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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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