Towards personalized cardiovascular risk management in renal transplant recipients

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Chapter 7

SUMMARY AND GENERAL DISCUSSION
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Prelude
Cardiovascular risk is greatly increased in renal transplant recipients (RTR), which likely is due to an interaction of traditional and transplantation-related cardiovascular risk factors. Many RTR already have several traditional cardiovascular risk factors before transplantation, which are only partially remitted following successful transplantation, and in fact, are more prevalent, more severe or less responsive to treatment than in non-transplanted patients. In addition, new transplantation-related risk factors emerge, such as remaining subnormal kidney function and the use of immunosuppressive drugs. Moreover, within the current immunosuppressive regimens, corticosteroids are still indispensable and dosed by a more or less ‘one-size-fits-all’ empirical schedule and therefore may result in higher administered doses than strictly necessary for an adequate therapeutic effect, which potentially adds to the already increased cardiovascular risk in these patients. Therefore, we are in need of comprehensive strategies to reduce the increased cardiovascular risk in RTR, ideally addressing both traditional and transplantation-related risk factors, and targeting the specific risk profile in this patient category rather than extrapolating from guidelines based on studies in other populations. In addition, we are in need of biomarkers that allow for better personalization of treatment. In this thesis, we focused on potential strategies to reduce hypertension, which is the most prevalent cardiovascular risk factor in RTR, specifically focusing on dietary sodium restriction (Chapter 2), the renin-angiotensin-aldosterone-system (RAAS), and their interaction (Chapter 3). In addition, we aimed to find biomarkers that allow for personalization of corticosteroid therapy, specifically focusing on pathways of endogenous cortisol synthesis (Chapters 4 and 5) and chronic inflammation (Chapter 6).

Treating post-transplantation hypertension: a role for dietary sodium restriction?
Hypertension is the most prevalent cardiovascular risk factor in RTR. Up to 90% of RTR have high blood pressure or are treated with antihypertensive drugs. However, antihypertensive treatment in RTR often remains suboptimal, with the vast majority of patients not meeting recommendations of a blood pressure below 130/80 mmHg, or even below 140/90 mmHg. Dietary sodium restriction is an important treatment strategy in chronic kidney disease (CKD), as recommended in guidelines for cardiovascular risk management in CKD. However, prior to our study, sodium restriction has not been investigated in an appropriately designed study in RTR. In our study, which is described in Chapter 2, we aimed to investigate the effects of dietary sodium restriction on blood pressure and albuminuria in stable RTR, in a two-center randomized cross-over clinical trial. To this end, we randomly assigned 23 stable RTR to treatment
with either a 6-week regular sodium diet (target: 150 mmol of sodium per day, equaling 9 grams of salt per day) or a 6-week low sodium diet (target: 50 mmol of sodium per day, equaling 3 grams of salt per day), after which they were crossed-over to the other treatment arm. Adherence to the sodium diet was monitored by measuring urinary sodium excretion in 24h urine samples, and adequate adherence was achieved in 86% of patients. We found that dietary sodium restriction reduced both systolic and diastolic blood pressure and, when adherence with the diet was adequate, also albuminuria, without materially affecting kidney function.

As already mentioned, our study is the first to investigate the effects of dietary sodium restriction in RTR, using a randomized trial design. However, it should be noted that this study in RTR is only the most recent in line of a long tradition of clinical trials on dietary sodium restriction in patients with non-diabetic and diabetic CKD by our\textsuperscript{5-10} and other groups.\textsuperscript{11} All these previous studies consistently showed that dietary sodium restriction significantly reduces blood pressure and albuminuria, and potentiates the efficacy of treatment with RAAS blockers. In addition, in CKD patients, adding dietary sodium restriction to treatment with RAAS blockade was shown to be as effective as adding hydrochlorothiazide\textsuperscript{7,9} and even more effective than treatment with a combination of two RAAS blocking agents.\textsuperscript{8} Moreover, several studies show that low sodium intake is associated with much better long-term renal and cardiovascular outcomes in CKD patients.\textsuperscript{12,13} In contrast, high sodium intake has been shown to blunt the antiproteinuric effects of RAAS blockade in CKD patients,\textsuperscript{14} and also recently in RTR.\textsuperscript{15}

