine AUC of 3250 ng·hr/mL (range, 3000-3500 ng·hr/mL) and tacrolimus AUC of 120 ng·hr/mL (range, 100-140 ng·h/mL) for the CNI-based therapy group. MMF or CNI doses were reduced with 50% every 2 weeks and completely withdrawn after 4 weeks. Concomitant with complete withdrawal, the prednisone dose was temporarily increased to 20 mg (1 week), 15 mg (1 week) and then back to baseline. Patients on tacrolimus were maintained on 5 mg per day prednisolone; all the other patients received 7.5 to 10 mg per day.

A total of 119 patients from the main study participated in the current cardiovascular substudy (NCT00169910). This study was designed to investigate the progression of surrogate CVD markers including echocardiographic parameters at baseline and 2 years after randomization. Patients with documented moderate or severe valvular disease on echocardiography were excluded from the study.

The study was performed in conformity to the Declaration of Helsinki, Good Clinical Practice guidelines. Informed consent was obtained from all patients. The study was approved by the Leiden University Medical Center Ethics Committee.

Clinical follow-up

All patients were followed in a dedicated outpatient clinic and treated according to predefined targets of care: hypertension [42]–SBP lower than 130 mm Hg and DBP lower than 85 mm Hg or, in case of proteinuria, SBP lower than 125 mm Hg and DBP lower than 75 mm Hg. The mean BP was recorded by averaging 3 repeated measurements in sitting position after a 5-minute rest, using a digital BP monitor. The target low density lipoprotein (LDL) cholesterol was less than 2.6 mmol/L. BP, weight, concomitant medication, eGFR, proteinuria, cholesterol and HbA1c levels were recorded at baseline, every 3 months during the first year and at 2 years.

Echocardiographic assessment

All patients in the cardiovascular substudy underwent transthoracic echocardiography before randomization and at 2 years. All patients were in sinus rhythm at the time of randomization. Images were performed using a commercially available system (Vingmed Vivid-7, General Electric Vingmed, Horten, Norway) equipped with a 3.5-MHz transducer. Standard LV dimensions were obtained from the parasternal long-axis view at end-diastole and end-systole. Subsequently, LV mass was calculated according to the Devereux method and indexed to body surface area (BSA) [43-44]. In addition, standard apical 2- and 4-chamber views were obtained. LVEDV and LVESV were measured and indexed to BSA, and LVEF was calculated using the biplane Simpson method [43]. Similarly, LA volumes were measured using the biplane Simpson method and indexed to BSA [43]. The assessment of LV diastolic function was performed by pulsed-wave Doppler examination of

the mitral inflow from the apical 4-chamber view. The ratio between the transmitral early (E-wave) and late (A-wave) diastolic filling velocities and deceleration time of the E-wave were obtained [45]. Tissue Doppler imaging of the mitral annulus was also performed and the early diastolic mitral annular velocities (e') were recorded [45]. The ratio of transmitral E-wave to e' (E/e' ratio), a marker of LV filling pressures, was obtained [46]. Finally, LV global function was evaluated using the myocardial performance index (a measure of both systolic and diastolic performance). This was obtained by measuring the Doppler time intervals between the mitral inflow and LV outflow tract velocity curves [47].

Outcome measures

The outcome of the present cardiovascular substudy was the change in echocardiographic parameters after 2 years of randomization, which served as surrogate markers of CVD. The primary outcome of the principal study was the change in Modification of Diet in Renal Disease (MDRD) clearance from baseline after randomization to CNI or MMF withdrawal. The secondary outcomes included incidence of acute rejection, proteinuria, patient death, kidney graft loss, BP and lipid profile.

Statistical analysis

The analyses were performed on an intention-to-treat basis. A sample size of 63 patients per group was calculated to have an 80% power to detect an increase in eGFR of 10 mL/min, assuming a standard deviation of 20 mL/min and $\alpha(2\text{-}z)=0.05$. The power of the actual sample size was 88% in the principal study. Continuous variables are presented as mean±standard deviation and categorical variables are presented as frequencies and percentages. For comparison between interval variables the Student *t* (parametric) or Mann-Whitney (nonparametric) test was used. Categorical variables were compared by a chi-square test, Fisher exact test or Kendall τ-b test. The changes in echocardiographic parameters over time were analyzed with linear mixed models adjusted initially for treatment group. The effect of changes in eGFR, SBP and DBP, and body mass index over time were subsequently introduced in the model as covariates to test whether these parameters had also an independent influence on the echocardiographic parameters. A linear mixed model was used to analyze the longitudinal data of renal function. The model assumed that the eGFR level of each patient at baseline might change after withdrawal and then follow a linear trend. The null hypothesis was tested that there were no differences in the change after withdrawal and the slopes of the subsequent trend of eGFR over time between the 2 groups. The interobserver and intraobserver reproducibility of the echocardiographic measurements were evaluated with the Bland-Altman analysis in 30 randomly selected echocardiography examinations. Two independent observers performed the measurements, blinded to test the interobserver variability. The same observer performed the measurements at two different time points. The mean

bias and the 2 standard deviations are provided. A $P<0.05$ was considered to be statistically significant. All analyses were performed using SPSS software (version 16.0; SPSS Inc, Chicago, IL).

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SUPPLEMENTAL DIGITAL CONTENT

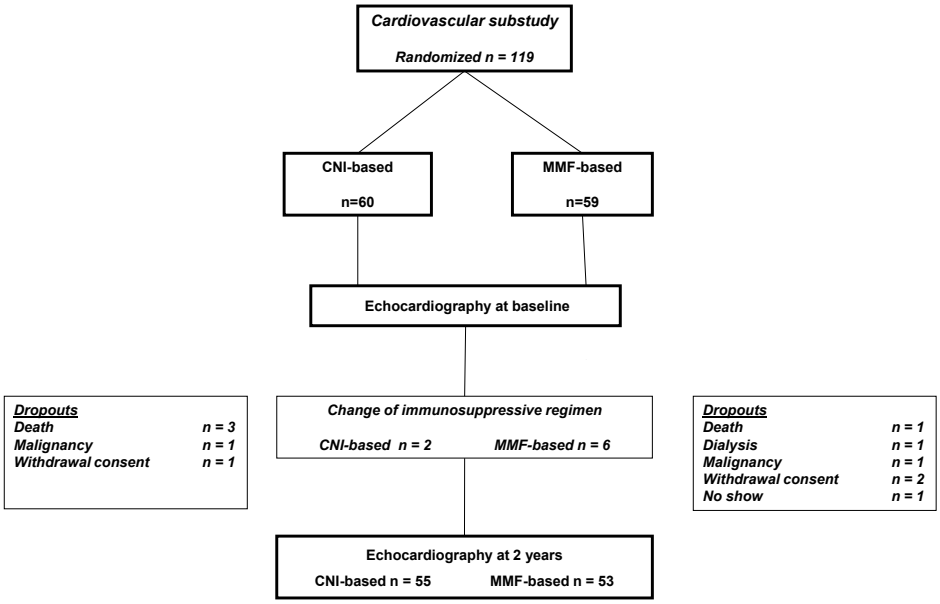

Figure SDC. Study flow diagram of the cardiovascular substudy
MMF, mycophenolate mofetil; CNI, calcineurin inhibitor.

CHAPTER 4

Impact of late calcineurin inhibitor withdrawal on ambulatory blood pressure and carotid intima media thickness in renal transplant recipients

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ABSTRACT

Introduction. Calcineurin inhibitors (CNIs) have an unfavorable cardiovascular risk profile in renal transplant recipients. The aim of this substudy was to assess the effects of late CNI or mycophenolate mofetil (MMF) withdrawal on ambulatory blood pressure monitoring and carotid intima media thickness.

Methods. A total of 119 stable renal transplant recipients on a triple regimen with steroids, a CNI and MMF were randomized into either the concentration-controlled CNI or MMF withdrawal groups. Patients were treated for traditional cardiovascular risk factors according to predefined targets. Ambulatory blood pressure monitoring and measurements of intima media thickness were performed at baseline and after 1, 2 and 3 years after randomization.

Results. CNI withdrawal resulted in a significant decline in both ambulatory day- and nighttime blood pressures (daytime: systolic blood pressure, -1.6 mm Hg/yr, *P*=0.018; diastolic blood pressure, -1.3 mm Hg/yr, *P*=0.002; nighttime: systolic blood pressure, -1.9 mm Hg/yr, *P*=0.008; diastolic blood pressure, -1.3 mm Hg/yr, *P*=0.014), which was not observed after MMF withdrawal. There was no difference in the proportion of nocturnal nondippers (both groups, 69%, *P*=0.95). Despite the reduction in ambulatory blood pressure, no effect of CNI withdrawal on carotid intima media thickness was found.

Conclusion. In stable renal transplant recipients, late CNI withdrawal from a triple-drug regimen decreased blood pressure in comparison with MMF withdrawal, but had no specific impact on carotid intima media thickness. Considering the high prevalence of hypertension in patients on CNI therapy, most stable renal transplant recipients may benefit from late CNI withdrawal by improved blood pressure control.

INTRODUCTION

Cardiovascular disease remains the leading cause of mortality in renal transplant recipients. Attention is increasingly focused on traditional and nontraditional cardiovascular risk factors to improve the long-term outcome after renal transplantation [1].

Arterial hypertension is highly prevalent in renal transplant recipients and has been associated with inferior graft outcome [2-3]. Ambulatory blood pressure monitoring (ABPM) is considered superior to office blood pressure measurements in predicting end-organ damage and cardiovascular events [4-5]. The absence of a nocturnal fall in blood pressure, known as nondipper pattern, is an additional prognostic factor and has been associated with left ventricular hypertrophy in patients with hypertension and in renal transplant recipients [6-9]. The intima media thickness (IMT) of the common carotid artery is a marker of atherosclerosis and has been associated with an increased incidence of cardiovascular disease in the general population [10-12] and mortality in renal transplant recipients [13]. In clinical trials, IMT is often used as a surrogate endpoint to evaluate the treatment efficacy of antihypertensive or lipid-lowering drugs.

Calcineurin inhibitors (CNIs) carry an unfavorable cardiovascular risk profile, including nephrotoxicity, hypertension, hypercholesterolemia and new-onset diabetes mellitus [14]. Mycophenolate mofetil (MMF), however, has none of these cardiovascular adverse effects and may even be advantageous by reducing atherosclerosis, and preventing intimal hyperplasia and smooth muscle cell proliferation [15-16]. Therefore, the withdrawal of CNIs from the immunosuppressive regimen could be a rational treatment approach to lower the cardiovascular risk. Early CNI withdrawal, however, has not become popular because of the increased risk of acute rejection [17]. In a randomized concentration-controlled study, the impact of late withdrawal of a CNI or MMF from a triple-drug regimen on renal function was recently evaluated [18]. CNI elimination resulted in better renal function, whereas the risk of acute rejection was low in both groups. Here, a cardiovascular substudy is reported, which investigated the impact of CNI withdrawal on ambulatory blood pressure and carotid IMT.

RESULTS

Study population

A total of 119 patients were enrolled in this cardiovascular substudy between December 2005 and September 2007. The baseline characteristics of the study population are summarized in Table 1. The results show comparable demographic characteristics, and recipient, kidney donor and transplantation procedure-related factors. There were also no differences in the baseline clinical or cardiovascular risk parameters.

Table 1. Baseline characteristics of study population at the time of randomization

		MMF withdrawal, N=60	CNI withdrawal, N=59
Recipient:			
Age (yrs)		52.3±13.1	53.6±10.5
Sex (M/F)		65	70
BMI (kg/m^2)		25.8±3.7	27.0±4.1
Time on dialysis (yrs)		3.2 (0.8-5.5)	2.9 (1.4-4.2)
Coronary vascular disease (%)		8	7
Cerebral ischemic disease (%)		8	10
Peripheral vascular disease (%)		3	2
DM (%)		17	20
Smoking (%)		8	17
Transplant:			
Donor age (yrs)		42.6±14.5	43.3±16.8
Donor sex (M/F)		47	51
Donor type (%LD/DD)		40/60	41/59
PRA>5% (%)		38	37
HLA mismatch	A locus	1.0±0.6	0.9±0.6
	B locus	1.0±0.6	1.0±0.7
	DR locus	0.9±0.6	0.8±0.6
Time posttransplantation (yrs)		1.9 (1.4-3.4)	2.1 (1.3-3.1)
Previous rejection (%)		8	9
Clinical parameters:			
Office SBP (mmHg)		139.4±15.8	144.1±17.9
Office DBP (mmHg)		79.6±11.0	81.6±10.8
Hemoglobin (mmol/L)		8.5±0.9	8.6±1.0
MDRD clearance (mL/min/1.73 m^2)		56.2±16.5	58.1±15.9
Albumin-to-creatinine ratio (mg/mmol)		2.4 (1.5-6.3)	2.3 (1.4-6.6)
Cholesterol (mmol/L)		5.3±1.3	5.2±0.9
LDL cholesterol (mmol/L)		3.3±1.0	3.0±0.8
HbA1c (%)		5.8±1.0	5.7±0.8
Immunosuppression:			
Cyclosporine/tacrolimus (%)		67/33	69/31
Cyclosporine dose (mg/d)		211±54	224±65
AUC cyclosporine (ng·hr/mL)		3622±942	3645±803
Tacrolimus dose (mg/d)		5.0±1.3	5.9±2.7
AUC tacrolimus (ng·hr/mL)		158±70	149±65
MMF dose (mg/d)		1540±511	1698±590
AUC MPA (µg·hr/mL)		42±18	49±24

Table 1. Baseline characteristics of study population at the time of randomization (continued)

	MMF withdrawal, N=60	CNI withdrawal, N=59
Concomitant medication:		
Antihypertensive drugs >2 (%)	48	36
Calcium entry blocker (%)	70	61
ACEi and/or ARB (%)	63	54
Diuretics (%)	35	25
β-blocker (%)	70	59
α-blocker (%)	5	8
Statin (%)	57	66
Ezetimibe (%)	0	3

Data presented as mean±SD, median and interquartile range or percentages.
ARB, angiotensin II receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; AUC, area under the concentration-over-time curve; BMI, body mass index; CNI, calcineurin inhibitor; DBP, diastolic blood pressure; DD, deceased donor; DM, diabetes mellitus; HbA1c, glycated hemoglobin; HLA, human leucocyte antigen; LDL, low-density cholesterol; MDRD, Modification of Diet in Renal Disease; MMF, mycophenolate mofetil; LD, living donor; MMF, mycophenolate mofetil; MPA, mycophenolic acid; PRA, panel reactive antibodies; SBP, systolic blood pressure.

