Risk factors for late graft failure and mortality in renal transplantation

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Stellingen behorende bij het proefschrift
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1. A majority of renal transplant recipients has the metabolic syndrome long-term after transplantation (*dit proefschrift*).

2. Women are more at risk for posttransplant weight gain and metabolic syndrome than men (*dit proefschrift*).

3. Metabolic syndrome after renal transplantation is associated with insulin resistance and impaired renal allograft function (*dit proefschrift*).

4. HOMA, Quicki, and McAuley's index are valid surrogate estimates of insulin resistance in the renal transplant population (*dit proefschrift*).

5. Obesity, in particular central obesity, and prednisolone are the most important determinants of insulin resistance beyond 1 year after renal transplantation (*dit proefschrift*).

6. Renal function and proteinuria are clinically useful, albeit suboptimal predictors of graft outcome; renal vascular resistance does not add significantly to these current predictors (*dit proefschrift*).

7. The excess risk of mortality in renal transplant recipients compared to the general population owes more to heart failure than to ischemic risk factors (*dit proefschrift*).

8. Latent CMV is an independent risk factor for graft outcome (*dit proefschrift*).

9. De hitserie '*House, MD*' biedt de internist m(w)eer identiteit.

10. Ook bankiers wachten hedentendage op een Eurotransplant.

11. Kwaliteit in de gezondheidszorg is als lucht: het is overal onopvallend aanwezig, maar naarmate je sneller vooruit wilt, neemt de weerstand exponentieel toe.

12. Veel dialysepatienten dromen van Cocagne; schransen en plassen kan je.

Aiko P.J. de Vries

rijksuniversiteit groningen

Risk factors for late graft failure and mortality in renal transplantation

Proefschrift

ter verkrijging van het doctoraat in de
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Rector Magnificus, dr. F. Zwarts,
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Most patients with end-stage renal disease view kidney transplantation to be the Land of Cockaigne, a medieval fantasy of the perfect life. A wonderful world free from dialysis, in which physical comforts come instantly and dietary restrictions are non-existent. A very understandable response to a previous paradise lost. As a true allegory of life, it may come at a price. Side-effects of chronic immunosuppression, posttransplant metabolic syndrome, and opportunistic infection may limit long-term patient and graft survival.

Content

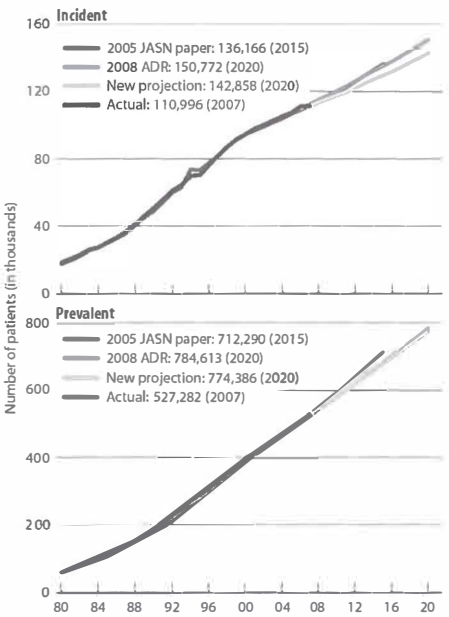
Chapter 1

Introduction and aims of thesis

Introduction

Epidemiological studies consistently demonstrate that chronic kidney disease (CKD) affects approximately 10% of population in Western industrialized nations.[1] In the U.S.A. alone; over 400.000 patients receive renal replacement treatment for end-stage renal disease (ESRD), the most serious stage of CKD. This number is expected to increase to over 700.000 by the year 2015 (Figure 1).[2] CKD reduces patients' quality of life and life expectancy dramatically.[3] Furthermore, it imposes significant health-economic burden on society. Although only 0.1% of the general population suffers from ESRD, it accounts for over 7% of total healthcare expenditure, exceeding 19 billion dollars annually in the U.S.A.[1] The prevalence of ESRD in Europe is less compared with the U.S.A. but the increase in prevalence parallels the U.S.A. unmistakably.[4]

Figure 1: Projected counts of incident & prevalent ESRD patients through 2020



Adapted from Figure 2.1 (Volume) United States Renal Data System. Counts projected using a Markov model. New projections (yellow) use data through 2007.

Renal transplantation is the preferred renal replacement treatment because of increased survival,[5] improved quality of life,[6] and lower healthcare costs[7] compared with dialysis. Over the past three decades, one-year graft survival has improved impressively from approximately 40% in the 1970's to over 90% in present days for deceased donors, and more than 97% for living donors (Figure 2a+b).[8] This improvement owes mainly to better prevention of acute rejection[9] by the introduction of cyclosporine and muromonab-CD3 (OKT3 monoclonal antibody) in the early 1980's in addition to advances in surgical techniques, organ procurement,[10] and treatment of opportunistic infection. The incidence of acute rejection was further reduced in the 1990's by the introduction of stronger immunosuppressive drugs such as mycophenolate mofetil,[11] tacrolimus,[12] and more recently by the introduction of mTOR (mammalian target of rapamycine) inhibitors sirolimus and everolimus.

Surprisingly, the improvement in short-term graft survival has not been paralleled by a substantial improvement in long-term graft survival (Figure 2).[13] Approximately half of all renal allografts from deceased donors are still lost within 10 to 12 years after transplantation. The main reasons why long-term graft survival remains behind are recipient mortality with a functioning graft and the development of chronic renal transplant dysfunction (CTD).[14, 15]

Figure 2a: Kaplan-Meier estimates of graft survival during the first year after transplantation for grafts from living donors (Panel A) and Cadaveric Donors (Panel B) from 1988-1996

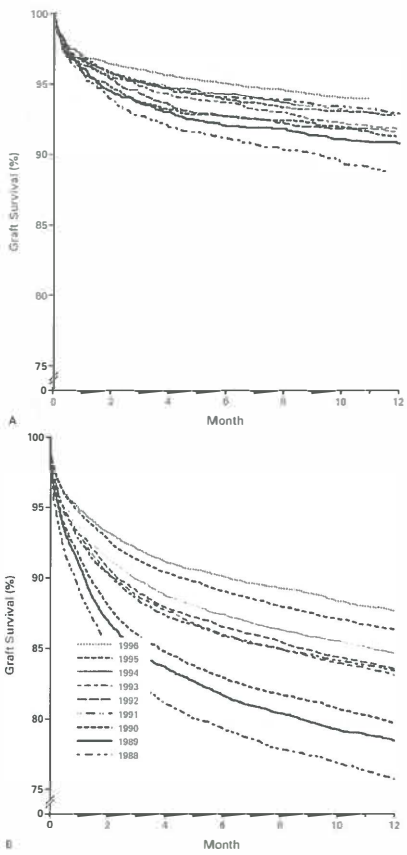
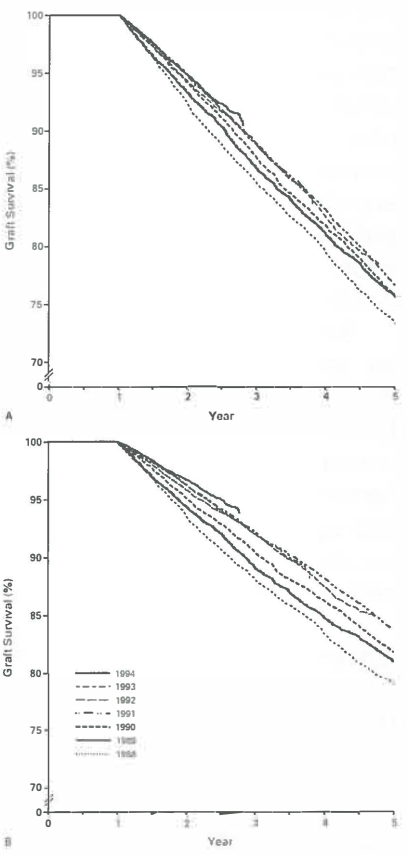


Figure 2b: Kaplan-Meier estimates of graft survival after one year for all grafts from cadaveric donors (Panel A) and after censoring of data for patients who died with functioning cadaveric allograft (Panel B)



From: Hariharan S et al. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 2000; 342: 605-12.

Recipient mortality

The main cause of death after renal transplantation is cardiovascular disease. Although renal transplantation lowers the risk for cardiovascular mortality compared with maintenance dialysis, renal transplant recipients remain at higher risk for cardiovascular mortality compared with the general population.[16] It is estimated to be three to five times higher than in the general population. Risk factors for cardiovascular disease are to a certain extent similar to those in the general population and include age,[17] hypertension,[18] diabetes,[19] dyslipidemia,[20] smoking,[21] impaired graft function,[22] and proteinuria,[23] but also include specific transplant-related factors such as acute rejection episodes[23] and cytomegalovirus.[24]

There is a tendency to transplant older recipients with more pre-transplant co-morbidity and cardiovascular disease. Diabetes and hypertension are nowadays main causes for end-stage renal disease.[2] Renal transplants recipients are also increasingly obese at time of transplantation,[25] reflecting the rise in obesity seen in the general population. In addition, most transplant recipients experience almost a ten-percent weight gain within the first year after transplantation,[26, 27] which is predominantly caused by an increase in fat mass.[28] The chronic use of corticosteroids is thought to contribute significantly to post-transplant weight gain and cardiovascular risk,[29, 30] but is hard to avoid as steroid-free immunosuppressive regimens have been associated in the past with increased risk of long-term graft failure.[31] Loss of uremia and ease of dietary restrictions while on dialysis are likely contributors to posttransplant weight gain as well.

Both posttransplant obesity and immunosuppressant medication such as calcineurin inhibitors and m-TOR (mammalian target of rapamycine) inhibitors add significantly to various posttransplant cardiovascular risk factors such as hypertension, dyslipidemia, and diabetes.[32-35] Many renal transplant recipients suffer from a constellation of cardiovascular risk factors that is consistent with the metabolic syndrome.[36] Unfortunately, therapeutic interventions aimed at decreasing cardiovascular disease, such as statins, do not necessarily convey similar protection in renal transplant recipients as in the general population.[37]

Chronic renal transplant dysfunction

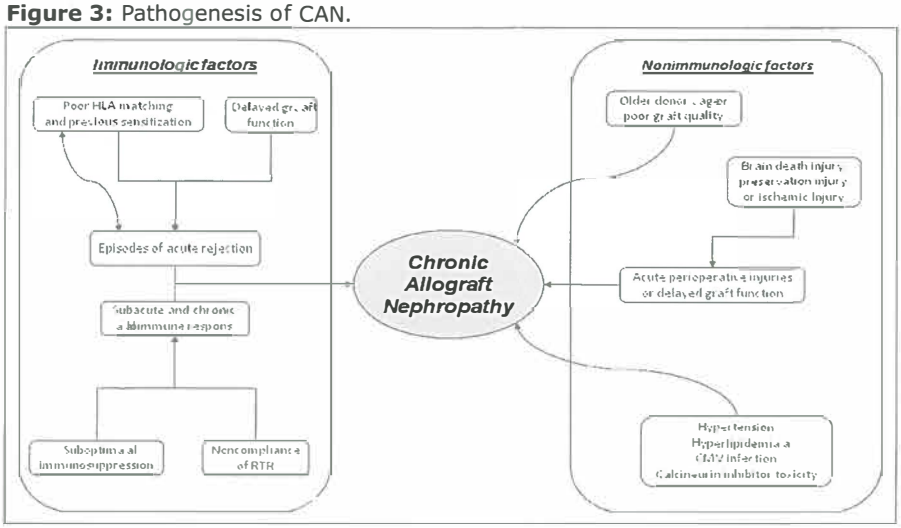
Chronic renal transplant dysfunction (CTD) is a nonspecific term describing a clinical syndrome which is defined as progressive graft dysfunction occurring beyond three months after transplantation which is independent of acute rejection and specific disease entities (such as recurrence of primary renal disease, subclinical rejection, or transplant glomerulopathy, etc.). Clinically, CTD is characterized by a gradual decline in renal function with slowly rising serum creatinine. The decline in renal function is often found in combination with proteinuria and *de novo* or accelerated hypertension. The prevalence of CTD varies, ranging from

23% at 5 years after transplantation to up to 60% of grafts at 10 yr after transplantation.[38]

CTD is often the functional consequence of chronic allograft nephropathy (CAN), which is a descriptive term for histological changes consisting of interstitial fibrosis (IF), tubular atrophy (TA), arteriosclerosis, and glomerulosclerosis.[39] Since 1991, there has been an ongoing effort to standardize renal transplant pathology interpretation, which lead to the Banff working scheme and subsequent adjustments in an attempt to promote uniform allograft biopsy grading for drug trials and routine diagnostic use.[40] During the last Banff conference, the term 'chronic allograft nephropathy' was struck from the classification and replaced by 'interstitial fibrosis and tubular atrophy not otherwise specified' (IF/TA NOS).[41] The rationale for this update of the Banff classification is the misusage of 'CAN' as a generic term for all causes of CTD with fibrosis, delaying accurate diagnosis of specific disease entities and delaying subsequent therapy; e.g. cessation of calcineurin inhibitors if signs of chronic toxicity are present (e.g. arteriolar hyalinosis).

CTD is a complex and multifactorial disorder with involvement of both alloantigen-dependent and alloantigen-independent factors. Alloantigen-dependent factors include acute rejection episodes, human leukocyte antigen (HLA) matching, donor-specific antibodies, delayed graft function, and inadequate immunosuppression or noncompliance. Alloantigen-independent risk factors include donor age, brain death, ischemia/reperfusion injury, hypertension, dyslipidemia, posttransplant diabetes, cytomegalovirus (CMV) or BK virus infection, and calcineurin inhibitors (CNI)-related nephrotoxicity (Figure 3).[14]

Figure 3: Pathogenesis of CAN.



Adapted from Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. N Engl J Med 2002: 346; 580-590

Many transplant centers are reluctant to perform surveillance or protocol biopsies, as biopsies carry the risk of complications such as intractable bleeding necessitating removal of the graft. Consequently, noninvasively estimates such as glomerular filtration rate (e.g., creatinine clearance or plasma creatinine) and proteinuria are used clinically for the identification of renal transplant recipients (RTR) at increased risk for CTD.[42] However, once serum creatinine rises or proteinuria occurs, chronic structural lesions are already present and intervention can only be aimed at slowing progression.[39] Unfortunately, parameters such as serum creatinine and proteinuria are not perfect predictors of graft loss.[43] Moreover, rates of allograft function decline vary substantially among recipients,[44] probably reflecting the heterogeneous pathogenesis of CTD. There is still a need to (non-invasively) identify recipients at increased risk for progression of renal function decline and graft loss at an early stage after transplantation.[42, 45]

Metabolic syndrome and insulin resistance: a missing link?

In recent years, the notion emerged that CTD and cardiovascular disease after transplantation share inflammation and accelerated atherogenesis in their pathogenesis.[15, 46, 47] This notion is supported by the histological features of CTD, in which equivalents of atherosclerosis are prominent. These equivalents of atherosclerosis are glomerulosclerosis, hyalinosis, and inflammation (perivascular and interstitial).[39] The notion is also supported by the fact that CTD and cardiovascular disease share many risk and progression factors such as age, smoking, obesity, hypertension, dyslipidemia, and diabetes, most of which are consistent with the metabolic syndrome, previously known as the insulin resistance syndrome.[48] Insulin resistance, i.e. the resistance to insulin-mediated glucose uptake in insulin sensitive tissues, such as muscle, is considered a central pathophysiological feature of this syndrome.[49] Metabolic syndrome and insulin resistance might contribute to accelerated atherogenesis via common pathways such as endothelial dysfunction, oxidative stress, and inflammation.[49]

Cytomegalovirus

Inflammation and oxidative stress also play a role in the (re)activation of cytomegalovirus (CMV). Cytomegalovirus (CMV) has been established as the single most important opportunistic pathogen after transplantation.[50, 51] Since CMV is a member of the genus herpes viridae it persists latently after infection, i.e. a dynamic balance exists between CMV reactivation/ replication and clearance by the immune system. Symptomatic primary infection via donor kidney or (secondary) reactivation from latency commonly occurs 1 to 4 months after transplantation owing mainly to high-dose immunosuppression in the immediate posttransplant period.[2] In addition to effects of immunosuppression, pro-inflammatory cytokines such as TNF-alpha (tumor necrosis factor alpha - via binding to TNF

receptor 1), Il-6 (interleukin 6) as well as reactive oxygen species and noradrenalin have shown to be able to activate the enhancer/promoter region of CMV via well-known cellular cascades such as PKC/NF-kB and CREB-1/ATF-1 pathways.[52-55]

Since risk factors of the metabolic syndrome are associated with a state of chronic low-grade inflammation, oxidative stress, (nor)adrenergic activation, and endothelial dysfunction, these entities may theoretically contribute to low-grade CMV replication/activation and accelerated atherogenesis in renal transplant recipients. Sites of in vivo latency of human CMV are endothelial cells, monocytes/macrophages, and probably fibroblasts and smooth muscle cells as well.[56, 57] These cell types have all been implicated in the pathophysiology of atherosclerosis.[58] Vice-versa, systemic (low-grade) CMV replication (by any cause) may lead to additional inflammation,[59] oxidative stress,[60] and endothelial dysfunction[61] thereby contributing perhaps to an insulin-resistant state. It is interesting to learn that CMV has been associated with post-transplant diabetes,[62] although it is difficult to exclude confounding effects of immunosuppression on this relationship. It has been shown that latent CMV can be locally active in a transplanted organ with ongoing low-grade alloreactivity, without signs of systemic activity long-term after transplantation.[102] Numerous studies have shown that both CMV disease and CMV reactivation early after transplantation are risk factors for rejection and mortality.[24, 63-66] The effects of chronic CMV infection beyond the first-year after transplantation on transplant outcomes, and to which extent CMV is associated with cardiovascular risk factors remain unclear.

Aim of this thesis

The aim of the present thesis is to investigate risk factors for late graft loss and mortality after renal transplantation with emphasis on insulin resistance, metabolic syndrome and CMV.
Chapter 2 goes into more detail on the hypothesis that insulin resistance and the metabolic syndrome may contribute to the pathogenesis of chronic renal transplant dysfunction.
In Chapter 3, we investigated whether surrogate measures of insulin resistance that are derived from non-transplant populations are valid measures of insulin resistance, as assessed by the hyperinsulinemic euglycemic clamp, the golden standard, in a stable renal transplant population.
In Chapter 4, surrogate estimates of insulin resistance were used to investigate to which extent both traditional (e.g. obesity) and transplant-related factors (e.g. immunosuppressants) may contribute to insulin resistance after renal transplantation.
In Chapter 5, we investigated prevalence of the metabolic syndrome in our renal transplant cohort and investigated to which extent metabolic syndrome is associated with impaired long-term renal allograft function.
Chapter 6 shows the predictive performance of intermediates such as renal allograft function, proteinuria, and renal vascular resistance for graft failure.
Chapter 7 investigates the impact of cardiovascular risk factors such as N-terminal pro-BNP and those associated with the metabolic syndrome on renal transplant recipient mortality in comparison with the general population.
In Chapter 8, we investigated the impact of CMV serology determined after one year after transplantation on graft failure and mortality as well as associations with cardiovascular risk factors.
Finally, Chapter 9 summarizes the results of previous chapters and puts it in recent perspectives. Furthermore, suggestions for future research are proposed.

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Chapter 2

Insulin resistance as putative cause of chronic renal transplant dysfunction

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Stephan J.L. Bakker
Willem J .van Son
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Abstract

Transplantation is the preferred organ replacement therapy for most patients with end-stage renal disease. Despite impressive improvements over recent years in the treatment of acute rejection, approximately half of all grafts will loose function within ten years after transplantation. Chronic renal transplant dysfunction, also known as transplant atherosclerosis, is a leading cause of late allograft loss. To date, no specific treatment for chronic renal transplant dysfunction is available. Although its precise pathophysiology remains unknown, it is believed that it involves a multifactorial process of alloantigen-dependent and alloantigen-independent risk factors. Obesity, posttransplant diabetes mellitus, dyslipidemia, hypertension, and proteinuria have all been identified as alloantigen-independent risk factors. Notably, these recipient-related risk factors are well-known risk factors for cardiovascular disease, which cluster within the insulin resistance syndrome in the general population. Insulin resistance is considered the central pathophysiological feature of this syndrome. It is therefore tempting to speculate that it is insulin resistance, which underlies the recipient-related risk factors for chronic renal transplant dysfunction. Recognition of insulin resistance as a central feature underlying many, if not all recipient-related risk factors would not only improve our understanding of the pathophysiology of chronic renal transplant dysfunction, but also stimulate development of new treatment and prevention strategies.

Introduction

Transplantation is the preferred treatment for most patients with end-stage renal disease. Improvements over the past thirty years in the prevention and treatment of acute rejection and opportunistic infection have raised the 1-year graft survival rate to over 90%. Surprisingly, the 10-year rate has not paralleled the increase in short-term survival.[1] Almost half of all grafts are lost within 10 years after transplantation.[2] Leading causes of late allograft loss are patient mortality due to cardiovascular disease and development of chronic renal transplant dysfunction (CRTD).[3] CRTD is characterized clinically by a slow decline in transplant function over time, albeit that onset and progression may vary among patients.[4] A multifactorial process of alloantigen-dependent and -independent risk factors is thought to cause CRTD.[5] Identified alloantigen-dependent factors include inadequacy of immunosuppression, human leukocyte antigen (HLA)-incompatibility between donor and recipient, and occurrence of acute rejection. Ischaemia and reperfusion injury have been implicated as alloantigen-independent risk factors that are related to the transplant procedure, while obesity, posttransplant diabetes mellitus (PTDM), dyslipidemia, hypertension, and proteinuria have been identified as alloantigen-independent factors.[6-8] Notably, these recipient-related factors are well-known risk factors for cardiovascular disease, which cluster within the insulin resistance syndrome (IRS) in the general population. In this paper, we propose that insulin resistance and the accompanying metabolic cluster underlie the recipient-related risk factors for CRTD.

Insulin resistance syndrome

Originally, Reaven described IRS as 'syndrome X', comprising a cluster of associated abnormalities including obesity, glucose intolerance, hyperinsulinemia, type 2 diabetes mellitus, dyslipidemia and hypertension.[9] IRS has been expanded since to include other features as well, such as a central body fat distribution, hyperuricemia, coagulation abnormalities, increased oxidative stress, chronic low-grade inflammation, and endothelial dysfunction.[10-12] The central feature thought to underlie this metabolic clustering is insulin resistance.[13,14]

Obesity, poor physical activity, and poor physical fitness – constituting a sedentary life style – are important independent causes of insulin resistance in the general population.[15-17] Many features of IRS, including glucose intolerance, resistance to insulin-mediated glucose disposal, hyperinsulinemia, hypertriglyceridemia, low levels of high-density-lipoprotein (HDL)-cholesterol, and small dense low-density-lipoprotein (LDL)-cholesterol have been related pathophysiologically to an increased mobilization of nonesterified fatty acids from intra-abdominal

and peripheral fat depots.[9,18-22] In central obesity, this mobilization is attributed to a mass effect of stored triglycerides on lipolysis and to a higher sensitivity to lipolytic stimuli.[23] This mobilization leads to an increased exposure of nonadipose tissue, such as skeletal muscle and the liver, to nonesterified fatty acids.[24] This increased exposure is though to underlie the association of central obesity with insulin resistance. Poor physical activity and physical fitness are associated with a diminished utilization and oxidation of nonesterified fatty acids by skeletal muscle, leading to an increased exposure of nonadipose tissue to nonesterified fatty acids as well.[25-28]

Nonesterified fatty acids and their metabolites have been shown to interfere with glucose oxidation as well as insulin signaling pathways, causing resistance to the action of insulin in insulin-dependent tissues.[29,30] An increased exposure to nonesterified fatty acids is also thought to underlie the association of IRS with a milieu of increased oxidative stress, chronic low-grade inflammation and endothelial dysfunction.[31,32] Both in-vitro studies and infusion studies with nonesterified fatty acids in humans support this lipotoxicity theory.[32,33]

Insulin resistance syndrome after renal transplantation

Evidence for a role of immunosuppressive drugs such as corticosteroids or calcineurin inhibitors in the development of insulin resistance after transplantation comes from experimental and observational research.[34,35,36] However, corticosteroid therapy is associated with insulin resistance in a dose-dependent matter.[37,38] It may be questioned therefore, whether the chronic use of low doses of corticosteroids (<7.5 mg/day) has any important clinical effect on development of insulin resistance.[37] Konrad et al[39] found no effect of low-dose (5-mg/day) prednisone therapy on development of insulin resistance in liver transplant recipients. The authors found the body mass index (BMI) to be the overall influencing factor instead.[40,41]

In renal transplant recipients, BMI is an important determinant of insulin resistance as well. In a study of 167 renal transplant recipients, Hjelmesaeth et al[42] showed that BMI explained approximately one-third of variability in insulin resistance at 10 weeks after transplantation. The impact of obesity on insulin resistance in renal transplant recipients seems therefore comparable to that in the general population.[43] Because overweight and obesity are highly prevalent in the renal transplant population,[44] it may be deduced from this study that insulin resistance is prevalent in the renal transplant population also. A study by Ekstrand et al[45] in which renal transplant recipients were compared with age- and BMI-matched controls provides additional evidence for the high prevalence of insulin resistance among renal transplant recipients. In this study, two times higher fasting insulin concentrations in renal transplant recipients

than in healthy controls (48 ± 12 vs. 24 ± 6, $p < 0.01$) indicate a more severe degree of insulin resistance in the transplant group. The additional finding in this study of a 34% lower glucose disposal rate in renal transplant recipients than in controls during a hyperinsulinemic euglycemic clamp, is compelling evidence that renal transplant recipients are less insulin sensitive than healthy subjects. This difference suggests that factors other than age and BMI underlie insulin resistance in renal transplant recipients.

First, it remains uncertain to what extent calcineurin inhibitors play a role in the induction of insulin resistance. Some clinical studies demonstrated a beneficial effect of lowering calcineurin inhibitors on insulin resistance; other studies did not.[36,40,41]

Second, BMI does not reflect the ratio of fat mass to muscle mass very well. Although both groups in the study of Ekstrand had comparable BMI, the authors showed that renal transplant recipients had higher fat mass and lower muscle mass than controls. It is thought that chronic glucocorticoid therapy has an important role in the increase of fat and decrease of muscle mass after transplantation.[46] However, a study by Van den Ham et al[46] demonstrated that fat and lean body mass were not associated with daily or cumulative prednisolone dose but with the level of physical activity.[47] Patients who depend on dialysis therapy usually exhibit poor physical fitness.[48] Although the majority of renal transplant recipients increase their physical activity spontaneously after transplantation, physical activity levels remain approximately 75% of levels found in the general population.[49] Physical fitness, as assessed by measurement of VO2 max, also remains lower in the renal transplant population than in the general population.[50] Therefore, differences in physical activity and physical fitness might constitute another explanation for the excess of insulin resistance in the renal transplant population compared with age- and BMI-matched controls.

Third, other factors such as differences in psychosocial stress or effects of certain commonly used medications (other than immunosuppressants e.g. diuretics and beta-blockers) may have a role.[51,52] However, these remain largely uninvestigated in the renal transplant population.

Additional support for a high prevalence of insulin resistance among renal transplant recipients is provided by studies on impaired glucose tolerance and posttransplant diabetes mellitus. In renal transplant recipients, it has been shown that insulin resistance characterizes these metabolic states.[53] Midtvedt et al[53] found a combined prevalence of impaired glucose tolerance and PTDM of 45.7 % in a random Norwegian renal transplant population; a considerably higher prevalence than typically found in the general Scandinavian population.[54,55] Moreover, Hjelmesaeth et al[56] showed that glucose intolerance (impaired glucose tolerance and PTDM combined) was present in about half of their population and correlated with lower HDL-cholesterol, higher serum triglyceride, apolipoprotein-B and 2-hour insulin concentrations; a

clustering typical of IRS. Above-mentioned studies all strongly suggest that insulin resistance and its associated abnormalities are highly prevalent in the renal transplant population, making it worthwhile to investigate insulin resistance as putative cause of CRTD. Valantine et al[57] found evidence that both insulin resistance and its cluster of cardiovascular risk factors were associated with the development of chronic heart transplant dysfunction.