We found that dietary sodium restriction reduced systolic blood pressure by 11 mmHg and diastolic blood pressure by 7 mmHg (Chapter 2). Compared to the 4 to 6 mmHg reduction in mean arterial pressure by dietary sodium restriction in CKD patients,\textsuperscript{5-10} blood pressure reduction in our study was quite impressive. Indeed, blood pressure is thought to be very sodium sensitive in RTR. This was also illustrated by a recent study in 660 Dutch RTR that showed an independent association of sodium intake with blood pressure.\textsuperscript{16} Contributing factors to sodium sensitivity of blood pressure may include factors which are also present in CKD patients, such as prevalent obesity,\textsuperscript{17,18} reduced kidney function, and proteinuria.\textsuperscript{7,19} In addition, treatment with immunosuppressive drugs, such as corticosteroids and/or calcineurin inhibitors (CNIs), may render blood pressure even more sodium sensitive.\textsuperscript{20} More specifically, corticosteroids are known to activate the mineralocorticoid receptor, resulting in increased water and sodium retention.\textsuperscript{21,22} In addition, CNIs increase sodium sensitivity by activation of the sodium-chloride co-transporter in the distal tubule.\textsuperscript{23,24}
Taking this into consideration, it may seem very surprising that dietary sodium restriction is not applied more systematically and effectively in clinical practice in RTR yet. This may be related to the overall lack of adequate lifestyle management relative to pharmacological management in clinical medicine, but there may also be transplant-specific barriers. One of these could be the reluctance of transplant physicians to apply antihypertensive treatment strategies which may give an initial reduction in kidney function, such as the use of RAAS blockers, (thiazide) diuretics, and dietary sodium restriction, or a combination thereof. Obviously, a treatment-related reduction in kidney function is less desirable shortly after transplantation, because it can confound the assessment of possible rejection. However, in patients who have stable transplant function but have inadequately treated hypertension and/or proteinuria with a combination of antihypertensive drugs, it may be a different story. Especially, since the reduction in kidney function is often temporary and not related to acute kidney injury. Moreover, a slight reduction in kidney function following treatment with RAAS blockade has been shown to predict better kidney outcomes in the long-term in CKD patients, possibly because it indicates amelioration of glomerular hypertension. Despite the rigorous reduction in sodium intake, serum creatinine rose only slightly in our study. Therefore, our study demonstrates that sodium restriction could be feasible in RTR, without compromising allograft function too severely.

Of note, choosing the most suitable antihypertensive treatment strategy in RTR remains a challenge to date, because few studies have directly compared antihypertensive agents in this population. The majority of studies compared a single antihypertensive drug, mostly a calcium channel blocker or angiotensin-converting enzyme inhibitor, with no treatment or placebo, while very often treatment with two or more antihypertensive drugs is required. In addition, most studies only assessed intermediate end points, whereas the choice of antihypertensive therapy should ideally be guided by hard end points, like cardiovascular events or allograft failure. More importantly, there are no large prospective studies investigating effects of lifestyle interventions, such as dietary sodium restriction, or multimodal lifestyle approaches in RTR. Such studies would be highly valuable, and remain sorely awaited. Meanwhile, valuable information can be obtained from well-documented observational cohorts, such as the TransplantLines cohort in our center, and from short-term interventions on intermediate end-points. Interestingly, when intermediate end-points are available, such as is the case for hypertension, or insulin resistance, rotation design studies allow to identify personalized treatment strategies. However, even in the general population, despite long-term availability of data and strategies to support personalized approaches in antihypertensive treatment, so far, the concept has not systematically been elaborated, and thus is considered to be still in its infancy.
Another potential obstacle to applying dietary sodium restriction in clinical practice may be adherence to the sodium restricted diet. Current sodium intake of stable RTR in the Netherlands is about 150-200 mmol (equaling about 9-12 grams of salt) per day,\textsuperscript{1,16} which is comparable to sodium intake in the general population, and by far exceeds the recommended intake of less than 100 mmol (or 6 grams of salt) per day.\textsuperscript{32,33} We and others showed that an 80- to 100-mmol reduction in sodium intake is feasible in a regular nephrology outpatient setting, at least for the duration of the studies (Chapter 2).\textsuperscript{7,8,10} However, when patients participated in these studies, they received intensive, individualized, dietary counseling by study physicians and/or dieticians and adherence to the study diet was checked by interim urinary sodium analysis. This most probably led to increased dietary adherence. In addition, there is mounting evidence that persistent lifestyle alterations necessitate a dedicated behavioral approach. Such strategies are not included in routine clinical care yet, but have recently been studied in e.g. the Sodium Burden Lowered by Lifestyle Intervention: Self-Management and E-Health Technology [SUBLIME] Study.\textsuperscript{34} This study in CKD patients and RTR showed that the addition of a program using e-health technology and self-management to regular dietary counseling was highly valued by the patients, and also the feedback on actual dietary intake of sodium by the on-line tool, as well as the 24h urine excretion of sodium was considered very useful. In addition, it resulted in a sustained reduction in dietary sodium intake and blood pressure. Therefore, to really achieve a persistent reduction in dietary sodium intake in RTR, we need comprehensive strategies, which encompass individualized dietary counseling and include a behavioral approach, including feedback on actual dietary intake.