Clinical follow-up

The clinical parameters of the patients at 3 years are shown in Table 2. The results on renal function and treatment of hyperlipidemia and diabetes mellitus have been reported previously [18]. In short, after 3 years of follow-up, the CNI withdrawal group had a better estimated glomerular filtration rate using the Modification of Diet in Renal Disease (MDRD) formula [19] as compared with the MMF withdrawal group (60.1±20.1 vs. 54.0±17.2 mL/min/1.73 m^2, *P*=0.10; Table 2). In both groups, a significant decrease in low-density lipoprotein (LDL) cholesterol was observed (MMF withdrawal, *P*<0.001; CNI withdrawal, *P*=0.001), but no significant difference between the groups (Table 2). The groups also did not differ in glycated hemoglobin (HbA1c) values or in the proportions of patients with diabetes mellitus (Table 2).

In the first 6 months after withdrawal, three patients in the CNI withdrawal group experienced acute rejection versus one patient in the MMF withdrawal group (*P*=0.36). At 3 years, one patient in each group had returned to dialysis because of chronic allograft nephropathy (MMF withdrawal group) and chronic rejection (CNI withdrawal group). During the follow-up period, coronary events occurred in one patient in the CNI withdrawal group and three patients in the MMF withdrawal group (*P*=0.62). None of the patients experienced a cerebrovascular event. Three patients in the CNI withdrawal group and one patient in the MMF withdrawal group were treated for peripheral arterial disease (*P*=0.36). Two patients in the CNI withdrawal group and four patients in the MMF withdrawal group died during the 3-year follow-up (*P*=0.68). Three patients dropped

Table 2. Clinical parameters and medication at 3 years after withdrawal

	MMF withdrawal, N=50	CNI withdrawal, N=52	*P*
Clinical parameters:			
Hemoglobin (mmol/L)	8.5±1.0	8.4±1.1	0.63
MDRD clearance (mL/min/1.73 m^2)	54.0±17.2	60.1±20.1	0.10
Albumin-to-creatinine ratio (mg/mmol)	1.3 (0.7-4.1)	1.6 (0.9-3.8)	0.37
Cholesterol (mmol/L)	4.7±0.9	4.7±1.2	0.78
LDL cholesterol (mmol/L)	2.5±0.7	2.6±0.9	0.85
HbA1c (%)	5.8±0.7	5.7±0.7	0.63
DM (%)	20	25	0.55
Immunosuppression:			
Cyclosporine dose (mg/d)	204±50		
AUC cyclosporine (ng·hr/mL)	3537±689		
Tacrolimus dose (mg/d)	4.5±1.4		
AUC tacrolimus (ng·hr/mL)	135±38		
MMF dose (mg/d)		2163±830	
AUC MPA (µg·hr/mL)		72±19	
Concomitant medication:			
Antihypertensive drugs >2 (%)	57	35	0.03
Calcium entry blocker (%)	59	57	0.81
ACEi and/or ARB (%)	88	75	0.09
Diuretics (%)	37	26	0.22
β-blocker (%)	59	39	0.05
α-blocker (%)	6	10	0.72
Statin (%)	78	80	0.73
Ezetimibe (%)	15	12	0.71

Data presented as mean±SD, median and interquartile range or percentages.
ARB, angiotensin II receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; AUC, area under the concentration-over-time curve; CNI, calcineurin inhibitor; DM, diabetes mellitus; HbA1c, glycated hemoglobin; MDRD, Modification of Diet in Renal Disease; MMF, mycophenolate mofetil; MPA, mycophenolic acid.

out of the study because of posttransplant lymphoproliferative disease (CNI withdrawal group), peripheral vascular disease (CNI withdrawal group) and a Merkel cell tumour (MMF withdrawal group). Two and four patients in the CNI withdrawal and MMF withdrawal groups, respectively, withdrew their informed consent because of the perceived intensity of the outpatient visits.

Ambulatory blood pressure and intima media thickness

The results of the ABPM at baseline and 3 years after withdrawal are summarized in Table 3. At baseline, there were no significant differences in the mean 24-hr, and the daytime

Table 3. Ambulatory blood pressure and carotid intima media thickness at baseline and 3 years

		MMF withdrawal	CNI withdrawal	*P*
24-hr ABPM				
SBP (mm Hg)	Baseline	128.1±13.5	127.8±12.1	0.89
	3 yrs	127.9±9.9	121.2±9.3	0.004
DBP (mm Hg)	Baseline	77.5±7.6	77.4±8.6	0.95
	3 yrs	78.5±5.3	72.2±6.9	<0.001
Daytime ABPM				
SBP (mm Hg)	Baseline	129.7±13.6	129.4±13.7	0.90
	3 yrs	128.3±9.6	122.7±9.9	0.017
Change in SBP (mm Hg/yr)		-0.13	-1.59	0.035
DBP (mm Hg)	Baseline	78.8±7.4	78.3±8.8	0.74
	3yrs	79.0±5.1	73.3±7.2	<0.001
Change in DBP (mm Hg/yr)		0.48	-1.26	0.001
Nighttime ABPM				
SBP (mmHg)	Baseline	123.6±15.4	123.9±14.0	0.98
	3 yrs	123.1±15.6	115.9±10.6	0.07
Change in SBP (mm Hg/ yr)		0.48	-1.93	0.015
DBP (mm Hg)	Baseline	74.1±8.7	73.5±9.5	0.50
	3 yrs	75.0±9.1	67.3±7.3	<0.001
Change in DBP (mm Hg/yr)		0.28	-1.28	0.003
Nondipping pattern (%)	Baseline	71	77	0.55
	3 yrs	69	69	0.95
IMT mean (mm)	Baseline	0.605±0.092	0.632±0.105	0.13
	3 yrs	0.591±0.070	0.611±0.074	0.27
Diameter (mm)	Baseline	7.892±0.796	7.973±0.805	0.52
	3 yrs	7.769±0.655	7.864±0.763	0.51

Data presented as mean±SD or percentages.
ABPM, ambulatory blood pressure monitoring; CNI, calcineurin inhibitor; DBP, diastolic blood pressure; IMT, intima media thickness; MMF, mycophenolate mofetil; SBP, systolic blood pressure.

or nighttime ambulatory blood pressures, in the CNI and MMF withdrawal groups. After 3 years, the mean 24-hr systolic blood pressure (SBP; 121.2±9.3 vs. 127.9±9.9 mm Hg, *P*=0.004) and diastolic blood pressure (DBP; 72.2±6.9 vs. 78.5±5.3 mm Hg, *P*<0.001; Table 3) were significantly lower in the CNI withdrawal group. This was observed for blood pressures recorded during both the daytime and nighttime, but the proportion of non-dippers remained similar in both groups (69%, *P*=0.95). The day- and nighttime blood pressures of the MMF withdrawal and CNI withdrawal groups are plotted in Figure 1. The CNI withdrawal group demonstrated a significant decline in ambulatory blood pressure during the follow-up of the study (slope daytime SBP, -1.6 mm Hg/yr, *P*=0.018; slope

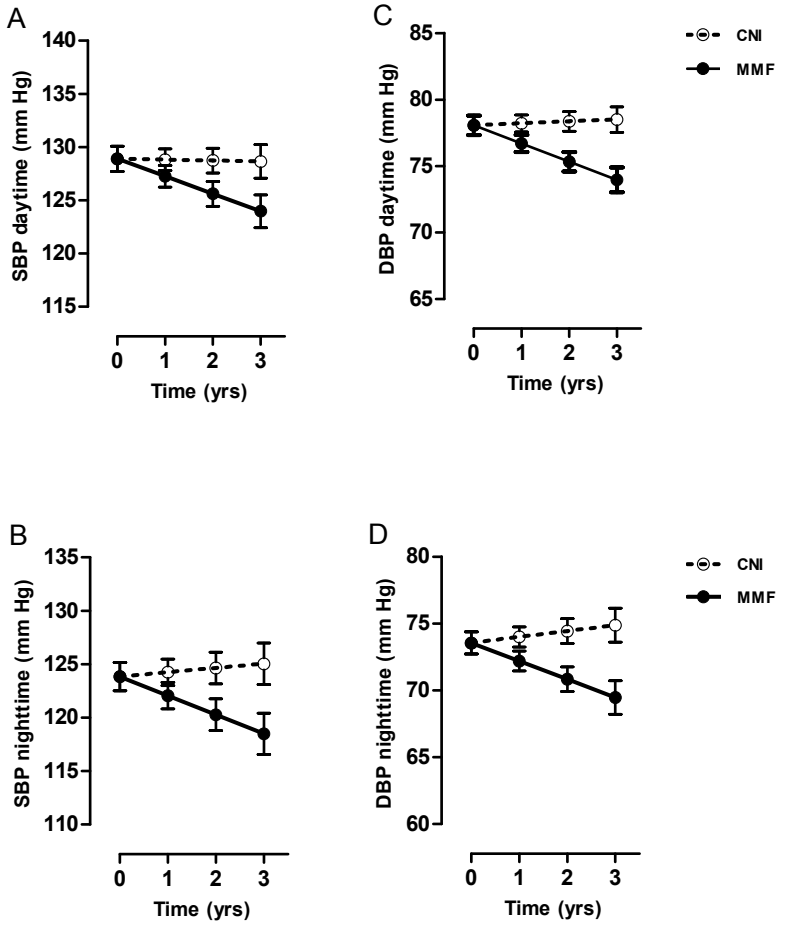

Figure 1. Ambulatory blood pressure monitoring

Intention-to-treat analysis of daytime and nighttime systolic (SBP) and diastolic (DBP) blood pressure. Plotted are the model-predicted means and standard errors at baseline, 1, 2 and 3 years after calcineurin inhibitor (CNI) (solid lines) or mycophenolate mofetil (MMF) (dotted lines) withdrawal.

(A) Daytime SBP **(B)** Nighttime SBP **(C)** Daytime DBP **(D)** Nighttime DBP.

daytime DBP, -1.3 mm Hg/yr; *P*=0.002; slope nighttime SBP, -1.9 mm Hg/yr, *P*=0.008; slope nighttime DBP, -1.3 mm Hg/yr, *P*=0.014), which was not present in the MMF withdrawal group (see Table 3). If linear trends were assumed, the slopes of ambulatory blood pressure were significantly different between the groups (daytime SBP, *P*=0.035; daytime DBP, *P*=0.001; nighttime SBP, *P*=0.015; nighttime DBP, *P*=0.003).

An analysis of the subgroups of patients, who were originally on cyclosporine (CsA) or tacrolimus, was also performed (see Table S1, SDC, http://links.lww.com/TP/A818). The CsA subgroup showed similar results 3 years after withdrawal as compared with the whole study population. Patients had a significantly lower mean 24-hr SBP (123.1±7.9

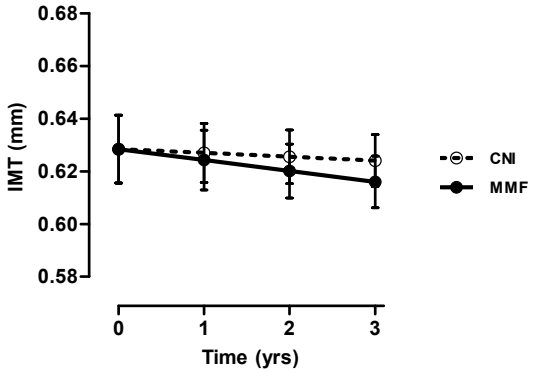

Figure 2. Carotid intima media thickness
Intention-to-treat analysis of mean common carotid intima media thickness (IMT). Plotted are the model-predicted means and standard errors at baseline, 1, 2 and 3 years after calcineurin inhibitor (CNI) (solid line) or mycophenolate mofetil (MMF) (dotted line) withdrawal.

vs. 128.3±9.5 mm Hg, *P*=0.05) and DBP (72.9±6.1 vs. 78.0±4.2 mm Hg, *P*=0.001) after CsA withdrawal. The slopes of daytime and nighttime ambulatory blood pressure showed a decline after CNI withdrawal and were significantly different from the slopes after MMF withdrawal (see Table S1, SDC, http://links.lww.com/TP/A818). Moreover, in the tacrolimus subgroup, mean 24-hr ambulatory blood pressures were lower after tacrolimus withdrawal compared with MMF withdrawal (24-hr SBP, 115.9±11.3 vs. 127.2±11.0 mm Hg, *P*=0.058; 24-hr DBP, 70.1±8.8 vs. 79.5±6.8 mm Hg, *P*=0.058). The slopes of daytime and nighttime ambulatory blood pressure over time were not statistically different between the groups.

At 3-year follow-up, a significantly lower proportion of patients used more than two antihypertensive drugs in the CNI withdrawal group than in the MMF withdrawal group (35% vs. 57%, *P*=0.03; Table 2). Compared with baseline, more patients used more than two antihypertensive drugs at 3 years after MMF withdrawal (from 48% to 57%, *P*=0.04), whereas the proportion of patients who were CNI withdrawn with more than two antihypertensive agents did not increase (from 36% to 35%, *P*=1.00). There was no difference in the type of antihypertensive medication used between the groups, except for more use of β-blockers (59% vs. 39%, *P*=0.05) in the MMF withdrawal group (Table 2).

At baseline, the mean IMT was not significantly different between the groups (MMF withdrawal, 0.605±0.092 mm; CNI withdrawal, 0.632±0.105 mm, *P*=0.13; Table 3). During the follow-up period, mean IMT did not significantly change over time in both groups (slope MMF withdrawal, -0.00145 mm/yr, *P*=0.50; slope CNI withdrawal, -0.00416 mm/yr, *P*=0.14; slope difference, *P*=0.54; Figure 2) and after 3 years there was no significant difference in mean IMT between the withdrawal groups (MMF withdrawal, 0.591±0.070 mm; CNI withdrawal, 0.611±0.074 mm, *P*=0.27; Table 3).

DISCUSSION

The results of this study demonstrate that, in stable renal transplant recipients, late CNI withdrawal significantly improved ambulatory blood pressures during both the daytime and nighttime recordings. No effect on intima media thickness was observed at 3 years after either late CNI or MMF withdrawal.

Early CNI withdrawal is not popular because of the increased risk of acute rejection [17]. The risk of acute rejection in studies with early CNI withdrawal has been associated with mycophenolic acid (MPA) exposure [20-22]. This study was conducted as a cardiovascular substudy of a randomized trial, which showed that late CNI withdrawal while providing adequate mycophenolate exposure resulted in better renal function with only a low risk of acute rejection [18].