Posttransplant obesity

To prevent fluid overload and uremia, dialysis patients are put on stringent diets with volume and protein restriction. To compensate for the caloric loss of protein restriction, intake of carbohydrates and fat is stimulated. Furthermore, fruit is frequently avoided for fear of hyperkalemia. In short, health care professionals stimulate patients to acquire poor dietary habits during dialysis therapy. It readily explains that after transplantation, when stringent diet restrictions disappear and appetite increases due to the disappearance of uremia, nearly all renal transplant recipients experience excessive weight gain. An average weight gain of more than 10-kg is not unusual in the first year after transplantation.[58] Risk factors implicated in posttransplant weight gain, such as age, gender, ethnicity, income, and diet seem to parallel those of weight gain in the general population.[58-60] Van den Ham et al[61] demonstrated that posttransplant weight gain is predominantly due to an increase in body fat mass.

Using the United States Renal Data System (USRDS), Meier-Kriesche et al[44] found a J-shaped curve between BMI at time of transplantation and the relative risk for death-censored CRTD. The relative risk was approximately 1.5 ($p<0.001$) for patients with a BMI >36 kg/m2. However, their analyses did not account for any posttransplant weight gain. Patients with identical body mass indices at time of transplantation will likely demonstrate different weight gains. It is conceivable that a stronger association exists between BMI at e.g. 1-year after transplantation and CRTD than between BMI at time of transplantation and CRTD. Furthermore, the authors used BMI and not waist-to-hip ratio as parameter for obesity. As central obesity clusters more strongly with IRS than overall obesity, the waist-to-hip ratio might reveal a stronger association with chronic renal transplant dysfunction.[62] Also, Meier-Kriesche used death-censored graft survival as parameter for CRTD. As cardiovascular mortality is the leading cause of death in the renal transplant population, death-censored subjects might have died from IRS-related cardiovascular disease before an IRS-related decline in graft function could have resulted in graft loss.[3] The use of the decline in graft function as parameter for CRTD until death or CRTD ensues, rather than the use of death-censored graft survival, might reveal a stronger association as well.

Posttransplant diabetes mellitus

Posttransplant diabetes is a consequence of insulin resistance. PTDM depends on both an impaired secretion of insulin and insulin resistance, similar to type-2 diabetes mellitus in the general population.[45,63] In order to compensate for the peripheral resistance to the action of insulin, the pancreas increases its insulin secretion, leading to hyperinsulinemia. Only when the pancreas cannot maintain the (increased) secretion rate, PTDM will become manifest.[53] Both transplant-related and traditional risk factors have been implicated in the development of PTDM. Transplant-related risk factors such as immunosuppression may add to the development of PTDM by influencing both insulin resistance and insulin secretion. Boots et al[36] found that steroid withdrawal (from 10-mg/day) resulted in increased insulin sensitivity, while tacrolimus trough-level reduction resulted in an improved insulin secretion capacity. More 'traditional' risk factors, such as African or Hispanic descent, and a family history of diabetes are all important risk factors for the development of PTDM,[38] similar to the development of type 2 diabetes mellitus.[64]

In a cohort of 2078 previously nondiabetic patients, Cosio et al[65] showed that the prevalence of PTDM increased linearly with time from 7% of recipients at one year to 21% of recipients at ten years after renal transplantation. They noticed a near doubling of prevalence in PTDM at one and three years after transplantation since 1995 compared with the period before. The authors explained the increased prevalence by the fact that recipients had become older and more obese at time of transplantation since 1995. Unfortunately, the authors did not study whether the increased prevalence of PTDM was associated with increased graft loss.

Posttransplant dyslipidemia

The largest prospective study (706 patients; mean follow-up of 7 years) investigating the role of posttransplant dyslipidemia in development of CRTD, demonstrated an association between increased serum triglyceride concentrations and graft loss, independent of age, gender, diabetes, hypertension, cholesterol levels, body mass index, proteinuria, serum albumin, and number and severity of acute rejection episodes.[66] In addition, an inverse trend for HDL-cholesterol with graft loss was shown, although not independent of serum triglycerides. However, no association between total cholesterol or LDL-cholesterol concentrations and graft loss was found. As hypertriglyceridemia and lower serum HDL-cholesterol rather than hypercholesterolemia and elevated LDL-cholesterol cluster within the insulin resistance syndrome, these results are in accordance with our hypothesis.

It has been shown that obesity is associated with posttransplant dyslipidemia.[67] Moreover, it has been shown that insulin resistance is associated with serum triglycerides in renal transplant recipients.[42] The association of the insulin resistance syndrome with low levels of HDL-

cholesterol and a preponderance of small dense LDL-particles is mainly due to the increased exchange of neutral lipid driven by hypertriglyceridemia and mediated by the cholesteryl ester transfer protein (CETP).[68] Although immunosuppressive agents have a clear effect on lipid metabolism,[69,70] a preclinical study suggested that a sedentary life-style may have additive or synergistic effect.[71]

Transplant atherosclerosis

CRTD is also known as 'transplant atherosclerosis'. A commonly held view is that CRTD results from 'response to injury',[7,8] similar to the 'response to injury' hypothesis of atherosclerosis.[72] Recent experimental studies at our center confirm the theory that an overshooting healing process following initial allograft injury is involved in the development of transplant atherosclerosis.[73,74] However, initial graft injury does not fully explain the development of late graft loss. Noxious effects of chronic irritating stimuli may superimpose on the initial injury of ischaemia, reperfusion, and acute rejection episodes. From this perspective, it is interesting to realize that risk factors influence graft survival in a time-dependent manner. Early graft failure (<5 yr.) correlates better with acute rejection and ischaemic injury, while late graft failure (>5 yr.) correlates better with recipient-related risk factors such as age and measures of body size.[75] These risk factors, and other renal transplant recipient-related risk factors for the development of CRTD, including dyslipidemia and diabetes, are cardiovascular risk factors that cluster within IRS as well. [76,77]

In transplanted kidneys, glomerulosclerosis and atherosclerosis of intragraft arterioles with perivasculitis are histopathological hallmarks of CRTD.[8] This histopathology has a striking resemblance with diabetic nephropathy in type 2 diabetes mellitus.[78] However, in the renal transplant population, these histological features often develop in the absence of (post-transplant) diabetes mellitus. The vascular wall of the allograft seems therefore more susceptible to risk factors for atherosclerosis than the vascular wall of native kidneys. Other risk factors for CRTD such as ischaemia/reperfusion injury, acute vascular rejection, active cytomegalovirus infection, or calcineurin inhibitors apparently prime the endothelium and the wall of intragraft arterioles to respond more vehemently when IRS-related risk factors are encountered.

Posttransplant hypertension, hyperfiltration, and proteinuria

A relative reduction in the number of functioning nephrons (due to a single functioning kidney and loss of nephrons caused by ischemic, reperfusion, and immunological injury) leading to compensatory

hyperfiltration, has been postulated to progress development of CRTD.[75,79] Obesity and insulin resistance may add to the compensatory hyperfiltration via posttransplant hypertension and changes in glomerular hemodynamics. Both obesity and insulin resistance have been associated with posttransplant hypertension.[42,80] Evidence from studies in the general population demonstrates that both obesity and insulin resistance are associated with glomerular hyperfiltration.[81,82] Hyperinsulinemia is thought to constitute the link between IRS and glomerular hyperfiltration.[83] This is supported by preclinical and clinical experiments with short-term insulin infusion, in which hyperinsulinemia causes a rise in glomerular filtration.[84,85] In the long-term however, posttransplant hypertension and hyperfiltration can cause micro-albuminuria, which may progress to macro-albuminuria. Macro-albuminuria is thought to induce renal damage such as interstitial fibrosis and tubular atrophy, leading to a progressive decline in renal function.[86] It is gaining rapid appreciation that nonesterified fatty acids have a role in this process as well.[87-89] Interstitial fibrosis and tubular atrophy are both histopathological features of CRTD.[8] Nelson et al[90] demonstrated that at the onset of type 2 diabetes, glomerular hyperfiltration was associated with normo- or micro-albuminuria. Once macro-albuminuria had ensued, the glomerular filtration rate declined rapidly. This parabolic pattern of renal function was recently found present in the general population as well, in which it was associated with IRS-features such as central obesity, hypertension, and fasting glucose concentrations.[91] This parabolic pattern might explain the paradoxical finding that an increase in weight is associated with a rise in creatinine clearance in the first 2 years after transplantation.[92]

Future studies

We have presented clinical evidence that insulin resistance and the accompanying syndrome might underlie the recipient-related risk factors for CRTD. Both traditional (a sedentary life-style) and transplant-related (immunosuppressive drugs) factors seem to have an important role in the high prevalence of insulin resistance in the renal transplant population. IRS may act in combined action or even in synergy with other risk factors for CRTD, making renal transplant recipients more susceptible to the consequences of a sedentary life-style. Kasiske[93] showed that the impact of smoking and posttransplant diabetes mellitus on development of ischemic heart disease was two to three times greater in the renal transplant population than in the general population, perhaps because he found acute rejection episodes to increase the risk for ischemic heart disease as well. A similar synergistic finding may be true for development of CRTD. However, whether insulin resistance and its typical metabolic cluster underlie the recipient-related risk factors for CRTD, remains to be investigated.

Cross-sectional, but preferably longitudinal clinical studies in which insulin sensitivity indices as well as its associated features can be related in multivariate analyses to declines in graft function are therefore needed.[94-97] In our opinion, the decline in graft function is the preferred parameter for CRTD as it allows inclusion of patients who die from IRS-related cardiovascular disease before an IRS-related decline in graft function could have resulted in graft loss. The slope of the regression line of reciprocal serum creatinine versus time appears to be a poor predictor of CRTD, possibly because it incorrectly assumes full linearity in development of CRTD.[98] Therefore we suggest that CRTD should be defined as the absolute decline in transplant function, corrected in multivariate linear regression analyses for baseline renal function and time elapsed since transplantation.

Furthermore, future research should focus in clinical trials on the prevention and treatment of IRS in renal transplant recipients. Prevention programs should not only focus on tailoring immunosuppressive regimens but also on diet, weight reduction, and exercise. In addition, intervention effects of insulin resistance lowering drugs (e.g. metformin and thiazolidinedion derivatives), and effects of anti-oxidants, blood pressure-, proteinuria- and lipid-lowering drugs (e.g. angiotensin converting enzyme inhibitors and statins) on progression of CRTD need careful investigation.
Now that a growing number of end-stage renal disease patients who are already insulin resistant due to obesity and type-2 diabetes undergo transplantation, putative effects of IRS on long-term graft survival are becoming of even more concern.[99]

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Chapter 3

Validation of insulin resistance indices in a stable renal transplant population

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Abstract

Objective

To investigate the validity of established insulin resistance indices, based on fasting blood parameters, in a stable renal transplant population.

Research Design and Methods

Fasting insulin, Homeostasis Model Assessment (HOMA), Quantitative Insulin Sensitivity Check Index (QUICKI), and McAuley's index were assessed for correlation and agreement with insulin resistance, as measured by the hyperinsulinaemic euglycemic clamp, in 51 stable renal transplant recipients, who were at a median of 7.5 years post-transplant. Multivariate linear regression analyses were used to determine independent risk factors for insulin resistance.

Results

Clamp-assessed insulin resistance correlated with fasting insulin (r=-0.56), HOMA (r=-0.53), QUICKI (r=0.52), and McAuley's index (r=0.61) (all P-values <0.01). Linear regression showed agreement between all indices and insulin resistance. However, McAuley's index showed the strongest agreement irrespective of age, gender, renal allograft function, and obesity. In multivariate analysis, fasting insulin (β= -0.59, P= 0.002), fasting triglycerides (β= -0.33, P=0.04), and body mass index (β= -1.22, P=0.05) were independently associated with clamp-assessed insulin resistance.

Conclusion

All investigated insulin resistance indices were valid estimates of insulin resistance. in the long-term stable renal transplant population. However, correlation and agreement were strongest for McAuley's index. In addition to fasting insulin and triglyceride concentrations, of which McAuley's index is composed, only body mass index seemed to be independently associated with insulin resistance in this population.

Introduction

The incidence and prevalence of cardiovascular disease have been estimated to be three to five times greater in the renal transplant population than in the general population.[1,2] A recent study showed that the majority of renal transplant out-patients suffers from a constellation of cardiovascular risk factors, i.e. obesity, dyslipidemia, hypertension, and post-transplant diabetes mellitus, that is consistent with the metabolic syndrome (MS).[3] According to preliminary data of the ALERT trial, MS is associated with an increased risk of cardiovascular mortality.[4]

Insulin resistance is thought to be the central pathophysiological feature underlying MS.[5] In order to study the role of insulin resistance in the high incidence of cardiovascular morbidity and mortality in this population validated insulin resistance indices are needed. Insulin resistance indices have not yet been validated in comparison to the hyperinsulinaemic euglycemic clamp in the stable renal transplant population. Indices that are based on fasting blood parameters alone, have the distinct advantages over other methods of quantifying insulin resistance in that they are less cumbersome and less time-consuming for large-scale epidemiological studies at outpatient clinics. However, established indices have been derived from correlates of insulin resistance in non-transplant populations. Evidence suggests that insulin resistance in the renal transplant population may be caused by other risk factors as well, such as immunosuppression and anti-hypertensive medication.[6] Consequently, it remains uncertain whether these indices are applicable to the stable renal transplantation population.

The primary objective of this study was therefore, to validate established insulin resistance indices based on fasting blood parameters in a stable renal transplant population. The second objective was to investigate which risk factors, both traditional and those specifically related to the transplant population, are associated with insulin resistance.

Research design and methods

Study population

The Institutional Review Board approved the study protocol (METc 01/039), which was in adherence with the Declaration of Helsinki.[7] Patients from the renal transplant out-patient population, who were part of a previous study cohort,[3] were randomly invited to participate. Recruitment was performed in a stratified manner so that similar numbers of males and females and similar numbers of participants with a high and a low waist hip ratio would be included. Subjects were eligible for participation in the present study if they had received a renal allograft at our center at least 2 years prior to the start of the study and used

cyclosporine micro-emulsion (Neoral®; in combination with prednisolone and/or azathioprine, mycophenolate mofetil, or rapamycin) as part of their immunosuppressive regimen. Inclusion required a stable allograft function, defined as a 24-hour urinary creatinine clearance of >30 ml/min, and a difference in 24-hour urinary creatinine clearance over the past year of ≤20 ml/min, to participate. Excluded from invitation were subjects with diabetes mellitus, defined as plasma glucose ≥ 7.0 mmol/L, and/or use of anti-diabetic medication. Sources funding this project did not play a role in either data collection or analysis or in submission and publication of the manuscript.

Procedure

Subjects were admitted at 8:00 am to our clinical research unit after an 8-hour overnight fasting period. Fasting blood was drawn first, after which patients were allowed to take their immunosuppressive medication. Weight, height, waist (midway between the iliac crest and the 10th rib), and hip (at the level of the trochanter major) circumference were measured secondly. Blood pressure was reported as the average of five automated measurements taken at 3-minute intervals (Dinamap; GE Medical Systems, Milwaukee, Wisconsin, USA).

Hyperinsulinaemic euglycemic clamp

Insulin resistance was measured using the hyperinsulinaemic euglycemic clamp technique. The clamps were performed as described by previous investigators.[8] To give a brief summary of the procedure, exogenous insulin (Velosulin, Novo Nordisk, Bagsvaerd, Denmark) was infused at a continuous rate of 50 mU/kg/hour for 120 minutes. Glucose concentration of 5 mmol/L was maintained by adjusting the rate of a 20% D-glucose and 1% KCl infusion based on plasma glucose measurements performed at 5-minute intervals. Whole body glucose uptake (M-value; mg/kg/min) was determined by the total amount of glucose infused during the last 60 minutes of the clamp. Steady-state insulin concentration (I-value; pmol/L) was determined as the mean of two plasma samples at 90 minutes and 120 minutes. Insulin sensitivity was defined as the whole body glucose uptake (M-value) divided by the prevailing serum insulin concentrations (I-value) during the clamp (mg/kg/min per pmol/L). Insulin resistance is the reciprocal of insulin sensitivity. For convenience, the M/I-value was multiplied by 100.

Insulin resistance indices

The following indices were validated against the clamp: fasting insulin (in μU/mL),[9] Homeostasis Model Assessment (HOMA): *glucose (in mmol/L) x insulin (in μU/mL)] / 22.5*,[10] Quantitative Insulin sensitivity Check Index (QUICKI): *1 / [log glucose (in mg/dL) + log insulin (in μU/mL)]*,[11] and McAuley's index: *exp[2.63 - 0.28 ln(insulin (μU/mL)) - 0.31ln(triglycerides (mmol/L))]*.[12]

Analytical methods

Fasting serum insulin and insulin levels during the clamp were determined using a radioactive immuno-assay (DSL-1600, Texas, USA). The intra- and inter-assay coefficients of variation at 16.9 μU/mL are 4.5% and 9.9% respectively, and at 53.4 μU/mL 6.4% and 4.7% respectively. Total cholesterol was assessed using the CHOD-PAP method and serum triglyceride level was measured using the GPO-PAP method (both on a MEGA AU 510, Merck Diagnostica, Darmstadt, Germany). High density lipoprotein (HDL) cholesterol was determined using the CHOD PAP method on a Technikon RA-1000 (Bayer Diagnostics b.v., Mijdrecht, The Netherlands). Low density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.[13] Total protein concentration was analyzed using the Biuret reaction (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany). Creatinine clearance was calculated using 24-hour urinary creatinine excretion and serum creatinine.

Transplant related factors

Relevant donor, recipient, and transplant characteristics were extracted from the Groningen Renal Transplant Database. This database holds information on all renal transplantations that have been performed at our center since 1968. Parameters used for analysis were donor and recipient age and gender, dialysis modality and duration, date of transplantation, delayed graft function (i.e. days of oliguria or necessity of dialysis treatment), weight 6 months after transplantation (to calculate post-transplant weight gain), human leukocyte antigen (HLA) mismatches, cold and warm ischaemia times, cytomegalovirus (CMV) seropositivity of donor and recipient, acute rejection treatment, and immunosuppressive medication.

Statistical analysis

Analyses were performed using SPSS version 12.0 software (SPSS Inc. Chicago IL). The Kolmogorov-Smirnov test was used to assess the normality assumption of continuous distribution. Parametric values are presented as mean ± standard deviation, whereas non-parametric values are displayed as median [interquartile range]. A two-sided p-value of 0.05 or less was considered to indicate statistical significance. All indices and M/I values were log transformed prior to analysis.

The study sample was compared to the population from which participants were recruited,[3] with regard to age, sex, time after transplantation, body mass index (BMI), blood pressure, renal allograft function, and proteinuria using Student's T-test for parametric variables, and the Mann-Whitney test for non-parametric variables.

Correlation between the indices and log transformed M/I values of the clamps were analyzed by Pearson's test for parametric variables. Agreement between the indices and the clamps was assessed by linear regression of the insulin resistance index under investigation against the

M/I-values with a 95% prediction interval, as suggested by Bland and Altman when methods have different units.[14]

To determine whether age, gender, BMI or renal allograft function influenced the association between the indices and clamp-assessed insulin resistance, correlation was re-assessed after stratification along the median of the above-mentioned variables. In case of difference in correlation, linear regression was performed to determine whether effect modification existed between the above-mentioned variables and the indices.

To determine which traditional and transplant-related risk factors were associated with insulin resistance, all putative factors that were univariately associated with log transformed M/I values at a p-value ≤0.1, were entered simultaneously in a backward linear regression model with log transformed M/I values as the dependent variable. The variables that were retained in the crude model were subsequently tested for interaction among covariates, goodness of fit, and higher-order (e.g. polynomial) regression by ANOVA. Residual terms were tested to determine if distribution was normal

Results

Table 1 shows baseline characteristics of the 51 subjects. Mean age was 53±11 years, 55% were male, median time after transplantation was 7.5 years, and the majority (90%) had received a cadaveric allograft. Fourty percent was overweight (BMI between 25-30 kg/m^2) and 20% was obese (BMI>30kg/m^2). Creatinine clearance was 65 [57-78] mL/min. Average blood pressure was 145/85 mmHg. The study sample did not differ significantly from the population from which it was recruited with respect to age, sex, time after transplantation, BMI, blood pressure, or renal allograft function (data not shown). Only proteinuria was significantly lower in the study sample (0.1 [0.0-0.2] vs 0.2 [0.0-0.5] g/24h, P=0.001).

The hyperinsulinaemic euglycaemic clamp was performed with glucose concentrations of 5.04±0.16 mmol/L during the last hour of the clamp. Insulin levels were raised to 550 [391 - 751] pmol/L, yielding an M-value of 4.9±1.8 mg/kg/min and an M/I-value of 0.83 [0.57-1.39] mg/kg/min per pmol/L.

Table 1 - Population characteristics

Characteristics	Value
n	51
Recipient demographics	
Age (Years)	53 ±11
Male sex	28(55)
Time since transplantation (years)	7.5(5.2-12.0)
Cadaveric donor	46(90)
Body composition	
BMI (kg/m2)	26.0 (23.8-28.6)
Waist circumference (cm)	101±12
Waist-to-hip ratio	1.03(0.92-1.09)
Renal function and proteinuria	
Creatinine clearance (ml/min)	65(57-78)
Serum creatinine (µmol/l)	134(106-149)
Proteinuria (g/24 h)	0.1(0.0-0.2)
Blood pressure	
Systolic blood pressure (mmHg))	145±15
Diastolic blood pressure (mmHg)	85±11
Lipids	
Total Cholesterol (mmol/l)	5.4±0.9
LDL cholesterol (mmol/l)	3.3±0.8
HDL cholesterol (mmol/l)	1.3(0.9-1.7)
Triglycerides (mmol/l)	1.7(1.1-2.4)
Medication	
Antihypertensive	
β-Blocker	6(12)
ACE inhibitor	20(40)
Angiotensin II antagonist	4(7)
Calcium antagonist	19(38)
Diuretics	22(44)
Lipid-lowering drugs	
Statine	37(72)
Immunosuppresion	
Prednisolone dose (mg/day)	10(7.5-10)
Cyclosporine	51(100)
Trough-level (µg/l)	109(78-143)
Azathioprine	10(20)
Mycophenolate mofetil	13(25)
Trough level (µg/l)	1.5(1.1-3.6)
Rapamycine	1(2)

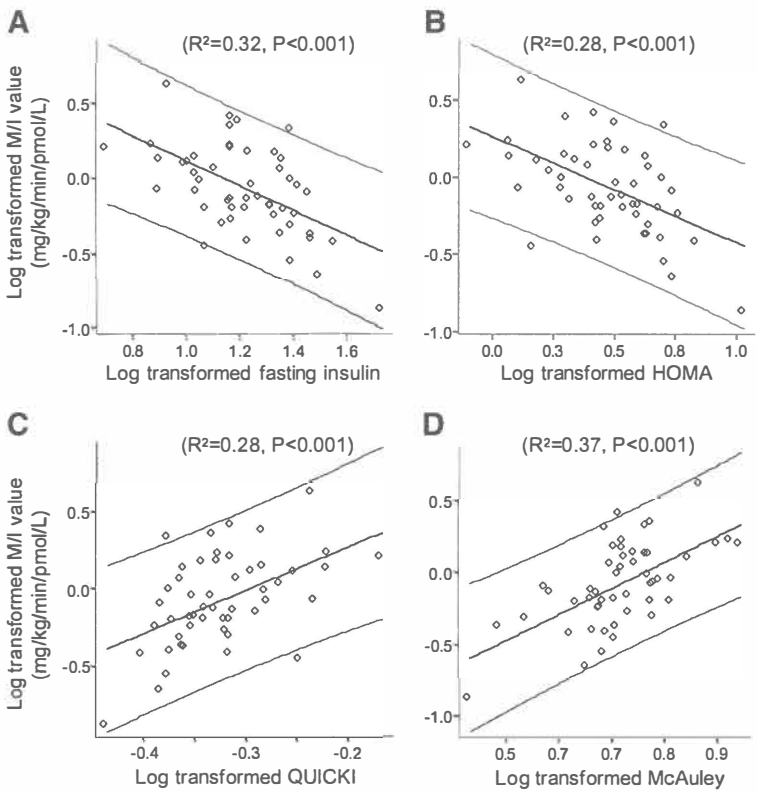
Data are means ± SD, η(%), or median (interquartile range)

Fasting insulin was 16.5 [12.0-23.5] µU/mL; fasting glucose was 4.5 ± 0.6 mmol/L; HOMA 6.4 [5.2-9.3]; QUICKI 0.32 [0.30-0.34]; and McAuley's index 5.4 ± 1.2. Correlation coefficients between the indices and clamp-assessed insulin resistance were r= -0.56 for fasting insulin, r= -0.53 for HOMA, r=0.52 for QUICKI and r=0.61 for McAuley's index, all at

P<0.01. Figures 1a-d show the regression analyses with 95% prediction intervals. Agreement was reached for all indices. HOMA and QUICKI had two (≅ 4%) subjects outside the prediction interval.

Fasting insulin and McAuley's index had one subject outside the interval.

Figure 1 : Regression analyses of fasting insulin, HOMA, QUICKI, and McAuley against M/I value. Data are presented as best-fit regression line with prediction interval



The correlation coefficients between the indices and clamp-assessed insulin resistance did not change significantly after stratification along the median of age and renal allograft function. However, a difference was observed after stratification for BMI and gender. In the lower BMI (<26.0 kg/m^2) and female sex groups, the correlations of fasting insulin, HOMA, and QUICKI with clamp-assessed insulin resistance lost statistical significance (data not shown). Only McAuley index remained significantly correlated with M/I-values in all subgroup analyses (low BMI group r=0.41, p<0.05; high BMI group r=0.63, p<0.01; males r=0.64, p<0.01; females r=0.60, p<0.01). No effect modification was found for BMI and gender in the linear regression analyses.

Putative determinants of insulin resistance were analyzed, first univariately and later multivariately in a backward linear regression

model. Table 2 shows that only fasting insulin, BMI, HDL-cholesterol, fasting triglycerides, and waist circumference were univariately associated with insulin resistance. All other putative variables did not reach the p≤0.10 level; specifically: gender, age, post-transplant weight gain, LDL-cholesterol, use of lipid lowering drugs, systolic and diastolic blood pressure, blood pressure medication (diuretics, β-blocker, angiotensin inhibitor or angiotensin receptor blocker and total number of anti-hypertensive drugs), fasting glucose, creatinine clearance, daily prednisolone dosage, cyclosporine trough-levels, mycophenolate mofetil or azathioprine use, cold and warm ischaemia times, delayed graft function, HLA- mismatches, cold and warm ischaemia times, CMV-seropositivity, and acute rejection treatment.

Table 2 Univariate and backward multivariate regression analyses

	β	95%CI	P value
Univariate analysis			
Log-transformed insuline	-0.83	-1.18 to -0.47	<0.001
log-transformed BMI	-2.24	-3.69 to -1.19	<0.001
Log-transformed HDL cholesterol	0.69	0.14-1.24	0.01
Log-transformed triglycerides	-0.55	-0.90 to -0.19	0.003
Waist circumference (cm)	-0.02	-0.04 to -0.01	0.004
Multivariate analysis			
Log-transformed insuline	-0.59	-0.96 to -0.22	0.002
Log-transformed triglycerides	-0.33	-0.64 to -0.01	0.04
Log-transformed BMI	-1.22	-2.27 to 0.00	0.05

Log-transformed M/I values were entered as the dependent variable in univariate and backward multivariate regression analysis. In univariate analysis only variables at P < 0.10 are shown. R^2=0.44, F=12.2, total df=47, P<0.001

Variables that were significantly associated with M/I values were entered together with age and gender in a backward linear regression model. The crude model was subsequently tested for interaction terms, higher order regression, and goodness-of-fit with ANOVA. These subsequent models were not significantly better, so the crude model was accepted as the final model. In this model, only log transformed insulin (β -0.59, 95%CI [-0.96, -0.22], p=0.002), log transformed fasting triglycerides (β -0.33 95%CI [-0.64, -0.01], p=0.04), and log transformed BMI (β -1.22, 95%CI [-2.27, 0.00], p=0.05) remained independently associated with M/I values (R^2=0.44, F-test=12.2, dF 47, p<0.001) as shown in table 2.