Sodium and aldosterone: evil twins in action

In Chapter 3 we reviewed the role of the mineralocorticoid hormone aldosterone in the pathophysiology of cardiovascular and renal damage. Aldosterone is classically known for its role in water and sodium retention via activation of the mineralocorticoid receptor (MR), and therefore for its role in inducing hypertension. We describe that its actions go far beyond these classical effects, via mechanisms involving oxidative stress, inflammation, and fibrosis, which occur both in the heart and kidneys. Furthermore, we provide evidence that detrimental effects of aldosterone are likely only observed in states of primary increase in aldosterone concentrations, rather than states in which increased aldosterone concentrations are secondary to volume depletion. For example, in patients with resistant hypertension, the effects of high sodium intake on proteinuria are most pronounced in patients with the highest aldosterone.\textsuperscript{35} In contrast, in case of hyperaldosteronism secondary to volume depletion, such as routine low-sodium intake in Yanomami Indians or Gitelman or Bartter syndrome with renal sodium loss, hypertension and cardiovascular damage are absent.\textsuperscript{36,37} Taken together,
these data suggest that deleterious effects of aldosterone are most pronounced when its serum concentration is inappropriately high for the prevailing sodium status.

Therefore, MR blockade may have great potential to halt the progressive end organ damage as observed during current treatments, especially in combination with dietary sodium restriction. Indeed, the effectiveness of MR blockade in heart failure has already been proven in large randomized clinical trials. In addition, numerous small-scale clinical studies have demonstrated increased treatment efficacy by the addition of MR blockade to RAAS blockade in patients with non-diabetic and diabetic CKD. However, long-term data on the efficacy and safety of MR blockade in preventing dialysis and/or cardiovascular endpoints in CKD are still lacking. Moreover, there are currently no studies available in RTR, which investigate the effects of MR blockade on blood pressure and/or albuminuria, or long-term patient and allograft survival. Therefore, it would be of great interest to further explore whether treatment with MR blockade, possibly in combination with dietary sodium restriction, may also be beneficial in RTR.

From mineralocorticoids to glucocorticoids: monitoring prednisolone therapy

Chronic prednisolone treatment after kidney transplantation is associated with numerous side effects, including hypertension and impaired insulin sensitivity, which contribute to increased cardiovascular risk in RTR. In addition, prednisolone dosing regimens remain empiric to date, usually with fixed doses, independent of either body size and/or steroid sensitivity. The main reason is that there is currently no way to guide intensity of treatment, potentially leading to even more unwanted side effects. Chronic prednisolone treatment is also known to suppress endogenous cortisol production, by negative feedback inhibition of the hypothalamus-pituitary-adrenal (HPA)-axis. In addition, treatment with exogenous corticosteroids has been suggested to alter systemic cortisol exposure by interfering in the enzymes that activate and inactivate cortisol, i.e. the 11-beta hydroxysteroid dehydrogenases (11β-HSDs). We hypothesized that the degree to which prednisolone affects HPA axis and/or 11β-HSD enzyme activity might reflect the pharmacological effects of prednisolone, and might, therefore, be related to prednisolone-related metabolic side effects and long-term outcome after kidney transplantation.

In Chapter 4 we investigated whether HPA axis function, as measured by 24h urinary free cortisol excretion, is altered in prednisolone-treated RTR, and whether the degree of HPA axis suppression is related to prednisolone-related metabolic side effects and the metabolic syndrome. In a large cohort of 563 stable RTR, we found that urinary free cortisol excretion was markedly decreased compared to known values for the general population. Interestingly, there was considerable inter-individual variation in urinary
free cortisol excretion, even in patients treated with the same prednisolone dose. Moreover, we found that a higher degree of HPA axis suppression by prednisolone, as reflected by lower 24h urinary free cortisol excretion, was associated with increased prevalence of the metabolic syndrome and its individual components (obesity, dyslipidemia, hypertension, and diabetes).