Arterial hypertension is highly prevalent in renal transplant recipients and has been associated with inferior graft outcome and increased cardiovascular mortality [2-3]. CNIs may raise arterial blood pressure in transplant recipients by several mechanisms [23-25], including arteriolar vasoconstriction, activation of the renin-angiotensin system, direct effects on juxtaglomerular cells and increased tubular sodium reabsorption [26-28]. In addition, CNIs disturb the balance between vasoconstriction and vasodilator factors and may directly activate the sympathetic nervous system [25, 29]. This study indicated that CNI withdrawal facilitated better blood pressure control compared with MMF withdrawal. Significantly less patients who had the CNI withdrawn required more than two antihypertensive drugs, which still resulted in better ambulatory blood pressure. The observed absolute decline in blood pressure was, however, small. The most likely explanation is that, despite a dedicated outpatient clinic setting and stringent treatment algorithm to achieve predefined blood pressure targets–SBP less than 130 mm Hg and DBP less than 85 mm Hg (in case of proteinuria, SBP less than 125 mm Hg and DBP less than 75 mm Hg)–it turned out more difficult to achieve these targets than anticipated.

Approximately one third of patients (MMF withdrawal, 33%; CNI withdrawal, 31%) were treated with tacrolimus before randomization. A subgroup analysis of these patients showed a clear decline in 24-hr ambulatory blood pressures after tacrolimus withdrawal as compared with MMF withdrawal, close to reaching statistical significance. Although the subgroup analysis was not predefined in the protocol and the numbers of patients were small, these results suggest that, although CsA and tacrolimus inhibit calcineurin activity by means of interaction with different immunophyllines, they share a dose-dependent adverse effect on arterial blood pressure. Previously, the nephrotoxic potential of these two CNIs was found to be indistinguishable clinically [30-31], histologically [32] or at the renal molecular level [33].

This study is the first to evaluate the impact of late CNI withdrawal on ABPM in stable renal transplant recipients. Compared with office blood pressure measurements, ABPM

is generally considered a more precise method to evaluate the control of hypertension [34]. In addition, in renal transplant recipients, ambulatory blood pressure is also a better predictor of end-organ damage, such as left ventricular hypertrophy and renal allograft dysfunction [8, 35-36]. A nondipper status, which is frequently found beyond the first year after renal transplantation (60-76%) [37-39], has been associated with inferior graft function and may, at least partially, also be related to the use of CNIs twice daily [38, 40].

Only a few studies have evaluated the effect of CNI-based immunosuppressive regimens on ABPM [8, 40-42]. Van den Dorpel et al. reported a decrease in mean 24-hr blood pressure and an increase in the nocturnal decline of mean blood pressure after the late conversion from CsA to azathioprine in 18 stable renal transplant recipients [40]. In contrast with this study, we did not observe any difference in the nocturnal fall in SBP (results not shown) or a higher proportion of nondippers in the MMF withdrawal group. Our findings compare well with a more recent study comparing ABPM in 38 patients on sirolimus or CNI-based maintenance immunosuppression [41]. In that study, patients on CNI maintenance therapy had a higher 24-hr SBP and a higher nighttime SBP and DBP. Two small cross-sectional studies observed more left ventricular hypertrophy in CsA vs. azathioprine-treated patients, which was associated with a higher ambulatory SBP [8, 42].

To our knowledge, no prospective studies have been performed to examine the influence of either CNI or MMF withdrawal on IMT in renal transplantation. The IMT reflects the overall degree of (subclinical) atherosclerosis. In patients on hemodialysis and in renal transplant recipients, IMT has been associated with increased mortality [13, 43-44]. Although IMT in renal transplant recipients is generally lower when compared with dialysis patients [45], the values are still increased compared with healthy control subjects [43, 45-47]. An increased IMT in renal transplant recipients has been associated with inferior graft outcome [48]. The data on the progression of IMT in renal transplant recipients in the long-term are, however, scarce and remain inconclusive, since previous studies in limited numbers of patients have reported either a reduction [49], no change [50] or a progression [51] with time.

In this study, the mean common carotid IMT of the whole study population at baseline was 0.618±0.099 mm, which is comparable to values reported in previous studies in renal transplant recipients [43, 51-53]. CNIs have widely documented adverse effects on blood pressure, cholesterol, glucose metabolism and renal function, but no specific effect of CNI withdrawal on IMT was found during the 3-year follow-up period in this study. Hypertension and ambulatory blood pressure have been found to be determinants of IMT in the general population and hypertensive patients [54-56]. In this study, however, a significant reduction in ambulatory blood pressure after CNI withdrawal was not associated with a change of IMT. One could hypothesize that a longer follow-up period is necessary to detect the beneficial effect of blood pressure on IMT progression or that the differences in blood pressures observed in this study were too small. In this context,

it is relevant to note that randomized trials evaluating the effect of antihypertensive medication have shown a reduction of IMT progression with smaller blood pressure differences [57]. In addition, the reduction in mean LDL cholesterol levels by statin treatment (from 3.1 to 2.6 mmol/L, 16% reduction) in the current study was associated with a stable IMT. Intensive lipid lowering has been associated with a reduction in the progression of IMT [58] and lipid lowering may have contributed to the stable IMT observed in this study. Only one small prospective randomized study has examined the effect of pravastatin on IMT in renal transplant recipients and reported less progression after 1 year after a 26% reduction in mean LDL cholesterol (from 3.8 to 2.8 mmol/L) [51].

A longer follow-up time and a larger sample size may be required to detect any significant alterations of IMT before robust conclusions can be made regarding the impact of either CNI or MMF withdrawal. Another potential shortcoming of the study is that it is a single-center study in mainly Caucasian transplant recipients, and the results may not be extrapolated to patients with another risk profile or those of different ethnic origin. Finally, this study did not include a control group, but it is not to be expected that blood pressure control would have improved in a control group continuing on CNI-based maintenance therapy. In conclusion, this study demonstrated that late CNI withdrawal from a triple-drug regimen facilitated better blood pressure control. No specific beneficial effect of either CNI or MMF withdrawal on carotid IMT was found. Considering the high cardiovascular risk in renal transplant recipients, most patients might benefit from better control of blood pressure by late CNI withdrawal.

MATERIALS AND METHODS

Patients and study design

The design of the main study and the detailed treatment plan have been published previously [18]. Briefly, the main study was a prospective, open-label, single-center, randomized controlled trial that enrolled 177 stable renal transplant recipients. In the first phase of the study, the safety of high-exposure dosing of MMF to an MPA target of 75 µg·hr/mL (range, 60-90 µg·hr/mL) was tested and 58 patients were randomized (1:1:1) into the CNI withdrawal, MMF withdrawal or continuation of triple therapy with steroids groups. All patients were randomized with stratification for previous acute rejection and prior cardiovascular events (myocardial infarction, coronary bypass surgery or percutaneous coronary interventions). Patients were dosed using a population-based pharmacokinetic model with a Bayesian estimator and limited sampling [59-60]. The targets for the area under the concentration-over-time curves (AUC_{0-12}) of CsA and tacrolimus were 3250 ng·hr/mL (range, 3000-3500 ng·hr/mL) and 120 ng·hr/mL (range, 100-140 ng·hr/mL), respectively. The MMF or CNI doses were reduced by 50% every 2 weeks and withdrawn after 4 weeks.

Patients on tacrolimus used prednisolone at 5 mg/day, and all other patients at 7.5 to 10 mg/day. Concomitant with withdrawal, the prednisone dose was temporarily increased to 20 mg (1 week), 15 mg (1 week) and then back to baseline.

Based on the favorable results in these 58 patients, another 119 patients were included in the second phase of the main study. These patients participated in the current cardiovascular substudy (NCT00169910). The substudy was designed to investigate the effect of CNI or MMF withdrawal on ambulatory blood pressure and IMT. All patients were followed in a dedicated outpatient clinic and treated according to predefined target standards of care: SBP less than 130 mm Hg and DBP less than 85 mm Hg; in cases of proteinuria, SBP less than 125 mm Hg and DBP less than 75 mm Hg [61]. The target LDL cholesterol was less than 2.6 mmol/L for the entire population. The treatment of hypertension was guided by office blood pressures only.

Study visits were scheduled every 3 months after trial entry during the first year, and subsequently, after 2 and 3 years. At baseline and on a yearly basis during the study, weight, blood pressure, laboratory parameters and concomitant medication were recorded. Office blood pressure was measured in a sitting position after 5 minutes of rest using a digital blood pressure monitor (OMRON; Kyoto, Japan). The mean was calculated from three measurements. Laboratory measurements included hemoglobin, serum creatinine, cholesterol, LDL cholesterol, HbA1c and urinary albumin-to-creatinine ratio. The trial was performed in conformity with the Declaration of Helsinki, Good Clinical Practice guidelines. Informed consent was obtained from all patients. The main study and the substudy were approved by the Leiden University Medical Center Ethics Committee.

Ambulatory blood pressure monitoring and intima media thickness

The main outcome parameters for the substudy were the change in ambulatory blood pressure and carotid IMT during a 3-year follow-up period. At baseline, and at 1,2 and 3 years after randomization, 24-hr ABPM were obtained and the IMT of the carotid arteries was assessed. 24-hr ABPMs were performed with a high-precision blood pressure monitor (Mobil-O-Graph; Vitalsys, Antwerp, Belgium). Measurements were performed every 20 minutes during daytime and every 30 minutes during nighttime. Dipping status was defined as a reduction in mean SBP of 10% or greater during the night compared with during the day. The common carotid artery was examined using high-resolution B-mode ultrasound with online radio frequency processing-based measurements (ArtLab; Esaota, Maastricht, The Netherlands). IMT was measured at the far wall of the common carotid artery 1 cm proximal to the bifurcation over a range of 15 mm in recumbent position and was defined as the distance between the leading edges of the lumen interface and the media-adventitia interface of the far wall. A total of six measurements of the left and right common carotid artery were performed at three predefined angles (left: 180°, 225° and 270°; right: 180°, 135°, 90°). The mean IMT was calculated from the measure-

ments of both arteries. The assessment of IMT for individual patients during the study was performed by the same sonographer according to standardized procedures [62]. The results of the ABPM and carotid IMT were not known to the physicians that treated the patients in the study and, therefore, did not influence treatment decisions.

Statistical analysis

The analysis was performed on the basis of the intention-to-treat principle. Continuous variables are presented as mean±SD and categorical variables are presented as frequencies and percentages. Differences of continuous numeric variables between the CNI withdrawal and MMF withdrawal groups were evaluated using the unpaired Student *t* test or Mann-Whitney test. Nominal categorical variables were compared by a chi-square test or Fisher exact test. Linear mixed models were used to analyze the longitudinal data of ambulatory blood pressure, IMT and renal function in both withdrawal groups. The models of ABPM and IMT assumed that blood pressure and IMT had a linear trend during the follow-up period with a random slope and intercept. By plotting the crude data, a linear approach on ABPM and IMT over time was demonstrated to be acceptable. Several other models (including nonlinear models) did not improve the fit of the linear model to the data significantly. Mixed modeling was chosen because it allows analysis of all data, including those of subjects with missing values (assuming the missing measurements occurred at random). Other advantages of using mixed procedure included allowing analysis of uneven-spaced repeated measurements and handling random effects effectively. First, the null hypothesis was tested that the slopes of the trends over time did not differ between the two withdrawal groups. Second, the null hypothesis was tested that the change from baseline was zero within each group. A *P*-value less than 0.05 was considered to be statistically significant. All analyses were performed using the statistical software package SSPS (SPSS Inc.; Chicago, Illinois, USA; version 16.0) and R.10.2 [63].

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SUPPLEMENTAL DIGITAL CONTENT

Table S1. Ambulatory blood pressure at baseline and 3 years

		MMF withdrawal Cyclosporine, N=40	CNI withdrawal Cyclosporine, N=41	*P*
24-hr ABPM				
SBP (mm Hg)	Baseline	128.3±14.0	129.8±11.8	0.65
	3 yrs	128.3±9.5	123.1±7.9	0.050
DBP (mm Hg)	Baseline	76.2±7.7	77.5±8.9	0.60
	3 yrs	78.0±4.2	72.9±6.1	0.001
Daytime				
SBP (mm Hg)	Baseline	130.3±13.8	131.5±13.9	0.84
	3 yrs	129.4±9.8	124.9±8.3	0.08
Change in SBP (mm Hg/yr)		0.25	-1.90	0.017
DBP (mm Hg)	Baseline	77.8±7.3	78.2±8.9	0.74
	3 yrs	78.6±4.5	74.0±6.4	0.006
Change in DBP (mm Hg/yr)		0.47	-1.31	0.002
Nighttime				
SBP (mm Hg)	Baseline	123.9±16.2	126.5±14.6	0.52
	3 yrs	122.4±15.8	116.4±10.2	0.20
Change in SBP (mm Hg/yr)		0.73	-2.54	0.004
DBP (mm Hg)	Baseline	72.9±8.5	73.5±10.2	0.88
	3 yrs	74.5±8.1	67.8±6.7	0.008
Change in DBP (mm Hg/yr)		0.48	-1.36	0.004
		Tacrolimus, N=20	**Tacrolimus, N=18**	
24-hr ABPM				
SBP (mm Hg)	Baseline	127.8±12.8	123.8±11.9	0.29
	3 yrs	127.2±11.0	115.9±11.3	0.058
DBP (mm Hg)	Baseline	80.2±7.0	77.1±8.0	0.10
	3 yrs	79.5±6.8	70.1±8.8	0.058
Daytime				
SBP (mm Hg)	Baseline	128.6±13.6	125.3±12.6	0.57
	3 yrs	126.4±9.4	116.4±11.7	0.10
Change in SBP (mm Hg/yr)		-0.80	-0.98	0.68
DBP (mm Hg)	Baseline	80.9±7.3	78.3±9.0	0.23
	3 yrs	79.6±6.3	71.4±9.3	0.041
Change in DBP (mm Hg/yr)		-0.46	-0.96	0.23
Nighttime				
SBP (mm Hg)	Baseline	122.9±13.6	118.5±11.3	0.29
	3 yrs	124.2±15.9	114.2±12.4	0.11
Change in SBP (mm Hg/yr)		-0.06	0.12	0.77
DBP (mm Hg)	Baseline	76.9±8.7	73.5±8.1	0.27
	3 yrs	75.8±10.9	65.9±9.2	0.025
Change in DBP (mm Hg/yr)		-0.20	-0.91	0.25

Data presented as mean±SD.

ABPM, ambulatory blood pressure monitoring; CNI, calcineurin inhibitor; DBP, diastolic blood pressure; IMT, intima media thickness; MMF, mycophenolate mofetil; SBP, systolic blood pressure.