Discussion

The present study shows that four commonly used insulin resistance indices, based on risk factors for insulin resistance in non-transplant populations, are valid estimates of clamp-assessed insulin resistance in a stable renal transplant outpatient population. Incidence and prevalence of

cardiovascular disease are high in the renal transplant population.[1,2] Insulin resistance is an independent risk factor for cardiovascular mortality in the general population[15] and has been hypothesized to play a role in the development of chronic renal allograft dysfunction as well.[16] Consequently, validated insulin resistance indices are needed to study the role of insulin resistance in the development of cardiovascular morbidity and mortality. Blood fasting based indices have the advantage that they are practical and easy to use for large-scale epidemiological studies.

The finding that the McAuley index, which consists of fasting triglycerides and fasting insulin, performed best was additionally supported by our multivariate linear regression analyses, which revealed that only fasting insulin, fasting triglycerides, and BMI were associated with insulin resistance in the long-term after renal transplantation. HOMA and QUICKI yielded weaker correlations and lesser agreement in comparison with both McAuley and fasting insulin, but did compare similarly to each other. This is most likely due to the fact that they are mathematically comparable. The presence of glucose in the HOMA and QUICKI indices clearly did not increase the strength of the association with insulin resistance compared to fasting insulin alone. This finding was additionally supported by the fact that glucose was not associated with clamp-assessed insulin resistance in the linear regression analysis. This lack of significant relationship is probably caused by the fact that the current study population was non-diabetic.

Correlations between the indices and M/I-values were significant, irrespective of age and renal allograft function. In contrast, fasting insulin, HOMA, and QUICKI did not correlate significantly with M/I-values in females and in the non-obese (low BMI) subgroups. However, further analyses by linear regression analyses could not demonstrate any significant effect modification of gender and degree of obesity. McAuley's index was the only index that remained significantly correlated with clamp-assessed insulin resistance in all stratified analyses; again showing that it performed best.

A previous study validated insulin resistance indices in renal transplant recipients at ten weeks post-transplant.[6] In that study, McAuley's index performed best of all indices based on fasting blood parameters as well.[17] That study did not only find BMI and triglycerides associated with insulin resistance, but daily prednisolone dose and active CMV-infection as well.[6] The explanation for this difference may lie in the time period after transplantation in which that study was performed. The period immediately after transplantation is characterized by high doses of immunosuppression to prevent and treat acute rejection. The consequence of high-doses immunosuppression is opportunistic infection. In that particular study at ten weeks post-transplant, cyclosporine trough-levels were more than double the levels compared to our study (242±60 vs 108±42 μg/L).[6] Cyclosporine is thought to increase insulin resistance and reduce insulin secretion.[18] Additionally, daily prednisolone dosage was

almost double in Hjelmesæth's study compared to ours (15±7 vs 8.7±2.0 mg/day). This difference may be of influence because the same group recently showed that a reduction in the daily prednisolone dose from 16 [10-30] mg/day to 9 [5-12.5] mg/day was accompanied by an average decrease in insulin resistance of 24%.[19] Moreover, the majority of participants in that study had received methylprednisolone boluses of 125 to 500 mg/day for 4 to 5 consecutive days for treatment of acute rejection episodes.[6] As mentioned before, Hjelmesæth found active CMV-infection to be associated with insulin resistance as well. Although this finding may constitute an epiphenomenon of immunosuppression, CMV may add directly to an insulin resistant state through release of cytokines such as TNF-α.[20,21]

When immunosuppression is tapered and opportunistic infections become less prevalent in the long-term after transplantation, obesity may become a more predominant factor that influences insulin resistance. Most renal transplant recipients experience at least a 10% weight gain after transplantation.[22] In Hjelmesæth's study, average BMI was 23.5±3.8 kg/m^2 at three months post-transplant. Our study subjects had a similar BMI of 23.7±3.4 kg/m^2 at 1 month post-transplant, which increased to 26.6 ±3.8 kg/m^2 at the time they participated in this study. Since obesity is an important determinant of insulin resistance, this weight gain might have a large effect on insulin resistance. The inclusion of BMI in an estimate of insulin resistance could further increase accuracy of such an index as was shown in our multivariate linear regression analyses.

This study had some limitations, however. All of our subjects had a cyclosporine-based immunosuppressive regimen and relatively preserved renal allograft function. We wanted to study a homogenous population because cyclosporine is thought to influence insulin secretion as well as resistance. It remains unknown whether our findings are applicable to subjects receiving other immunosuppressive regimens and those with less preserved renal allograft function. Both cyclosporine treatment and impaired renal function have been shown to be associated with hypertriglyceridemia.[23,24] Consequently, our finding that McAuley's index, which includes triglyceride levels in its equation, correlated and agreed strongest with clamp-assessed insulin resistance may only hold true for renal transplant recipients receiving a cyclosporine-based immunosuppressive regimen with relatively preserved renal allograft function. However, because both cyclosporine trough levels and renal allograft function were not found to be associated with M/I values in this study and because fasting insulin concentration, BMI, and triglyceride levels appeared to be the only determinants of insulin resistance in the long term, we hypothesize that our results may be generalized to subjects receiving other immunosuppressive regimens and to subjects with less preserved renal allograft function.

In summary, all insulin resistance indexes investigated in this study were valid estimates of clamp-assessed insulin resistance in a stable renal

transplant population. Only fasting insulin concentration, triglyceride levels, and BMI were independently associated with insulin resistance. These results underscore our finding that McAuley's index performed best in the present population with a cyclosporine-based immunosuppressive regimen and relatively preserved renal function

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Chapter 4

Determinants of insulin resistance in renal transplant recipients

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Abstract

Background
Insulin resistance is considered to play an important role in the development of cardiovascular disease, which limits long-term renal transplant survival. Renal transplant recipients are more insulin resistant compared to healthy controls. It is not known to date which factors relate to this excess insulin resistance. Therefore we investigated which factors are related to insulin resistance long-term after renal transplantation.

Methods
All renal transplant recipients at our out-patient clinic with a functioning graft for more than 1-year were invited to participate. We excluded diabetic recipients. Recipient, donor, and transplant characteristics were collected as putative determinants. We used fasting insulin, homeostasis model assessment index, and McAuley's index as valid estimates of insulin resistance. Linear regression models were created to investigate independent determinants of all indexes.

Results
A total of 483 recipients (57% male, 50±12 years) were analysed at a median [interquartile range] time of 6.0 [2.6-11.6] years post-transplant. The most consistent determinants across all three indexes were body mass index ($P<0.001$), waist-to-hip ratio ($P<0.001$), and prednisolone dose ($P<0.05$). Independent associations were present for total cholesterol ($P<0.001$), HDL-cholesterol ($P<0.001$), creatinine clearance ($P<0.05$), recipient age ($P<0.001$), and gender ($P\leq0.002$). No independent associations were present for transplant-related factors such as acute rejection treatment or CMV seropositivity.

Conclusions
Our results indicate that obesity, distribution of obesity, and prednisolone treatment are the predominant determinants of insulin resistance long-term after transplantation. Insulin resistance after renal transplantation could be managed favorably by weight and prednisolone dose reduction, which may reduce cardiovascular disease.

Introduction

Cardiovascular disease (CVD) after renal transplantation limits long-term patient survival.[1] Both incidence and prevalence of CVD are estimated to be five times higher than in the general population.[2,3] Insulin resistance is considered to play an important role in the development of CVD.[4]
Obesity has been identified as an important determinant of insulin resistance in renal transplant recipients shortly after transplantation.[5] However, renal transplant recipients are still more insulin resistant than healthy controls when matched for body mass index (BMI) and age.[6] This suggests that additional transplant-specific determinants contribute to insulin resistance in renal transplant recipients. These include poor physical activity, chronic use of immunosuppressive drugs such as corticosteroids and calcineurin inhibitors, and persistent low-grade inflammatory activity of viral infections, in particular cytomegalovirus (CMV).[5,7,8]
A recent study indicated that corticosteroid dose as well as active CMV infections are associated with insulin resistance immediately after transplantation.[5] However, it is unknown to which extent both traditional, non-transplant related factors (such as obesity) and transplant-related factors contribute to insulin resistance long-term after transplantation. Therefore, we investigated the determinants of insulin resistance in renal transplant recipients using validated insulin resistance indexes.[5,9,10]

Research Design and Methods

Research design and subjects

To investigate determinants of insulin resistance we invited all adult renal transplant recipients from our out-patient clinic who survived the first year after transplantation with a functioning allograft, as described in detail elsewhere.[11,12] A total of 606 renal transplant recipients signed written informed consent, from an eligible 847 (72% consent rate). The group that did not sign informed consent was comparable with the group that signed informed consent with respect to age, gender, BMI, serum creatinine, creatinine clearance, and proteinuria. Excluded from analysis were 106 recipients with diabetes mellitus (defined as a fasting plasma glucose ≥7.0 or use of anti-diabetic medication).[13] Also excluded were an additional 17 recipients who had received a combined transplantation (kidney-pancreas or kidney-liver), leaving 483 non-diabetic renal transplant recipients for analysis. The Institutional Review Board approved the study protocol (METc 2001/039). Funding sources had neither a role in the collection and analysis of data, nor in publication of the manuscript.

Renal transplant characteristics
Relevant transplant characteristics were taken from the Groningen Renal Transplant Database. This database holds information on all renal transplantations performed at our center since 1968, including primary and secondary infection with CMV. Current medication was taken from the medical record. Standard immunosuppressive treatment was described previously.[11, 12]

Putative determinants of insulin resistance
BMI, waist circumference, and blood pressure were measured as described previously.[11,12] Smoking status and alcohol consumption were assessed with a self-report questionnaire.[14] Physical activity was assessed during the past 6 months with the Tecumseh Occupational Activity Questionnaire and Minnesota Leisure Time Physical Activity Questionnaire.[15] Physical activity is expressed as metabolic equivalents (METs) per week. One MET is approximately 3.5 ml of oxygen per kilogram body weight per minute, the energy expenditure of the average adult for sitting quietly.

Insulin resistance Indexes
We used fasting insulin, HOMA, and McAuley's index as surrogate estimates of insulin resistance.[16,17,18] These indexes have recently been validated in our own renal transplant population.[10] as well as in a renal transplant population comparable to ours.[5,9] Homeostasis Model Assessment (HOMA) was calculated as: [glucose (mmol/L) x insulin (μU/mL)] / 22.5 and McAuley's index was calculated as: exp[2.63 - 0.28 ln (insulin (μU/mL)) - 0.31ln(triglycerides (mmol/L))].
Fasting insulin was determined using an AxSym auto-analyzer (Abbott Diagnostics, Hoofddorp, the Netherlands). The intra- and interassay coefficients of variation at 8.7 mU/L are 2.6% and 2.9% respectively. At 42.2 mU/L the intra- and interassay coefficients are 4.1% and 2.1% respectively. The assay shows virtually no cross-reactivity with pro-insulin (0.016% at 10^6 pg/mL).

Laboratory measurements
Blood was drawn after an 8-12h overnight fasting period. Serum total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), were assessed as described previously.[11] Non-HDL cholesterol was calculated by subtracting HDL-cholesterol from total cholesterol.[19] High-sensitivity C-reactive protein (CRP) was assessed as described previously.[12] Renal allograft function was assessed by 24h urinary creatinine clearance. Muscle mass was assessed by 24h creatinine excretion. Cytomegalovirus (CMV) IgG was assessed by routine ELISA assay.[20] A detectable CMV IgG titer indicated seropositivity.

Statistical analysis

Data was analyzed with SPSS version 12.0 (SPSS Inc. Chicago, IL). Parametric parameters are given as means ± standard deviation, whereas non-parametric parameters are given as median [interquartile range]. Skewed data were normalized by logarithmic transformation in all analyses. Chi-square and Student's T-test were used to test gender differences among categorical and continuous variables. A two-sided P-value less than $P<0.05$ indicated statistical significance. Regression coefficients are given as standardized beta's. Tolerance statistics >0.20 for variables in multivariate analyses were considered to indicate that assumptions of co-linearity were not violated.

Univariate linear regression analyses were used to explore the impact of each putative determinant on fasting insulin, HOMA, and McAuley's index. We additionally analyzed the determinants of insulin resistance for gender interaction, for such an interaction has been noted previously.[21] Finally, we performed multivariate linear regression analyses to investigate which determinants were independently associated with the insulin resistance indexes. To this purpose, all putative factors that were univariately associated with any index (at a P-value <0.1) were included in a backward linear regression model. Also included in multivariate analyses were age and gender of the recipient as well as any significant interaction term with gender. The impact of the determinants was compared by the magnitude of the standardized regression coefficients.

Results

We investigated 483 renal transplant recipients at a median time of 6.0 [2.6-11.6] years post-transplant (57% male, 50±12 years, 85% cadaveric transplants). Table 1 shows the further characteristics of the study population.

Table 2 shows the associations between the study characteristics and the insulin resistance indexes. Measures of obesity had the strongest associations with all three indexes. Obesity was negatively associated with McAuley's index as it reflects insulin sensitivity, and is an inverse measure of insulin resistance. Figures 1A and 1B show that both increasing BMI and increasing waist-to-hip ratio are associated with increasing fasting insulin concentrations. An interaction was present between gender and BMI for all indexes, showing that the effect of BMI on insulin resistance was stronger in men. The three indexes were also univariately associated with HDL-cholesterol, total cholesterol, triglycerides, and fasting glucose concentrations. Blood pressure was not associated with insulin resistance, in contrast to the use of angiotensin converting enzyme (ACE) inhibitors. Use of beta-blockers was only associated with McAuley's index. Inflammation, as reflected by CRP concentrations, was only associated with McAuley's index (β =-0.10, P=0.03).

Table 2 also shows that transplant-related characteristics were not associated with insulin resistance with the exception of prednisolone dose, which was univariately associated with all three indexes. Figures 1C shows that fasting insulin concentrations increase with higher prednisolone dose. Renal allograft function, as reflected by creatinine clearance, was significantly associated with both fasting insulin concentrations and HOMA. This was also true for creatinine excretion, a marker of muscle mass. McAuley's index was associated with serum creatinine. Previous CMV infection and use of tacrolimus tended to be associated with McAuley's index, but did not reach statistical significance. Other transplant-related characteristics did not show any association or any gender interaction.

Figure 1. Determinants of fasting insulin concentrations.

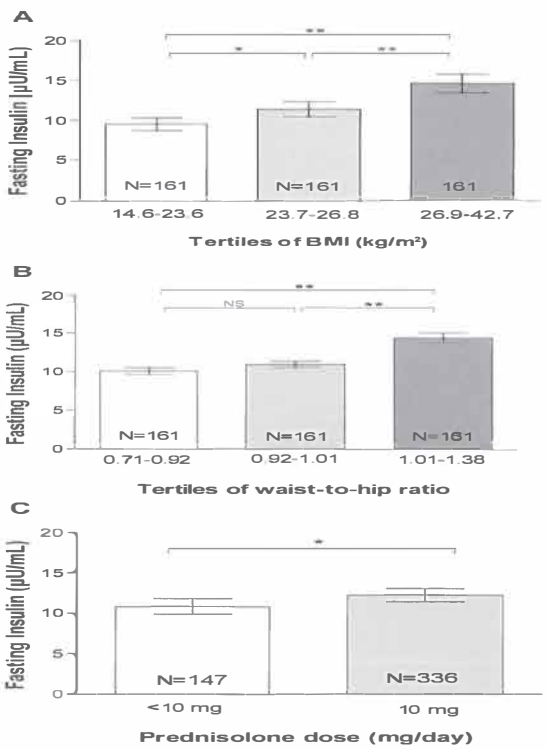

Figure 1A shows tertiles of BMI, Figure 1B shows tertiles of waist-to-hip ratio, and Figure 1C shows prednisolone dose. Results were similar for HOMA and McAuley's index. NS=not significant, * P<0.05, **P<0.001. Tested with Mann-Whitney test

Table 1. Recipient and transplant related characteristics

N=		483
Recipient demographics	Age, y	50±12
	Male gender, n (%)	275 (57)
Prior history of CVD[a]	MI[b], n (%)	37 (8)
	TIA[c] or CVA[d], n (%)	21 (4)
Body composition	BMI, kg/m^2	25.6±4.1
	Waist circumference, cm	95.6±13.1
	Waist to hip ratio	0.97±0.10
	Posttransplant weight gain, kg	2.2±6.3
Physical exercise	METs, per week	7204 [1620-17402]
Blood pressure and medication	Systolic blood pressure, mmHg	151±22
	Diastolic blood pressure, mmHg	90±10
	ACE-inhibitor, n (%)	159 (33)
	ß-blocker, n (%)	299(62)
Lipids	Cholesterol, mmol/L	5.6 [5.0-6.2]
	HDLc, mmol/L	1.1 [0.9-1.3]
	Non-HDLc, mmol/L	4.5 [3.9-5.2]
	Triglycerides, mmol/L	1.9 [1.4-2.5]
	Statin, n (%)	229 (47)
Substance use	Alcohol, n (%)	
	None,	218 (45)
	1-4/month	72 (15)
	>2/week	193 (40)
	Smoking, n (%)	
	Current smokers	112 (23)
	Non-smokers	371 (77)
Inflammation	CRP, mg/L	1.9 [0.8-4.4]
Insulin resistance indexes	Fasting insulin, µU/mL	10.3 [7.7-14.1]
	HOMA	2.02 [1.49-2.92]
	McAuley's index	6.1±1.2
	Glucose, mmol/L	4.5±0.6
Donor demographics	Age, y	38±16
	Male gender, n (%)	260 (54)
Renal allograft function	Serum creatinine, µmol/L	150±60
	Creatinine clearance, mL/min	62±23
	Creatinine excretion, mmol/24h	12.2±3.6
	Proteinuria, g/24h	0.2 [0.0-0.5]
Acute rejection treatment	High-dose corticosteroids, n (%)	150 (31)
	Other rejection therapy, n (%)	72 (15)
CMV status	CMV seropositivity before transplantation	
	Recipient, n (%)	223 (46)
	Donor, n (%)	255 (53)
	CMV infection	
	No infection, n (%)	255 (53)
	Primary infection, n (%)	95 (20)
	Secondary infection, n (%)	113 (27)
	CMV status at inclusion	
	Seropositive recipients, n (%)	349 (72)
Immunosuppresion	Prednisolone dose, mg/d	10.0 [7.5-10.0]
	Cyclosporine, n (%)	304 (63)
	Tacrolimus, n (%)	68 (14)
	Mycophenolate mofetil, n (%)	207 (43)
	Azathioprine, n (%)	159 (33)

a) CVD: Cardiovascular Disease
b) MI: Myocard Infarct
c) TIA: Transient Ischemic Attack
d) CVA: Cerebrovascular Accident

Table 2. Univariate analyses of renal recipient-related characteristics with fasting insulin, HOMA, and McAuley's index

		Fasting Insulin		HOMA		McAuley	
		β	P-value	β	P-value	β	P-value
Recipient demographics	Age, y	-0.10	0.04	-0.09	0.06	0.03	0.58
	Gender male, %	-0.03	0.46	0.01	0.97	-0.01	0.75
Prior history of CVD[a]	MI[b], n (%)	0.01	0.78	0.02	0.74	-0.03	0.54
	TIA[c] or CVA[d], n (%)	0.08	0.07	0.07	0.11	-0.08	0.08
Body composition	BMI, kg/m^2	0.40	<0.001	0.40	<0.001	-0.40*	<0.001*
	Waist circumference, cm	0.36	<0.001	0.36	<0.001	-0.42	<0.001
	WHR	0.29	<0.001	0.31	<0.001	-0.40	<0.001
	Posttransplant weight gain, kg	0.20	<0.001	0.20	<0.001	-0.20*	<0.001*
Physical exercise	METs, per week	-0.06	0.20	-0.05	0.26	0.08	0.11
Blood pressure	Systolic blood pressure, mmHg	-0.05	0.29	-0.04	0.35	-0.06	0.19
	Diastolic blood pressure, mmHg	-0.01	0.88	-0.01	0.85	-0.07	0.12
	ACE-inhibitor, %	0.15	0.001	0.15	<0.001	-0.15	0.001
	ß-blocker, %	0.05	0.23	0.08	0.08	-0.13	0.004
Lipids	Cholesterol, mmol/L	-0.11	0.01	-0.10	0.04	-0.14	0.002
	HDLc, mmol/L	-0.28	<0.001	-0.28	<0.001	0.40	<0.001
	Non-HDLc, mmol/L	-0.03	0.59	-0.01	0.86	-0.27	<0.001
	Triglycerides, mmol/L	0.21	<0.001	0.24	<0.001	-	-
	Statin, %	0.07	0.13	0.07	0.13	-0.19	<0.001
Substance use	Alcohol, %	0.12	0.007	-0.07	0.16	0.09	0.06
	Current smoking, %	-0.05	0.25	-0.02	0.67	-0.01	0.88
	Glucose, mmol/L	0.21	<0.001	-	-	-0.23	<0.001
Inflammation	CRP, mg/L	0.05	0.27	0.07	0.14	-0.10	0.03
Donor demographics	Age, y	-0.01	0.87	0.01	0.94	-0.02	0.66
	Male sex, %	0.03	0.52	0.03	0.55	-0.02	0.63
Renal allograft function	Serum creatinine, µmol/L	-0.02	0.79	0.01	0.88	-0.10	0.03
	Creatinine clearance, mL/min	0.10	0.02	0.09	0.05	-0.08	0.10
	Creatinine excretion, mmol/24h	0.11	0.02	0.10	0.02	-0.02	0.61
	Proteinuria, g/24h	0.02	0.69	0.06	0.23	-0.09	0.04
Acute rejection treatment	High-dose corticosteroids, %	-0.08	0.10	-0.05	0.28	-0.01	0.88
	Other rejection therapy, %	0.04	0.38	0.03	0.48	-0.04	0.36
CMV status	CMV seropositivity before transplantation	-0.03	0.47	0.04	0.42	-0.01	0.76
	Recipient, %	-0.01	0.94	0.01	0.82	-0.04	0.40
	Donor, %	0.05	0.23	0.05	0.23	-0.07	0.14
	Primary or secondary CMV infection, %	-0.05	0.27	-0.05	0.28	-0.05	0.31
	CMV seropositive at inclusion, %						
Immunosuppresion	Prednisolone dose, mg/d	0.09	0.06	0.10	0.03	-0.09	0.05
	Cyclosporine, %	-0.03	0.50	-0.04	0.40	-0.07	0.13
	Trough-level, µg/L	0.05	0.37	0.05	0.40	-0.04	0.47
	Tacrolimus, %	0.07	0.11	0.08	0.07	0.05	0.24
	Trough-level, µg/L	-0.11	0.39	-0.07	0.58	0.17	0.17
	Mycophenolate mofetil, %	0.01	0.90	0.01	0.87	0.06	0.18
	Azathioprine, %	0.04	0.35	0.04	0.37	-0.05	0.32

Beta's are reported as standardized Beta's. Significant effect modification by gender is indicated by an asterisk (*) for BMI and post transplant weight gain for McAuley's index.

a) CVD: Cardiovascular Disease
b) MI: Myocard Infarct
c) TIA: Transient Ischemic Attack
d) CVA: Cerebrovascular Accident

Table 3 shows the factors that were independently associated with the insulin resistance indexes. Independent determinants for all three indexes were both obesity (BMI) and central obesity (waist-to-hip ratio), prednisolone dose, male gender, recipient age, HDL-cholesterol, and total cholesterol. Other determinants differed among the indexes. The univariate interaction between gender and BMI did not remain statistically significant in multivariate analyses for McAuley's index. Triglyceride concentrations, and creatinine clearance were independently associated with HOMA and fasting insulin concentrations respectively.

Regression analyses were repeated without inclusion of lipid concentrations and use of statins, because triglyceride concentrations are incorporated in McAuley's index (Model 2). This could bias the analyses in Model 1 towards an association with HDL-cholesterol, owing to the close relationship between HDL-cholesterol and triglycerides. However, the adjusted analyses showed similar results as the primary analysis, with the distinction exception that tacrolimus use was independently associated with HOMA. Tolerance coefficients indicated that the assumption of co-linearity was not violated in all models.

Table 3. Multivariate regression analyses of independent determinants of fasting insulin, HOMA, and McAuley's index.

		Fasting Insulin		HOMA		McAuley's index	
	Characteristics	β	P-value	β	P-value	β	P-value
Model 1							
	Age, years	-0.21	<0.001	-0.20	<0.001	0.16	<0.001
	Male gender	-0.20	<0.001	-0.15	0.002	0.19	<0.001
	BMI, kg/m²	0.26	<0.001	0.28	<0.001	-0.19	<0.001
	Waist to hip ratio	0.29	<0.001	0.27	<0.001	-0.33	<0.001
	Total cholesterol, mmol/L	-0.09	0.02	-0.13	0.004	-0.20	<0.001
	HDL cholesterol, mmol/L	-0.11	0.001	-0.09	0.04	0.34	<0.001
	Triglyceriden, mmol/L			0.09	0.05		
	Statin use, %					-0.16	<0.001
	Creatinine clearance, ml/min	0.08	0.048				
	Prednisolone dose, mg/day	0.10	0.015	0.09	0.026	-0.07	0.045
	R², %	30		29		39	
Model 2							
	Age, years	-0.24	<0.001	-0.22	<0.001	0.21	<0.001
	Male gender	-0.18	<0.001	-0.15	0.002	0.15	0.001
	BMI, kg/m²	0.26	<0.001	0.28	<0.001	-0.25	0.001
	Waist to hip ratio	0.34	<0.001	0.35	<0.001	-0.45	<0.001
	Creatine clearance, ml/min	0.09	0.038			0.08	0.038
	Prednisolone dose, mg/day	0.10	0.011	0.09	0.038		
	Tacrolimus use, %			0.08	0.049		
	R², %	27		27		31	

Beta's are reported as standardized beta's.
Model 1 is the full model. Model 2 was repeated without lipid concentrations and without statin use.

Conclusions

We investigated which recipient- and transplant-related factors were associated with insulin resistance in renal transplant recipients long-term after transplantation. As judged from the magnitude of the standardised regression coefficients, the most important and consistent factors associated with insulin resistance were BMI, waist-to-hip ratio, and current prednisolone dose. Also independently associated were male gender, recipient age, HDL-cholesterol, total cholesterol, and renal function. No independent associations were present for many transplant related factors such as donor characteristics, acute rejection treatment, and CMV seropositivity.

Our results indicate that obesity is the most important determinant of insulin resistance in renal transplant recipients, as it is in the general population.[22] This is important as trends in the epidemic of obesity among the general population are paralleled by the renal transplant population.[23] The majority (60%) of transplant recipients in the United States are currently overweight or obese at time of transplantation. Furthermore, many renal transplant recipients suffer from a ten percent weight gain after transplantation,[24] predominantly because of an increase in fat mass.[25]

Not only was overall obesity (BMI) a determinant of insulin resistance, we also found that the distribution of obesity (waist-to-hip ratio) is an independent determinant of insulin resistance, even after adjustment for overall obesity. A possible explanation for waist-to-hip ratio as determinant in addition to BMI could be because it better reflects abdominal fat that is thought to cause insulin resistance.

Another important determinant of insulin resistance was prednisolone dose. Midtvedt et al[26] showed that lowering prednisolone dose towards 5 mg/day decreased insulin resistance. Our data are in accordance with this because recipients using <10 mg/day compared to 10 mg/day were less insulin resistant. Studies indicate that tacrolimus is an independent risk factor for post-transplant diabetes,[27] but in our study only in Model 2 HOMA was associated with tacrolimus use. This could be because tacrolimus is beta-cell toxic, decreasing insulin secretion more than increasing insulin resistance.[28]

Some characteristics that were not associated with insulin resistance in our study are also important to note. Especially transplant-related characteristics have been suggested to explain why transplant recipients are more insulin resistant than BMI and age matched non-transplant subjects. CMV is of particular interest in this respect, because shortly after transplantation active CMV disease has been associated with insulin resistance.[5] We found that CMV seropositivity does not determine insulin resistance longer after transplantation. Possibly CMV infection shortly after hospitalization reduces physical activity of the recipient either because of malaise or because of the fact that the obligatory intravenous treatment with ganciclovir restricts mobility of a patient. Also, CMV causes inflammation, and inflammation has been shown to cause insulin resistance.[29]

We also found creatinine clearance positively associated with fasting insulin concentrations and McAuly's index. The positive association between insulin resistance and renal function is by itself not a new finding because glomerular filtration rate is known to increase under hyperinsulinemic conditions in non-transplanted kidneys.[30] Possibly this phenomenon is also present in transplanted kidneys.