In Chapter 5 we went one step further into cortisol metabolism, and investigated not only HPA axis activity, but also 11β-HSD enzyme activities in 693 stable prednisolone-treated RTR and compared these to 275 healthy controls. In addition, we investigated whether altered HPA axis and 11β-HSD enzyme activities were related to long-term outcome after kidney transplantation. To this end, we measured 24h urinary excretion of total cortisol, but also of its inactive counterpart cortisone, and their metabolites tetrahydrocortisol (THF), allo-tetrahydrocortisol (alloTHF), and tetrahydrocortisone (THE). Urinary total cortisol excretion and summated excretion of cortisol, cortisone and their metabolites were used as estimates of HPA axis activity. Urinary (THF+alloTHF)/THE and cortisol/cortisone ratios were used as estimates for 11β-HSD enzyme activities. We found that urinary cortisol excretion and summated cortisol and metabolite excretion were significantly lower in RTR compared with healthy controls, whereas urinary (THF+alloTHF)/THE and cortisol/cortisone ratios were significantly higher. Again, there was considerable variation in these parameters in patients treated with the same prednisolone dose. In addition, we found that a higher degree of HPA axis suppression, as reflected by lower urinary cortisol and metabolite excretion, and also higher (THF+alloTHF)/THE and cortisol/cortisone ratios, were associated with increased risk of mortality, independent of potential confounders.

HPA axis activity in this thesis was estimated by 24h urinary excretion of free cortisol in Chapter 4, and by 24h urinary excretion of both total cortisol and summated urinary excretion of cortisol and its metabolites in Chapter 5. We chose to measure urinary excretion of free cortisol in Chapter 4, because this has often been used as a proxy for HPA axis activity and is considered to be the biologically active fraction. However, endogenous glucocorticoids are present in urine largely as conjugated derivatives (95% as glucuronides, and 3-4% as sulfates). Therefore, we think that measuring total cortisol, as we did in Chapter 5, instead of free urinary cortisol could potentially provide a more reliable reflection of total cortisol production. In addition, cortisol is mainly secreted in the urine in the form of its metabolites. As a result, the sum of urinary THF, alloTHF, and THE encompasses about 50% of total endogenous glucocorticoid secretion. Thus, summation of urinary cortisol and its metabolites, as we additionally did in Chapter 5, might even better reflect HPA axis activity than cortisol alone. We found that HPA axis activity was suppressed in prednisolone-treated RTR compared
to subjects from the general population. This is in line with previous studies showing that chronic prednisolone treatment suppresses HPA axis activity after kidney transplantation.\textsuperscript{48-50} However, substantial residual endogenous cortisol synthesis was still present in the majority of patients, despite treatment with prednisolone. Daily cortisol production rate in healthy subjects has been estimated to be approximately 6-10 mg/m\textsuperscript{2}/day,\textsuperscript{58,59} which is equivalent to 3-5 mg of prednisolone per day. When the equivalent exogenous glucocorticoid dose is higher than endogenous cortisol production, endogenous cortisol production is usually suppressed. Thus, theoretically, when a patient is chronically treated with a daily dose of prednisolone greater than 5 mg/day, and certainly greater than 7.5 mg/day, one might expect that HPA axis activity is entirely suppressed, with no residual endogenous cortisol synthesis present. However, our data, showing considerable endogenous cortisol synthesis in patients with daily prednisolone doses of 7.5 mg and higher, indicate that this is not what actually happens. Indeed, biological half-life of prednisolone is only 2-4 hours in stable outpatient RTR.\textsuperscript{60-64} Thus, one daily dose of prednisolone, like conventionally prescribed in our center and many other transplant centers, may not fully cover the normal diurnal pattern of endogenous cortisol requirement and thus may leave room for endogenous production.