CHAPTER 5

Long-term renal function after late complete calcineurin inhibitor withdrawal with mycophenolate mofetil

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ABSTRACT

Background. Prolonged calcineurin inhibitor (CNI) use is associated with nephrotoxicity. Early CNI withdrawal has not become routine practice, because of the risk of acute rejection. We assessed the safety of late CNI withdrawal and intermediate- to long-term impact on renal function in renal transplant recipients, including those with declining function.

Methods. 139 renal transplant recipients (age: 51±13 yrs, 65% male, median time post-transplantation: 4.8 yrs (IQR 1.8-7.8 yrs), eGFR: 44±17 mL/min/1.73 m^2), converted from a CNI-based regime to dual therapy with mycophenolate mofetil (MMF), were identified. Eighty-three patients (60%) were converted because of reasons unrelated to renal function (group 1) and 56 (40%) because of deteriorating renal function (group 2). Renal function was evaluated with a median follow-up of 3.4 yrs (IQR 2.9-5.1 yrs).

Results. In both groups eGFR improved immediately after CNI withdrawal (group 1: 4.9±2.4 mL/min/1.73 m^2, p=0.04; group 2: 5.4±1.8 mL/min/1.73 m^2, p=0.004). Group 1 had a stable eGFR before and after conversion (slopes: before: 0.11±0.09 mL/min/1.73 m^2/month; after: 0.04±0.06 mL/min/1.73 m^2/month, p=0.52). Group 2 had a declining eGFR slope (-0.34±0.07 mL/min/1.73 m^2/month), that stabilized after conversion (0.01±0.05 mL/min/1.73 m^2/month, p<0.001). One patient in each group experienced acute rejection.

Conclusion. Late CNI elimination to dual therapy with MMF was safe and improved renal function on the intermediate- to long-term, also in recipients with already deteriorating renal function.

INTRODUCTION

The introduction of calcineurin inhibitors (CNIs) in renal transplantation significantly reduced acute rejection rates, but to date long-term graft outcome has not improved accordingly [1-3]. Prolonged CNI use is associated with more hypertension, diabetes mellitus and hyperlipidemia; all factors known to contribute to the observed excess cardiovascular morbidity and mortality. In addition, CNI-related nephrotoxicity is a significant predictor of chronic allograft nephropathy (CAN), currently defined by interstitial fibrosis and tubular atrophy (IF/TA), the dominant cause of progressive graft dysfunction and late renal allograft loss [4-5].

A meta-analysis including 15 randomized, controlled, trials evaluating early CNI sparing regimens, either minimization or elimination, in stable kidney transplant recipients receiving mycophenolate mofetil (MMF), found that CNI sparing was associated with significantly better renal function and a tendency towards prolonged graft survival [6]. There was however an increased risk of acute rejection after CNI elimination, favoring CNI minimization to preserve renal function [7]. The largest multicenter study with cyclosporine reported a 50% increase in the incidence of acute rejection after early CNI withdrawal and no difference in renal function at one year [8]. Posthoc analysis showed that the risk of rejection was highest in patients with low mycophenolic acid (MPA) exposure, and that the optimal range of MPA exposure was close to 60 µg·h/mL [8-9]. Late elective complete CNI withdrawal (beyond month 12) also resulted in better renal function with a modest increase of acute rejection [10]. Long-term results, however, indicated a higher incidence of late rejection, probably explained by underdosing of steroids or MMF [11]. Therefore, early CNI minimization with MMF has become the preferred strategy, providing adequate protection against rejection and better renal function with reduced tacrolimus exposure. Disappointingly, follow-up failed to demonstrate a significant difference in graft function between the different treatment groups three years after transplantation [12].

Late CNI withdrawal in stable renal transplant patients with prospective therapeutic drug monitoring resulted in significantly better renal function compared to MMF withdrawal with only a low risk of acute rejection [13]. In accordance with the results after late conversion to mammalian target of rapamycin (mTOR) inhibitors [14-15], a limited benefit for patients with preserved renal function was found [13] . However, in contrast to the experience with mTOR inhibitors, the subgroup of patients with an estimated glomerular filtration rate (eGFR)<50 mL/min/1.73 m^2, but still ≥30 mL/min/1.73 m^2, benefitted most from late CNI elimination with MMF [13]. Two prospective studies, examining late CNI withdrawal in renal transplant recipients with deteriorating graft function and biopsy-confirmed CAN, observed an improvement in the course of renal function without the risk of acute rejection. Follow-up time of these trials was however limited with

only 6 months [16], while the other showed very promising results, but had to be stopped prematurely after 32 weeks for ethical reasons [17]. Therefore, we initiated an explorative study to assess our experience from daily clinical practice concerning the safety and the long-term impact on renal function of late complete CNI withdrawal with MMF and steroids in renal transplant recipients, including those with deteriorating renal function.

SUBJECTS AND METHODS

Study design

This study was conducted at the renal transplant outpatient clinic of the Leiden University Medical Center. All kidney transplant recipients, initially treated with CNI-based therapy and later (between 1997 and 2007) converted by their treating physician to dual therapy with MMF and steroids, were identified. Patients were followed 3 to 4 monthly either in Leiden or by an experienced nephrologist in one of the affiliated dialysis centers. The general policy over the years has been to consider complete CNI elimination in case of an otherwise unexplained gradual deterioration of renal graft function. A renal biopsy was encouraged to exclude recurrent glomerular disease, but not mandatory. The CNI dose was reduced by 50% every 2 weeks and withdrawn after 4 weeks with a temporary increase in steroid dose. Prednisolone was increased to 20 mg for 1 week, 15 mg the next week and returned to the maintenance dose of 7.5 to 10 mg daily. CNI withdrawal with therapeutic drug monitoring of MPA was optional from 2004 and included MPA levels predose and 1, 2 and 3 hours postdose. The MMF dose during and after withdrawal was adjusted to reach a target MPA-area under the concentration-time curve ($AUC_{0\text{-}12}$) of 75 µg·h/mL (range 60-90 µg·h/mL).

The primary outcome parameter of the current study was renal graft function. The eGFR was estimated using the 4-variable Modification of Diet in Renal Disease (MDRD) equation [18]. Secondary parameters included proteinuria, number of acute rejection episodes and graft loss. Recipient, donor and transplant characteristics, including immunologic risk profile (HLA mismatches, % panel reactive antibodies, rejection history), timing/reason for complete CNI withdrawal, the occurrence of rejection after withdrawal, blood pressure and number of antihypertensive drugs were retrieved from the Leiden Kidney Transplant Data system. The eGFR and degree of proteinuria were collected: (a) 3, 6, 12 and 24 months before CNI withdrawal; (b) at the time of CNI withdrawal; (c) 3, 6, 12, 18, 24, 30 and 36 months after CNI withdrawal; and (d) at the last visit of the patient to the hospital or outpatient clinic. To calculate the eGFR at the time of CNI withdrawal the last serum creatinine before CNI dose reduction was used.

Statistical analysis

Two subpopulations among our patients with CNI elimination were defined before examining the data. Group 1 consisted of a control group of patients switched for reasons not related to renal function (n=83) and group 2 consisted of patients with deteriorating renal function (n=56). All continuous variables are presented as mean±standard deviation or median with interquartile range and categorical variables as numbers and percentages. Continuous numeric variables were compared between the groups by the unpaired two-sample *t* test (parametric) or Mann-Whitney test (nonparametric) and nominal categorical variables by the chi-square test. Changes in renal function in the two groups were assessed using linear mixed models. Briefly, our model assumed that every patient experienced a linear trend in renal function before switching, a rapid increase at the time of CNI withdrawal, followed by again another linear trend after withdrawal. We tested if the rapid increase in renal function was significant and if the slopes (before and after) were different in both groups. Proteinuria measured at the conversion, at the first visit after CNI withdrawal and at the last visit was compared within the groups by a Friedman related samples test. A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS software version 20.0.

RESULTS

Characteristics of the patient population

Between 1997 and 2007, a total of 139 kidney transplant recipients had been converted to dual maintenance therapy with steroids and MMF after complete CNI withdrawal. Demographics, biometrics and the most relevant transplant-related factors are summarized in Table 1. These patients were transplanted between 1984 and 2005. We compared the most relevant characteristics with the reference population, being all single kidney transplants performed in our center in the same time period (Table 2). In the more recent cohort, there were less recipients of kidneys from deceased donors (69.6% vs. 78.8%, p=0.02) and less retransplants (6.5% vs. 15.8%, p=0.003). There were no differences with respect to the degree of immunization or HLA class II mismatches. Less patients experienced an acute rejection episode (25.9% vs. 47.9%, p<0.001) in more recent eras.

Eighty-three patients (59.7%) were converted for reasons not related to renal function: preference of the treating nephrologist or patient (25/83), a HLA-identical transplant (10/83) or adverse effects: gout (18/83), hypertension, diabetes and/or hypercholesterolemia (11/83), gingival hyperplasia (6/83), tremor (2/83), infection (4/83), bone or muscular pain (3/83) or miscellaneous (4/83). As expected, 56 patients (40.3%) converted because of a gradual deterioration of renal function had a significantly lower eGFR (32.1±9.0 vs. 52.0±15.6 mL/min/1.73 m^2, p<0.001), more proteinuria (0.37 g/24 h (IQR 0.15-1.28 g/24 h)

Table 1. Patient and transplant characteristics of study population

Characteristics	All patients N=139	Group 1 Stable renal function N=83	Group 2 Deteriorating renal function N=56	p
At conversion:				
Age (yrs)	50.6±12.6	50.7±12.3	50.5±13.2	0.93
Sex (% male)	65.5	63.9	67.9	0.63
Previous acute rejection (%)	25.9	19.3	35.7	0.03
BMI (kg/m^2)	25.2±4.7	26.3±5.0	23.8±3.6	0.001
eGFR (mL/min/1.73 m^2)	44.0±16.5	52.0±15.6	32.1±9.0	<0.001
Proteinuria (g/24 h)	0.22(0.15-0.46)	0.19(0.13-0.33)	0.37(0.15-1.28)	<0.001
Systolic blood pressure (mm Hg)	144±21	140±21	149±21	0.01
Diastolic blood pressure (mm Hg)	83±11	82±10	85±12	0.06
Time posttransplant (yrs)	4.8 (1.8-7.8)	3.6 (1.0-7.2)	6.0 (4.1-11.5)	<0.001
Immunosuppression: CsA:Tac (%)	85.6/14.4	80.7/19.3	92.9/7.1	0.05
CsA dose (mg/d)	199±81	197±82	200±81	0.75
Tac dose (mg/d)	6.3±4.7	7.1±4.9	3.3±2.2	0.13
MMF dose (mg/d)	1774±486	1786±481	1750±504	0.60
Antihypertensive drugs >2 (%)	43.2	36.1	53.6	0.04
ACE inhibitors (%)	47.8	43.9	53.6	0.26
Angiotensin II receptor blockers (%)	21.0	14.6	30.4	0.03
Diuretics (%)	42.8	37.8	50.0	0.16
At transplantation:				
Primary kidney disease:				
Glomerular disease/glomerulonephritis (%)	38.8	33.7	46.4	0.07
Pyelonephritis/interstitial nephritis (%)	15.1	14.5	16.1	
Hypertension/nephrosclerosis (%)	15.1	21.7	5.4	
APDKD (%)	16.5	14.5	19.6	
Other (%)	14.4	15.7	12.5	
Donor age (yrs)	43.3±12.9	42.5±12.9	44.7±13.0	0.32
Donor sex (% male)	51.8	45.8	60.7	0.08
Deceased donor (%)	69.6	59.8	83.9	0.002
Delayed graft function (%)	27.5	22.9	34.5	0.13
First transplantation (%)	93.5	92.8	94.6	0.74
PRA>5% (%)	53.2	47.0	62.5	0.07

Data are presented as mean±standard deviation, median and interquartile range or percentages.
BMI , body mass index; eGFR, estimated glomerular filtration rate; CsA, cyclosporine; Tac, tacrolimus; MMF, mycophenolate mofetil; ACE, angiotensin converting enzyme; ADPKD, autosomal dominant polycystic kidney disease; PRA, panel reactive antibodies.

Table 2.Transplant characteristics of reference and study population

Variable	Reference population (1984-2005) N = 1415	Study population N = 139	p
Year of transplantation (%):			
1984 – 1994	48.8	28.1	< 0.001
1995 – 1999	19.7	30.2	
2000 – 2005	31.5	41.7	
Acceptor age (yrs)	46.9±13.1	44.9±12.6	0.10
Sex (% male)	62.1	65.5	0.46
Donor age (yrs)	41.5±15.2	43.3±12.9	0.12
Donor sex (% male)	53.3	51.8	0.79
Deceased donor (%)	78.8	69.6	0.02
Retransplantation (%)	15.8	6.5	0.003
Pre-emptive transplantation (%)	5.2	3.6	0.54
Dialysis posttransplantation (%)	31.5	25.7	0.20
PRA>5% (%)	59.8	53.2	0.15
HLA mismatch (%):			
Class I	1.6±1.1	1.3±1.1	0.003
Class II	0.5±0.6	0.4±0.5	0.21
Acute rejection (%)	47.9	25.9	<0.001

Data are presented as mean±standard deviation or percentages.
PRA, panel reactive antibodies; HLA, human leucocyte antigen.

vs. 0.19 g/24 h (IQR 0.13-0.33 g/24 h), p<0.001) and a higher mean systolic (149±21 vs. 140±21 mm Hg, p=0.01) and diastolic blood pressure (85±12 vs. 82±10 mm Hg, p=0.06). A higher proportion of these patients had received a deceased donor kidney (83.9% vs. 59.8%, p=0.002). Patients with declining renal function were converted at a later median time point after transplantation (6.0 yrs (IQR 4.1-11.5 yrs) vs. 3.6 yrs (IQR 1.0-7.2 yrs) p<0.001) and a higher proportion was on cyclosporine-based therapy (92.9% vs. 80.7%, p=0.05) and treated with >2 types of antihypertensive drugs (53.6% vs. 36.1%, p=0.04). The median time of complete CNI withdrawal after transplantation was 4.8 yrs (IQR 1.8-7.8 yrs) and the median follow-up time after CNI withdrawal was 3.4 yrs (IQR 2.9-5.1 yrs).

Of the 56 patients converted because of deteriorating renal function, 25 (44.6%) had undergone a renal biopsy within 1 year before switch. One biopsy could not be evaluated due to insufficient amount of cortex. Chronic structural changes compatible with mild to moderate CAN-IF/TA were demonstrated in 24/24 biopsies in combination with arteriolar hyalinosis (20/24) and chronic transplant glomerulopathy (1/24). Five biopsies also identified recurrent disease: IgA nephropathy (4/24) and diabetic nephropathy (1/24).