Male gender was associated with less insulin resistance than female gender in multivariate analyses, but not in univariate analyses. This is a discrepancy which we can not fully explain. It should be noted that women

were more obese, but that BMI had a greater impact on McAuley's index in men than in women. This could explain why there was no gender difference in insulin resistance. However, the gender and BMI interaction did not retain statistical significance in the multivariate model. Physiologically, it has been suggested that a gender difference in insulin resistance could be due to different effects of sex hormones on body fat distribution and fat cell size.[31]

A limitation of the present study is that insulin resistance was not measured using the hyperinsulinaemic euglycaemic clamp technique, but with indexes based on fasting blood samples. However, the indexes used have recently been validated in renal transplant recipients.[10] Another limitation is that some known determinants of insulin resistance could not be taken into account, such as free fatty acids, birth-weight, or genetic factors, because we did not have information on these variables.[21,32]

It is important to known what the predominant determinants of insulin resistance are to reduce insulin resistance in renal transplant recipients. With reduction of insulin resistance, renal transplant recipients could possibly suffer from less cardiovascular morbidity and less chronic transplant dysfunction.[33] In analogy to the general population, reduction of insulin resistance could perhaps be achieved by weight management. Furthermore, tapering of prednisolone dose could decrease insulin resistance as well, but prednisone withdrawal negatively impacts long-term graft failure.[34]

In conclusion this study shows that obesity, the distribution of obesity, and prednisolone dose are predominant determinants of insulin resistance long-term after transplantation. Secondly, male gender, recipient age, HDL-cholesterol and total-HDL-cholesterol concentrations, and creatinine clearance were also independent determinants of insulin resistance. Transplant related characteristics such as CMV status, did not determine insulin resistance long-term after renal transplantation. Insulin resistance after renal transplantation, and perhaps cardiovascular mortality, may be managed favorably by weight reduction and prednisolone dose reduction.

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Chapter 5

The metabolic syndrome is associated with impaired renal allograft function; not all component criteria contribute equally.

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Abstract

Chronic renal transplant dysfunction (CRTD) remains a leading cause of renal allograft loss. Evidence suggests that immunological and ischemic insults are mainly associated with CRTD that occurs within the first year after transplantation, whereas non-immunological insults are predominantly associated with CRTD beyond the first year. Several cardiovascular risk factors, such as obesity, dyslipidemia, hypertension, and diabetes mellitus have been identified as important non-immunological risk factors for CRTD. These risk factors constitute the metabolic syndrome (MS). As renal allograft function is a surrogate marker of renal allograft loss, we investigated the association of MS with impairment of renal allograft function beyond the first year after transplantation in a cross-sectional study of 606 renal transplant outpatients. MS was defined using the definition of the National Cholesterol Education Program. Renal allograft function was assessed as the 24h-urinary creatinine clearance. A total of 383 out of 606 patients (63%) suffered from MS at a median time of 6 years [2.6-11.4] posttransplant. Presence of MS was associated with impaired renal allograft function beyond 1-year posttransplant (-4.1 mL/min, 95%CI [-7.1, -1.1]). The impact of MS did not change appreciably after adjustment for established risk factors for CRTD (-3.1 mL/min, 95%CI [-6.0, -0.2]). However, not all component-criteria of MS contributed equally. Only systolic blood pressure and hypertriglyceridemia were independently associated with impaired renal allograft function beyond 1-year posttransplant in multivariate analyses.

Introduction

Despite impressive improvements in short-term renal allograft survival, long-term allograft survival remains behind.[1] Approximately half of all cadaveric renal allografts are lost within 10 to 12 years after transplantation. A leading cause of late allograft loss is chronic renal transplant dysfunction (CRTD).[2] The development of CRTD is clinically characterized by a slow but steady decline in allograft function over time, albeit that onset and progression of CRTD may vary substantially among individuals.[3] To prevent or attenuate the development of chronic renal transplant dysfunction, a better understanding of its pathophysiology is imperative.[4] Similar to the process of atherosclerosis, CRTD is thought to result from a continuous 'response to injury' from various immunological and non-immunological insults.[5,6] Several clinical and histopathological studies have demonstrated a time-dependency of risk factors.[7-11] Immunological and ischemic factors are mainly associated with CRTD occurring within the first year posttransplant, whereas non-immunological factors such as donor age, recipient body size, and calcineurin-inhibitor toxicity are predominantly associated with CRTD beyond the first year.[7-11] In the past years, several traditional risk factors for cardiovascular disease, i.e. obesity, dyslipidemia, hypertension, and (posttransplant) diabetes mellitus have also been identified as important non-immunological risk factors for CRTD.[12] These risk factors constitute the metabolic syndrome (MS).[13] It is therefore tempting to speculate that the metabolic syndrome is a prominent risk factor for CRTD beyond the first year.[12] As renal allograft function is a surrogate marker of renal allograft loss,[14] we investigated the association of the metabolic syndrome with impairment of renal allograft function beyond the first year after transplantation in a cross-sectional study.

Materials and methods

Study design and patients

The Institutional Review Board approved the study protocol (METc 2001/039), which was incorporated in the outpatient follow-up of the Groningen Renal Transplant Program. The outpatient follow-up constitutes a continuous surveillance system in which patients visit the outpatient clinic with declining frequency, in accordance with American Transplantation Society guidelines i.e. ranging from twice a week just after hospital discharge to twice a year long-term after transplantation.[15] Between August 2001 and July 2003, all adult allograft recipients who survived the first year after transplantation with a functioning allograft (1-year posttransplant was considered baseline) were eligible to participate at their next visit to the outpatient clinic (*index date*). Patients who had received a combined transplantation (i.e. kidney/pancreas or kidney/liver) were invited to participate as well. Patients with known or

apparent systemic illnesses at index date (e.g. malignancies or opportunistic infections) were excluded from participation. A total of 606 out of 847 (72%) eligible renal transplant recipients signed written informed consent. Funding sources had neither a role in the collection and analysis of data, nor in the submission and publication of the manuscript.

Recipient and transplant characteristics

Relevant donor, recipient, and transplant characteristics were extracted from the Groningen Renal Transplant Database. This database holds information of all renal transplantations that have been performed at our center since 1968. In addition, the database contains the outcomes of outpatient visits (e.g. body weight, serum creatinine, creatinine clearance based on 24h urine collection, and proteinuria) at 1 month, 6 months, 1 year, 2 years, and each following fifth year after transplantation. Extracted from the database were donor and recipient age, gender, ethnicity, primary renal disease, type and duration of dialysis therapy, type and date of transplantation, number of previous transplants, cold and warm ischemia times, number of human leukocyte antigen (HLA) mismatches, delayed graft function (i.e. days of posttransplant oliguria), cytomegalovirus (CMV) status, type of acute rejection treatment, body weight at baseline, 24h-creatinine clearance, and proteinuria at baseline. Smoking status at index date was obtained through a self-report questionnaire[16] that had been sent to the participants via mail.

Standard immunosuppression consisted of the following. Azathioprine (100 mg/d) and prednisolone from 1968 until 1989. Cyclosporine standard formulation (Sandimmune, Novartis; 10 mg/kg; trough-levels of 175 - 200 µg/L in first 3 months, 150 µg/L between 3 and 12 months posttransplant, and 100 µg/L thereafter) and prednisolone (starting with 20 mg/d, rapidly tapered to 10 mg/d) from January 1989 until February 1993. Cyclosporine microemulsion (Neoral, Novartis; 10 mg/kg; trough-levels idem) and prednisolone from March 1993 until May 1996. Mycophenolate mofetil (Cellcept, Roche; 2 g/d) was added from May 1997 to date. Current medication was extracted from the medical record. Calcineurin-inhibitor nephrotoxicity was defined as the discontinuation of calcineurin-inhibitor use or the conversion from one calcineurin-inhibitor to the other between baseline and index date.

Measurements

Blood was drawn after an 8-12h overnight fasting period to determine serum creatinine, triglyceride, high-density lipoprotein cholesterol (HDLc), and plasma glucose concentrations. Serum creatinine concentrations were determined using the Jaffé-method, and serum triglycerides were determined with the GPO-PAP method (both on a MEGA AU 510, Merck Diagnostica, Darmstadt, Germany). HDLc was determined using the CHOD-PAP method on a Technikon RA-1000 (Bayer Diagnostics b.v.,

Mijdrecht, The Netherlands). Plasma glucose was determined by the glucose-oxidase method (YSI 2300 Stat plus, Yellow Springs, OH, USA). 24h-creatinine and protein excretions were determined from a 24h-urine sample. Total protein concentration was determined with the Biuret reaction (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany).

Waist circumference was measured midway the iliac crest and the 10th rib. Blood pressure was measured as the average of three automated (Omron M4; Omron Europe B.V., The Netherlands) measurements with 1-minute intervals after a 6-minute rest in supine position.

Definitions

The metabolic syndrome was defined using the working definition of the National Cholesterol Education Program Expert Panel (NCEP-ATP III).[17] In short, an individual has the metabolic syndrome, if he or she suffers from three or more of the following criteria: 1) a waist circumference > 102 cm in men and > 88 cm in women, 2) serum triglycerides ≥ 1.70 mmol/L, 3) serum HDLc < 1.03 mmol/L in men and < 1.29 mmol/L in women, 4) blood pressure ≥ 130/85 mmHg or use of antihypertensive medication, 5) fasting plasma glucose ≥ 6.1 mmol/L or use of antidiabetic medication (including insulin).

The American Diabetes Association (ADA) recently reduced the cut-off point for impaired fasting glucose to ≥ 5.5 mmol/L.[18] Therefore, we also analyzed prevalence of MS and the impact of MS on long-term allograft function using the new ADA definition.

Renal allograft function was assessed as the 24h-urinary creatinine clearance (24h-CrCl), i.e. the 24h-urinary creatinine excretion divided by the serum creatinine concentration. The 24h-creatinine clearance was not adjusted for body surface area (BSA) for the following reasons. First, renal transplant recipients often experience a ten percent weight gain and a change in body composition after transplantation, even beyond the first year after transplantation.[19-22] This is mainly due to an increase in body fat mass.[22] The resulting change in body surface area would confound the investigation of allograft function over time if allograft function was indexed for BSA. Second, if we indexed the dependent variable for BSA in linear regression analysis with MS as independent variable, bias could be introduced as BSA is most likely associated with MS.

Because of errors in the collection of 24h-urine, creatinine clearance may lack precision compared to serum creatinine-based equations such as the abbreviated MDRD- equation.[23] However, we preferred the 24h-urinary creatinine clearance as estimate of glomerular filtration rate (GFR) because of the following methodological consideration.[24] First, use of serum creatinine-based equations may be subject to systematic error over time as muscle mass decreases over time in renal transplant recipients.[25] As a result, serum creatinine-based equations may overestimate glomerular filtration rate progressively over time in transplant populations.[26-27] Second, if creatinine-based equations that also incorporate age and

weight are used in linear regression analyses as dependent variable together with MS as independent variable, bias could occur as both age and body weight are strongly associated with MS.[28] Third, the MDRD-equation implicitly adjusts for body surface area. Nonetheless, we repeated all analyses with estimated glomerular filtration rate as determined by the abbreviated MDRD-equation [i.e. GFR (mL/min per $1.73m^2$) = 186 x serum creatinine$^{-1.154}$ (mg/dL) x Age$^{-0.203}$ x 0.742 (if female)] to check the robustness of our findings.[29] Renal allograft function at 1-year posttransplant (serum creatinine and 24h-creatinine clearance) was considered baseline and extracted from the renal transplant database.

Statistical analyses

Analyses were performed with SPSS version 11.0 (SPSS Inc., Chicago, IL). Parametric parameters are expressed as mean ± standard deviation, whereas non-parametric parameters are expressed as median [interquartile range]. Student's t-test was used to compare means of parametric parameters between patients with and without MS, whereas the Mann-Whitney test was used for non-parametric variables. The Pearson χ^2-test was used to test distributions of categorical variables among patients with and without MS. The null hypothesis was defined as no difference between patients with and without the metabolic syndrome. A chance for wrongly rejecting the null hypothesis on repeated testing (the alpha or type 1 error) of 5% or less indicated statistical significance.

To study the association of MS with impaired renal allograft function beyond 1-year posttransplant, we performed a linear regression analysis (model 1) with presence of MS, renal allograft function at baseline, and time elapsed since baseline as explaining (independent) variables for renal allograft function at index date (dependent variable). This way, we aimed to adjust associations for the fact that transplant recipients were included at different time points in our study, and for the fact that patients started-off with different allograft function at baseline. Consequently, the model may allow interpretation of independent associations with allograft function at index date, as associations with the slope of renal allograft function over time.

To study whether MS was independently associated with impaired renal allograft function beyond 1-year posttransplant, we ran multiple linear regression analyses (model 2). Presence of MS, baseline allograft function, time elapsed since baseline, and all risk factors for CRTD from table 2 that showed at least a tendency ($p \leq 0.10$) to be univariately associated with renal allograft function at index date (dependent variable) were all entered together as putative explaining (independent) variables. Variables that were not significantly associated with renal allograft function at index date were subsequently excluded from the model (so-called *backward selection*). This method is most suitable for cross-sectional data.

To study which component-criteria were most strongly associated with impairment of renal allograft function at index date, we substituted the metabolic syndrome for its component-criteria and repeated the analysis with all component-criteria entered simultaneously (model 3) as well as separately. To compare the effects of MS-criteria amongst each other, we standardized the regression coefficients by multiplying regression coefficients with the standard deviation of the criterion divided by the standard deviation of the dependent variable. The standardized regression coefficient is a standardized score (like a Z-score) and is a way to compare the relative contributions of covariates amongst each other.

To test whether the models were appropriate and whether statistical assumptions for use of linear regression analyses were met, the models were tested for collinearity and interaction between independent variables, overall regression and lack-of-fit with ANOVA. Residuals were tested for the normality assumption.

Results

A total of 383 out of 606 patients (63%) suffered from the metabolic syndrome at a median time of 6 years posttransplant [2.6-11.4]. If we had used the new ADA definition for impaired fasting glucose, a total of 388 (64%) would have suffered from MS.

Table 1. Mean or median level of MS-criteria and percentage of patients with and without the metabolic syndrome meeting MS-criteria[a]

Criterion	With MS n=383		Without MS n=223	
	Level	% meeting criterion	Level	% meeting criterion
Waist circumference, cm				
Male	105 ± 12	64%	92 ± 10	9%
Female	99 ± 13	82%	84 ± 11	22%
Serum triglycerides, mmol/L	2.29 [1.83 - 2.99]	82%	1.40 [1.10 - 1.65]	22%
Serum HDLc, mmol/L				
Male	0.74 [0.64 - 0.87]	88%	1.05 [0.85 - 1.20]	22%
Female	0.91 [0.77 - 1.07]	81%	1.25 [1.04 - 1.41]	28%
Blood pressure, mmHg				
Systolic	155 ± 23	91%	149 ± 22	83%
Diastolic	90 ± 10		89 ± 10	
+ use of antihypertensives	-	100%	-	95%
Plasma glucose, mmol/L	4.2 [3.9 - 4.7]	15%	4.0 [3.7 - 4.3]	0.9%
+ use of antidiabetic drugs	-	26%	-	1.3%

[a]MS-criteria: 1) waist circumference > 102 cm in men; > 88 cm in women 2) serum triglycerides ≥ 1.70 mmol/L 3) serum HDLc < 1.03 mmol/L in men, < 1.29 mmol/L in women, 4) blood pressure ≥ 130/85 mmHg or use of antihypertensive medication, 5) fasting plasma glucose ≥ 6.1 mmol/L or use of antidiabetic medication.

Table 1 shows the average or median level of MS-criteria and the percentage of patients meeting criteria for both patients with and without the metabolic syndrome. Women met the waist circumference-criterion more frequently than men, even if they did not have the metabolic syndrome. Patients with and without the metabolic syndrome had almost comparable rates of hypertension. Furthermore, a quarter of patients with the metabolic syndrome had impaired fasting glucose or diabetes mellitus after transplantation, whereas only one percent of patients without the metabolic syndrome suffered from these conditions. Patients with the metabolic syndrome were older, more often female, and suffered more frequently from polycystic renal disease than patients without the metabolic syndrome (table 2). Patients with the metabolic syndrome were more frequent users of cyclosporine, statins, angiotensin converting enzyme (ACE)-inhibitors, and angiotensin II (AII)-antagonists at index date. Our results indicate that patients with the metabolic syndrome weighed on average 9-kg more at index date than patients without the metabolic syndrome. Moreover, patients with the metabolic syndrome at index date weighed already more at 1-month posttransplant and showed larger posttransplant weight gain. In addition, women showed larger posttransplant weight gain than men (14±15 vs. 11±12%, p=0.01).
Renal allograft function at baseline as well as time elapsed between baseline and index date, were comparable for patients with and without the metabolic syndrome. In spite of this, patients with the metabolic syndrome had a significantly lower 24h-creatinine clearance at index date (table 3). We visualized these data in figure 1. The association of MS with impaired renal allograft function at index date became even stronger after adjustment for baseline allograft function and time elapsed since baseline in linear regression analysis (table 4, model 1). If we had used the new ADA definition for impaired fasting glucose, the impact of MS on long-term renal allograft function in model 1 would have become even stronger (-4.4 mL/min 95% CI [-7.4, -1.4], p=0.004).

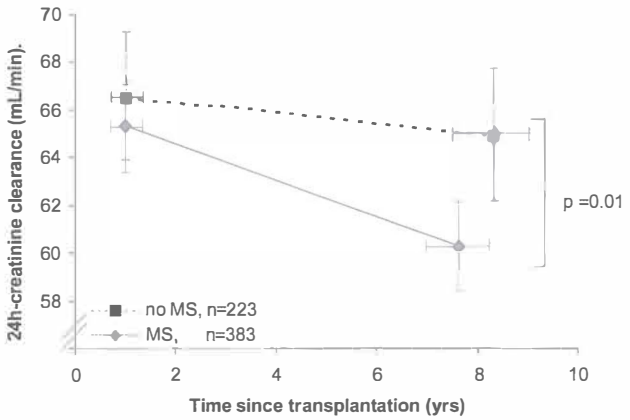
Table 2. Demographic and transplant characteristics of patients with and without the metabolic syndrome.

	With MS n=383	Without MS n=223	p-value
Age, yr			
recipient	53 ± 12	49 ± 13	<0.001
donor	37 ± 16	36 ± 15	n.s.
Male gender, %			
recipient	50	62	<0.01
donor	57	50	n.s.
Ethnicity of recipient, %	97	96	n.s.
Caucasian			
Primary renal disease, %			
primary glomerular disease	27	30	n.s.
glomerular disease of vascular/autoimmune origin	6	8	n.s.
tubular interstitial disease	15	17	n.s.
polycystic renal disease	21	12	<0.01
dysplasia and hypoplasia	3	4	n.s.
renovascular disease	5	6	n.s.
diabetes mellitus	3	5	n.s.
other or unknown cause	21	18	
Prior dialysis modality, %			
hemodialysis	54	52	
peritoneal dialysis (CAPD)	39	39	n.s.
none	7	10	
Prior dialysis duration, mo	29 [15 - 50]	25 [11 - 47]	0.03
Transplantation type, %			
post-mortem donor	85	79	
living donor	12	17	n.s.
combined transplantation	3	4	
Number of previous transplants, %			
0	90	89	n.s.
1 or more	10	11	
Ischemia times			
warm ischemia times, min	36 [30 - 45]	35 [30 - 43]	n.s.
cold ischemia time, h	22 [15 - 28]	21 [14 - 26]	n.s.
HLA mismatches			
HLA-AB	1 [0 - 2]	1 [0 - 2]	n.s.
HLA-DR	0 [0 - 1]	0 [0 - 2]	n.s.
Delayed graft function, days of oliguria	0 [0 - 0]	0 [0 - 0]	n.s.
Acute rejection treatment, %			
high dosage corticosteroids	30	33	
antilymphocyte antibodies	15	12	n.s.
CMV seropositivity, %			
donor	56	51	n.s.
recipient	50	44	n.s.
Weight, kg			
at 1 month posttransplant	70 ± 12	65 ± 11	<0.0001
at 1y posttransplant (baseline)	78 ± 13	70 ± 12	<0.0001
at index date	80 ± 13	71 ± 11	<0.0001
Height, m	1.72 ± 0.10	1.73 ± 0.10	n.s
Smoking, %			
currently	21	25	
formerly	41	43	n.s.
never	38	32	
Use of ACE-inhibitor or AII-antagonist, %	37	27	<0.01
Use of statin at index, %	55	40	<0.001

	With MS n=383	Without MS n=223	p-value
Calcineurin inhibitor, %			
cyclosporine	68	58	
tacrolimus	12	17	0.03
Cyclosporine trough-level, µg/L	106 [74 - 140]	110 [83 - 138]	n.s.
Tacrolimus trough-level, µg/L	8.3 [6 - 9.7]	8.9 [6 - 11.1]	n.s.
Proliferation inhibitor, %			
azathioprine	33	32	
mycophenolate mofetil	40	44	n.s.
Prednisolone dose, mg/day	10 [7.5 - 10]	10 [7.5 - 10]	n.s.

To determine whether MS was independently associated with impaired renal allograft function, we performed a backward linear regression analysis with renal allograft function at index date as the dependent variable. Included covariates (that were univariately associated with the dependent variable with a p-level ≤ 0.10) were MS, time elapsed since baseline, baseline renal allograft function, donor and recipient age, recipient gender, glomerular disease of vascular or autoimmune origin, duration of dialysis therapy, cold ischemia time, number of HLA-DR mismatches, days of posttransplant oliguria (delayed graft function), acute rejection treatment with high-dosage corticosteroids, calcineurin-inhibitor toxicity, cyclosporine trough-level, use of mycophenolate mofetil at index date, proteinuria, and use of ACE-inhibitors or AII-antagonists.

Figure 1. Course of 24h-creatinine clearance over time since transplantation of renal transplant recipients with and without metabolic syndrome (MS) at index date.



Data given as means of time and renal allograft function with 95% confidence interval. Renal allograft function at index date differs significantly for patients with and without MS (p=0.01).

Table 3. Renal allograft function at baseline and index date for patients with and without the metabolic syndrome.

	With MS N=383	without MS n=223	p-value
Time elapsed between baseline and index date, yr	4.8 [1.8-2.9]	5.6 [1.6-11.3]	n.s.
Serum creatinine, µmol/L			
baseline	134 [112-168]	130 [110-156]	n.s.
index date	137 [114-172]	130 [110-150]	0.02
24h-creatinine clearance, mL/min			
baseline	65±20	66±19	n.s.
index date	60±23	65±22	0.01
Urinary protein excretion, g/24h			
baseline	0.2 [0-0.5]	0.2 [0-0.4]	n.s.
index date	0.2 [0-0.5]	0.2 [0-0.5]	n.s.

n.s.= not significant

Backward elimination revealed that presence of MS was associated with impaired long-term renal allograft function independent of baseline allograft function, time elapsed since baseline, patient gender, donor age, number of HLA-DR mismatches, cyclosporine trough-level, and proteinuria (table 4, model 2). With a regression coefficient of -3.1 mL/min (95%CI [-6.0, -0.2]), the clinical impact of MS on long-term allograft function was e.g. comparable to the effect of cyclosporine trough-level (regression coefficient of -4.4 mL/min; i.e. average cyclosporine trough-level of 147 µg/L x -0.03 mL/min per µg/L). If we had used the new ADA definition for impaired fasting glucose, the impact of MS on long-term renal allograft function in model 2 would have become stronger (-3.4 mL/min 95% CI [-6.3, -0.6], p=0.02). Surprisingly, we found the number of HLA-DR mismatches associated with improvement of long-term renal allograft function. This association reflected the better performance of the living-unrelated transplant group, which had significantly more mismatches in HLA-DR than other groups, as it disappeared after adjusting the regression analysis for living-unrelated transplantation (data not shown).

To investigate which of the component-criteria constituting the metabolic syndrome contributed most to impairment of allograft function at index date, we substituted the metabolic syndrome for its separate continuous criteria, i.e. waist circumference (WC), systolic (SBP) and diastolic blood pressure (DBP), log-transformed fasting serum triglycerides (log_TG), log-transformed HDLc (log_HDLc), and log-transformed plasma glucose (log_gluc). All component-criteria, with the exception of HDLc, correlated positively with each other (data not shown). The third model (table 4, model 3) revealed that serum triglycerides (β=-2.5x10^{-12} ml min^{-1} per mmol L^{-1}, 95%CI [10^{-20}, 5.0x10^{-5}]) and systolic blood pressure (β=-0.13

ml min^{-1} per mmHg, 95%CI [-0.21, -0.05]) were strongly associated with impaired renal allograft function at index date. Decreased serum HDLc only showed a tendency towards impaired allograft function at index date.

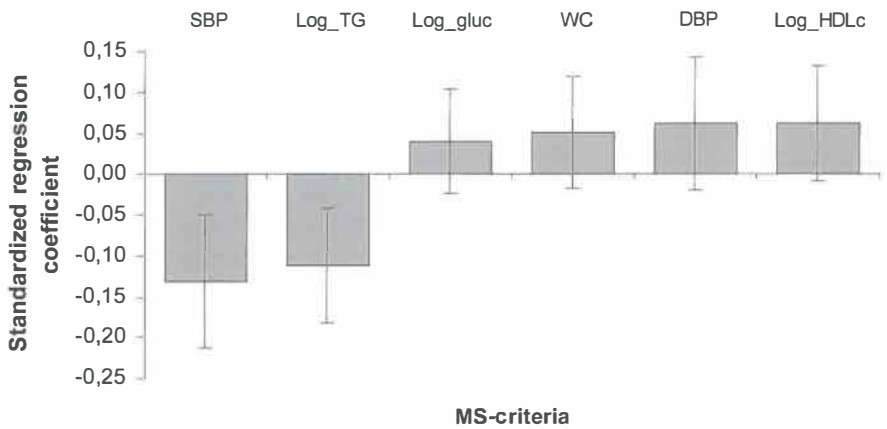
Table 4. Risk factors for impaired renal allograft function from (backward) linear regression analyses with 24h-creatinine clearance at index as dependent variable.