Interestingly, we found that there was considerable inter-individual variation in HPA axis activity, even in patients treated with the same prednisolone dose (Chapters 4 and 5). At first this seemed a surprising result, but when we examined both prednisolone pharmacokinetics and individual glucocorticoid sensitivity in more detail, this seemed not so surprising after all. First, from literature it is known that there is a greater than 3-fold variability in dose-adjusted prednisolone exposure in solid organ transplant recipients.\textsuperscript{47} Many factors contribute to this variability, of which the most well-studied are patient age, sex, and kidney function.\textsuperscript{47} Female sex has consistently been associated with increased prednisolone exposure, with a typical female having more than half the clearance of a typical male.\textsuperscript{64} In addition, prednisolone clearance is reduced in patients with reduced kidney function.\textsuperscript{65-68} Finally, in elderly transplant recipients, despite reduced metabolism of prednisolone, endogenous cortisol production has also been reported to be higher, suggesting less suppression of endogenous cortisol production by prednisolone.\textsuperscript{69} In line with this, we found higher age, male sex, and better kidney function to be independently associated with higher urinary cortisol and metabolite excretion (Chapter 5). Still, a greater understanding of the influence of various patient factors on prednisolone exposure in RTR is needed.

Moreover, the high inter-individual variation in HPA axis activity under prednisolone treatment we found, might also be related to the degree of individual sensitivity to
(exogenous and/or endogenous) glucocorticoids. Glucocorticoids are involved in countless metabolic and immune-modulatory processes. Therefore, it comes to no surprise that their regulation is extremely complex, involving a myriad of regulatory steps, which are often tissue and cell-specific. \(^\text{70}\) Individual variation in any of these steps, therefore, contributes to large variability in individual glucocorticoid sensitivity, and also to variability in glucocorticoid sensitivity of different tissues. It is beyond the scope of this discussion to go in great detail into this subject. In brief, glucocorticoids bind to the glucocorticoid receptor (GR) to exert their effects. Availability of glucocorticoids for the GR depends on the degree to which they are free or bound to cortisol binding globulin (CBG) or albumin in plasma. \(^\text{71}\) In addition, glucocorticoid availability is regulated by the degree to which glucocorticoids are inactivated to their inactive counterparts by the 11β-HSD2 enzyme and regenerated back by 11β-HSD1. \(^\text{53,72}\) Therefore, individual variation in CBG and 11β-HSD enzyme activity could contribute to individual glucocorticoid sensitivity. In addition, the number and affinity of available binding sites for glucocorticoids to the GR vary within the healthy population. \(^\text{73}\) This is caused by, among others, alternative splicing of the gene coding for the GR, giving rise to GR isoforms, \(^\text{74}\) and genetic polymorphisms in the GR. \(^\text{75}\) Thus, inter-individual variation in innate glucocorticoid sensitivity, in combination with differences in prednisolone exposure, could potentially lead to considerable variation in HPA axis activity in patients treated with the same prednisolone dose. Therefore, it is conceivable that under a standard once-daily dosing regimen with relatively low dosages, there can be incomplete suppression of the HPA axis and that there is considerable variation in the extent to which the HPA axis is suppressed.

We found that (THF+alloTHF)/THE and cortisol/cortisone ratios were increased in RTR compared to healthy controls (Chapter 5). Higher urinary (THF+alloTHF)/THE and cortisol/cortisone ratios suggest that cortisol balance as maintained by 11β-HSD enzymes has shifted towards cortisol production rather than metabolism. Since 11β-HSD2 metabolizes cortisol to cortisone and 11β-HSD1 regenerates cortisol back from cortisone, a shift towards cortisol production could either be attributable to a relative decrease in enzymatic activity of 11β-HSD2 or relative increase in enzymatic activity of 11β-HSD1. The 11β-HSD2 enzyme is mainly localized in mineralocorticoid sensitive tissues such as the kidney, where it protects the MR from cortisol. Previous studies showed that expression of 11β-HSD2 is reduced in kidney failure. \(^\text{76-79}\) We found a strong inverse association of (THF+alloTHF)/THE and cortisol/cortisone ratios with kidney function, so this could also be the case in our study. However, it has recently been suggested that exogenous steroids, such as prednisolone, could also influence peripheral cortisol balance by induction of 11β-HSD1, thereby creating a feed-forward mechanism enhancing systemic cortisol exposure. \(^\text{51-53}\) Taking this into consideration,
maybe not only the degree of HPA axis suppression, as estimated by 24h urinary cortisol and metabolite excretion, but also \((\text{THF+alloTHF})/\text{THE}\) and cortisol/cortisone ratios in our study could be a reflection of the pharmacological effects of prednisolone. This is supported by the very strong inverse, kidney function independent, association of urinary summated cortisol and metabolite excretion with \((\text{THF+alloTHF})/\text{THE}\) ratio, which we found in our study. Moreover, both decreased 24h urinary cortisol and metabolite excretion and increased \((\text{THF+alloTHF})/\text{THE}\) and ratio were associated with increased risk of all-cause mortality, and mortality of cardiovascular and infectious causes, which associations were also all independent of kidney function.