Renal function

The effects of complete CNI withdrawal with MMF on renal function were evaluated by sequential eGFR measurements, ranging from 2 years before until at least 3 years after conversion to MMF. The CNI withdrawal was performed stepwise in four weeks. Figure 1 shows renal function at different time points and trends in renal function over time for both groups. The eGFR significantly increased immediately after conversion in both groups (group 1: 4.9±2.4 mL/min/1.73 m^2, p=0.04; group 2: 5.4±1.8 mL/min/1.73 m^2, p=0.004). Group 1 had a stable renal function before and after conversion (slope before : 0.11±0.09 mL/min/1.73 m^2/month; after: 0.04±0.06 mL/min/1.73 m^2/month, p=0.52). Group 2 demonstrated a progressive gradual loss of eGFR towards conversion (-0.34±0.07 mL/min/1.73 m^2/month), that stabilized after conversion (0.01±0.05 mL/min/1.73 m^2/month) with a significant difference between the slopes (p<0.001).

Proteinuria did not differ significantly at the time of conversion, at the first visit after CNI withdrawal and at the last visit within both groups (group 1: 0.19 g/24 h (IQR 0.13-0.33 g/24 h) vs. 0.23 g/24 h (IQR 0.16-0.37 g/24 h) vs. 0.21 g/24 h (IQR 0.16-0.33 g/24 h), respectively, p=0.12; group 2: 0.37 g/24 h (IQR 0.15-1.28 g/24 h) vs. 0.42 g/24 h (IQR 0.24-0.69 g/24 h) vs. 0.31 g/24 h (IQR 0.18-0.60 g/24 h), respectively, p=0.54).

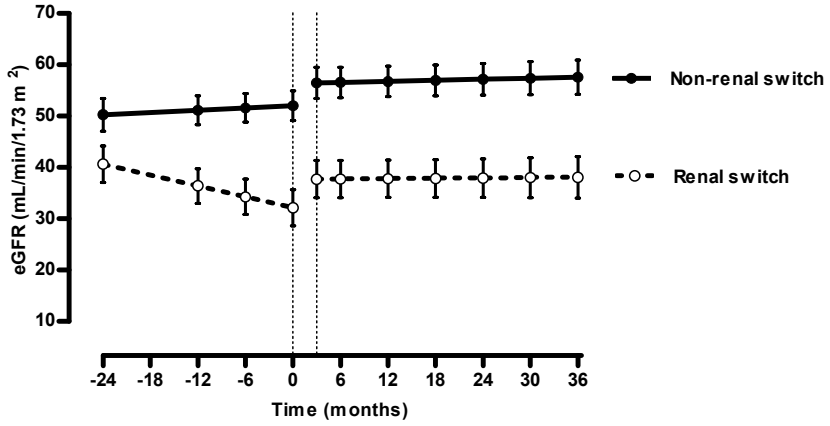

Figure 1. Renal follow-up before and after conversion to mycophenolate mofetil-based regimen
Linear mixed model showing the trends in eGFR-slopes before and after late CNI elimination.
Plotted are the model-predicted means and 95% confidence intervals. Solid lines: patients switched for nonrenal reasons (group 1); dotted lines: patients switched because of deteriorating renal function (group 2).

Acute rejection and graft loss

One patient in each group experienced an acute rejection episode. In group 1, acute interstitial rejection occurred in a stable male recipient of a 1-2-1 HLA-antigen mismatched kidney with a previous acute rejection episode and an MPA-AUC of 79 µg·h/mL at day 10

after CNI discontinuation. After successful treatment with steroids and antithymocyte globulin the CNI was reintroduced. In group 2, a female recipient of a 1-1-0 HLA-antigen mismatched kidney experienced graft loss (2.5 yrs after conversion) after reduction of immunosuppression with acute vascular and interstitial rejection in the context of severe abdominal tuberculosis. Three other patients in group 2 returned to dialysis: two due to CAN-IF/TA with progressive renal failure after 1.2 and 3.1 yrs after conversion to MMF and one after ICU-admission for pneumocystis jiroveci pneumonia complicated by irreversible acute tubular necrosis at 6.2 yrs after CNI withdrawal.

Four biopsies (4.8%) were performed (range 3.0-3.7 yrs after CNI withdrawal), because of a deterioration of renal function or increase in proteinuria in group 1. Three biopsies showed mild to moderate CAN-IF/TA, two biopsies signs of chronic transplant glomerulopathy and one recurrent IgA nephropathy. The MPA-AUCs were <60 µg·h/mL in all patients at the time of the biopsy.

MMF dosing and change of immunosuppressive regimen

The mean daily MMF dose was comparable at the time of conversion in both groups (group 1: 1786±481 mg; group 2: 1750±504 mg, p=0.60). The mean daily dose of MMF was higher in group 1 at 3 months after conversion (2228±666 mg vs. 1737±456 mg, p<0.001) and 3 years after conversion (1862±868 mg vs. 1427±729 mg, p=0.005). The MPA exposure was measured in 68 patients in group 1 (81.9%) and 41 patients in group 2 (73.2%) during and/or after conversion. AUC monitoring of MPA was infrequently performed (median 2.0 times during and/or after withdrawal). The mean MPA-AUC was between 60-90 µg·h/mL in 56.0% of cases in both groups, >90 µg·h/mL in 9.2% and <60 µg·h/mL in 34.9% of cases.

The immunosuppressive regimen was changed after the initial conversion in eleven patients in group 1 (13.3%) and nine patients in group 2 (16.1%). In group 1, the maintenance regimen was converted to triple therapy, consisting of a combination of prednisone, MMF, with either a CNI or an mTOR inhibitor in two and one patients, respectively. MMF was substituted for a CNI in three patients in group 1 and five patients in group 2, or for an mTOR inhibitor in four patients in group 1 and one patient in group 2, or for azathioprine in one patient in group 1 and three patients in group 2. There were various reasons for switching the immunosuppressive treatment; in group 1: diarrhea (2/11), leucopenia (1/11), acute rejection (1/11), transplant glomerulopathy (2/11), infection (1/11), malignancy (3/11) and pregnancy wish (1/11); in group 2: diarrhea (4/9), leucopenia (1/9) and infection (4/9).

DISCUSSION

The results of this retrospective evaluation of late complete CNI withdrawal in daily clinical practice showed an immediate and significant improvement in renal function after elective withdrawal in patients with stable renal function and those with progressive loss of function. More importantly, in the latter patient group, the slope of renal function over time improved significantly and stabilized after CNI withdrawal. Late CNI withdrawal and conversion to MMF in this observational cohort was safe, and not accompanied by excess acute rejection. The largest retrospective study on CNI reduction in transplant recipients with chronic allograft nephropathy had a median follow-up time of 651 days, but in only a minority of the patients (15%) the CNI was completely stopped [19]. In the Creeping Creatinine Study, elimination of the CNI in the presence of MMF resulted in improved renal function at 6 months in patients with biopsy-confirmed CAN and there was no risk of acute rejection [16]. A smaller trial randomized only 39 patients with biopsy-proven CAN and had to be stopped prematurely after 32 weeks. The reason was significantly better graft function after complete CNI withdrawal without the occurrence of acute rejection [17]. The present observational study confirms these previous data and the successful implementation of late CNI elimination in daily practice with only a low risk of acute rejection, while extending the observed beneficial effect on renal function to the intermediate- to long-term.

The immediate improvement in graft function is most likely explained by intrarenal hemodynamic changes [20]. CNIs cause renal vasoconstriction, especially of the afferent arterioles, followed by a decrease in GFR, renal plasma flow and increased renal vascular resistance [20]. Prolonged CNI use and intermittent daily vasoconstriction may lead to chronic tubulointerstitial lesions as well as impaired regional perfusion in case of progressive vascular hyalinosis. The stabilization of renal function for at least 3 years after CNI withdrawal also suggests an impact on chronic CNI-related nephrotoxic effects. Normalization of nitric oxide synthesis and reduced expression of transforming growth factor-β may also contribute to these changes [21].

In recent years, the role of chronic CNI nephrotoxicity in late renal graft failure has been questioned [22]. A retrospective study from the Mayo clinic identified glomerular disease and cellular and antibody-mediated immunologic injury as important causes of death-censored graft loss, while CNI-related toxicity only rarely attributed [23]. A recent prospective study following patients after for cause biopsies, indicated antibody-mediated or mixed rejection as the major cause of late graft failure, whereas CNIs did not play a role [24]. In the DeKAF study, patients with late allograft dysfunction with evidence of antibody-mediated injury had an increased risk of graft loss [25]. Patients only showing signs of CNI toxicity were less likely to experience graft failure [25]. Our results suggest that CNI use may have been the underlying cause of progressive deterioration of renal

function in this cohort of patients, and that CNI-related nephrotoxicity should not be neglected as a potentially important determinant of late and progressive graft dysfunction.

Most randomized studies on CNI withdrawal have shown an improvement in renal function, but mainly early after transplantation there is an increased risk of acute rejection [8, 10-11, 26-28]. The CAESAR trial found no difference in renal function at 12 months, despite a 50% increase in acute rejection after CNI withdrawal at 4 to 6 months after transplantation [8]. Abramowicz et al. compared CNI withdrawal or continuation of triple-drug therapy in stable renal transplant recipients 12 to 30 months after transplantation [10-11]. A significant improvement in renal function was documented after CNI withdrawal, but also a higher incidence of (chronic) rejection was noted at 5 years [11]. It appears that CNI elimination is associated with an increased acute rejection rate, especially early after transplantation and in case of inadequate dosing of MMF or steroids [8, 11, 28]. In a concentration-controlled randomized study, late CNI withdrawal resulted in significant improvement in renal function as compared to MMF withdrawal, especially in patients with an eGFR<50 mL/min/1.73 m^2, but ≥30 mL/min/1.73 m^2, up to 3 years [13]. These findings suggested that AUC monitoring of MPA, while preventing underdosing of MMF, reduced the occurrence of acute rejection after CNI withdrawal.

The beneficial effect of conversion to MMF on renal function, in patients with deteriorating renal function in this study, are in contrast with the CONVERT and ASCERTAIN studies evaluating late CNI withdrawal, but with conversion to an mTOR inhibitor [14-15]. Both studies failed to show better renal function in the intention-to-treat analysis. Only patients with an eGFR>40 mL/min in the CONVERT trial or GFR>50 mL/min in the ASCERTAIN study, who continued mTOR inhibitor treatment during 2 years, had a moderate but significant benefit in renal function with a low risk of acute rejection. These data may indicate that patients should be converted to an mTOR inhibitor well before they developed irreversible (structural) changes and a gradual decline in renal function. The lack of benefit, in patients with an eGFR<40 mL/min or <50 mL/min in the CONVERT and ASCERTAIN studies, respectively, may also relate to the relatively high rates of mTOR inhibitor discontinuation and reintroduction of CNI-based therapy. A randomized study comparing CNI withdrawal with adequate exposure of either MMF or an mTOR inhibitor would be needed to draw more definite conclusions on possible distinct effects in patients with impaired or deteriorating renal allograft function.

The main obvious limitation of our study is the observational character, but it does allow evaluation of a perceived beneficial strategy in daily clinical practice and provides long-term data on the course of renal function after complete CNI withdrawal. Although the study has no data on donor specific antibodies or (repeat) protocol biopsies, the observed results suggest a beneficial effect of CNI elimination on the course of renal function. Another limitation may be that the large majority of patients used cyclosporine,

whereas tacrolimus is currently the most frequently prescribed CNI after renal transplantation. Cyclosporine and tacrolimus, however, share comparable vasoconstrictive and nephrotoxic properties. Further, CNI withdrawal with therapeutic drug monitoring of MPA was only optional, therefore from the current study no conclusions can be drawn on the benefit of concentration-controlled dosing of MMF.

In summary, in our clinical experience, late CNI withdrawal with adequate MMF dosing was safe in the large majority of patients with a low risk of acute rejection. The immediate improvement in renal function after withdrawal was sustained on the intermediate- to long-term, with a significant deviation and stabilization of the eGFR slope over time, in those converted because of progressive loss of renal function. Within the multifactorial pathogenesis and progression of chronic renal allograft dysfunction, the impact of prolonged CNI exposure should not be disregarded.

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None.

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CHAPTER 6

Summary and general discussion

Kidney transplantation is the preferred treatment for patients with end-stage renal disease (ESRD). However, the shortage of donor kidneys remains a major obstacle. Despite more deceased donor transplants with extended donor criteria and more (unrelated and older) living donors, waiting lists are still growing. Living donor transplants currently account for 50% of the total renal transplants in the Netherlands (RENINE data). Considering the lack of donor organs, extending graft survival as long as possible, and thereby preventing a return to the waiting list, has become a critical issue in renal transplantation.

Long-term graft survival is determined by death with a functioning graft and late graft loss [1-2]. The major causes of mortality are cardiovascular disease, malignancy and infectious disease [3]. Calcineurin inhibitors (CNIs) increase the cardiovascular risk via hypertension, hyperlipidaemia, diabetes mellitus and chronic renal failure by nephrotoxicity [4]. Late allograft loss is mainly caused by chronic transplant dysfunction (CTD) [5]. CTD is a clinical syndrome of gradually deteriorating renal function, combined with hypertension and proteinuria, starting early after transplantation and ultimately ending in graft failure [6]. Chronic allograft nephropathy (CAN), its histological counterpart, is currently defined by interstitial fibrosis and tubular atrophy (IF/TA) [7]. Immunological factors, such as chronic cellular- or antibody-mediated rejection, and non-immunological factors, including CNI nephrotoxicity, contribute to the evolution of CAN-IF/TA [5].

Intervention strategies to improve long-term graft survival include early CNI sparing to reduce cardiovascular adverse effects and nephrotoxicity. Although early CNI withdrawal with mycophenolate mofetil (MMF)-based therapy may improve renal allograft function, it is associated with an increased risk of acute rejection [8]. Therefore, early minimisation of CNIs has become the trend, as low dose tacrolimus may ameliorate renal function and still protect against acute rejection [9]. However, the 3-year follow-up data of the Symphony study did not demonstrate a significant difference in renal function in favour of the low dose tacrolimus group [10]. Risk factors for acute rejection after CNI elimination are early withdrawal, subclinical rejection or underdosing of MMF [11-14]. The optimal range of mycophenolic acid (MPA) exposure in patients who were withdrawn of cyclosporine (CsA) was found to be higher than the usual target of 30-60 µg·h/ml in combination with CNIs [12, 14]. Therapeutic drug monitoring (TDM) might prevent underexposure to MPA.