Model		B	95% CI	p-value
Model 1	Constant			
Creatinine clearance baseline, mL/min		0.70	0.62, 0.77	<0.0001
Time elapsed since baseline, yr.		-0.52	-0.75, -0.29	<0.0001
Metabolic syndrome, y/n		-4.1	-7.1, -1.1	0.007
R^2=0.36, F-statistic=111.4, total df=605, p<0.0001				
Model 2	Constant	34.6	26.3, 42.9	<0.0001
Creatinine clearance baseline, mL/min		0.65	0.57, 0.72	<0.0001
Proteinuria, g/24h		-4.7	-6.2, -3.1	<0.0001
Time elapsed since baseline, yr.		-0.63	-0.90, -0.36	<0.0001
Donor age, yr.		-0.2	-0.3, -0.1	<0.001
Cyclosporine trough-level, µg/L		-0.03	-0.06, -0.01	0.005
Patient gender, m/f		3.4	0.6, 6.3	0.02
Number of HLA-DR mismatches		2.8	0.3, 5.3	0.03
Metabolic syndrome, y/n		-3.1	-6.0, -0.2	0.04
R^2=0.43, F-statistic=54.0, total df=590, p<0.0001				
Model 3 (= model 2 with MS substituted for its component-criteria)				
Waist circumference, cm		0.08	-0.03, 0.20	0.11
Log_triglycerides, mmol/L		-11.6	-20.0, -4.3	0.002
Log_HDL cholesterol, mmol/L		11.0	-1.4, 23.4	0.08
Systolic blood pressure, mmHg		-0.13	-0.21, -0.05	0.002
Diastolic blood pressure, mmHg		0.14	-0.04, 0.33	0.14
Log_glucose, mmol/L		9.6	-5.4, 24.7	0.21
Remaining covariates of model 2 did not change appreciably in model 3				
R^2=0.45, F-statistic 33.4, total df=589, p<0.0001				

Surprisingly, WC, DBP, and log_gluc showed tendency to be associated with improvement of renal allograft function at index date (model 3). As this was in contrast with expectation, we analyzed the impact of each criterion separately (instead of simultaneously) in model 3. Higher serum triglycerides and systolic blood pressure remained significantly associated with impairment of renal allograft function, whereas waist circumference (-0.01 mL/min per cm, p=0.79), diastolic blood pressure (-0.06 mL/min per mmHg, p=0.44), and plasma glucose (-2.8 mL/min per mmol/L, p=0.71) remained not-significantly associated, albeit with impaired rather than improved renal allograft function when analyzed separately. The substitution of MS for its component-criteria did not change the effect of other covariates appreciably (data not shown). To

compare the effects of MS-criteria amongst each other, we standardized the regression coefficients by multiplying regression coefficients with the standard deviation of the criterion divided by the standard deviation of the dependent variable (see figure 2). To test the robustness of our findings, all analyses were repeated using the abbreviated MDRD-equation. Use of the abbreviated MDRD-equation did not change our findings essentially. MS was associated with a 2.6 mL/min per 1.73 m^2, 95% CI [-4.5, -0.8], p=0.007 decrease of GFR as estimated by the abbreviated MDRD-equation. In multivariate analysis, MS was associated with a decrease in long-term allograft function of 2.3 mL/min per 1.73 m^2, 95% CI [-4.0, -0.5], p=0.01. Furthermore, we repeated the analyses after exclusion of renal transplant recipients that had received a combined transplantation (15 kidney/pancreas and 5 kidney/liver transplantations) and after exclusion of recipients with pretransplant diabetes mellitus (22 type-1 and one recipient with type-2 diabetes). These secondary analyses did not alter our findings also (data not shown).

Figure 2. Standardized regression coefficients of MS-criteria. Data shown as means with



95% confidence interval. Standardization was done by multiplying the regression coefficient of a component-criteria with the quotient of the standard deviation of that component-criterion and the standard deviation of 24h-creatinine clearance at index date.

Discussion

This study demonstrates that the metabolic syndrome is independently associated with impaired renal allograft function beyond the first year after transplantation. As renal allograft function is a well-established surrogate estimate of long-term renal allograft survival,[14,30] it is in line with the hypothesis that MS can accelerate the development of CRTD. The pathogenetic concept that a 'metabolic milieu' may modify the process of chronic transplant dysfunction has been established previously by Hannah Valantine et al[31] in heart transplant recipients. This study is the first to use a consensus-definition of the metabolic syndrome in a transplant population. The merit of understanding that a clustering of already-established risk factors for CRTD constitutes the metabolic syndrome is not a matter of mere semantics. The metabolic syndrome is considered to be more than the sum of its current component-criteria.[32] The metabolic syndrome is associated with an array of other cardiovascular risk factors such as coagulation abnormalities, chronic inflammation, increased oxidative stress, endothelial dysfunction, and insulin resistance.[33,34] Consequently, the identification of the metabolic syndrome as a risk factor for chronic renal transplant dysfunction may yield new treatment modalities to prevent or attenuate it.

Not all component-criteria of the metabolic syndrome, however, contributed equally to the impairment of long-term renal allograft function. Only systolic blood pressure and hypertriglyceridemia were independently associated with impaired renal allograft function. These findings were expected as systolic blood pressure and hypertriglyceridemia have been associated with renal dysfunction in the general population as well.[35,36] Decreased HDLc only showed a tendency towards impairment of renal allograft function. The tendency of waist circumference, diastolic blood pressure, and plasma glucose to be associated with improvement of long-term renal allograft function were in contrast with expectation. However, when these component-criteria were analyzed separately, each component-criterion was associated negatively, albeit not-significantly, with renal allograft function. The fact that the direction of the associations of these three factors appeared positively associated with renal allograft function in model 3, must therefore be interpreted as inherent to the multivariate model in which all component-criteria are entered simultaneously to adjust for the effects of each other.

We found other risk factors for CRTD independently associated with impaired long-term allograft function as well. Not surprisingly, renal allograft function at 1-year posttransplant (baseline), and time elapsed since baseline were strongly associated with impaired renal allograft function. Increasing donor age was associated with impaired allograft function as well. This confirms other studies that identified donor age as a risk factor for late allograft survival.[8,9] Donor age may reflect the

functional transplanted nephron mass that constitutes the functional reserve or redundancy to buffer cumulative damage to nephrons from immunologic and non-immunologic insults. In addition, we found women, with men as reference, to have impaired long-term allograft function. Halloran et al[38] found female recipients at risk for impaired renal allograft function as well. According to our data, women were more at risk for posttransplant weight gain and the metabolic syndrome than men. If women suffered more frequently from the metabolic syndrome, it is likely that they also suffer more frequently from MS-associated risk factors (e.g. insulin resistance) for which our analyses did not adjust. Several studies reported larger posttransplant weight gain in women.[19-21] Women seem also more resistant to dietary intervention to reduce posttransplant obesity and hyperlipidemia.[39] Thus, the larger posttransplant weight gain might indicate a greater susceptibility of women to side effects of immunosuppressants such as corticosteroids. Immunosuppressants are known to contribute to MS-criteria.[40-42] However, we found no association between MS and daily or cumulative prednisolone dose for maintenance therapy or with high-dose corticosteroids for acute rejection treatment.

A recent histopathological study suggested that chronic calcineurin-inhibitor nephrotoxicity is the predominant cause of late CRTD.[10] Our data indicate that cyclosporine trough-level was associated with impairment of long-term allograft function independent from MS and its components. However, the association likely reflects the acute renal hemodynamic effects of cyclosporine at time of measurement rather than chronic dose-dependent nephrotoxicity.[43] We tried to account for calcineurin-inhibitor nephrotoxicity by adjusting our model for the discontinuation or conversion of calcineurin-inhibitor between baseline and index date. In our hospital, clinical or histopathological signs of chronic nephrotoxicity are the predominant motive for the discontinuation or conversion of calcineurin-inhibitors. However, backward elimination did not retain this parameter in the regression model. Furthermore, we found proteinuria associated with impaired long-term allograft function. Proteinuria is an established risk factor for both graft and patient survival.[44] Finally, we found neither acute rejection treatment, nor HLA-mismatches associated with lower long-term allograft function. Overall, our findings support the evidence that non-immunological insults, such as donor age, recipient body size, and calcineurin-inhibitor effects rather than immunological and ischemic insults are the predominant factors associated with CRTD beyond the first year after transplantation.

The present study has several limitations. As the study was cross-sectional in nature, directions of causality can not be inferred. Renal transplant recipients may well have developed dyslipidemia and hypertension at index date because of lower allograft function at baseline. In addition, the generalizability of our results to more racially diverse renal transplant

populations remains limited as our study population consisted almost solely of subjects of Caucasian ethnicity. Component-criteria of MS are weighed equally in the NCEP-definition, but both prevalence, impact, and even cut-off points of single MS component-criteria may vary substantially among ethnic groups.[45] Furthermore, it is likely that our study underestimated the effect of the metabolic syndrome on renal allograft function. First, cardiovascular risk factors, which cluster within the metabolic syndrome, may not only confer risk for the development of CRTD but also for cardiovascular mortality. Cardiovascular mortality is the most important cause of graft loss in addition to CRTD.[2] It is therefore conceivable that people with the highest susceptibility to consequences of MS already deceased before start of our study. As a result, the study may have suffered from a healthy-survivor bias. Second, the NCEP-definition does not incorporate use of statins in the definition. If we adjusted the definition for use of statins, an additional 5% of our population would have suffered from the metabolic syndrome. Third, the present study used renal allograft function as surrogate endpoint. Recently, Nankivell et al[10] showed that allograft function may underestimate the histopathological development of chronic renal allograft dysfunction.[10]

In conclusion, this study showed that the metabolic syndrome as defined by the NCEP is independently associated with impairment of long-term renal allograft function. However, not all component-criteria of the metabolic syndrome contributed equally. Longitudinal and intervention studies are needed to assess the full impact of the metabolic syndrome, its component-criteria, and MS-associated risk factors such as insulin resistance on long-term renal allograft loss. Future studies should also focus on the causes of the high prevalence of MS in this particular population.

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Chapter 6

The predictive value of renal vascular resistance for late renal allograft loss

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Abstract

The renal artery resistance index (RI), assessed by Doppler ultrasonography, was recently identified as a new risk marker for late renal allograft loss. This finding requires confirmation, since RI in that study was not measured at predetermined time points and since ultrasonography is operator-dependent. We investigated the predictive value of renal vascular resistance (RVR), a less operator-dependent method as assessed by mean arterial pressure divided by renal blood flow, for the prediction of recipient mortality and death-censored graft loss. RVR was compared to commonly used risk markers such as creatinine clearance, serum creatinine, and proteinuria (UProt) in 793 first-time cadaveric renal transplant recipients at predetermined time points after transplantation using ROC and Cox survival analyses. The present study showed that RVR is a prominent risk marker for recipient mortality and death-censored graft loss. However, the predictive value of RVR for recipient mortality owed mainly to the impact of mean arterial blood pressure. In contrast, RVR constituted more than the sum of its components for death-censored graft loss, but showed less predictive value than serum creatinine in univariate analysis. As the assessment of RVR is expensive and time-consuming, we believe that RVR holds no clinical merit for the follow-up of renal transplant recipients.

Introduction

Long-term renal allograft survival has not paralleled improvements made over the past three decades in short-term survival.[1] Almost half of all cadaveric allografts is lost within 10 years after transplantation. The predominant causes of late renal allograft loss are factual graft loss and recipient mortality, the latter often from cardiovascular disease.[2] To identify transplant recipients at risk for late renal allograft loss, who may benefit from preventive and therapeutic strategies at an early stage after transplantation, renal allograft function and proteinuria are applied in clinical practice as early risk markers.[3] Unfortunately, renal allograft function[4,5] and proteinuria[6] have limited predictive value. A pressing need exists for better early risk markers.[7]

The renal artery resistance index (RI), as determined by Doppler ultrasonography, was recently identified by Radermacher et al[8] as a new risk marker with high sensitivity and specificity for renal allograft loss. The high predictive value found in that study, has been debated to result from time bias.[9] RI in that study was measured within a cross-sectional time period ranging from 3 to 317 months after transplantation. The predictive value of RI might have increased if RI were measured closer to factual graft loss. Ideally, the predictive value of RI needs confirmation at predetermined time points after transplantation before widespread use of RI can be advocated. Moreover, implementation of RI in the follow-up of renal transplant outpatients might proof troublesome for many centers as the applicability of Doppler ultrasonography depends heavily on operators' skills.[10]

For the above mentioned reasons, we investigated the value of renal vascular resistance (RVR) for the prediction of recipient mortality and death-censored graft loss in a prospectively collected database of 793 first-time cadaveric renal transplantations. RVR is considered a less operator-dependent technique and is assumed to correlate well with RI as shown by experimental studies.[11] RVR was compared to commonly used risk markers such as 24-h urinary creatinine clearance, serum creatinine, and proteinuria at predetermined time points after transplantation.

Materials and Methods

Study design and population

The current study population was drawn from the Groningen Renal Transplant Database. This database holds information pertaining to the transplant procedure as well as status of all patients who have received a renal transplantation at the University Medical Center Groningen. After transplantation, all recipients are prospectively monitored within the renal transplant outpatient program. The outpatient program comprises a continuous surveillance system, in which patients visit the outpatient clinic with declining frequency in accordance with American Transplantation Society guidelines (i.e. ranging from twice a week just after hospital discharge to twice a year long-term after transplantation).[12] At 3 months, 6 months, 1 year, 2 years, and each subsequent fifth year after transplantation, routine visits to the outpatient clinic are extended by radio-isotope studies to measure glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and filtration fraction (FF, i.e. GFR/ERPF).

We investigated the predictive value of RVR at 1 year after transplantation in comparison to commonly used predictors such as 24-hour urinary creatinine clearance (CrCl), serum creatinine (SCreat), and proteinuria (UProt). RVR at 1 year is less confounded by delayed graft function, acute rejection, urological complications, or opportunistic infections than RVR measured at 3 and 6 months post-transplant, whereas 1 year post-transplant is still close enough to transplantation to start preventive therapy. The predictive values of RVR at 2 and 5 years after transplantation were investigated to study the extent to which the predictive value of RVR was influenced by time elapsed since transplantation.

From March 1968 to March 2004, the Groningen Renal Transplant Program performed a total of 2034 renal transplantations. For the present study, only patients who received a first time cadaveric renal transplant were eligible in order to obtain a homogenous study population. Excluded were 287 second-time transplants, and 225 living-donor or combined (kidney/pancreas or kidney/liver) transplantations. Furthermore, 340 transplants did not reach the first year post-transplant, and 389 patients had missing data on RVR at 1 year post-transplant, leaving a total of 793 consecutive first-time cadaveric renal transplants for analysis.

Measurement of renal function and renal vascular resistance

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by constant infusion of the radio-labeled tracers 125 I-iothalamate and 131I-hippurate respectively, as described previously.[13] In short, a priming solution containing 0.4 ml/kg body weight of the infusion solution (0.04 MBq of ^{125}I-iothalamate and 0.03 MBq of ^{131}I-hippurate)

plus an extra of 0.6 MBq of ^{125}I-iothalamate were given at 8:00 am after a blank blood sample was taken. This was followed by infusion of the solution at a rate of 12, 9, or 6 mL/h, depending on the level of renal function. To reach stable plasma concentrations of both tracers, a 2-hour stabilization period followed, after which baseline measurements started at 10:00 am. Clearances were calculated as (U*V) / P and (I*V) / P respectively. U*V represents the urinary excretion of the tracer, I*V represents the infusion rate of the tracer; P represents the tracer value in plasma at the end of each clearance period. This method corrects for incomplete bladder emptying and dead space, by multiplying the urinary clearance of ^{125}I-iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippuran. The filtration fraction (FF) was calculated as the ratio of GFR and ERPF and expressed as a percentage. This method has a day-to-day variation coefficient of 2.5% for GFR and 5% for ERPF.

Renal vascular resistance was calculated by dividing the mean brachial arterial pressure (MAP) by effective renal blood flow (ERBF). Mean arterial pressure, i.e. (systolic blood pressure + 2 x diastolic blood pressure) / 3, was determined from the average of three automated measurements (Dynamap, GE Medical Systems, Milwaukee, USA) taken with 5-minute intervals after an one-hour rest in semi-supine position. ERBF was calculated by dividing ERPF by (1-hematocrit).

Putative predictors of graft loss

The following transplant-related predictors for renal allograft loss were taken from the database: recipient and donor age and gender, primary renal disease, type and duration of dialysis therapy, date of transplantation, type of preservation solution, cold and warm ischemia times, number of human leukocyte antigen (HLA) mismatches, delayed graft function (i.e. days of post-transplant oliguria), cytomegalovirus (CMV) status, immunosuppressive maintenance therapy, and type of acute rejection treatment. The following recipient-related predictors were assessed at 1-year post-transplant and taken from the database as well: body weight and height, blood pressure, number of anti-hypertensive drugs, number of diuretics, use of angiotensin converting enzyme (ACE) inhibitors, serum creatinine, hematocrit, presence of post-transplant diabetes mellitus, 24-hour urinary creatinine clearance, proteinuria, GFR, ERPF, and FF. In addition, 24-hour urinary creatinine clearance and proteinuria at 2 years, and 5 years after transplantation were extracted.

Endpoints

The primary endpoints of the study were recipient mortality and death-censored graft loss. Death-censored graft loss was defined as return to dialysis or re-transplantation. The continuous surveillance system of the outpatient program, which operates in close collaboration with referral hospitals in our catchment area, ensures up-to-date information on

patient status. In case a recipient had moved out of the catchment area or lacked follow-up information, he or she was censored from time of last recorded visit.

Sensitivity analyses
We performed the following sensitivity analyses: 1) inclusion of the entire Groningen renal transplant population (including second time, combined or living-donor transplantations) and 2) the use of radio-isotope measured glomerular filtration rate in line of 24-h urinary creatinine clearance.

Statistical analyses
To compare RVR to other risk markers such as creatinine clearance, serum creatinine, and proteinuria, we performed the following statistical analyses.[3] First, RVR and other risk markers were tested for association with the endpoints. Student's t-test was used to compare means of parametric predictors, whereas the Mann-Whitney test was used for non-parametric predictors. The Pearson χ^2-test and Fisher's exact test (if single cell count was ≤ 5) were used to test the distribution of categorical predictors.

Second, the predictive test characteristics of RVR, creatinine clearance, serum creatinine, and proteinuria for renal allograft loss were assessed by plotting receiver operating characteristics (ROC) curves.[14] The area under the ROC curve (AUC) serves as a diagnostic test for graft loss irrespective of time to loss. The AUC's of RVR, 24-hour creatinine clearance, serum creatinine, and proteinuria were compared non-parametrically by the method of DeLong, DeLong, and Clarke-Pearson.[15] As a dichotomous endpoint such as graft loss ignores the fact that all recipients will develop graft loss eventually, and that it is actually time to graft loss which may be important to clinicians and patients, we also performed survival analyses. First, Kaplan-Meier survival plots were drawn to visualize recipient survival as well as death-censored graft survival for quartiles of RVR. Second, we performed univariate and multivariate Cox proportional hazard models.[16] In the first multivariate model, RVR was adjusted for the possible confounding effects of CrCl and UProt. In the second multivariate model, RVR was adjusted for CrCl, UProt, MAP, and ERBF. This way, we could investigate the merit of RVR as an interaction term of its components MAP and (1/)ERBF. Units were chosen in such a way that each hazard ratio represented a ten-percent increase in risk marker in order for hazard ratios to be better compared amongst each other. The proportional hazard assumption of the Cox model was tested visually with log minus log plots.

Analyses were performed with SPSS version 12.0 (SPSS Inc., Chicago, IL) with the exception of ROC analyses, which were performed with AccuRoc 2.5 (Accumetric Corporation, Montreal, Canada). Parametric variables are expressed as mean $\pm$ standard deviation, whereas non-parametric

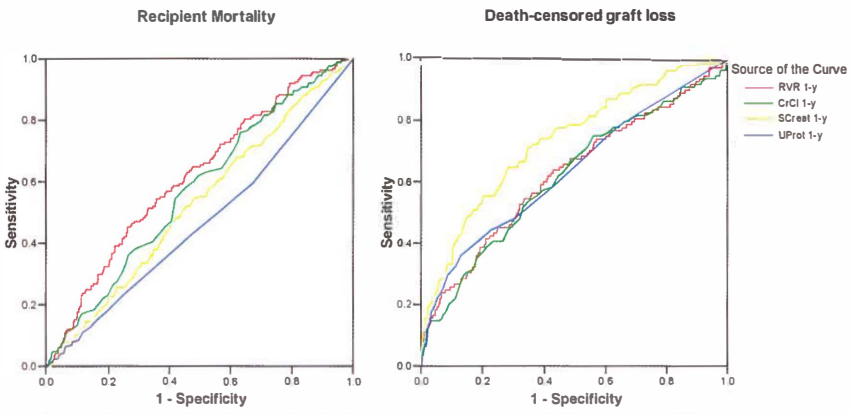
variables are expressed as median [interquartile range]. A two-sided p-value of 5% or less was considered to indicate statistical significance.

Results

A total of 163 out of 793 (21%) first-time cadaveric renal transplant recipients, who had survived the first year after transplantation with a functioning graft, deceased after an average follow-up of 7.5 (± 5.1) years. In addition, a total of 109 (14%) subjects experienced death-censored (factual) graft loss. The resultant median overall graft survival was 15.3 years (95%CI [13.4-17.3]). Almost 30% of the cohort had developed end-stage renal disease because of primary glomerular disease, 21% because of unknown causes, 18% because of polycystic renal disease, 15% of tubular interstitial disease, 6% of renal vascular disease, and 3% of diabetes mellitus. Table 1 shows potential predictors of graft loss at 1 year postransplant and their association with recipient mortality and death-censored graft loss. Recipients who deceased during follow-up were older, more often transplanted between 1980 and 1990 (rather than after 1990), had received hemodialysis rather than peritoneal dialysis therapy prior to transplantation, had more induction therapy, were prescribed less tacrolimus and mycophenolate mofetil, but suffered more often from post-transplant diabetes mellitus, hypertension, as well as lower renal allograft function and higher renal vascular resistance. Similar associations were found for death-censored graft loss. In addition, recipients with death-censored graft loss were younger, more often male, had more often acute rejection therapy with high-dose corticosteroids, as well as more proteinuria, higher serum creatinine, and lower creatinine clearance. Figure 1 shows the ROC curves for renal vascular resistance, creatinine clearance, serum creatinine, and proteinuria at 1 year post-transplant. RVR predicted recipient mortality better than creatinine clearance, serum creatinine, and proteinuria. However, RVR did not predict death-censored graft loss better than creatinine clearance or proteinuria at 1 year post-transplant. Moreover, RVR predicted death-censored graft loss worse than serum creatinine. Importantly, all variables under study were moderate predictors of the endpoint as evidenced by the relatively low AUC values. The maximum sensitivity and specificity of RVR at 1 year post-transplant for recipient mortality were 57% and 40% respectively at a RVR cut-off value of 0.33 mmHg per mL/min. A similar cut-off value was attained for death-censored graft survival with a maximum sensitivity of 62% and specificity of 59%. The predictive value of all markers increased with time elapsed since transplantation (Table 2). RVR remained a better predictor of recipient mortality than creatinine clearance and proteinuria at 2 and 5 years post-transplant, but not of death-censored graft loss. RVR seemed to predict death-censored graft loss worse than serum creatinine at 2 and 5 years post-transplant, although the observed tendency was not

statistically significant. Stratification of ROC analyses for gender and dichotomously along the median of age, RVR, CrCl, SCreat, and UProt did not change the notion of our findings (data not shown).

Figure 1. Area under the receiver operating curves (AUG) with standard error (SE) of RVR. CrCL SCreat. and UProt at 1-year post-transplant for recipient mortality and death censored graft loss. P-Value was calculated versus RVR by method of DeLong. Delong and Clarke-Pearson a=P<0.05



163 events (n=793)	AUC ± SE
RVR 1-y	0.62 ± 0.02
CrCl 1-y	0.57 ± 0.02[a]
SCreat 1-y	0.53 ± 0.03[a]
UProt 1-y	0.46 ± 0.03[a]

109 events (n=793)	AUC ± SE
RVR 1-y	0.63 ± 0.03
CrCl 1-y	0.62 ± 0.03
SCreat 1-y	0.78 ± 0.03[a]
UProt 1-y	0.63 ± 0.03

Figure 2 shows the Kaplan-Meier curves of RVR quartiles at 1-year post-transplant. Subjects in Q4 had significantly lower recipient survival compared to the others (p<0.001). Subjects in Q3 and Q4 had significantly lower death-censored graft survival than subjects in Q1 and Q2 (p<0.001).

Table 3 shows the results of the Cox proportional hazard analyses. Units were chosen in such a way that each hazard ratio represented a ten-percent increase in risk marker in order for hazard ratios to be better compared amongst each other. RVR had a higher univariate hazard ratio for recipient mortality than creatinine clearance, serum creatinine, or proteinuria at 1 year post-transplant. This notion remained unchanged after adjustment for CrCl, and UProt in the first multivariate model. However, the impact of RVR on recipient mortality owed mainly to the impact of MAP on recipient mortality, according to the second multivariate model.

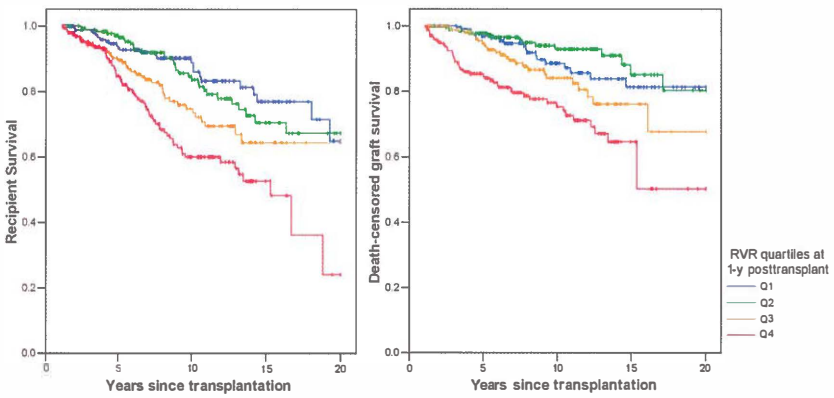
Tabel 1. Association of putative predictors at time of transplantation or at 1-year post-transplant with recipient mortality and death-censored graft loss.

	Recipient mortality	Death-censored graft loss	All included subjects
N =	163	109	793
Age, yr			
recipient	54 ± 10 [d]	40 ± 13 [d]	47 ± 13
donor	38 ± 16	38 ± 17	37 ± 16
Male gender, n (%)			
recipient	94 (58)	74 (68) [a]	460 (58)
donor	95 (58)	65 (60)	476 (60)
Prior dialysis modality, n (%)			
hemodialysis	114 (70) [b]	73 (67)	468 (59)
peritoneal dialysis (CAPD)	41 (25) [a]	27 (25) [a]	262 (33)
Duration of dialysis therapy, mo	36 [20-57] [b]	19 [10-33] [d]	29 [16-50]
Decade of transplantation, n (%)			
1970 – 1980	2 (1)	3 (3)	12 (2)
1980 – 1990	65 (40) [d]	53 (49) [d]	192 (24)
1990 – 2000	95 (58)	53 (49) [c]	496 (63)
2000 to date	1 (1) [d]	0 (0) [d]	93 (12)
Ischemia times			
warm ischemia times, min	39 [32-47]	40 [32-47]	38 [30-46]
cold ischemia time, h	24 [19-29]	23 [19-28]	23 [19-28]
Preservation medium, n (%)			
Euro-Collins	71 (46) [d]	53 (49) [d]	213 (27)
University of Wisconsin	77 (44) [b]	43 (39) [d]	456 (56)
Delayed graft function, n (%)	39 (24)	25 (23)	198 (25)
CMV seropositivity, n (%)			
donor	85 (52) [b]	71 (65) [b]	364 (54)
recipient	94 (50)	43 (39) [b]	402 (51)
Number of HLA mismatches			
HLA-AB	1 [1-2]	1 [1-2]	1 [0-2]
HLA-DR	0 [0-1]	0 [0-1]	0 [0-1]
Induction therapy, n (%)			
antilymphocyte antibodies	17 (10) [b]	5 (5)	71 (9)
IL-2 receptor antagonists	0 (0)	0 (0)	10 (1)
Calcineurin inhibitor at 1-y, n (%)			
cyclosporine	136 (83)	80 (73)	626 (79)
tacrolimus	3 (2) [a]	2 (2) [a]	48 (6)
Proliferation inhibitor at 1-y, n (%)			
azathioprine	65 (40) [a]	58 (53) [d]	262 (33)
mycophenolate mofetil	17 (10) [d]	15 (14) [c]	214 (27)
Acute rejection treatment, n (%)			
high dosage corticosteroids	49 (30)	41 (38) [a]	230 (29)
antilymphocyte antibodies	17 (10)	18 (17)	41 (14)
BMI at 1-y, kg/m^2	25.9 ± 3.6	25.8 ± 4.5	25.8 ± 4.0
Post-transplant diabetes at 1-y, n (%)	38 (23) [b]	25 (23) [a]	127 (16)
Mean arterial pressure at 1-y, mmHg	112 ± 14 [d]	111 ± 12 [a]	108 ± 12
number of anti-hypertensives	1 [0-2] [b]	1 [1-2] [a]	1 [0-2]
number of diuretics	0 [0-1] [a]	0 [0-1]	0 [0-1]
use of ACE-inhibitor, n (%)	12 (7)	16 (15)	79 (10)
Proteinuria at 1-y, g/24h	0.2 [0-0.4]	0.3 [0.2-1] [d]	0.2 [0-0.5]
24-h creatinine clearance at 1-y, mL/min	58 ± 19 [c]	57 ± 26 [c]	62 ± 22
Serum creatinine, µmol/L	155 ± 48	205 ± 97 [d]	154 ± 59

	Recipient mortality	Death-censored graft loss	All included subjects
N =	163	109	793
Renal clearance studies at 1-y			
GFR, mL/min	50 ± 17 [b]	44 ± 18 [d]	53 ± 18
ERPF, mL/min	193 ± 62 [d]	189 ± 77 [c]	210 ± 67
ERBF, mL/min	333 ± 110 [d]	309 ± 136 [d]	356 ± 123
FF	0.26 ± 0.04	0.24 ± 0.06 [a]	0.25 ± 0.05
Hematocrit at 1-y	0.42 ± 0.05	0.39 ± 0.06 [d]	0.41 ± 0.05
RVR at 1-y, mmHg per mL/min	0.38 ± 0.15 [c]	0.46 ± 0.32 [d]	0.34 ± 0.17

Data are shown as mean ± SD, Median [IQR], or N (%). P-values were calculated versus patients not reaching the endpoint under study.
[a] = $p \leq 0.05$
[b] = $p \leq 0.01$
[c] = $p \leq 0.001$
[d] = $p \leq 0.0001$

Figure 2. Kaplan-Meier survival plots of quartiles of RBR at 1 year port-transplant for recipient mortality and death-censored survival. Both with p-value <0.0001 according to log-rank test for trend.