Although individual glucocorticoid sensitivity is more and more acknowledged as important determinant of potential success and toxicity of corticosteroid treatment\(^{70,75}\), there are few to no clinical read-out parameters by which it can be assessed. We chose to measure 24h urinary excretion of cortisol, cortisone, and their metabolites to measure prednisolone-related effects on the HPA axis. In addition, we measured \((\text{THF+alloTHF})/\text{THE}\) and cortisol/cortisone ratio to estimate systemic 11β-HSD enzyme activities. We realize that these are very crude measures to assess something as delicate is glucocorticoid sensitivity, but they are – in our opinion – the best available clinical read-out parameters available yet. Indeed, we could have measured prednisolone exposure by means of pharmacokinetic models. Nevertheless, measuring exposure to prednisolone would not have given us information on its effects. Measurement of urinary excretion of cortisol and/or its metabolites has the advantage of being non-invasive, relatively easy, and more feasible for large cohort studies and future application in patient care. In addition, it is relatively unaffected by the circadian rhythm of cortisol or by varying plasma protein binding capacities compared to serum cortisol. Thus, although we made a first step towards measuring individual sensitivity to the effects of prednisolone, it would be of great interest to explore alternative ways to measure individual glucocorticoid sensitivity to personalize prednisolone therapy.

One of the main drawbacks of the studies presented in Chapters 4 and 5, is that they are observational in nature. Observational studies can only establish associations between observations and outcome variables, but cannot establish whether the associations identified represent cause-and-effect relationships, leaving room for potential confounding. Although we adjusted for several potential confounding factors, such as age and sex, daily prednisolone dose, kidney function and body composition, residual confounding cannot be excluded. In addition, medication intake was not monitored in these studies. Therefore, we did not have information on adherence to treatment with corticosteroids or other immunosuppressive drugs, or the time of the day patients took these drugs. This is especially important since prednisolone half-life is typically
2-4 hours. In addition, the rate of non-adherence to immunosuppressive medication has been reported to range from 15% to even 45% in RTR. Due to the lack of information on adherence to prednisolone intake, this is something we could not take into account and could have affected our results. Moreover, all but two patients in our studies were treated with prednisolone as part of their maintenance immunosuppressive regimen, which makes it difficult to dissect whether alterations in HPA axis and 11β-HSD enzyme activities were related to treatment with prednisolone or are innate to the situation of kidney transplantation itself. Thus, to address all these questions, we are currently investigating cortisol metabolism in a post-hoc analysis of a randomized clinical trial in which intake of immunosuppressive medication was closely monitored, including intake of prednisolone, and which also had a prednisolone-free arm.

The tryptophan-kynurenine pathway: friend or foe in transplantation?
A major effect of corticosteroids is to suppress inflammation, not only locally in transplanted organs, but also systemically, in the organism hosting the transplanted organ. In RTR, systemic inflammation is associated with reduced long-term patient and allograft survival. An interesting pathway that is tightly linked to systemic inflammation and corticosteroid exposure is the kynurenine pathway, which is the major metabolic pathway of the essential amino-acid tryptophan. Under inflammatory conditions, tryptophan is metabolized by the enzyme indoleamine 2,3-dioxygenase (IDO) to kynurenine, which is subsequently metabolized by the enzyme kynurenine 3-monooxygenase (KMO) to cytotoxic 3-hydroxykynurenine. Therefore, in Chapter 6, we aimed to investigate whether activation of the tryptophan-kynurenine pathway is associated with systemic inflammation in stable RTR, and whether it is also associated with long-term patient and allograft survival.