This thesis studied the impact of late CNI withdrawal from a triple-drug regimen with corticosteroids, CNIs and MMF in renal transplant recipients, while providing adequate immunosuppression by using TDM of MPA, on renal function, the risk of acute rejection and surrogate markers for cardiovascular disease, including echocardiographic parameters, 24-hour ambulatory blood pressure and carotid intima media thickness (IMT).

In **chapter 2** the safety of either late concentration-controlled CNI or MMF withdrawal, in stable renal transplant recipients on a triple-drug regimen with corticosteroids, CNIs and MMF, was evaluated in a prospective randomised controlled study. In the safety phase, 58 patients were randomised (1:1:1) to CNI or MMF withdrawal or continuation of their immunosuppressive regimen. Subsequently, in the extension phase, another 119 patients were randomised (1:1) to either CNI or MMF withdrawal. The target exposures were 3250 ng·h/ml for CsA, 120 ng·h/ml for tacrolimus and 75 µg·h/ml for MPA. Only 1/79 patients (1.3%) in the MMF withdrawal group and 3/79 (3.8%) in the CNI withdrawal group experienced acute rejection during the first 6 months after withdrawal (p=0.62). The occurrence of acute rejection episodes was not significantly different between both groups at 3 years (MMF withdrawal: 2.5% vs. CNI withdrawal: 5.1%, p=0.68).

Late CNI withdrawal resulted in an immediate and significant improvement in renal function, which was maintained during the study follow-up of 3 years (59.5±2.1 ml/min/1.73 m^2 vs. 51.1±2.1 ml/min/1.73 m^2, p=0.006). Especially patients with an estimated glomerular filtration rate (eGFR)<50 ml/min/1.73 m^2 (but ≥30 ml/min/1.73 m^2) benefitted from the late elimination of a CNI, with significant less decline in renal function compared to MMF withdrawal (slope: +0.008 ml/min/1.73 m^2/month vs. -0.096 ml/min/1.73 m^2/month, p=0.03). The defined higher MPA exposure was well tolerated. It was concluded, therefore, that either late concentration-controlled CNI or MMF withdrawal was safe, with a low probability of acute rejection in the large majority of stable renal transplant recipients. Area under the concentration-time curve (AUC) monitoring might prevent inadequate immunosuppression and CNI withdrawal had the advantage of improved renal function.

In the cardiovascular substudies, the impact of either concentration-controlled CNI or MMF withdrawal on surrogate markers of cardiovascular disease, including echocardiographic parameters, was investigated in **chapter 3** and the same for ambulatory blood pressure and common carotid IMT in **chapter 4**. Patients were treated for cardiovascular risk factors according to stringent predefined targets: goals for systolic blood pressure (SBP)<130 mm Hg and diastolic blood pressure (DBP)<85 mm Hg; in case of proteinuria SBP<125 mm Hg and DBP<75 mm Hg [15]; for low-density lipoprotein (LDL)-cholesterol<2.6 mmol/L. Study visits were scheduled 3-monthly after trial entry during the first year, and subsequently after 2 and 3 years in a dedicated outpatient clinic, alternating with 3-monthly visits to their treating nephrologist in a regional outpatient clinic.

Echocardiographic measurements were performed in 108 patients at baseline and 2 years after withdrawal. The assessment of left ventricular (LV) diastolic function showed a significant prolongation of E-wave deceleration time after MMF withdrawal, whereas the E-wave deceleration time remained unchanged after CNI withdrawal at 2 years (p=0.013). Mitral annular e' velocity improved in the CNI withdrawal group at 2 years,

but remained unchanged in the MMF withdrawal group (p=0.16). The left atrial volume index, an indicator of chronic LV diastolic dysfunction, was significantly increased in the MMF withdrawal group after 2 years (p<0.001). In addition, CNI withdrawal resulted in a lower SBP (135±18 vs. 141±14 mm Hg, p=0.06) and DBP (77±9 vs. 84±10 mm Hg, p=0.001) with the use of less antihypertensive drugs. A higher proportion of patients in the CNI withdrawal group achieved the target blood pressure (<130/85 mm Hg: 41.5% vs. 12.7%, p=0.001). Change in E-wave deceleration time was significantly associated with withdrawal group (p=0.025), but not with changes in SBP, DBP or renal function over time. Change in left atrial volume index was significantly associated with withdrawal group (p=0.03), changes in SBP (p=0.005) and DBP (p=0.005), and not with change in renal function over time. These findings indicated that CNI withdrawal prevented the progressive development of LV diastolic dysfunction and facilitated better treatment of hypertension.

In **chapter 4** the results of ambulatory blood pressure monitoring (ABPM) and IMT of 119 patients were reported. Both measurements were performed at baseline and repeated every year. At 3 years, the CNI withdrawal group had significantly lower 24-hour ambulatory SBP (121.2±9.3 vs. 127.9±9.9 mm Hg, p=0.004) and DBP (72.2±6.9 vs. 78.5±5.3 mm Hg, p<0.001). During the 3-year follow-up, the CNI withdrawal group demonstrated a significant decline in ambulatory blood pressure (day-time: SBP: -1.6 mm Hg/yr, p=0.018; DBP: -1.3 mm Hg/yr, p=0.002; night-time SBP: -1.9 mm Hg/yr, p=0.008; DBP: -1.3 mm Hg/yr, p=0.014), whereas the MMF withdrawal group did not. There was a significant difference between the slopes of ambulatory blood pressure of both groups (day-time SBP: p=0.035; day-time DBP: p=0.001; night-time SBP: p=0.015; night-time DBP: p=0.003). The proportion of patients with >2 antihypertensive drugs was significantly higher after MMF withdrawal vs. CNI withdrawal (57% vs. 35%, p=0.03). A subgroup analysis of patients, who were treated with either CsA or tacrolimus at randomisation, demonstrated comparable results after CsA withdrawal (24-hour SBP: 123.1±7.9 vs. 128.3±9.5 mm Hg, p=0.05; 24-hour DBP: 72.9±6.1 vs. 78.0±4.2 mm Hg, p=0.001) and tacrolimus withdrawal (24-hour SBP: 115.9±11.3 vs. 127.2±11.0 mm Hg, p=0.058; 24-hour DBP: 70.1±8.8 vs. 79.5±6.8 mm Hg, p=0.058) . The mean IMT did not change over time in both groups with no significant difference in IMT between the patients who had been withdrawn from either a CNI or MMF after 3 years (CNI withdrawal: 0.611±0.074 mm; MMF withdrawal: 0.591±0.070 mm, p=0.27). These results showed that late CNI withdrawal improved ambulatory blood pressure during both day-time and night-time. The significant reduction in ambulatory blood pressure after CNI withdrawal was not associated with a change in IMT after 3 years.

In **chapter 5** a retrospective study was described investigating the safety and intermediate- to long-term impact on renal function of late CNI withdrawal and conversion to an immunosuppressive regimen with corticosteroids and MMF in renal transplant recipients, including those with deteriorating renal function. This study confirmed our clinical experience that late CNI withdrawal was not associated with an increased rate of acute rejection in the outpatient setting in the long-term. A total of 139 patients, who had been converted by their treating physician in our outpatient clinic, were included. Eighty-three (60%) patients had been withdrawn of the CNI because of reasons not related to renal function (group 1) and 56 (40%) because of declining renal function (group 2). The median follow-up time was 3.4 years (IQR 2.9-5.1 years). Conversion to MMF resulted in an immediate increase in renal function in both groups (group 1: 4.9±2.4 ml/min/1.73 m^2, p=0.04; group 2: 5.4±1.8 ml/min/1.73 m^2, p=0.004). Group 1 had a stable renal function before and after conversion. Group 2 demonstrated a progressive and gradual loss of eGFR towards conversion (−0.34±0.07 ml/min/1.73 m^2/month), that stabilised after conversion (0.01±0.05 ml/min/1.73 m^2/month) with a significant difference between the slopes (p<0.001). One patient in each group experienced an acute rejection episode. These results demonstrated that late CNI withdrawal was safe and led to improved renal function, with the stabilisation of renal function in the intermediate to long-term, in patients with stable function and in those with an already progressive loss of renal function.

LATE CNI WITHDRAWAL AND RENAL ALLOGRAFT OUTCOME

The introduction of CNIs and MMF has tremendously improved short-term graft survival due to the reduction of early acute rejection by better control of the alloimmune response in the immediate post-transplant period [16-20]. However, long-term survival has not equivalently improved [21-23], which has partly been attributed to the adverse effects of CNIs, especially nephrotoxicity [24]. Consequently, there has been an ongoing interest in interventions to eliminate or minimise CNI-based therapy. CNI withdrawal with MMF-based therapy has not become common practice, because of the documented increased risk of acute rejection [8].

In this study, late CNI withdrawal with TDM resulted in significantly better renal function in stable renal transplant patients with a low risk of acute rejection. The improvement in renal function directly after CNI withdrawal was sustained over time and most likely resulted from a reversal of renal vasoconstriction, since no change in the slopes of renal function could be demonstrated after 3 years. When comparing the change in eGFR from baseline to 3 years between the groups, a difference of 4.4±2.2 ml/min/1.73 m^2 (MMF withdrawal: -2.9±1.6 ml/min/1.73 m^2, CNI withdrawal: 1.5±1.6 ml/

min/1.73 m^2, p<0.05) in favour of the CNI withdrawal group was rather limited, but still clinically important and in accordance with a recent meta-analysis [8]. Furthermore, the current study found a greater difference in the change in renal function from baseline of 8.7±2.3 ml/min/1.73 m^2 between the withdrawal groups in the subpopulation with an eGFR<50 ml/min/1.73 m^2 and ≥30 ml/min/1.73 m^2 after CNI withdrawal at 3 years (MMF withdrawal: -2.3±1.6 ml/min/1.73 m^2, CNI withdrawal: 6.4±1.7 ml/min/1.73 m^2, p<0.001), with a significant difference in the slopes of renal function over time. This finding suggested the elimination or reversal of chronic nephrotoxicity by the CNI in a subgroup with worse renal function and consequently more renal damage. These results are in line with 2 prospective trials investigating the effects of late CNI withdrawal with conversion to MMF (>5 years post-transplantation) in patients with deteriorating renal function [25-26].

The current results are in contrast with the CONVERT and ASCERTAIN studies, which have both evaluated late CNI withdrawal (3.2 and 5.4 years post-transplantation, respectively), but with conversion to a mammalian target of rapamycin (mTOR) inhibitor [27-28]. Both studies failed to show better renal function in the intention-to-treat analysis. Only patients with an eGFR>40 ml/min in the CONVERT trial or a GFR>50 ml/min in the ASCERTAIN study and who carried on with mTOR inhibitor treatment for 2 years had a significant benefit in renal function, with a low risk of acute rejection [27-28]. These data indicated that patients should be converted to an mTOR inhibitor before they develop chronic and irreversible structural (nephrotoxic) changes. Given the high rates of discontinuation of mTOR inhibitors in these studies, it is tempting to speculate that late CNI withdrawal with MMF may be more attractive than with an mTOR inhibitor, especially in patients with an eGFR between 30 and 50 ml/min/1.73 m^2 or deteriorating renal function. On the other hand, conversion to an mTOR inhibitor has the benefit of a lower incidence of malignancies [29]. However, a randomised study comparing CNI withdrawal with adequate exposure of either MMF or an mTOR inhibitor would be needed to analyse this hypothesis. In general, it is desirable to stop or minimise CNI treatment before it is too late and irreversible damage has been done.

We hypothesise that concentration-controlled CNI withdrawal also might have been safe, if it had been performed prior to 3 years post-transplantation, but at least beyond the first year in view of the higher immunological risk after early elimination. The risk of acute rejection was low, but numerically higher after CNI withdrawal than MMF withdrawal at 3 years (5.1% vs. 2.5%, p=0.68). However, the current study was not designed to show a difference in this outcome. The rate of rejection was still lower than the incidence of between 11% and 22% reported in previous withdrawal studies [8, 11-13, 30-32]. Also, numerically more patients developed a chronic transplant glomerulopathy after the CNI was withdrawn (2.5% vs. 0%, p=0.50). Most of the rejection episodes occurred during the first 6 months after either CNI or MMF withdrawal and could not have been predicted by the immunological risk profile of the patients.

TDM may have prevented inadequate exposure to MPA and consequently an excess rate of rejection after CNI withdrawal, in both the short- and long-term. The two patients in our study who experienced acute rejection after ≥1 year after either MMF or CNI withdrawal had underexposure to the remaining drug, due to therapeutic non-compliance and an intercurrent illness, respectively. Physicians (and patients) should be aware of the risk of rejection, in particular during the first 3-6 months after withdrawal and in case of an intercurrent illness, even when concentration-controlled dosing of the remaining drug is used. During these episodes, renal function and the AUC have to be monitored more frequently. TDM does not invariably detect non-compliance. In case there is serious doubt regarding the compliance, the patient should not be switched to dual therapy.

Although a high exposure to MPA was provided, the donor specific antibodies (DSA) or protocol biopsies were not included at the time of randomisation or during follow-up in order to identify patients with subclinical (chronic) rejection and CAN-IF/TA. According to the protocol, a renal biopsy was performed in case serum creatinine increased >15%. As serum creatinine is an insensitive marker, the rate of subclinical (chronic) rejection or CAN-IF/TA may have been underestimated. The follow-up time was 3 years, but it may still not be long enough to reveal the functional consequences of these subclinical histological changes.

The present study has several other limitations. It is a single-centre study in a selected group of renal transplant recipients with stable renal function and a relatively low immunological risk profile. Therefore, the results of the present study may not be directly extrapolated to populations with a higher immunological risk. Outside the feasibility phase, there was no control group, but it is not likely that renal function would have improved in a control group with a CNI-based regimen with corticosteroids and MMF. Another shortcoming is that 75% of patients were treated with CsA in the MMF withdrawal group, whereas tacrolimus has become the most frequently prescribed CNI after renal transplantation.

LATE CNI WITHDRAWAL AND CARDIOVASCULAR RISK

Blood pressure

Hypertension is common in renal transplant recipients and an independent predictor of graft failure and cardiovascular mortality [33-34]. CNIs cause hypertension in transplant recipients by renal vasoconstriction by the activation of vasoconstrictive factors, including the renin-angiotensin-aldosterone system, endothelin and thromboxane A2, and the reduction of vasodilator factors, such as nitric oxide and prostacyclin [35]. Activation of the sympathetic nervous system may also play a role [35]. Furthermore, sodium and

water retention can be increased by the activation of the renin-angiotensin-aldosterone system and inactivation of the natriuretic peptide [35].