Numbers at Risk	0-y	5-y	10-y	15-y	20-y
Q1 (RVR 0.12 - 0.23)	190	143	64	29	8
Q2 (RVR 0.24 - 0.30)	198	159	82	28	11
Q3 (RVR 0.30 - 0.39)	195	137	59	14	5
Q4 (RVR 0.39 - 1.89)	212	130	59	13	1

RVR also predicted death-censored graft loss better than creatinine clearance and proteinuria at 1 year post-transplant in both univariate and multivariate analyses, but RVR was a worse predictor than serum creatinine in the univariate analysis. Although the impact of RVR on death-censored allograft loss attenuated in the first multivariate model, RVR remained an independent predictor after adjustment for its components MAP and ERBF in the second multivariate model.

Table 2. Predictive values of renal vascular resistance (RVR), 24-hour urinary creatinine clearance (CrCl), serum creatinine (SCreat), and proteinuria (UProt) at 1-, 2-, and 5-years post-transplant for recipient mortality and death-censored graft survival.

	RVR	CrCl	Screat	UProt
at 1-year post-transplant				
Recipient mortality	0.62 ± 0.02	0.57 ± 0.02[a]	0.53 ± 0.03[a]	0.46 ± 0.03[a]
Death-censored loss	0.63 ± 0.03	0.62 ± 0.03	0.78 ± 0.03[a]	0.63 ± 0.03
at 2-years post-transplant				
Recipient mortality	0.64 ± 0.03	0.57 ± 0.02[b]	0.51 ± 0.03[b]	0.53 ± 0.03[b]
Death-censored loss	0.70 ± 0.04[a]	0.69 ± 0.05	0.74 ± 0.03	0.70 ± 0.03
at 5-years post-transplant				
Recipient mortality	0.67 ± 0.03[a]	0.63 ± 0.03	0.55 ± 0.03[c]	0.55 ± 0.04[c]
Death-censored loss	0.75 ± 0.04[a]	0.67 ± 0.05	0.77 ± 0.04	0.72 ± 0.04

Data given as areas under the ROC-curve with standard errors. ROC analyses were performed according to DeLong, DeLong, and Clark-Pearson

[a] = $p \leq 0.05$ versus RVR at 1-y post-transplant

[b] = $p \leq 0.05$ versus RVR at 2-y post-transplant

[c] = $p \leq 0.05$ versus RVR at 5-y post-transplant

All analyses were repeated with glomerular filtration rate as measured by ^{125}I-iothalamate instead of creatinine clearance, and also repeated for the entire Groningen renal transplant cohort (i.e. including second time, combined, or living-donor renal transplantations). Both sensitivity analyses did not change the notion of our findings (data not shown).

Table 3. Predictive values renal vascular resistance (RVR), 24-hour urinary creatinine clearance (CrCl), serum creatinine (SCreat), and proteinuria (UProt) at 1-year post-transplant for recipient mortality and death-censored graft survival according to univariate and multivariate Cox proportional hazard analyses.

Predictor at 1-y post-transplant	Univariate Analysis	Multivariate Analyses	
Recipient mortality			
RVR *per 0.2 mmHg mL^{-1} min^{-1}*	1.67 (1.53 - 1.82)	1.34 (1.12 - 1.61)	1.02 (0.69 - 1.52)
- MAP *per 10 mmHg*		.20	1.23 (1.07 - 1.42)
- ERBF *per 50 mL min^{-1}*			0.91 (0.78 - 1.06)
CrCl *per 14 mL min^{-1}*	0.73 (0.66 - 0.80)	0.83 (0.73 - 0.95)	0.84 (0.72 - 0.97)
SCreat *per 60 µmol/L*	1.20 (1.01 - 1.41)		
UProt *per 1 g 24h^{-1}*	1.24 (1.14 - 1.35)	1.00 (0.80 - 1.24)	1.01 (0.80 - 1.25)
Death-censored graft loss			
RVR *per 0.2 mmHg mL^{-1} min^{-1}*	1.79 (1.60 - 2.00)	1.63 (1.42 - 1.87)	1.61 (1.34 - 1.93)
- MAP *per 10 mmHg*			1.01 (0.86 - 1.19)
- ERBF *per 50 mL min^{-1}*			0.99 (0.87 - 1.13)
CrCl *per 14 mL min^{-1}*	0.70 (0.68 - 0.80)	0.89 (0.77 - 1.04)	0.90 (0.76 - 1.07)
SCreat *per 60 µmol/L*	1.92 (1.73 - 2.13)		
UProt *per 1 g 24h^{-1}*	1.40 (1.28 - 1.52)	1.38 (1.24 - 1.54)	1.38 (1.23 - 1.55)

Discussion

The present study showed that RVR is one of the most prominent, albeit still modest, predictors of both recipient mortality and death-censored graft loss. RVR was a better risk factor for recipient mortality than renal function and proteinuria, but this owed largely to the effect of mean arterial blood pressure. In contrast, RVR seemed to constitute more than the sum of its components for the prediction of death-censored graft loss, but showed less predictive value than a simple tool such as serum creatinine.

Although we found RVR to be one of the most prominent predictors of recipient mortality and death-censored renal allograft loss, our results were not as pronounced as Radermacher et al found using the renal artery resistance index (RI) as assessed by Doppler ultrasonography. One explanation for this may be that RVR in our study was measured at fixed times points after transplantation. RI in the study of Radermacher was measured in a cross-sectional time period ranging from 3 to 317 months after transplantation. Consequently, the high predictive value found in that study has been debated to result from time bias.[9] The predictive value of RI could have increased if RI were measured closer to the point of graft loss. Our ROC analyses, which showed that the predictive value of all risk markers increased with time elapsed since transplantation, supports this hypothesis. However, the benefit of a risk marker identified close to the point of graft loss is likely to be limited. Ideally, clinicians need an early risk marker (e.g. at 1-year post-transplant) for the prediction of late renal allograft loss to warrant benefit from preventive therapy. A second explanation for the fact that RVR was not as pronounced as RI may lie in the fact that Radermacher et al used relatively arbitrary cut-off points for all risk markers (with the exception of RI) in their Cox survival analyses. We used a ten-percent increase in risk marker as unit of hazard ratio in our Cox survival analyses for less arbitrary comparison. The final and most likely explanation is that renal vascular resistance as estimated by Ohm's law for fluidics using renal radioisotope clearance and brachial blood pressure measurements, does not equal the renal artery resistance index as assessed by Doppler ultrasonography. Although very early work on this topic suggested that both measures could be interchangeable, solely on the basis that they were highly correlated,[11] a few recent well-designed experiments show that the relationship between RVR and RI is more complex. A series of in-vitro experiments showed that RI depends on vascular resistance as well as compliance; becoming less dependent on vascular resistance as compliance decreases.[17] The importance of vascular compliance for RI was underlined further by an experimental study of perfused rabbit kidneys. That study showed only weak association between RI and RVR, whereas strong association was found between RI and the pulse pressure index.[18] Pulse pressure is determined in large part

by arterial compliance. Although RVR and RI are correlated in certain conditions, they may be different manifestations of 'vascular resistance'. It is also important to note that the mean arterial pressure was measured at the brachial artery, which may be at variance with the renal transplant artery. However, as the prevalence of transplant renal artery stenosis has been estimated to be as low as 1 to 2%,[19] we estimate any difference to be small.

The present study had limitations. First, we had to exclude 389 subjects from our analyses because of missing RVR data. Missing RVR data were most in almost all cases due to logistic problems with supply of radio-isotopes. The fact that missing values were not scattered throughout the transplant database, but clustered around certain time periods instead, is in agreement with this observation. This is expected to result in random bias, rather than systematic bias. The finding that patients with missing values had comparable serum creatinine concentrations, creatinine clearance, and proteinuria at 1 year post-transplant, as well as similar patient and graft survival as the analyzed cohort, strengthens this notion. We therefore estimate any putative bias to be limited.
In conclusion, the present study showed that RVR is a prominent, albeit still modest, risk marker for both recipient mortality and death-censored graft loss. The predictive value of RVR for recipient mortality owed mainly to the impact of mean arterial blood pressure , one of the defining components of RVR. In contrast, RVR constituted more than the sum of its components for the prediction of death-censored graft loss, but showed less predictive value than serum creatinine. As the assessment of RVR is expensive and time-consuming, we believe that RVR therefore holds no merit for the follow-up of renal transplant recipients. Although the previously identified RI might be a better early risk marker, we suggest this to be evaluated at predetermined time points early after transplantation in light of our findings that the predictive value of RVR is time-dependent, and increases with time elapsed since transplantation.

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Chapter 7

N-terminal pro-B-type natriuretic peptide and mortality in renal transplant recipients versus the general population.

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Abstract

Background
Mortality rates are higher in renal transplant recipients (RTR) than in the general population (GP). It is unknown which risk factors account for this difference.

Methods
We prospectively followed a cohort of 606 RTR for 3,026 person-years, during which 95 died. A GP cohort of 3,234 subjects was followed for 24,940 person-years, during which 130 died.

Results
All investigated risk factors, except ethnicity, BMI and total cholesterol, differed significantly between cohorts, with an adverse risk profile in the RTR. The age and sex adjusted hazard ratio (HR) for mortality in RTR was 6.2 [95%CI 4.6-8.3] compared to GP, which was reduced to 2.4 [95%CI 1.6-3.6], 4.3 [95%CI 3.0-6.1] and 5.0 [95%CI 3.5- 7.3] after additional adjustment for differences in NT-proBNP, creatinine clearance and need for anti-hypertensive medication respectively (all $P<0.001$). Associations of NT-proBNP, creatinine clearance and the use of anti-hypertensive medication with mortality were significantly steeper in RTR than in GP. Risk for mortality was similar for RTR and GP with low NT-proBNP (<100 pg/ml).

Conclusions
Elevated NT-proBNP, low creatinine clearance and need for anti-hypertensive
medication are stronger risk factors for mortality in RTR than in GP. The most important targets to reduce the increased mortality in RTR seem to be both cardiac and renal function.

Introduction

Renal transplantation is the therapy of choice for patients with end stage renal disease. Quality of life improves after transplantation and life expectancy of renal transplant recipients (RTR) exceeds that of dialysis patients.[1,2] However, mortality rates of RTR are still high compared with the general population, which likely has a multifactorial background, since many risk factors are highly prevalent in RTR. Cardiac dysfunction and heart failure are prevalent after transplantation, and associated with poor prognosis.[3-5] NT-proBNP is a marker of cardiac dysfunction and is a very strong predictor of mortality in various populations, including the general population[6] and the dialysis population.[7] However, little is known about the relation between NT-proBNP and mortality in RTR, and whether and to what extend NT-proBNP accounts for the greater risk of mortality in RTR compared to the general population. Other risk factors for increased mortality in RTR include impaired renal function,[8,9] hypertension,[11-12] persistent low grade inflammation,[13-14] impaired glucose tolerance[15] and obesity.[16,17] It is unknown whether the increased mortality in RTR compared to the general population is due to the high prevalence or severity of risk factors, because a detailed comparison between RTR and the general population is lacking. Such a comparison would be important, not only for risk stratification, but also to design targeted intervention strategies in RTR aimed to reduce the high mortality rate. Therefore, we compared the prevalence and severity of risk factors and their contribution to the difference in mortality between a RTR cohort and a representative sample from the general population from the same region. In particular, we were interested in NT-proBNP, a measure of cardiac dysfunction.

Matherials and Methods

Renal transplant cohort

For the RTR cohort we used data of a prospective cohort study for which we invited all RTR who visited our outpatient clinic between August 2001 and July 2003. RTR were asked to participate if they had a functioning graft for ≥ 1 year. A total of 606 RTR signed written informed consent, from an eligible 847 (72% consent rate). The group that did not consent was comparable with the group that participated with respect to age, sex, BMI, serum creatinine, creatinine clearance and proteinuria.

General population cohort

For the general population cohort we used data of the PREVEND study (Prevention of Renal and Vascular End-stage Disease). The PREVEND study is an ongoing longitudinal cohort study based on the general population in the Netherlands, between the ages of 28 and 75 years. In brief, 8,592 individuals completed the baseline survey (1997-1998). For

the present study we used a random sample (n=3,432) representative of the general population because those with albuminuria are overrepresented in the PREVEND study as a whole.

Endpoint
The endpoint of the study was all-cause mortality. We explicitly choose to analyse all-cause mortality and not different causes of mortality, because the cause of death is often difficult to ascertain.[21-23] Furthermore, it is plausible that the accuracy of cause of death may differ between RTR and the general population, which could introduce unwanted bias. For the RTR cohort, the continuous surveillance system of the outpatient program ensures up-to-date information on patient status. Follow-up of RTR was complete until August 2007. For the general population cohort, data on mortality was received from the municipal register until December 2005 and in case someone moved to an unknown destination, the date on which the person was removed from the municipal registry was used as censor date.

Renal transplant characteristics and immunosuppression
Relevant transplant characteristics were taken from the Groningen Renal Transplant Database. This database holds information on all renal transplantations performed at our center since 1968, including dialysis history.

Renal function
In both populations, renal function was assessed as the 24h urinary creatinine clearance, i.e. the 24h creatinine excretion divided by the serum creatinine concentration. We also estimated the glomular filtration rate (eGFR) with the MDRD formula.[24]

Anthropometrics
Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in metres squared. In both cohorts, weight was measured after shoes and heavy clothing were removed. In both cohorts waist circumference was measured midway between the iliac crest and the 10th rib. Body surface area was calculated according to DuBois and DuBois.

Blood pressure
In the RTR cohort, blood pressure was measured after morning medications (including anti-hypertensive medication) were not taken. In both cohorts, blood pressure was measured with an automatic device.

Definitions, medication, and questionnaire information

Diabetes was defined as a fasting plasma glucose level of ≥7.0 mmol/l and/or use of anti-diabetic drugs or insulin according to the criteria of the ADA.[25] In the RTR cohort, current medication was extracted from medical records. In the general population cohort, information on drug use was self reported and substantiated with the use of pharmacy dispensing data from all community pharmacies in the city of Groningen, which has complete information on drug-use of 80% of the PREVEND subjects. Ethnicity, smoking status, and cardiovascular disease history were recorded with a similar questionnaire in both populations.

Laboratory measurements

In both the RTR cohort and the general population cohort blood was drawn after an 8-12h overnight fasting period. In both cohorts, NT-proBNP was determined by immunoassays (ELECSYS® proBNP, Roche Diagnostics, Germany) using an ELECSYS®2010 instrument. In both cohorts insulin was measured using an AxSym auto-analyzer (Abbott Diagnostics, the Netherlands) in both cohorts. In the RTR cohort, creatinine in serum and urine were determined using the Jaffé method on a MEGA AU 510 (Merck Diagnostica, Germany). In the general population cohort, creatinine in serum and urine were determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY). There is a difference between serum creatinine measured by the Jaffé method (RTR cohort) and by the dry chemistry method (general population cohort).[26] To correct for this difference, we used the correction formula that we previously determined: serum creatinine by dry chemistry=1.027 × serum creatinine by Jaffé–8.243.[26] In the RTR cohort high sensitivity CRP was measured using a double plated ELISA assay.[18] In the general population high sensitive CRP was determined by nephelometry (BNII N; Dade Behring Diagnostic, Germany). Plasma glucose was determined in the RTR cohort by the glucose-oxidase method (YSI 2300 Stat plus; Yellow Springs, OH, USA) and in the general population by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY). HDLc was determined in the RTR cohort using the CHOD-PAP method on a Technikon RA-1000 (Bayer Diagnostics, the Netherlands) and in the general population with a homogeneous method (direct HDL, AEROSETTM System, Abbott Laboratories, Abbott Park). In the general population urinary albumin concentration was determined by nephelometry (BNTMII Dade Behring Diagnostic, Germany).

Statistical analysis

Data were analyzed with SPSS version 14.0 (SPSS Inc. Chicago, IL). Normally distributed variables are given as means ± standard deviation, whereas skewed variables are given as median [interquartile range]. Hazard ratio's (HR) are reported with 95% confidence interval [95%CI]. Differences between variables with a normal distribution were tested with students t-tests; differences between variables with a skewed distribution

with Mann-Witney U-test and differences between categorical variables with χ-square tests. The 95% confidence intervals of mortality rates were calculated based on the table provided by Haeszel and colleagues.[28] For each cohort, we calculated the age and sex adjusted HR's of potential risk factors for mortality using Cox-regression analyses. A Kaplan-Meier survival curve was constructed comparing mortality between cohorts and tested Log-rank test. We proceeded with a crude Cox-regression analysis and with an adjusted for age and sex to determine the HR for mortality for RTR, compared to the general population. We then separately adjusted for each potential risk factors to discern how much the hazard ratio for mortality in RTR was changed. Percentage change in HR was calculated as: ((HR before adjustment) – (HR after adjustment)) / (HR before adjustment – 1) x 100. To determine whether risk factors have a significant different association with mortality between the cohorts, interaction terms between cohort and all investigated risk factors were also analysed a two sided P value <0.05 considered to indicate statistical significance.

Results

Baseline characteristics and risk factors in RTR and the general population are shown in Table 1. All characteristics and risk factors differed significantly between the two cohorts, except ethnicity, weight, height, BMI and total cholesterol. All risk factors that differed between the cohorts were consistent with a higher risk for renal transplant recipients (i.e. more often a history of CVD, higher blood pressure, etc.), except current smoking. Remarkably, the prevalence of overweight and obesity was similar between the cohorts (P=0.2 and P=0.1 respectively), but RTR were more often centrally obese (51% versus 24% in the general population, P<0.001). Of the 606 RTR, 95 (16%) died during the follow-up of 3,026 person-years, with a mortality rate of 31.4 [95%CI 25.5-38.6] per 1,000 person-years. Of the 3,432 persons of the general population cohort, 130 (4%) died during the follow-up of 24,904 person-years, with a mortality rate of 5.2 per [95%CI 4.4-6.3] 1,000 person-years. Mortality differed significantly (P<0.001) between the cohorts according to the Log rank test.

Table 2 shows the age and sex adjusted risk for mortality associated with risk factors in RTR and the general population. All risk factors were significantly associated with mortality in RTR except sex, fasting insulin, total cholesterol and BMI.

In the general population age, sex, NT-proBNP, waist circumference, CRP, a history of CVD and current smoking were significantly associated with mortality. Male sex was the only risk factor that was associated with mortality in the general population but not in RTR. In RTR, time since transplantation was not a risk factor for mortality (univariate analysis: HR:

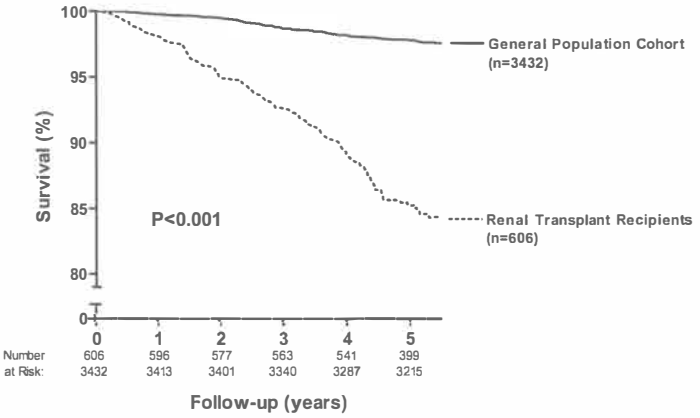
1.01 [0.98-1.04], P=0.5, adjusted for age and sex: HR: 1.00 [0.96-1.03], P=0.7).

Table 1. Baseline characteristics and risk factors for mortality in renal transplant recipients and the general population.

	Renal Transplant Recipients	General Population	
n=	606	3432	P-value
Age, yrs	51±12	49±12	<0.001
Male sex, n (%)	332 (55)	1548 (45)	<0.001
Caucasian ethnicity, n (%)	584 (96)	3240 (94)	0.1
Dialysis time, months	27 [13-49]	-	-
Time since transplantation, yrs	6.0 [2.6-11.4]	-	-
Transplantation era			
Transplanted after 2000, n (%)	123 (20)	-	-
Transplanted between 1990-2000, n (%)	354 (58)	-	-
Transplanted prior to 1990, n (%)	129 (22)	-	-
Post-mortem donor, n (%)	523 (86)	-	-
History of CVD, n (%)	75 (12)	145 (4)	<0.001
Current Smoking, n (%)	133 (22)	1067 (31)	<0.001
Previous Smoking, n (%)	255 (42)	1244 (36)	0.004
Weight, kg	77±14	77±14	0.9
Height, m	1.72±0.10	1.73±0.10	0.2
Body surface area, m^2	1.90±0.19	1.90±0.19	0.5
BMI, kg/m^2	26.0±4.3	25.9±4.1	0.3
Overweight (25-30 kg/m^2), n (%)	250 (41)	1333 (39)	0.2
Obesity (>30 kg/m^2), n (%)	96 (16)	470 (14)	0.1
Waist circumference men, cm	100 [91-108]	93 [86-100]	<0.001
Waist circumference women, cm	93 [83-104]	81 [74-90]	<0.001
Central Obesity (waist>102/88 cm in men/women)	308 (51)	812 (24)	<0.001
Systolic blood pressure, mmHg	153±23	126±19	<0.001
Diastolic blood pressure, mmHg	90±10	72±9	<0.001
Any antihypertensive (AHT) medication, n (%)	529 (87)	508 (15)	<0.001
1 AHT, n (%)	147 (24)	334 (10)	<0.001
2 AHT, n (%)	199 (33)	136 (4)	<0.001
3 AHT, n (%)	133 (22)	32 (0.9)	<0.001
≥4 AHT, n (%)	50 (8)	6 (0.1)	<0.001
NT-proBNP, pg/ml	296 [131-669]	38 [17-73]	<0.001
Total cholesterol, mmol/l	5.6 [4.9-6.2]	5.5 [4.8-6.3]	0.8
HDL cholesterol, mmol/l	1.0 [0.9-1.3]	1.3 [1.0-1.6]	<0.001
Triglycerides, mmol/l	1.9 [1.4-2.6]	1.1 [0.8-1.6]	<0.001
Statin use, n (%)	300 (50)	126 (4)	<0.001
Glucose, mmol/l	4.8±1.4	4.7±1.0	0.02
Insulin, pmol/l	11.2 [8.0-16.3]	7.8 [5.4-11.7]	<0.001
HOMA	2.3 [1.6-3.6]	1.6 [1.1-2.5]	<0.001
Diabetes, n (%)	107 (18)	89 (3)	<0.001
hsCRP, mg/dl	2.0 [0.8-4.8]	1.1 [0.5-2.7]	<0.001
Creatinine clearance, ml/min	62±22	100±26	<0.001
Serum creatinine, µmol/	130 [107-162]	82 [73-91]	<0.001
eGFR ml/min/$1.73m^2$	49±17	81±15	<0.001
Proteinuria, g/24hr	0.2 [0-0.5]	-	-
Proteinuria (≥0.5 g/24hr), n (%)	169 (28)	-	-
Albuminuria, mg/24hr	-	7 [5-11]	-
Macro-albuminuria (≥0.5 g/24hr), n (%)	-	16 (0.5)	-

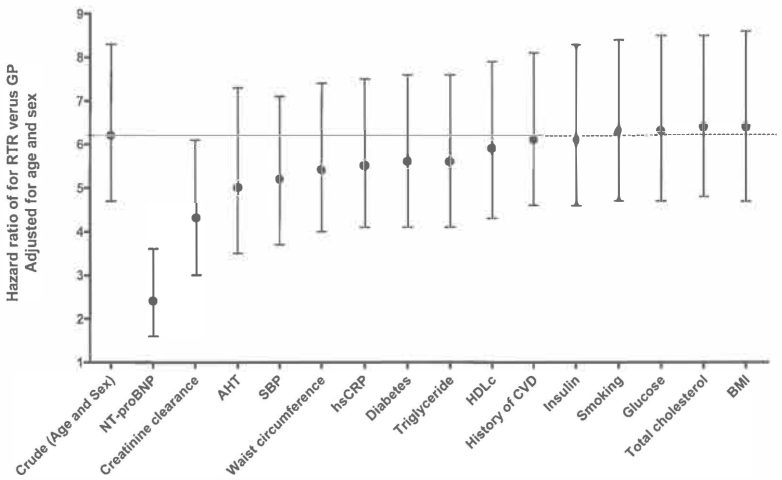
Normally distributed variables are given as mean ± standard deviation. Differences between normally distributed variables was tested with a students t-test. Variables with a skewed distribution are given as median [interquartile range]. Differences between variables with a skewed distribution was tested with a Mann-Witney U test. Differences between categorical variables was tested using χ-square test.

Figure 1. Kaplan-Meier survival analysis for renal transplant recipients and general population.



Likewise the era of transplantation (ie. after 2000, between 1990-2000 or prior to 1990) was not associated with mortality (data not shown). The crude, unadjusted HR for mortality for RTR compared to the general population was 6.9 [5.2-9.3] (P<0.001). Adjustment for age and sex slightly lowered the HR to 6.2 [4.6-8.3] (P<0.001). Next, we separately adjusted for other risk factors investigated in Table 2 .

Figure 2. Age and sex adjusted hazard ratios of individual risk factors for excess mortality in renal transplant recipients compared with general population.



The HR for mortality was affected most by adjustment for differences in NT-proBNP, which resulted in a HR of 2.4 [1.6-3.6], which was a 73% reduction of the age and sex adjusted HR. The next second greatest effect was after adjustment for differences in creatinine clearance, which resulted in a HR of 4.3 [3.0- 6.1], which is a 37% reduction of the age and sex adjusted HR. With adjustment for both NT-proBNP and creatinine clearance, the HR for mortality of RTR was reduced to 2.2 [1.4-3.3]

(P<0.001).The model with NT-proBNP and creatinine clearance was not significantly better than with NT-proBNP alone (HR 2.4 versus 2.2, P=0.2). In contrast, additionally adjustment of the model of creatinine clearance with NT-proBNP further halved the HR, which was significantly different (HR 4.3 versus 2.2, P<0.001). Other factors that reduced the HR for mortality were amongst others the need for antihypertensive medication (HR: 5.0 [3.5-7.3], 23% reduction of HR), systolic blood pressure (HR: 5.2 [3.7-7.1], 19% reduction of the HR), waist circumference (HR: 5.4 [4.0-7.4], 15% reduction of the HR) and CRP (HR: 5.5 [4.1-7.5], 13% reduction of the HR) (all P<0.001).