To this end, we measured serum and urinary tryptophan, kynurenine, and 3-hydroxykynurenine in a cohort of 561 stable RTR. In addition, IDO enzyme activity was estimated by the kynurenine-to-tryptophan ratio and KMO enzyme activity was estimated by the 3-hydroxykynurenine-to-kynurenine ratio. We found that serum kynurenine and particularly cytotoxic serum 3-hydroxykynurenine, and also serum kynurenine-to-tryptophan and 3-hydroxykynurenine-to-kynurenine ratios, were strongly associated with parameters of systemic inflammation. In addition, they were independently associated with increased risk of allograft failure long-term after kidney transplantation. Serum 3-hydroxykynurenine and 3-hydroxykynurenine-to-kynurenine ratio were also independently associated with mortality. There were no independent associations of urinary parameters of the tryptophan-kynurenine pathway with allograft failure or mortality.
The tryptophan-kynurenine pathway was initially thought to play an important role in preventing allograft rejection after transplantation. This was based on findings in experimental transplantation models in small animals, where the tryptophan-kynurenine pathway activation was shown to induce immune tolerance. Surprisingly, however, our study and other observational studies in human kidney transplantation consistently demonstrated that tryptophan-kynurenine pathway activation is associated with reduced allograft survival. For example, IDO activity, as measured by kynurenine-to-tryptophan ratio, was increased right before and during acute kidney allograft rejection. In addition, increased pre-transplantation serum kynurenine concentration was associated with the occurrence of acute allograft rejection after kidney transplantation. Moreover, increased serum and urinary kynurenine early after kidney transplantation was associated with increased serum creatinine and albuminuria two years post-transplantation. Finally, activation of the tryptophan-kynurenine pathway has been associated with increased acute rejection rates in heart transplantation and increased prevalence and severity of graft-versus-host disease after bone marrow transplantation.

We found that serum kynurenine and 3-hydroxykynurenine were strongly associated with parameters of systemic inflammation. Indeed, the tryptophan-kynurenine pathway has been implicated in many disease states in which systemic inflammation is present. Via its role in systemic inflammation, it has also been implicated in the development of atherosclerosis and cardiovascular disease, especially in patients with impaired kidney function. In these patients, tryptophan-kynurenine pathway metabolites are known to accumulate. It has been hypothesized that the increase in serum kynurenine metabolites in patients with impaired kidney function is caused not only by reduced renal clearance of the metabolites themselves, but also by increased tryptophan break-down to kynurenine by the pro-inflammatory uremic environment. Therefore, interfering in the kynurenine pathway by blocking different steps of the pathway is currently being evaluated as potential therapeutic strategy. Initially, inhibitors of IDO gained most interest, because this enzyme catalyzes the first and rate-limiting step in the pathway. Importantly, it recently became apparent that the IDO product kynurenine is not only metabolized to cytotoxic 3-hydroxykynurenine, but also to (neuro)protective kynurenic acid. Therefore, inhibitors of the down-stream enzyme KMO were developed with the aim of reducing production of 3-hydroxykynurenine and shifting the pathway towards production of kynurenic acid.
The question remains whether blocking of IDO and/or KMO could be a useful therapeutic strategy in RTR. For this, we have to dive a bit deeper into kynurenine pathway physiology. The kynurenine pathway was originally found to play an important role in the immune response against intracellular viruses and parasites. By depleting the essential amino acid tryptophan, which is required for growth, it protected the host against these pathogens. However, IDO was also found to suppress the immune response in experimental models. Two distinct mechanisms have been proposed to explain the immunosuppressive function of IDO. In the first mechanism – called the “tryptophan starvation or depletion” theory, IDO activity directly reduces local tryptophan concentrations, thereby impeding normal proliferation in a variety of cell types and increasing their susceptibility to apoptosis. T lymphocytes in particular have been shown to be susceptible to tryptophan starvation. In the second mechanism – called the “tryptophan utilization” theory, IDO activation indirectly produces immunosuppressive effects via increased formation of downstream metabolites, such as kynurenine and 3-hydroxykynurenine. These metabolites have been implicated in reduced proliferation and increased apoptosis of both T helper cells and natural killer cells. Additionally, the combined effects of tryptophan depletion and metabolite interaction with CD8+ effector T lymphocytes reduce the capabilities of these immune response cells.