Late CNI withdrawal resulted in a significant decline in ambulatory blood pressure after 3 years, while less patients had to be treated with >2 antihypertensive drugs (35% vs. 57%, p=0.03). A subgroup analysis of patients, who were on either on CsA (68.1%) or tacrolimus (31.9%), showed comparable results with lower ambulatory blood pressures after CsA as well as tacrolimus withdrawal at 3 years. Although the study protocol did not include a subgroup analysis and the number of patients was small, these results suggest that both CsA and tacrolimus have adverse effects on arterial blood pressure, though CsA and tacrolimus inhibit calcineurin activity by binding to different immunophyllins [36].

Despite the treatment of hypertension according to a predefined target (blood pressure <130/85 mm Hg) and a treatment protocol, blood pressure was still insufficiently controlled in a relatively large proportion of patients. These findings are in accordance with a large cohort study, which reported that 46% of renal recipients had an SBP≥140 mm Hg at 1 year after transplantation [34]. In the current cardiovascular substudy, only 12.7% of the patients in the MMF withdrawal group and 41.5% in the CNI withdrawal group had a blood pressure below the target of 130/85 mm Hg at 2 years (p=0.001).

The vigorous regulation of hypertension merits more attention, since it would confer a benefit in graft survival [33-34]. After the first year, the majority of patients were followed by their treating physician 3-monthly, alternating with yearly visits to the dedicated outpatient clinic. Part of the disappointing results may be explained by non-adherence to the treatment protocol, although these were based on commonly accepted practice guidelines for renal transplant recipients. On the other hand, non-compliance and the tendency of many patients to bargain for as few antihypertensive drugs as possible, may have contributed to the poor control of blood pressure. A greater awareness of the importance and implications for long-term outcome among patients and their treating physicians, as well as the use of collective treatment protocols by physicians or their nurse practitioners/physician assistants, may help to achieve targets in a higher proportion of patients. Measures such as self-management may provide better patient understanding and promote compliance.

Left ventricular function

Patients with ESRD already have a high cardiovascular burden at the start of dialysis therapy with LV hypertrophy (LVH) as the most prevalent cardiac alteration [37]. LVH and also higher left atrial volume are independent predictors of mortality and cardiovascular outcome in dialysis patients [37-39]. Renal transplantation can reverse the structural cardiac changes associated with ESRD [40-42] and reduce cardiovascular mortality, but the cardiovascular risk remains high [43].

There have been conflicting reports concerning the effects of CNIs on the myocardium [44-49]. An observational study reported worsening of LV diastolic function after renal transplantation, despite LVH regression and improvement in LV systolic function [41]. The authors speculated that CsA treatment might cause the progression of LV diastolic dysfunction in renal transplant recipients [41]. In cardiac and renal transplant recipients, replacing the CNI with sirolimus resulted in LV mass regression [44-45] and improvement in LV diastolic function [44]. However, in experimental animals, calcineurin activation appeared to be a key player in mediating the development of LVH and calcineurin inhibition could prevent LVH without affecting LV systolic function [46-48]. Genetic inhibition of calcineurin reduced LVH development in a mouse model, but resulted in LV diastolic dysfunction [49].

In the present cardiovascular substudy, deterioration of LV diastolic function, as determined by mitral deceleration time and mitral annular e' velocity, was prevented by CNI withdrawal. Increase in E-wave deceleration time was significantly associated with MMF withdrawal. Additionally, left atrial volume index, an indicator of chronic LV diastolic dysfunction, significantly increased in the MMF withdrawal group, which was associated with MMF withdrawal and SBP and DBP. These findings indicated that CNI withdrawal may stabilise LV diastolic dysfunction, probably by influencing blood pressure control. However, this study was not designed to discriminate between the indirect effects of better blood pressure control and improved renal function or the direct effect of CNI elimination on the myocardium.

Furthermore, CNI withdrawal had no effect on LVH, despite better regulation of blood pressure. Another possible explanation for this finding may be that only a minority of the study population (22.2% of female patients and 11.1% of male patients) had LVH at baseline and, therefore, it is not surprising that no significant reduction in left ventricular mass was documented. In addition, the use of angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) was high, with the majority of patients (70.4%) using ≥2 antihypertensive drugs at baseline. As the renin-angiotensin-aldosterone system is activated in CNI-treated patients [50], treatment with an ACEi or ARB may have suppressed the renin-angiotensin-aldosterone system-mediated cell growth and the development of LVH.

Intima media thickness

Intima media thickness of the common carotid artery reflects atherosclerosis and is associated with an increased incidence of cardiovascular and cerebrovascular events in the general population [51-53]. Consequently, it is increasingly used as a surrogate cardiovascular endpoint in interventional trials and for risk assessment in individual patients. In renal transplant recipients, IMT is increased compared to healthy controls [54-57], but lower than in haemodialysis patients [57-58]. Increased IMT is a risk factor for mortality in renal transplant recipients [59] and has been associated with inferior graft outcome [60].

Previous studies remain inconclusive regarding the progression of IMT long-term after renal transplantation and have reported either a reduction [61], no change [62] or progression [63] in limited numbers of patients.

Although CNI withdrawal resulted in a significant reduction in ambulatory blood pressure, sequential IMT measurements did not show a significant alteration in IMT during 3 years. Theoretically, the observed difference in blood pressures in this study may have been too small or a longer follow-up period may be required to detect the beneficial effect of blood pressure lowering on IMT progression. However, randomised trials investigating the effect of antihypertensive medication have shown a reduction of IMT progression with smaller blood pressure differences [64]. Furthermore, the reduction in LDL-cholesterol by treatment with lipid lowering drugs (from 3.1 to 2.6 mmol/L, 16% reduction) in this study was not associated with a change in IMT. As intensive lipid lowering is known to reduce the progression of IMT [65], statin treatment may have attributed to the stable IMT. One small prospective randomised study has evaluated the impact of pravastatin on IMT in renal transplant recipients and found less progression after 1 year (LDL-cholesterol fell from 3.8 to 2.8 mmol/L, 26% reduction) [63].

It may be necessary to include a larger number of patients with a longer follow-up time to draw definite conclusions on the influence of either CNI or MMF withdrawal on carotid atherosclerosis. The sample size calculation of the main study was based on the difference in renal function between the groups. Since data on the rate of IMT progression in patients long-term after transplantation are limited and indefinite and no studies have assessed the impact of CNI elimination on IMT change over time, a sample size calculation based on a change in IMT progression for our intervention would have a high level of uncertainty. A review by Bots provided sample size calculations based on a pooled common carotid IMT progression rate of 0.0147 mm/year with a median SD of 0.053, with a 2-sided α and a power of 90% for a period of 2 and 3 years [66]. The number needed for each arm in a randomised controlled trial, varied from 339 (30% effect in 3 years) to 30 (100% effect in 3 years) [66]. Therefore, the current study may have been underpowered to find a smaller effect in 3 years.

FUTURE PERSPECTIVES

Late CNI withdrawal, while providing adequate exposure, is a potential approach to reduce CNI-related nephrotoxicity and cardiovascular side-effects, but earlier interventions within the first 6 months after renal transplantation are more preferable, since patients already show features of nephrotoxicity within the first year [67]. There are several potential pre-emptive strategies to decrease CNI exposure:

1) De novo CNI minimisation with MMF.

The CAESAR and Symphony studies have evaluated de novo CNI minimisation under the umbrella of MMF [9, 13]. The CAESAR study demonstrated that renal function and acute rejection rates were similar in either the low-dose or standard-dose CsA groups at 12 months after transplantation [13]. In the Symphony study, the low-dose tacrolimus regimen was superior to the low- and standard-dose CsA regimen with respect to the eGFR and the occurrence of acute rejection at 12 months post-transplantation [9], although the observed benefit in renal function in favour of the low-dose tacrolimus treatment arm did not reach statistical significance at 3 years, suggesting less, but consistent nephrotoxicity, even at low doses of tacrolimus [10]. Further dose reduction to very low doses of tacrolimus with concentration-controlled dosing of MMF may be a future strategy, which has yet to be evaluated in randomised controlled trials.

2) De novo CNI minimisation or early CNI elimination with an mTOR inhibitor.

This strategy includes the additional benefits on long-term outcome by the inhibition of angiogenesis/vascular remodeling and proliferation associated with the use of mTOR inhibitors (sirolimus and everolimus) [68]. They may have the capacity to prevent or delay the progression of CAN-IF/TA at an early stage, as previously demonstrated in rat models [69-70]. Additionally, the anti-tumour properties of mTOR inhibitors have been shown in clinical trials, reporting a decreased incidence of new malignancies among patients with sirolimus [29, 71]. Furthermore, mTOR inhibitors protect against viral infections, which was demonstrated by a decrease in the incidence of cytomegalovirus (CMV) [72-74] and BK virus infections [75] in randomised studies comparing everolimus with MPA in de novo renal transplant recipients. CMV infection may play a role in the development of CAN-IF/TA [76] and BK virus-associated nephropathy is an important cause of CTD [77]. There may be beneficial effects on cardiovascular outcome, as de novo everolimus reduced the incidence of cardiac allograft vasculopathy and its associated major cardiac adverse events in comparison to azathioprine in cardiac transplant recipients [78-79]. On the other hand, mTOR inhibitors may contribute to the elevated cardiovascular risk in transplant recipients by increasing the incidence of hypercholesterolaemia and new-onset diabetes [80]. Finally, mTOR inhibitors could potentially induce immunological tolerance, as indicated by in vitro experiments [81].

Efficacy and safety has been demonstrated for de novo everolimus with CNI minimisation [75, 82-86], although renal function did not improve significantly compared to standard dose CNI in several randomised studies [75, 84-86], probably owing to relatively small differences in CNI blood levels between the groups [84-86]. De novo MPA-based therapy with a CNI followed by early conversion to an mTOR inhibitor with CNI elimination (between month 2 and 6) resulted in improved renal function in recently reported randomised trials [87-91], but was associated with an increase in acute rejection rates [87, 89-91]. Furthermore, the toxicity of mTOR inhibitors limits their usability. Considerable proportions of patients discontinued mTOR inhibitors in these trials (20-40%) due to various serious ad-

verse effects, including mucocutaneous and gastro-intestinal side-effects, proteinuria, delayed wound healing, bone marrow depression and pneumonitis [92-93].

3) CNI avoidance with belatacept.

Belatacept is a CTLA-4 antibody that blocks T-cell co-stimulation by binding to CD80/CD86 on antigen presenting cells [94]. Two phase III multicentre trials, BENEFIT and BENEFIT-EXT, in recipients with extended criteria donor kidneys, evaluated CNI-free more and less intensive belatacept-based immunosuppressive regimens with a standard CsA-based regimen [95-99]. Although more acute rejection episodes occurred in the BENEFIT study during the first year (more intensive belatacept: 22%; less intensive belatacept: 17%; CsA: 7%) [95], renal function was significantly better in the patients with belatacept-based therapy vs. CsA at 3 years (BENEFIT: 21 ml/min/1.73 m^2) [98]. In the BENEFIT-EXT trial, the incidence of acute rejection was comparable in the belatacept-treated and the CsA-treated groups after 1 year [96, 99]. At 3 years, the eGFR was 11 ml/min/1.73 m^2 higher in the belatacept-treated groups [99]. However, the risk of post-transplant lymphoproliferative disorder, especially with central nervous system involvement, was increased in both studies [95-97, 99-100]. Associated risk factors were primary Epstein Barr virus (EBV)-infection, T-cell depleting therapy and CMV disease [100] . Belatacept is therefore only recommended in EBV-seropositive patients [101]. The cardiovascular risk profile improved in the belatacept-treated groups at 1 year [102], and showed similar trends at 2 and 3 years [97, 99]. One of the limitations of these studies was the high target trough level range of CsA (100-250 ng/ml), which may have worsened renal function in the CsA group [103].

In recent years, several studies have questioned the dominant role of CNI-related nephrotoxicity in late renal graft failure [104-107]. The application of the immunological markers C4d and DSA has revealed that chronic humoral alloreactivity is common in transplant recipients with new-onset late transplant dysfunction [104]. In the DeKAF study, patients with for cause biopsies with C4d+ staining and DSA had an increased risk of losing their graft, whereas patients with only CNI toxicity had a lower risk of experiencing graft loss [104]. A retrospective study from the Mayo clinic demonstrated cellular and antibody-mediated alloimmune and autoimmune injury to be important factors in graft loss, whereas CNI-related toxicity only rarely attributed [105]. Two prospective studies following patients after for cause biopsies, indicated antibody-mediated rejection and glomerular disease as the major causes of late allograft failure, while CNIs did not play a role [106-107].

Nevertheless, chronic CNI nephrotoxicity remains a serious problem, as shown in previous studies [67, 108] and in patients with non-renal transplantations and autoimmune diseases [109-112], but questions have risen regarding the consequence of reducing CNIs on chronic (humoral) immunity and what the best immunosuppressive protocol to prevent it would be [113]. Therefore, improving the monitoring of the immunological status has become even more important for the individualisation of immunosuppression [113]. There

has been an extensive search for biomarkers that are able to predict rejection or organ tolerance in transplant recipients and could reduce the risk during CNI minimisation or withdrawal [114-115]. Potential biomarker assays include analysis of gene expression profiles of either rejection or tolerance, assessment of regulatory T-cells, detection of urinary biomarkers of acute rejection (at the protein, peptide or mRNA level) and functional assays to monitor humoral or effector and memory T-cell alloimmune responses [114]. Currently, none of these biomarkers or assays have been standardised and validated in prospective trials [114].

CNI-induced nephrotoxicity might be reduced by strategies for advanced TDM, including pharmacodynamic analysis and pharmacogenetic testing [116-118]. Pharmacodynamic monitoring assessing calcineurin enzyme activity [116] and determination of the genotype of drug metabolising genes as CYP3A5 and ABCB1 (encoding the efflux pump transporter P-glycoprotein) [117-118] could further individualise and optimise CNI dosing. However, at present, the only parameter that may be clinically relevant is CYP3A5 genotyping and solely in relation to the initial dosing of tacrolimus [118-119].

In summary, CNI withdrawal using TDM of MPA may result in improved outcome. In this context, the timing of the intervention, early vs. late, and the immunological risk profile appear the most relevant parameters. It is not known whether comparable results can be achieved in patients with a higher immunological risk profile and who will develop DSA.

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NEDERLANDSE SAMENVATTING

Niertransplantatie is de beste behandeling voor patiënten met eindstadium nierfalen. Hoewel steeds meer marginale postmortale en levende donoren (ook niet-verwante donoren) worden geaccepteerd, blijft het tekort aan donornieren een groot probleem. De nierfunctie en de overleving van transplantaatnieren op de korte termijn is sterk verbeterd door de introductie van afweerremmende medicatie, zoals calcineurineremmers (CNIs) en mycofenolaat mofetil (MMF), die heeft geleid tot minder acute afstotingsreacties, en de verbetering van chirurgische technieken. De resultaten van niertransplantaties op de lange termijn zouden nog verder verbeterd kunnen worden.