To investigate whether risk factors were differently associated with mortality between the cohorts, we tested for significant interactions between cohort and risk factors (P-values shown in Table 2). There was a significant interaction between cohort and age (P<0.001), indicating that the risk for mortality increased more with older age in the general population than in RTR. However, at all ages RTR had a higher absolute mortality rate (data not shown). There was also a significant interaction between cohort and sex (P<0.001) indicating that in the general population men had a significant higher risk for mortality than women, but not in the RTR cohort (Figure 3). As such, men had a higher mortality rate compared to the women in the general population (7.4 [95%CI 5.9-9.3] versus 3.5 [95%CI 2.6-4.6] per 1000 person-years), but in RTR, men and women had a similar mortality rate (31.8 [95%CI 22.7-43.2] versus 31.1 [95%CI 23.1-41.0] per 1000 person-years). We subsequently tested for other interactions between risk factors and cohort that were independent of age and sex. There were only significant interactions between cohort and NT-proBNP, renal function (as reflected by creatinine clearance and eGFR) and use of antihypertensive medication. Therefore we further investigated these relations in and between the two cohorts. The interaction of NT-proBNP was consistent that with higher NT-proBNP the risk for mortality increased more in RTR than in the general population (Figure 4A). Importantly, RTR with NT-proBNP levels <100 pg/ml did not have higher risk for mortality compared to subjects in the general population that also had NT-proBNP <100 pg/ml (HR: 0.8 [0.1-5.9], P=0.7), even after adjustment for age, sex, and creatinine clearance (HR: 0.7 [0.1-5.2], P=0.7). The interaction between creatinine clearance and cohort was consistent that with a lower creatinine clearance, the risk for mortality increased more in RTR than in the general population (Figure 4B). In RTR, the risk for mortality increased from 3.0 [1.1-8.3] (P=0.03) in RTR with creatinine clearance >90 ml/min to 9.4 [6.5-13.7] (P<0.001) in RTR with creatinine clearance <60 ml/min, compared to the general population with creatinine clearance >90 ml/min as reference. In the general population those with lower creatinine clearance did not have a significant higher risk for mortality. Figure 4B also highlights the difference in prevalence of creatinine clearance <60 ml/min in RTR and the general

population (50% versus 4%, P<0.001). Results for eGFR were similar to that of creatinine clearance (data not shown).

Table 2. Risk for mortality associated of common risk factors in renal transplant recipients and the general population.

	Renal Transplant Recipients		General Population		Interaction
	HR [95%CI]	P-value	HR [95%CI]	P-value	P-value
Crude					
Age, 10 yrs	2.1 [1.7-2.6]	<0.001	2.7 [2.3-3.2]	<0.001	<0.001
Age adjusted					
Sex, (women=0, men=1)	1.1 [0.7-1.6]	0.7	2.0 [1.4-2.8]	<0.001	<0.001
Age and sex adjusted					
Log NT-proBNP, pg/l [a]	1.5 [1.3-1.6]	<0.001	1.3 [1.1-1.4]	<0.001	0.04
Creatinine clearance, 10 ml/min	0.8 [0.7-0.8]	<0.001	1.0 [0.9-1.1]	0.5	<0.001
Systolic blood pressure, 10 mmHg	1.1 [1.0-1.2]	0.02	1.1 [1.0-1.1]	0.1	0.4
Antihypertensive medication (AHT) use, no	1.0 (reference)	-	1.0 (reference)	-	
RTR = 1 AHT / GP = Any AHT	2.5 [0.8-7.1]	0.1	1.3 [0.8-1.4]	0.3	
RTR=2 AHT	2.6 [0.9-7.3]	0.08			<0.001
RTR=3 AHT	3.1 [1.1-9.0]	0.04			
RTR≥4 AHT	5.4 [1.8-16.5]	0.003			
Waist circumference, 5 cm	1.1 [1.0-1.2]	0.02	1.1 [1.0-1.2]	0.05	0.8
Log hsCRP, mg/dl [a]	1.2 [1.1-1.3]	0.002	1.3 [1.1-1.4]	<0.001	0.4
Diabetes, (no=0, yes=1)	1.8 [1.1-2.7]	0.01	1.5 [0.8-3.0]	0.2	0.7
Log Triglyceride, mmol/l [a]	1.3 [1.0-1.7]	0.07	1.2 [0.9-1.5]	0.2	0.6
HDL-cholesterol, mmol/l	0.5 [0.2-0.9]	0.03	0.9 [0.5-1.5]	0.6	0.2
Statin use, (no=0, yes=1)	1.0 [0.7-1.5]	0.9	1.0 [0.8-1.3]	0.9	0.9
History of CVD, (no=0, positive=1)	1.9 [1.2-3.1]	0.007	1.6 [1.0-2.6]	0.05	0.6
Log Insulin, pmol/l [a]	1.1 [0.8-1.4]	0.7	1.1 [0.9-1.3]	0.6	0.9
Glucose, mmol/l	1.1 [1.0-1.2]	0.05	1.1 [0.9-1.2]	0.3	0.6
Smoking, none	1.0 (reference)	-	1.0 (reference)	-	
Current smoking, (yes=1)	2.5 [1.4-4.6]	0.003	2.5 [1.6-4.2]	<0.001	0.1
Previous smoking, (yes=1)	2.4 [1.4-4.1]	0.002	1.3 [0.8-2.2]	0.3	
Total cholesterol, mmol/l	1.0 [0.8-1.2]	0.9	1.1 [0.9-1.3]	0.3	0.4
BMI, 5 kg/m^2	1.1 [0.9-1.4]	0.3	1.2 [0.9-1.5]	0.2	0.8
Proteinuria, g/24hr [a]	1.3 [1.2-1.5]	<0.001	-	-	-
Albuminuria, mg/24hr [a]	-	-	1.4 [1.3-1.4]	<0.001	-

a: Log transformed with a base two (i.e. the HR per a doubling of the variable)
All analyses that were performed for the risk factors were performed in univariate analyses after adjustment for age and sex.

The interaction between antihypertensive medication and cohort was consistent that with a greater need for antihypertensive medication, the risk for mortality increased more in RTR than in the general population (Figure 4C). The hazard ratio for mortality increased from 2.7 (P=0.05) in RTR who required no antihypertensive medication, to 6.4, 6.9, 8.0 and 13.9 (all P<0.001) in RTR that respectively required 1, 2, 3 and 4 or more antihypertensive medication, compared to those in the general population who required no antihypertensive medication. Finally, all results remained similar when the time after transplantation for RTR was taken into account (data not shown).

Figure 3. Interaction between cohort and sex graph

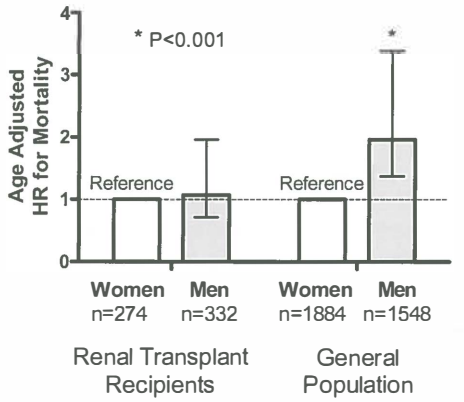
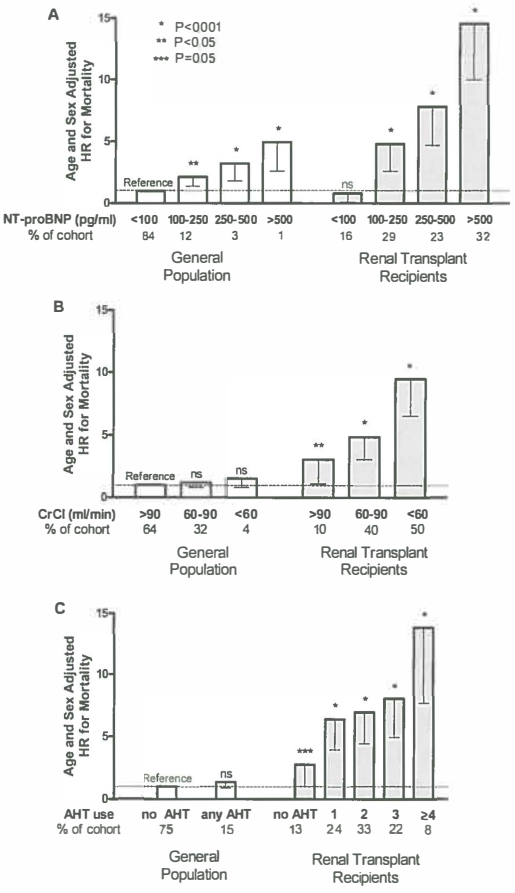


Figure 4. Age and sex-adjusted hazard ratios for NT-pro BNP, creating clearance, and use of antihypertensive medication in renal transplant recipients and the general population.



Discussion

This study compared the prevalence and severity of risk factors and their contribution to mortality in RTR and the general population. RTR had a six fold greater risk for mortality compared to the general population and most risk factors were more prevalent or severe in RTR. Despite their greater prevalence or severity, many risk factors did not account for the excess risk for mortality in RTR. The three risk factors that accounted for most of the difference in mortality and that were stronger risk factors in RTR than in the general population were NT-proBNP, renal function and the need for antihypertensive medication. In fact, RTR with low NT-proBNP had a similar risk for mortality as their counterparts in the general population, even after adjustment for age, sex and creatinine clearance. There are only a few studies that have compared RTR with the general population, but these studies lack detailed information of either the RTR and/or general population cohort.[29-31] Aakhus and colleagues investigated cardiovascular disease prevalence in a Norwegian cohort of RTR and made a comparison with national data, in a cross-sectional study.[29] However, the analysis was limited to an age and sex comparison of prevalent angina pectoris.[29] A study Foley and colleagues consisted of longitudinal data of several registry studies, indicating a two-fold higher annual cardiovascular mortality rate in RTR compared to the general population.[30] However, the authors noted that the number of RTR with cardiovascular mortality was under reported, which likely resulted in an underestimation of the mortality rate in RTR. The study of Foley showed that age was a greater risk for cardiovascular mortality in the general population than to RTR, similar to it's input on all cause mortality in this present study.[30] In another longitudinal study of several registry studies, Sarnak and colleagues investigated mortality caused by sepsis and infection in RTR and the general population.[31] RTR had approximately a 20-fold higher annual mortality rate due to infections than observed in general population, but again this combination of registry studies did not investigate the impact of other characteristics besides age. Results NT-proBNP are intriguing because the increased risk for mortality for RTR compared to the general population more than halved after adjustment of NT- proBNP. Indeed, studies have shown that cardiac dysfunction is highly prevalent and an important risk factor for mortality in RTR.[3-4,10] Lentine and colleagues showed that de novo congestive heart failure predicts mortality in RTR.[4] Furthermore, systolic dysfunction measured prior to transplantation is associated with mortality after transplantation.[5] Intriguingly, Rigatto et al. reported that the incidence of congestive heart failure in a cohort of RTR was considerably higher than in the Framingham cohort, while this was not the case for ischemic heart disease.[3] This suggests that renal transplantation might correspond more to a state of "accelerated heart failure" than to "accelerated atherosclerosis".[3] Our study supports this notion, as a marker of cardiac dysfunction (NT-

proBNP) determined for a larger part the increased mortality in RTR than a maker of accelerated atherosclerosis (hsCRP). Renal dysfunction was more common in RTR than in the general population and our study showed that renal dysfunction was a greater risk factor for mortality in RTR than in the general population. In parallel with a lower renal function, proteinuria was also more prevalent in RTR compared to macro-albuminuria in the general population. However, this was not associated with a greater risk for mortality in RTR, but since proteinuria and albuminuria can not be directly compared, we could not make a comparison as we did for other risk factors. Considering renal function, numerous trials have been performed to improve allograft function in the short term after renal transplantation through optimization of immunosuppression. However, in the long term after transplantation, renal protective treatments which are successful in renal disease in native kidney's have been little investigated in clinical trials in RTR. Targeting to preserve renal function could be achieved through reduction of hypertension, suppression of the renin angiotensin system, reduction of salt and protein intake and smoking cessation. Unfortunately in RTR, it is unknown whether some strategies are more advantageous than others. Our finding that RTR women lose their sex advantage with lower risk for mortality, as they have in the general population, is in accordance with literature.[33] In the general population the female advantage is also lost in diabetes and insulin resistance.[34-37] Intriguingly, in an earlier study we showed in this RTR cohort that women were more insulin resistant than men.[19] Furthermore, 22% of female RTR had diabetes compared to only 14% of male RTR (difference $P<0.001$). Whether a disturbed glucose homeostasis underlies the sex difference between RTR and the general population is unknown. It is known that a disturbed glucose homeostasis is a profound problem in RTR.[38,39] In this study, diabetes was six time as prevalent in RTR and RTR were more insulin resistant compared to the general population. Also, higher fasting glucose increased the risk for mortality in RTR, but not in the general population. However, adjustment for either diabetes, insulin or glucose accounted little for the difference in mortality between RTR and the general population. A possible reason for the high prevalence of diabetes in RTR could be due to central obesity. Waist circumference in RTR was on average 7 cm greater in men and 12 cm greater in women compared to their counterparts in the general population. Remarkably, BMI was similar in both cohorts. A likely a reason for the greater central obesity but similar BMI observed in RTR, could be due to chronic use of corticosteroids in RTR resulting in loss of muscle mass and a gain in fat mass with perhaps no change in BMI. A consequence of the greater central obesity, next to the higher prevalence of diabetes, could be the higher CRP levels in RTR.[18,19] In summary, it seems that central obesity plays an important role in the increased risk for mortality in RTR, possibly in relation with glucose homeostasis or inflammation. A limitation of this study is that both the cohorts were not set-up to be compared in this manner. Therefore some

measurement differed between the cohorts. For example, blood pressure can not be directly compared between the two cohorts because of the difference in measurement. However, the far greater need of anti-hypertensive medication, which was carefully documented in both cohorts, indicating that blood pressure control was more difficult to achieve in RTR. Other differences between the cohorts are that not all lab techniques were identical (i.e. CRP, lipids), however other techniques were (i.e. NT-proBNP, insulin). Another limitation may be that all RTR consented to participate, which could bias the results if younger, healthy RTR were inclined to participate and elderly RTR with poor health declined. However, there was no difference between RTR that consented and did not consent concerning age, sex, BMI, renal function and proteinuria. An important strength of this study is that to the best of our knowledge, no other study directly compared a RTR cohort with it's reference population population with such detailed and comprehensive data. Finally it is also important to recognize that all persons of the two cohorts reside predominantly in one province of a small country, thus excluding geography and geography associated factors as a confounder.[41] Perhaps the results of NT-proBNP as measure of cardiac dysfunction could justify more attention in the future because the increased mortality was accounted for the largest part by NT-proBNP in RTR. Importantly these results were independent of renal function. It is known that renal transplantation improves cardiac function, even in end-stage renal disease patients with congestive heart failure and that regression of left ventricular hypertrophy (LVH) after transplantation continues for at least 2 years.[42,43] Never the less, the prevalence of LVH remains high and is estimated between 50-70%.[30] In heart failure patients, (NT-pro)BNP guided therapy improved outcome compared to clinically based therapy alone, mostly through stronger inhibition of the renin angiotensin system[44,45] and increase of β-blockers dosage.[45] A (NT-pro)BNP guided therapy could perhaps limit cardiac dysfunction in RTR through greater use of ACE inhibition, which is thought to be beneficial for patient and graft survival.[46] In conclusion, the age and sex adjusted risk for mortality was 6 times higher for RTR compared to the general population. Importantly, both cohorts had detailed information to discern that despite their greater prevalence, many risk factors did not account for the greater risk for mortality in RTR. The risk for morality of RTR compared to the general population was reduced most with adjustment by NT-proBNP, creatinine clearance and the need for anti-hypertensive medication. In fact, transplant recipients with low NT-proBNP (<100 pg/ml) had a similar risk for mortality as their counterparts in the general population, even after adjustment for age, sex and creatinine clearance.

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Chapter 8

Latent cytomegalovirus infection is an independent risk factor for late graft failure in renal transplant recipients

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Abstract

Cytomegalovirus (CMV) is a risk factor for rejection and mortality shortly after renal transplantation. Little is known about its consequences longer after transplantation. We prospectively investigated whether latent CMV infection is a risk factor for graft failure and mortality late after transplantation. 606 renal transplant recipients (RTR) with a functioning graft for >1 year. CMV serology was determined using ELISA. RTR were divided into CMV seronegative and latent CMV (seropositive + seroconverted) group. We measured CMV IgG (median [IQR] 72.0 [0.0-154.5] U/mL) at 6.0 [2.6-11.4] years post-transplant. During follow-up (5.3 [4.7-5.7] years), 42 (7%) RTR experienced graft failure and 95 (16%) RTR died. Risk for graft failure and mortality was significantly higher in RTR with latent CMV compared to CMV seronegative RTR (HR=3.3, *P*=0.01 and HR=2.2, *P*<0.005, respectively). After adjustment for potential confounders, latent CMV infection remained an independent risk factor for graft failure (HR=4.0, *P*=0.009), but not for mortality (HR=1.5, *P*=0.2). Latent CMV is an independent risk factor for graft failure long term after renal transplantation and carries higher risk for graft failure than for mortality. These findings confirm the notion that latent CMV mat contribute to the development of chronic renal transplant disfunction, possibly in conjunction with chronic low-grade alloreactivity.

Introduction

Cytomegalovirus (CMV) has been established as the single most important pathogen after transplantation.[1-3] Several studies have shown that primary CMV infection were as reactivation from latency shortly after transplantation are risk factors for both immunological rejection and mortality in the first year after transplantation.[4-9] The reactivation from latency that commonly occurs shortly after transplantation is the consequence of a temporary disruption of an otherwise existing balance between immunological surveillance and low-grade viral replication by treatment with cytotoxic drugs and antilymphocyte antibody therapy and by systemic infection and inflammation .[10] Both in case of primary infection and reactivation, CMV as a medical problem slowly diminishes with time after transplantation in conjunction with return to latency. It is seldom seen that CMV latency has not been achieved within one year after transplantation. However, the virus may continuously smoulder in the vascular wall, in particular in inflamed tissues under conditions of chronic immunosuppression.[11,12] It has indeed been shown that latent CMV can be locally active in a transplanted organ with ongoing low-grade alloreactivity, without systemic signs of activity in the chronic phase after transplantation.[13] As a consequence, investigation of CMV reactivation and primary infection shortly after transplantation as a risk factor for graft loss or mortality may have negated the possibility that the situation in which CMV remains in latency in the early phase after transplantation can be accompanied by ongoing CMV-related inflammation locally in tissues later after transplantation, in the transplanted kidney in particular. To investigate the late impact of latent CMV infection versus a persistent CMV negative state on late outcome, we prospectively investigated the relation between CMV serology determined more than one year after transplantation with graft failure and mortality late after renal transplantation.

Materials and methods

Research design and subject

In this prospective cohort study, all renal transplant recipients (RTR) who visited our out-patient clinic between August 2001 and July 2003 and had a functioning graft for at least 1 year were eligible to participate at their next visit to the out-patient clinic. Recipients were asked to participate at a later visit to the out-patient clinic if they were ill or had an infection. A total of 606 RTR signed written informed consent, from an eligible 847 (72% consent rate). The group that did not sign informed consent was comparable with the group that signed informed consent with respect to age, sex, body mass index (BMI), serum creatinine, creatinine clearance, and proteinuria. Further details of this study have been published

previously.[14] The Institutional Review Board approved the study protocol (METc 01/039) which was in adherence to the Declaration of Helsinki.[15]

Outcome events

All participating subjects visited the out-patient clinic at least once a year. Information on mortality and graft loss is recorded by our renal transplant center through close contact with general practitioners and referring nephrologists. Graft failure was defined as return to dialysis or re-transplantation and was censored for death. Mortality and graft failure of all RTR were recorded until August 2007. There was no loss to follow-up.

Renal transplant characteristics

Relevant transplant characteristics were taken from the Groningen Renal Transplant Database. This database holds information on all renal transplantations performed at our center since 1968, including dialysis history of individual RTR. Standard immunosuppression consisted of the following: from 1968 until 1989, prednisolone and Azathioprine (100 mg/day); from January 1989 to February 1993, cyclosporin standard formulation (Sandimmune, Novartis Pharma b.v., Arnhem, The Netherlands; 10 mg/kg; trough levels of 175–200 mg/l in first 3 months, 150 mg/l between 3 and 12 months post-transplant, and 100 mg/l thereafter) combined with prednisolone (starting with 20 mg/day, rapidly tapered to 10 mg/day). From March 1993 to May 1997, cyclosporin microemulsion (Neoral; Novartis Pharma b.v., Arnhem, The Netherlands; 10 mg/kg; trough levels idem) and prednisolone. From May 1997 to date, mycophenolate mofetil (Cellcept; Roche b.v., Woerden, The Netherlands; 2 g/day) was added. Current medication was extracted from the medical record. BMI, waist circumference, body surface area (BSA), and blood pressure were measured as described previously.[14] Smoking status and cardiovascular history were recorded with a self-report questionnaire. Cardiovascular disease history was considered positive if there was a previous myocardial infarction (MI), transient ischemic attack (TIA) or cerebrovascular accident (CVA). In our center we do not apply routine CMV prophylaxis. Prophylaxis for CMV is only applied in case of combined transplantation or use of anti-thymocyte globulin (ATG). Instead we perform frequent monitoring for CMV in blood, formerly – before and during the days that we performed the baseline measurements for the current study – by measuring CMV pp65 antigenaemia and nowadays by PCR. Guided by this monitoring we start pre-emptive treatment, formerly by i.v. ganciclovir and nowadays by oral valganciclovir preferentially. CMV disease was defined by detection of CMV in a clinical specimen accompanied either by CMV syndrome with fever, muscle pain, leucopenia, and/or thrombocytopenia without other known causes, or by organ involvement such as hepatitis, gastrointestinal ulceration, pneumonitis, or retinitis. Leukopenia was defined as leukocyte count less than 4×10^9 /L and thrombocytopenia when the cell count was less than 100×10^9 /L in

peripheral blood. Hepatitis was defined as a rise in liver enzymes of at least twice the initial values without other known cause. Gastrointestinal CMV ulceration was confirmed by endoscopy and biopsy. Presence of CMV in tissue biopsies was detected by immunohistochemistry or growth of virus in cell cultures.

Laboratory measurements

Blood was drawn after an 8-12h overnight fasting period. Anti-CMV IgG antibody levels were assessed by routine ELISA assay as described previously.[16] A detectable anti-CMV IgG titer indicated seropositivity. CMV in blood was monitored by measuring CMV pp65 antigenaemia as described previously.[17] Serum creatinine levels were determined using a modified version of the Jaffé method (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany). Serum total cholesterol, HDL cholesterol, triglycerides, high-sensitivity C-reactive protein (hsCRP), and urinary protein excretion were assessed as described previously.[14] Proteinuria was defined as urinary protein excretion ≥0.5 g/24 hr.

Statistical analysis

Analyses were performed with SPSS version 14.0 (SPSS Inc., Chicago, IL) and Sigma Plot version 10 (Systat software Inc., Germany). Parametric variables are expressed as mean±SD, whereas non-parametric variables are given as median [interquartile range]. A two-sided *P*-value less than $P<0.05$ indicated statistical significance. Recipient characteristics are shown according groups of CMV serostatus >1 year after transplantation: CMV seronegative (CMV IgG ≤1 U/ml at transplantation and beyond one year after transplantation), CMV seroconverted (CMV IgG ≤1 U/mL at time of transplantation and CMV IgG >1 U/ml beyond one year after transplantation) and CMV seropositive (CMV IgG >1 U/mL at time of transplantation and beyond one year after transplantation). Latent CMV infection was defined as CMV IgG > 1 U/mL beyond one year after transplantation (= CMV seroconverted + CMV seropositive). In time to event analyses, we first investigated CMV serostatus (seronegative, seroconverted, and seropositive) as potential predictor of graft failure and mortality using Kaplan-Meier analyses. Statistical significance was tested by Log-Rank test. Finally, univariate and multivariate Cox-proportional hazard regression analyses were performed to judge whether the potential effect of latent CMV infection on graft failure and mortality was independent of potential confounders. In the multivariate analyses, the associations of latent CMV infection with graft failure and mortality were adjusted for recipient age and sex (Model 2) and for time between transplantation and inclusion date, creatinine clearance, and immunosuppressive era (Model 3). We, subsequently, adjusted for all other characteristics which were significantly associated with CMV serostatus >1 year after transplantation (table 1 and 2, $P<0.05$, Model 4). As secondary analysis, the analysis was repeated with inclusion of

variables with a *P*-value >0.05 and ≤0.1 (Model 5). Also as a secondary analysis, we investigated whether log-transformed quantitative anti-CMV antibody titers were associated with occurrence of graft failure of out bounce.

Table 1. Recipient-related characteristics of renal transplant recipients according to CMV serostatus > 1 year after transplantation.

	CMV serostatus >1 year after transplantation			*P*
	Negative	Seroconverted	Seropositive	
n (%)	174 (29)	152 (25)	280 (46)	
Recipient demographics				
Age (years)	47.9 ± 13.1	52.5 ± 11.4	53.1 ± 11.5	<0.0001
Male, n (%)	103 (59)	85 (56)	144 (51)	0.3
Body composition measurements				
BMI (kg/m^2)	25.2 ± 4.04	26.2 ± 4.45	26.5 ± 4.30	0.01
Waist circumference (cm)	94.8 ± 13.5	97.8 ± 14.5	98.3 ± 13.2	0.03
Blood pressure				
Systolic pressure (mmHg)	151 ± 21.4	149 ± 23.1	157 ± 23.0	0.001
Diastolic pressure (mmHg)	90.1 ± 10.1	88.0 ± 10.4	90.9 ± 9.34	0.01
Prior history of cardiovascular disease				
MI[a], *n* (%)	10 (6)	20 (13)	18 (6)	0.02
TIA/CVA[b], *n* (%)	6 (3)	12 (8)	15 (5)	0.2
Lipids				
Total cholesterol (mmol/L)	5.6 [4.9-6.2]	5.7 [4.9-6.3]	5.5 [4.9-6.2]	0.6
LDL (mmol/L)	3.6 [3.0-4.2]	3.6 [3.0-4.2]	3.5 [2.9-4.0]	0.2
HDL (mmol/L)	1.0 [0.9-1.3]	1.0 [0.8-1.3]	1.1 [0.9-1.3]	0.2
Triglycerides (mmol/L)	1.8 [1.3-2.4]	1.9 [1.4-2.8]	2.0 [1.4-2.6]	0.02
Use of statin, *n* (%)	79 (45)	68 (45)	153 (55)	0.06
CRP (mg/L)	2.0 [0.7-4.4]	2.1 [0.8-4.9]	2.0 [1.0-5.5]	0.4
CMV				
CMV IgG (U/mL)	0 [0-0]	110 [62-191]	110 [62-198]	<0.0001
CMV disease, *n* (%)	0 (0)	66 (43%)	66 (24)	<0.0001

[a]MI, myocardial infarction.

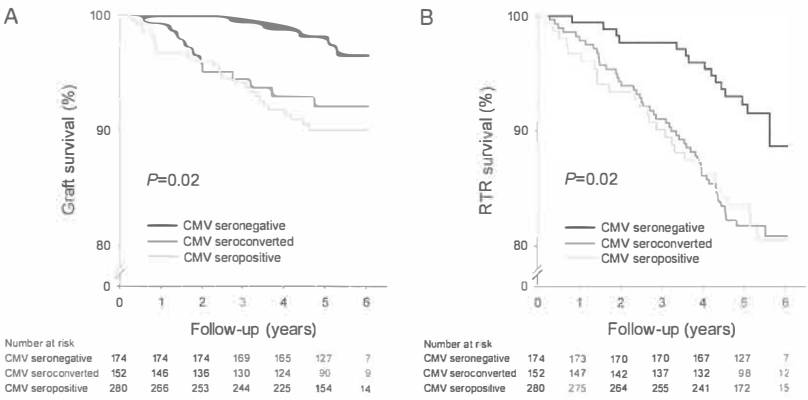
[b]TIA/CVA, transient ischaemic attack/cerebrovascular accident.

Table 2. Transplant-related characteristics of renal transplant recipients according to CMV serostatus > 1 year after transplantation.