Taken together, these proposed mechanisms suggest that activation of the kynurenine pathway could also have immunosuppressive effects in vivo. Therefore, it came as a surprise that every single clinical study in kidney and other transplant recipients showed that kynurenine pathway activation was associated with reduced allograft survival. To date, no solid explanation for this controversy has been found. One of the possible explanations could be that, in contrast to the ‘closed’ setting of experimental cell models, the human body is an open system, so tryptophan can easily be replenished and depletion will never take place. Moreover, in the case of inflammation, it is likely that continuously dying cells release their intracellular stocks, thereby providing additional supplies of tryptophan. In addition, in order to provide adequate immunosuppression, tryptophan concentrations would have to fall below 1 µmol/L, which is about one fiftieth of normal tryptophan concentrations in plasma. The other way around, the toxic effects of kynurenine metabolites are only apparent at concentrations that are 5 to 10 times higher than the expected physiological concentration. Although kynurenine and 3-hydroxykynurenine were increased in RTR with more adverse outcome in our study, their median concentrations were only 2 times higher than in RTR with more favorable outcome. Another possible explanation would be that the potentially suppressive function of kynurenine metabolites is outcompeted by the
overwhelming immune response during allograft rejection. However, although this maybe could be true for the event of acute rejection, this would be less likely in the case of chronic systemic inflammation, which is more smoldering in nature. The third possible explanation would be that activation of the kynurenine pathway by IDO is merely a marker of ongoing systemic inflammation or immune activation. Numerous pro-inflammatory cytokines, including interferons (IFN-α, IFN-β, and IFN-γ), interleukins (IL-1 and IL-2), and tumor necrosis factor (TNF-α) induce the expression of IDO and also KMO. Therefore, measuring parameters of the kynurenine pathway may only be useful in assessing chronic inflammation, but interfering in it would not have the expected effects. To conclude, it remains to be elucidated whether interference in or, conversely, active induction of the kynurenine pathway would be beneficial in the clinical setting of kidney transplantation, and whether we are dealing with a friend or foe, or maybe even an innocent bystander.

Towards personalized treatment in renal transplant recipients
With this thesis, we tried to make first steps towards personalized treatment of prednisolone-treated RTR. Although the concept of personalized medicine has received great interest over the past years, the idea dates from ancient times. Already in 400 BC, the father of early medicine, Hippocrates, said: “it is far more important to know what person the disease has, than what disease the person has”. Thus, the concept that individuals with the same disease and a similar clinical presentation may have very different outcomes and need very different therapies is not novel. In modern medicine, personalized – or precision or stratified – medicine encompasses a medical model that proposes the customization of health, with medical decisions, practices and/or products being tailored to the individual patient, based on their predicted response or risk of disease. This is opposed to the ‘one-size-fits-all’ approach, which applies decisions from average treatment effects observed in clinical trials to all patients. Prediction modeling, using information on patients’ genetic and molecular make-up, can be applied to predict different outcomes, such as the duration of survival, potential complications, response to therapy, and other outcomes. Individual genetic and molecular make-up would be assessed by personal ‘omics’ profiling, which uses molecular diagnostic tools to combine information on, for example, the (epi)genome, transcriptome, proteome, metabolome, lipidome, and microbiome.

In the field of kidney transplantation, personalized medicine, by means of assessing individual genetic and molecular make-up, could have great potential in, for example, selecting optimal donor-recipient pairs and individualizing immunosuppressive therapy, but also in predicting the probability of more complex outcomes, such as the risk of acute or chronic rejection, infections, and malignancies, which may help to
individualize immunosuppressive treatment even further.¹³¹,¹³² Therefore, personalized immunosuppressive treatment, based on molecular profiling of each individual transplant recipient, holds great promise for optimizing the balance between therapeutic efficacy and toxicity of treatment with these drugs, thereby potentially reducing unwanted side effects and concomitant cardiovascular risk.¹³¹ However, many challenges have to be met, such as problems with standardization, means of analyzing and interpretation of the enormous amounts of data which will come available, before the use of these complex molecular techniques can really be implemented in clinical kidney transplant care.

In the meantime, we should strive for personalized cardiovascular risk management in RTR, which encompasses the development and application of adequate transplantation-specific guidelines, using knowledge on the pathophysiology and risk factors specific to this patient group, rather than extrapolating guidelines from the general population or other high risk populations, in order to reduce the cardiovascular burden after kidney transplantation. In addition, as we learned from studying lifestyle interventions in RTR, not only a patient’s molecular or genetic make-up determines the potential success or failure of treatment, but also his or her social environment, and beliefs and attitudes towards this treatment. Therefore, to truly personalize treatment in RTR in the future, we need an integrative systems approach which addresses both the needs and specific characteristics of this patient population, at both individual and group level. With this thesis, we show that combining lifestyle interventions with individual monitoring of immunosuppressive (i.e. corticosteroid) treatment may serve as an important first step towards personalized cardiovascular risk management in prednisolone-treated RTR.
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