Sterfte van de patiënt met een functionerende donornier en verlies van het transplantaat bepalen de lange termijn overleving van de transplantatienier. De belangrijkste doodsoorzaken zijn hart- en vaatziekten, kanker en infectieziekten. De CNIs, zoals ciclosporine en tacrolimus, verhogen het risico op hart- en vaatziekten, doordat zij hoge bloeddruk, een verhoogd cholesterol en suikerziekte kunnen veroorzaken. Tevens kunnen zij schadelijk (toxisch) zijn voor de transplantaatnier. Verlies van het transplantaat op de langere termijn wordt met name veroorzaakt door chronische transplantaatdysfunctie (CTD). Dit is een klinisch syndroom, dat gepaard gaat met een geleidelijke achteruitgang van de nierfunctie, hoge bloeddruk en eiwitverlies in de urine, en kan uiteindelijk leiden tot nierfalen. In het nierweefsel ontstaan afwijkingen, zoals toename van bindweefsel en verschrompeling van nierbuisjes, hetgeen ook wel chronische allograft nefropathie (CAN-IF/TA) wordt genoemd. Zowel immunologische factoren, zoals chronische afstoting via T-lymfocyten of antistoffen, als andere factoren, zoals niertoxiciteit ten gevolge van CNIs, kunnen bijdragen aan de ontwikkeling van CAN-IF/TA.

Een manier om de transplantaatoverleving op de lange termijn te verlengen kan derhalve het verminderen of stoppen van de CNI zijn, vroeg na transplantatie (minder dan 1 jaar), om de nierschade en de bijwerkingen op hart en vaten door CNIs te beperken. Hoewel het onttrekken van de CNI van een combinatie van afweerremmende medicijnen met prednison, een CNI en MMF de nierfunctie kan verbeteren, is het geassocieerd met een toegenomen risico op acute afstoting. Risicofactoren voor het optreden van acute afstoting na CNI onttrekking zijn: afbouwen kort na transplantatie, aanwezigheid van afstoting zonder klinische verschijnselen (subklinisch) of onderdosering van MMF.

De blootstelling aan CNIs en mycofenolzuur (MPA), de actieve component van MMF, gedurende 12 uur kan, na afname van een dalspiegel en concentraties na 2 en 3 uur (CNI) of na 1, 2 en 3 uur (MPA) na inname, met modellen berekend worden met de oppervlakte onder de concentratie-tijd curve (AUC). Door tijdens en na het stoppen van een CNI de MPA-AUC te meten en de dosering aan te passen op geleide hiervan, zou onderdosering van MMF voorkomen kunnen worden. De optimale AUC van MPA gedurende CNI ont-

trekking is op basis van studies in de literatuur hoger dan de gebruikelijke streef AUC van 30-60 µg·h/ml bij een combinatiebehandeling met prednison, een CNI en MMF.

In **hoofdstuk 2** werd het effect van late CNI- of MMF onttrekking op de nierfunctie en het optreden van acute afstoting bij stabiele niertransplantatiepatiënten met een regime van afweeronderdrukkende medicatie met prednison, een CNI en MMF in een prospectieve gerandomiseerde studie geëvalueerd. Onttrekking werd gecombineerd met monitoring van de AUC van het resterende medicament (CNI of MMF). In de eerste fase van de studie werden 58 patiënten gerandomiseerd (1:1:1) naar CNI- of MMF onttrekking of continuering van triple therapie. Vervolgens werden in de tweede fase 119 patiënten gerandomiseerd (1:1) naar CNI- of MMF onttrekking. De streef AUC was 3250 ng·h/ml voor ciclosporine, 120 ng·h/ml voor tacrolimus en 75 µg·h/ml voor MPA.

Slechts 1 van de 79 patiënten (1.3%) in de MMF onttrekkinggroep en 3 van de 79 patiënten (3.8%) in de CNI onttrekkinggroep ontwikkelden een acute afstotingsreactie in de eerste 6 maanden na het afbouwen (p=0.62). Na 3 jaar follow-up was het aantal acute afstotingen, dat optrad, niet significant verschillend in beide groepen (MMF onttrekking: 2.5% vs. CNI onttrekking: 5.1%; p=0.68). CNI onttrekking leidde direct tot een verbetering van de nierfunctie, die gedurende 3 jaar aanhield (59.5 ± 2.1 ml/min/1.73 m^2 vs. 51.1 ± 2.1 ml/min/1.73 m^2, p=0.006). Met name patiënten met een lagere nierfunctie (tussen 30 en 50 ml/min/1.73 m^2) profiteerden van het stoppen van de CNI en vertoonden minder achteruitgang van de nierfunctie dan na het staken van MMF. De hogere streef MPA-AUC werd goed door de patiënten verdragen. Concluderend ging zowel het laat staken van een CNI als van MMF met AUC bepalingen gepaard met een lage kans op acute afstoting bij de meerderheid van stabiele niertransplantatiepatiënten. CNI onttrekking had het voordeel van een verbeterde nierfunctie.

In de cardiovasculaire substudies van deze gerandomiseerde studie werd het effect onderzocht van CNI- of MMF onttrekking op indirecte maten van hart- en vaatziekten, namelijk parameters gemeten met (doppler)echografieën van het hart in **hoofdstuk 3** en de 24-uurs bloeddruk en de vaatwanddikte van de halsslagader (intima-media dikte) in **hoofdstuk 4**. De risicofactoren voor hart- en vaatziekten werden bij alle patiënten behandeld volgens van tevoren vastgestelde streefwaarden voor de bloeddruk en het LDL-cholesterol.

Bij 108 patiënten werden (doppler)echografieën van het hart aan het begin van de studie en 2 jaar na randomisatie verricht, waarbij de dikte van de linker kamerwand en de diastolische functie (relaxatie) en de systolische functie (pompfunctie) van de linker kamer werden gemeten. De deceleratietijd van de vroeg diastolische instroomsnelheid over de mitralisklep (E-wave deceleration time, maat voor de diastolische functie) bleef onveranderd na onttrekking van de CNI, terwijl deze verslechterde na afbouwen van

MMF. De toename van de deceleratietijd was geassocieerd met het onttrekken van MMF. De vroeg diastolische relaxatiesnelheid van de hartspier ter hoogte van de mitralisklepring (mitral annular e'velocity, maat voor de diastolische functie) verbeterde na staken van de CNI en veranderde niet na onttrekking van MMF. De volume index van de linker boezem (left atrial volume index, maat voor chronische diastolische dysfunctie) nam significant meer toe na het afbouwen van MMF. Deze toename was geassocieerd met MMF onttrekking en met de systolische en de diastolische bloeddruk. In de groep waar de CNI gestopt werd, waren de systolische bloeddruk (135 ± 18 vs. 141 ± 14 mm Hg, p=0.06) en de diastolische bloeddruk (77 ± 9 vs. 84 ± 10 mm Hg, p=0.001) na 2 jaar lager en werden minder bloeddrukverlagende medicijnen gebruikt. Meer patiënten in de CNI onttrekkinggroep hadden een bloeddruk conform de streefwaarde (lager dan 130/85 mm Hg: 41.5% vs. 12.7%, p=0.001) 2 jaar na randomisatie. Deze resultaten tonen aan dat late onttrekking van een CNI de ontwikkeling van diastolische dysfunctie van de linker kamer kan voorkomen en tot een betere regulatie van hoge bloeddruk kan leiden bij niertransplantatiepatiënten.

In **hoofdstuk 4** werden de resultaten van de metingen van de bloeddruk gedurende 24 uur en van de intima-media dikte bij 119 patiënten gepresenteerd. Met de intima-media dikte van de halsslagader kan de mate van aderverkalking en het risico op hart- en vaatziekten beoordeeld worden. Beide metingen werden bij de start van de studie verricht en jaarlijks herhaald. Na 3 jaar had de groep, waarin de CNI werd afgebouwd, een lagere 24-uurs systolische (121.2 ± 9.3 vs. 127.9 ± 9.9 mm Hg, p=0.004) en diastolische bloeddruk (72.2 ± 6.9 vs. 78.5 ± 5.3 mm Hg, p<0.001). De onttrekking van de CNI resulteerde in een afname van de gemiddelde bloeddrukwaarden gedurende de dag en de nacht, maar na het stoppen van MMF was dat niet het geval. Een subgroepanalyse van patiënten, die bij baseline ciclosporine of tacrolimus gebruikten, liet in beide subgroepen vergelijkbare resultaten zien 3 jaar na CNI onttrekking met betrekking tot de 24-uurs systolische en diastolische bloeddrukmetingen. Drie jaar na het staken van de CNI gebruikte een kleiner percentage van de patiënten meer dan 2 medicijnen tegen hoge bloeddruk dan na het stoppen van MMF (35% vs. 57%, p=0.03). De intima-media dikte veranderde niet significant na afbouwen van de CNI of MMF en was 3 jaar na randomisatie niet verschillend in beide groepen (CNI onttrekking: 0.611 ± 0.074 mm; MMF onttrekking: 0.591 ± 0.070 mm, p=0.27). Uit deze resultaten kan geconcludeerd worden, dat late CNI onttrekking de gemiddelde bloeddruk, zowel gedurende de dag als de nacht, kan verbeteren. De significante verlaging van de 24-uurs bloeddruk leidde niet tot een meetbare afname van de intima-media dikte na 3 jaar.

In **hoofdstuk 5** werd een retrospectieve studie beschreven, waarin het effect op de nierfunctie van het laat afbouwen van de CNI en omzetten naar een afweerremmende

behandeling met prednison en MMF werd onderzocht bij niertransplantatiepatiënten met een stabiele of verslechterende transplantaatfunctie. Tussen 1997 en 2007 werden 139 patiënten op de polikliniek overgezet op prednison en MMF door hun behandelend internist-nefroloog. Bij 83 patiënten (60%) werd de CNI onttrokken om redenen die niet gerelateerd waren aan de nierfunctie (groep 1) en bij 56 patiënten (40%) vanwege afname van de nierfunctie (groep 2). De mediane follow-up periode was 3.4 jaar. Na het afbouwen van de CNI ontstond direct een toename van de nierfunctie in beide groepen (groep 1: 4.9 ± 2.4 ml/min/1.73 m^2, p=0.04; groep 2: 5.4 ± 1.8 ml/min/1.73 m^2, p=0.004). Groep 1 had voor en na de conversie een stabiele nierfunctie, terwijl bij groep 2 de achteruitgaande nierfunctie na conversie stabiliseerde. In beide groepen ontwikkelde 1 patiënt een acute afstotingsreactie. Deze resultaten laten zien dat CNI onttrekking, laat na transplantatie, niet geassocieerd is met een verhoogd risico op acute afstoting. Conversie naar prednison en MMF kan ook de nierfunctie bij niertransplantatiepatiënten met een reeds achteruitgaande nierfunctie stabiliseren.

Samenvattend kan het laat onttrekken van een CNI van onderhoudstherapie met prednison, een CNI en MMF met AUC monitoring van MPA de uitkomsten van niertransplantaties op de langere termijn gunstig beïnvloeden door verbetering van de nierfunctie en verlaging van het risico op hart- en vaatziekten. De kans op acute afstoting was laag, maar de onderzochte patiëntengroep was een geselecteerde groep met een relatief lagere risico-inschatting op afstoting. Derhalve kunnen de resultaten niet geëxtrapoleerd worden naar populaties met een hoger immunologisch risicoprofiel.

LIST OF ABBREVIATIONS

ABPM	Ambulatory blood pressure monitoring
ACE	Angiotensin converting enzyme
ACEi	Angiotensin-converting enzyme inhibitor
ARB	Angiotensin II receptor blockers
AUC	Area under the concentration-time curve
AZA	Azathioprine
BMI	Body mass index
BP	Blood pressure
BPAR	Biopsy-proven acute rejection
BSA	Body surface area
C_2	Concentration at 2 hours postdose
CAN	Chronic allograft nephropathy
CMV	Cytomegalovirus
CNI	Calcineurin inhibitor
CS	Corticosteroids
CsA	Cyclosporine
CTD	Chronic transplant dysfunction
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DD	Deceased donor
DSA	Donor specific antibodies
EBV	Epstein Barr virus
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
HbA1c	Glycated hemoglobin
HLA	Human leucocyte antigen
IF/TA	Interstitial fibrosis and tubular atrophy
IMT	Intima media thickness
LA	Left atrial
LD	Living donor
LDL	Low-density lipoprotein
LV	Left ventricle/left ventricular
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end-systolic volume

LVH	Left ventricular hypertrophy
MACE	Major adverse cardiac events
MDRD	Modification of Diet in Renal Disease
MMF	Mycophenolate mofetil
MPA	Mycophenolic acid
MPAG	7-*O*-MPA-glucuronide
MRP-2	Multidrug resistance-associated protein-2
mTOR	Mammalian target of rapamycin
NODAT	New-onset diabetes after transplantation
PRA	Panel reactive antibodies
SBP	Systolic blood pressure
SDC	Supplemental digital content
Tac	Tacrolimus
TDM	Therapeutic drug monitoring

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 18 maart 1971. In 1989 deed zij eindexamen aan het ongedeeld atheneum van het Sint-Laurenscollege in Rotterdam. Na het cum laude halen van de propedeuse Biomedische wetenschappen aan de Universiteit Leiden, stapte zij over naar de studie Geneeskunde in 1990. In 1997 behaalde zij het artsexamen. Zij werkte in 1997 en 1998 als AGNIO interne geneeskunde in het Andreas Ziekenhuis in Amsterdam en het LUMC in Leiden. Van 1999 t/m 2001 deed zij de perifere opleiding interne geneeskunde in het Rode Kruis Ziekenhuis (opleider: dr. R.M. Valentijn). Van 2002 t/m 2004 werd de opleiding interne geneeskunde voortgezet in het LUMC (opleider: prof. dr. A.E. Meinders). Van 2005 t/m 2009 was de auteur werkzaam als fellow nierziekten in het LUMC (opleider: prof. dr. T.J. Rabelink en prof. dr. J.W. de Fijter) en specialiseerde tot internist-nefroloog. Vanaf 2005 werkte zij aan het onderzoek, dat tot dit proefschrift heeft geleid. Sinds 2012 werkt zij als medisch adviseur bij het College voor Zorgverzekeringen.

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MYCOPHENOLATE MOFETIL IN RENAL TRANSPLANT RECIPIENTS LATE CONCENTRATION-CONTROLLED CALCINEURIN INHIBITOR WITHDRAWAL WITH MYCO