	CMV serostatus >1 year after transplantation			*P*
	Negative	Seroconverted	Seropositive	
n (%)	174 (29)	152 (25)	280 (46)	
Donor demographics				
Age (years)	35.9 ± 15.4	34.8 ± 14.9	38.7 ± 15.6	0.02
Male, *n* (%)	98 (56)	82 (54)	148 (53)	0.8
Renal allograft function				
Serum creatinine concentration (µmol/L)	136 [112-162]	129 [111-170]	134 [114-166]	0.8
Creatinine clearance (mL/min)	66.5 ± 21.2	59.6 ± 21.3	60.5 ± 23.5	0.007
Proteinuria (g/24hr)	0.2 [0.0-0.4]	0.2 [0.0-0.5]	0.2 [0.0-0.5]	0.7
Prior dialysis duration (mo)	25 [12-47]	26 [15-29]	29 [16-53]	0.09
Transplantation type, *n* (%)				
Postmortem donor	137 (79)	134 (88)	232 (83)	
Living donor	33 (19)	15 (10)	35 (12)	0.06
Combined transplantation	4 (2)	3 (2)	13 (5)	
Number of previous transplants, *n* (%)				
0	163 (94)	134 (88)	245 (88)	0.01
1 or more	11 (6)	18 (12)	35 (12)	
Acute rejection, *n* (%)	77 (44)	77 (51)	118 (42)	0.2
Immunosuppressive era, *n* (%)				
from 1968 to January 1989	30 (17)	65 (43)	17 (6)	
from January 1989 to February 1993	19 (11)	17 (11)	55 (20)	<0.0001
from March 1993 to May 1997	42 (24)	32 (21)	82 (29)	
from May 1997 to date	83 (48)	38 (25)	126 (45)	
Immunosuppresion				
Prednisolone dose, (mg/day)	10.0 [7.5-10.0]	10.0 [7.5-10.0]	10.0 [7.5-10.0]	0.04
Calcineurine inhibitor, *n* (%)	140 (81)	94 (62)	241 (86)	<0.0001
Cyclosporin, *n* (%)	112 (65)	81 (53)	197 (70)	0.5
Trough-level (µg/L)	114 [82-140]	101 [75-128]	108 [80-143]	0.2
Tacrolimus, *n* (%)	28 (16)	13 (9)	44 (16)	0.5
Trough-level (µg/L)	8 [6-11]	10 [7-11]	9 [6-10]	0.7
Proliferation inhibitor, *n* (%)				
Azathioprine or Mycophenolate mofetil	133 (76)	107 (70)	208 (74)	0.5

Results

Figure 1. Kaplan-Meier analysis of (A) graft and (B) RTR survival according to CMV serostatus > 1 year after transplantation. Tested with Log-Rank test.



A total of 606 RTR (55% male, aged 51±12 years, 83% post-mortem donor transplants) were analyzed. Median time between transplantation and baseline measurements was 6.0 [2.6-11.4] years. Baseline median anti-CMV IgG was 72.0 [0.0-154.5] U/mL. Baseline characteristics according to CMV serostatus >1 year after transplantation are shown in tables 1 and 2; 174 (29%) RTR were CMV seronegative, 152 (25%) RTR were CMV seroconverted, and 280 (46%) RTR were CMV seropositive. CMV serostatus was significantly associated with recipient age, BMI, waist circumference, systolic and diastolic blood pressure, myocardial infarction, triglyceride concentration, donor age, creatinine clearance, immunosuppressive era, dose of prednisolone, and use of calcineurine inhibitors. CMV disease was significantly associated with CMV serostatus ($P<0.0001$). In total 132 RTR experienced CMV disease, 66 (43%) of the 152 CMV seroconverted RTR and 66 (24%) of the CMV seropositive RTR. Median follow up was 5.3 [4.5-5.7] years for graft failure and 5.3 [4.7-5.7] years for mortality. During follow up, 42 (7%) RTR experienced graft failure and 95 (16%) RTR died. In the CMV seronegative group 5 (3%) RTR experienced graft failure and 16 (9%) died, while these numbers were 11 (7%) and 28 (18%) for the CMV seroconverted RTR and 26 (9%) and 51 (18%) for the CMV seropositive RTR (both Log-Rank test: $P=0.02$, figure 1 A and B). Further analyses were performed for latent CMV infection (= CMV seroconverted + CMV seropositive) versus CMV seronegative RTR. RTR with latent CMV infection were at significantly higher risk for graft failure (hazard ratio (HR)=3.3, 95% confidence interval (CI) 1.3-8.5, $P=0.01$) and death (HR=2.2, 95% CI 1.3-3.7, $P=0.005$) than CMV seronegative RTR (Model 1, table 3).

Table 3. Univariate and multivariate Cox-proportional hazards analyses of the effect of latent CMV infection on graft failure and mortality in RTR.

	Graft failure			Death		
	HR	95% CI	*P*	HR	95% CI	*P*
Model 1	3.3	1.3-8.5	0.01	2.2	1.3-3.7	<0.005
Model 2	3.9	1.5-10.0	<0.005	1.8	1.1-3.1	0.03
Model 3	3.3	1.2-8.8	0.02	1.6	0.9-2.7	0.1
Model 4	3.4	1.2-.9.3	0.02	1.5	0.8-2.5	0.2
Model 5	4.0	1.4-11.2	0.009	1.5	0.8-2.5	0.2

CI: confidence interval
Model 1: crude model.
Model 2: model 1 + recipient age and sex.
Model 3: model 2 + time between transplantation and inclusion date, creatinine clearance, use of cyclosporin, tacrolimus, their trough levels and immunosuppressive era.
Model 4: model 3 + BMI, systolic blood pressure, myocardial infarction, concentration triglycerides, donor age, prednisolone dose, and calcineurine inhibitor.
Model 5: model 4 + use of statin, prior dialysis duration, transplantation type, and number of previous transplants.

Adjustment for recipient age and recipient sex did not materially change these associations (Model 2, table 3). After further adjustment for time between transplantation and inclusion date and creatinine clearance, use of cyclosporine, tacrolimus, their trough levels and immunosuppressive era (Model 3, table 3), CMV latency remained significantly associated with graft failure (HR=3.3, 95% CI 1.2-8.8, P=0.02), while the association of CMV latency with death lost significance (HR=1.6, 95% CI 0.9-2.7, P=0.1). Additional adjustment for variables which were significantly associated with CMV serostatus (see table 1 and 2, all variables with a P<0.05) did not materially change the outcomes (Model 4, table 3). Subsequent adjustment for variables which were borderline significant associated with CMV serostatus (0.05<P<0.10, table 1 and 2) did not materially change the outcomes (Model 5, table 3). After multivariate analyses the risk for graft failure (HR=4.0, 95% CI 1.4-11.2, P=0.009) in CMV IgG positive RTR was 2.7 times higher than the risk for death (HR=1.5, 95% CI 0.8-2.5, P=0.2, Model 5, table 3). Final adjustment for CMV disease did not materially change the outcomes (data not shown). As a secondary analysis, we investigated in RTR with latent CMV infection whether quantitative anti-CMV antibody titers were associated with graft outcome. This appeared not the case (HR 1.2 (95%CI 0.5-2.6), P = 0.6).

Discussion

To the best of our knowledge, our study is the first to prospectively investigate the impact of CMV serology determined >1 year after transplantation on graft and RTR survival late after renal transplantation. The main finding is that graft survival is significantly better in CMV seronegative RTR than in those with latent CMV infection. We furthermore found that RTR with latent CMV infection are at 2.7 times higher at risk for graft failure than for death. CMV has been established as a major pathogen after renal transplantation and as such as an important cause of morbidity and mortality after renal transplantation. CMV infection is highly prevalent in RTR (up to around 80% in the western countries), whereas 25-33% of the infected RTR develop a clinically overt disease after renal transplantation.[18] Numerous studies have shown that CMV infection and disease occurring in the first months after transplantation are risk factors for immunological rejection and mortality, both early and late after transplantation.[4-9] However, use of CMV disease and infection early after transplantation as predictors of late graft failure and mortality may lead to underestimation of risk held by CMV if it is the CMV positive state itself rather than the severity of CMV disease or infection in the first phase after transplantation that is the risk factor. Inclusion in the control group of CMV positive recipients that not exhibit early CMV disease or infection will dilute the group of CMV seronegative controls with subjects that are at increased risk. Results of our study are consistent with this notion because the CMV seroconverted and the CMV seropositive groups had similar increases in risk for late graft failure and mortality compared to recipients that remained CMV seronegative. Although latent CMV infection lost significance as a risk factor for mortality after adjustment for other variables, it can not be excluded that CMV actually acts on mortality, in part through these variables. Potential mechanisms underlying an association of latent CMV infection with mortality may lie in CMV causing accelerated decline of renal function and/or accelerated atherosclerosis in RTR. A potential role for CMV-related decline of renal function is supported by loss of significance of the association of CMV with mortality after adjustment for creatinine clearance. Active, but also latent CMV infection, may not only be associated with overexpression of major histocompatibility complex molecules and altered expression of growth factors and cytokines, but also with upregulation of pro-inflammatory adhesion molecules, which might lead to accelerated atherosclerosis in association with CMV.[2,11,19] The finding that CMV DNA is present in atherosclerotic plaques supports a role for CMV in atherogenesis,[20-22] although some have failed to detect CMV in atherosclerotic tissue.[23,24] In a study performed shortly after transplantation, CMV has been suggested to play a role in the pathogenesis of post-transplant diabetes mellitus,[25] which may also exert a pro-atherogenic effect. The fact that we found that RTR with latent CMV infection are at 2.7-fold higher at risk for graft failure

than for death is consistent with the recent finding that latent CMV may be locally active in a transplanted organ, without systemic signs of activity or consequences.[13] Latent CMV may be particularly active in organs and tissues with ongoing inflammation not directly related to CMV.[12] In transplantation, the allo-surrounding may provide the background inflammation on which CMV comes to expression above levels of innocence. After cardiac transplantation, it has been shown that CMV is associated with development of accelerated coronary artery sclerosis.[26] A similar process has been observed in transplanted kidneys in association with CMV infection.[27,28] In studies in rats, the interaction between CMV and the alloreactive response on the development of chronic rejection and transplant vascular sclerosis was investigated in small bowel and heart transplantation models.[13] It was shown that CMV infection accelerated the time to graft chronic rejection and increased the severity of transplant vascular sclerosis in both small bowel and heart allografts. In our study CMV serostatus >1 year after transplantation was not associated with acute rejection. Most of the studies investigating the impact of CMV on acute rejection found an association of CMV infection or disease with acute rejection early after transplantation.[4,7,8,29] The absence of an association in our study may be explained by the fact that our study was designed to investigate the impact of CMV determined >1 year after transplantation on long-term graft and RTR survival. As a consequence of the fact that we only invited RTR with a kidney functioning for >1 year, RTR who lost their kidney due to acute rejection in the first year(s) after transplantation were not invited to participate in this study. Therefore, in this study the number of RTR who had an acute rejection is probably underestimated compared to studies in which RTR were included from the moment of transplantation. Currently, two strategies are considered acceptable for CMV prevention: (1) universal prophylaxis and (2) preemptive therapy.

Prophylaxis is associated with the risk of late-onset CMV disease and toxicity/costs,[30] whereas preemptive therapy requires frequent monitoring of CMV activity using sensitive methods and patient compliance.[31] Furthermore, the impact of preemptive therapy on reducing the indirect effects of CMV is questionable.[32] CMV resistance has been observed with both strategies.[33,34] We can not address the question whether prophylaxis would alter the associations we found. However, since we found an association between latency of CMV more than one year after transplantation and subsequent development of graft failure, while CMV prophylaxis is only applied in the first months after transplantation, it seems unlikely that this initial treatment would importantly alter the biology of a state of latency late after transplantation. The present study has several limitations. First, because the study population almost entirely consisted of patients of Caucasian ethnicity, the applicability of our results to more racially diverse renal transplant population may be limited. Furthermore, this study was a single centre study and the findings need to

be confirmed in other centra and/or multicenter studies. Third, our study includes RTR that were transplanted in multiple immunosuppressive eras. Although, immunosuppressive therapy was associated with CMV serostatus at baseline, adjustment for immunosuppressive era in the multivariate analyses did not materially change the association of CMV serostatus with outcomes. Furthermore, we did not perform surveillance biopsies as this is an invasive procedure, with an ever existing risk of complications, including intractable bleeding necessitating removal of the graft. It may also be seen as a limitation that the fraction of CMV seronegative RTR of 54% at the time of transplantation in our population is higher than in other studies, in which for instance fractions of 49%, 45% and 52% have been reported.[5,35,36] It should, however, be noted that these studies included patients at the moment of transplantation, while we included our patients at a median time of 6.0 years after transplantation. Because CMV disease is an acknowledged risk factor for mortality early after transplantation, and we included patients at a moment beyond the first year after transplantation, the relatively high fraction of CMV seronegative RTR at the time of transplantation is consistent with the notion that CMV is indeed a risk factor for mortality early after transplantation rather than an indication that our population is not representative of an average renal transplant population. Finally, two other members of the β-herpesvirus family, human herpesvirus-6 (HHV-6) and HHV-7, are increasingly recognized as important pathogens in transplant recipients.[37] Reactivation of HHV-6 and HHV-7 occurs in up to 66% and 46% of the RTR.[38,39] Several studies have further demonstrated that HHV-6 and HHV-7 reactivation occurs prior to that of CMV, and that HHV-6 and HHV-7 have been implicated as factors for subsequent CMV reactivation and disease.[39-42] It has, however, recently been reported that correlation between CMV and HHV-antigens in biopsies of kidney transplant recipients with cytomegalovirus infection is low.[43] Thus, it is unlikely that concurrent HHV-6 reactivation explains our finding of latent CMV infection as a risk factor for graft failure. An important strength of this study is that follow-up was complete for all patients. In conclusion, graft and recipient survival is significantly better in RTR who are CMV seronegative when compared to RTR with latent CMV infection. Our results are consistent with the notion that it is not severity of infection early after transplantation, but rather the CMV positive state itself. Furthermore, RTR with latent CMV infection are at 2.7-fold higher risk for graft failure than for death. This suggests that latent CMV is more active in a transplanted organ, potentially in association with chronic ongoing low-grade alloreactivity, or in kidneys in general. Future studies are needed to elucidate the mechanism underlying the link of CMV with graft failure and mortality late after renal transplantation.

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Chapter 9

Summary, discussion and future studies

The aim of the present thesis was to investigate risk factors for late graft loss and mortality after renal transplantation with emphasis on insulin resistance, metabolic syndrome and CMV. Transplant research has focused traditionally on immunological (alloantigen-dependent) issues to avoid (hyper)acute and chronic rejection. With the advent of stronger immunosuppressive drugs, such as calcineurin inhibitors and mycophenolate, the transplant community became to realize that the success in reduction of acute rejection and short-term graft survival did not improve long-term survival inherently.[1] The observed slow deterioration of graft function over time, most often without specific histological diagnosis other than chronic scarring and/or arteriolar hyalinosis, could not be overcome by more and stronger immunosuppression. On the contrary, the development of long-term side-effects of this immunosuppression including obesity, hypertension, diabetes, calcineurin nephrotoxicity in addition to opportunistic infection (first CMV, later BK virus nephropathy), as well as the acceptance of older, more marginal donors & recipients, shifted attention also towards alloantigen-independent risk factors for chronic renal transplant dysfunction. Nowadays, it is e.g. known that new-onset diabetes after transplantation carries almost a similar burden on graft outcome as e.g. an acute rejection episode. Many rejection episodes formerly classified as treatment resistant, may perhaps in hindsight be attributed to BK virus nephropathy.

Summary and discussion

At the turn of the century, insights on the development of chronic renal transplant dysfunction coincided with the explosion on papers on the insulin resistance syndrome in the general population. The purpose of **Chapter 2** was to increase awareness among the transplant community that many of the known alloantigen-independent risk factors for chronic renal transplant dysfunction share analogy with the cardiovascular risk factors that cluster within the insulin resistance syndrome. Chapter 2 built a case for the insulin resistance syndrome as a unifying pathophysiological entity that underlies a majority of the alloantigen-independent risk factors for CTD. Furthermore, this chapter stressed the need for longitudinal cohort studies investigating insulin resistance as a putative cause of CTD. Both complex disorders (insulin resistance syndrome and chronic renal transplant dysfunction) are thought to have some degree of overlap. The term "insulin resistance" syndrome demonstrates the initial glucocentric or diabetogenic view on the metabolic syndrome with a central role for insulin resistance in the clustering of various metabolic derangements. Presently, "metabolic syndrome" (as coined in 1998 by the World Health Organisation) is the most popular nomenclature and underscores the more recent lipocentric view with a role of (central) obesity and increased exposure of nonesterified fatty acids to nonadipose tissue as the central feature linking the clustering of various metabolic factors.[2]

The hyperinsulinemic euglycemic clamp is the gold standard to study insulin resistance, but this technique is too cumbersome and time-consuming for large cohorts. Insulin resistance indices based on fasting parameters are useful surrogate measures of insulin resistance for epidemiological studies but are derived from non-transplant populations. In **Chapter 3**, we aimed to validate some frequently used insulin resistance indices that are based on fasting blood parameters alone in 51 stable non-diabetic renal transplant recipients, who were on a cyclosporine-based immunosuppressive regimen. A secondary objective was to investigate which risk factors - both traditional and more transplant-related (e.g. immunosuppression) - were associated with clamp-assessed insulin resistance. It was shown that all three investigated indices were acceptable surrogate estimates of insulin resistance, but that the index, which incorporated fasting serum triglycerides in addition to glucose and insulin (i.e. McAuley's index), had best correlation and agreement with the gold standard.

In addition, we showed that BMI, triglycerides, and insulin were the predominant determinants of insulin resistance in this non-diabetic cohort on cyclosporine. We do not have a ready explanation why BMI correlated better with insulin resistance than waist-circumference. Most epidemiological studies show a better correlation of central obesity (waist circumference) with insulin resistance than a measure of general obesity (BMI). The fact that we did not adjust our analyses for muscle mass might have contributed to a stronger association for BMI.[3] Furthermore, we could not demonstrate any association between insulin resistance and transplant-related factors. This might be caused by the fact that the population was too homogenous among the transplant-related factors to have enough discriminatory power for the relatively small sample size. We did not find CMV seropositivity of the donor or recipient to be related with clamp-assessed insulin resistance long-term after transplantation.

Since publication of the ELITE-Symphony trial,[4] in which the tacrolimus arm proved most efficacious with regards to graft outcomes at 1 year posttransplant, the initial use of tacrolimus has become almost standard. Both this trial and the DIRECT trial[5] showed that calcineurin inhibitors cyclosporine and tacrolimus might have different diabetogenic propensities, as well as different associations with serum triglycerides. Therefore, concerns might rise whether our results are applicable to patients on tacrolimus-based regimens. Very recently, Sharif et al[6] showed that these indices are also valid for recipients on tacrolimus-based regimens and corroborated our finding that McAuley's index was most strongly associated with insulin resistance. It has also been questioned whether these indices are valid in transplant populations with worse renal function than in these relatively stable transplant cohorts. Although they did not investigate the McAuley index, Crutchlow et al[7] investigated the applicability of HOMA and Quicki indices in various stages of CKD and

found no major influence of impaired renal function on the validity of these indices over the spectrum of CKD.

In **Chapter 4**, we used surrogate estimates of insulin resistance that were validated in Chapter 3 to investigate to which extent both traditional (e.g. obesity) and transplant-related factors (e.g. immunosuppressants) may contribute to insulin resistance after renal transplantation in a large cohort of renal transplant outpatients. We found that obesity, in particular a central distribution of obesity, as well as prednisolone dose were important determinants of insulin resistance after 1 year posttransplant. In addition, we found recipient age, and HDL- and total cholesterol to be independently associated with the indices as well. These findings were in accordance with expectation. Again, we did not find CMV positivity to be related with insulin resistance longer after transplantation. Renal function, however, as assessed by 24-h creatinine clearance, was positively related to fasting insulin and HOMA, suggesting that insulin resistance was associated with better renal function. We stated that this relationship by itself did not constitute a new finding because glomerular filtration rate is known to increase under acute hyperinsulinemic conditions in nontransplanted kidneys[8] and we postulated that this phenomenon might also be present in transplanted kidneys. Unfortunately, we did not perform polynomial (higher order) regression analysis of renal function and insulin resistance to assess a putative parabolic relationship between these two variables. Later, we confirmed that this type of relationship is present in the general Groningen population.[9] However, it remained unknown whether a single (transplanted) kidney, which already undergoes compensatory hyperfiltration after transplantation, demonstrates (additional) hyperfiltration owing to hyperinsulinemia. The notion that transplanted kidneys may undergo hyperfiltration owing to hyperinsulinemia was supported by the finding of Bosma et al[10] using the overall Groningen Renal Transplant Database, that obesity (BMI) was indeed associated with an increased glomerular filtration rate as well as filtration fraction at 1 year posttransplant. It is not unlikely that hyperinsulinemia may underlie this relationship. Recently, Porrini et al[11] demonstrated in a Spanish multicenter cohort of 202 transplant recipients that obesity-associated hyperinsulinemia is independently associated with increasing renal function within the first year after transplantation, suggesting that hyperinsulinemia may indeed add to the compensatory hyperfiltration in kidneys in the first year after transplantation. After adjusting for hyperinsulinemia, the impact of obesity attenuated in their multivariate analyses.

In **Chapter 5,** we investigated the prevalence of the metabolic syndrome in our renal transplant cohort and investigated to which extent metabolic syndrome (MS) was associated with impaired long-term renal allograft function. We found that a majority (63%) of our renal transplant cohort suffered from the metabolic syndrome as defined by ATPIII criteria. In

2001, the National Cholesterol Educational Program – Adult Treatment Panel III devised a new definition of the metabolic syndrome that is comprised of 5 simple criteria.[12] Before 2001, there were only the WHO and EGIR definitions which included etiological but more abstract and impractical parameters such as insulin resistance and/or hyperinsulinemia.[2] As insulin assays are not standardized, it is impossible to define cut-off points or compare results among studies. The simplicity of the ATPIII criteria caused it to be the most widely utilized definition to date. Using this definition, we found age and gender adjusted odds of having MS in the renal transplant population to be 7 compared with the general Groningen population.[13] Since this publication, similar prevalence rates have been found by other groups using the same (or a slightly adjusted) definition.[14,15] We found presence of MS independently associated with impaired long-term renal allograft function, which was corroborated by Porrini et al.[15]

Of the MS component criteria, we found systolic blood pressure and serum triglycerides to be independently associated with renal function. We could not demonstrate independent relationship between waist circumference and serum glucose. Moreover, the direction of these two associations changed from univariate to multivariate analyses. They might have been confounded by a putative hyperfiltration relationship between hyperinsulinemia and renal function, owing to the cross-sectional nature of our analyses. Unfortunately, insulin concentrations were not available at that time to investigate this hypothesis. The group of Porrini did not find any association between fasting glucose and long-term allograft function either.[15] A similar finding has been reported in the general population.[16] Fasting glucose may not be sensitive enough (compared e.g. to hyperinsulinemia) to analyze the impact of metabolic syndrome/insulin resistance in cohorts of which the majority is nondiabetic.

Furthermore, it remains a matter of debate whether the metabolic syndrome in renal transplant recipients is similar to metabolic syndrome in the general population as any combination of three criteria may 'diagnose' MS. We demonstrated that the increased prevalence of MS (using ATPIII criteria) in renal transplant recipients is largely explained by an increased prevalence of hypertension and dyslipidemia compared with the general population.[13] This might be an additional explanation why we found systolic blood pressure and triglycerides (with HDL cholesterol borderline) to be associated with impaired renal allograft function. Also, we cannot fully exclude the possibility that hypertension and triglycerides were the consequence of impaired renal allograft function rather than the cause. However, our analyses suggest a putative causal relationship as the multivariate analyses were adjusted for renal function at 1-year posttransplant (baseline) as well as time elapsed since transplantation.

A cause for the increased prevalence of hypertension and dyslipidemia might lie both in the immunosuppression that transplant recipients receive as well as in obesity. RTR were more often central obese than the general population (51 vs 24%). Armstrong et al[17] showed that as body mass

increases after transplantation, the prevalence of metabolic syndrome increases proportionally. It might be hypothesized that the metabolic syndrome in renal transplant recipients is characterized by varying 'phenotypes' which may to a certain extent be time-dependent: one phenotype in non-overweight transplant recipients that clusters around hypertension and dyslipidemia owing mainly to initial immunosuppression (e.g. calcineurin inhibitors) and perhaps relatively impaired renal allograft function; and another that clusters more around posttransplant weight gain and obesity with obesity-superimposed dyslipidemia and hypertension. Maybe even a third phenotype may exist that clusters around pre-existing or new onset diabetes after transplantation in which both obesity and immunosuppression play a role.

In 2005, the International Diabetes Federation[18] published a definition very similar to ATPIII, but inclusion of central obesity is required. Another difference with ATPIII is that IDF also includes overweight people by defining a lower waist circumference cut-off to identify men and women with a BMI ≥ 25 kg/m^2.[19] Although there has been some debate about returning to a more etiological based definition, a clear benefit of the IDF definition is that it standardizes etiological fenotype of metabolic syndrome the ATPIII definition. Although they excluded diabetics, Sharif compared both ATPIII and IDF definitions of MS (using similar waist-to-hip ratio cut-offs) in renal transplant recipients.[14] They found a prevalence of 59% using ATPIII vs 43% using the IDF definition, suggesting that 16% of ATPIII diagnosed MS does not cluster around central obesity. They did not find important differences in immunosuppressive drugs between definitions, probably because their sample size was too small (only 58 individuals). Furthermore, they found MS by IDF criteria to correlate more closely with insulin resistance and subclinical inflammation than ATPIII. Furthermore, impaired renal function was associated with IDF only, and not significantly with ATPIII. However, larger studies have confirmed our finding that ATPIII defined MS is associated with impaired renal allograft function, suggesting a sample size effect.[15, 20]
Finally, we found women to suffer more from postttransplant weight gain, metabolic syndrome and impaired renal function than men. This is in accordance with previous studies which found increased weight gain for women as well[21-23] Unfortunately, we could not stratify our analyses for sex without loosing power and association.

Chapter 6 shows the predictive performance of intermediates such as renal vascular resistance, renal allograft function, and proteinuria for graft failure. A pressing need exists for better intermediate endpoints to predict graft and patient survival.[24] Intermediate or surrogate markers can be used in lieu of conventional clinical endpoints such as graft survival or death. In 2003, Radermacher et al[25] published an article in the NEJM that the renal resistance index as assessed by ultrasonography was an excellent intermediate endpoint of transplant outcome. It remains uncertain to what extend ultrasonographic measurement of renal

transplant arteries represents truly intrarenal resistance or merely vascular compliance. The paper by Radermacher provided opportunity to compare renal vascular resistance (based on Ohm's law for fluidics) with renal function and proteinuria - which we used in previous chapters as intermediates for graft outcome - in the Groningen Renal Transplant Database. This database is unique as it contains isotope clearance studies of all transplant recipients at fixed time points after transplantation. The study showed that RVR is a prominent risk marker for recipient mortality and death-censored graft loss. However, the predictive value of RVR for recipient mortality owed mainly to the impact of mean arterial blood pressure. In contrast, RVR constituted more than the sum of its components for death-censored graft loss, but showed less predictive value than serum creatinine in univariate analysis.

There has been some dispute over the validity of serum creatinine and GFR as a predictor of long-term graft survival. Poor positive and negative predictive values were found with (early post transplant) serum creatinine, GFR, and even delta GFR for long-term graft failure with and without censoring for death.[26] Short-term renal function might not reflect optimally what will happen with long-term graft function as well as patient death. The suboptimal predictive value of renal function and proteinuria separately may be overcome by using a composite intermediate that contains information of both.[27] We did not test this hypothesis further. Its limitations notwithstanding, renal allograft function and proteinuria remain among the most important intermediate endpoints currently available.

Certainly, obesity, hypertension, dyslipidemia, and posttransplant diabetes can be viewed as risk markers as well. MS could therefore be considered a composite intermediate of these factors. However, MS is neither a diagnosis, nor a risk score on its own merit. The prognostic value of MS in nontransplant populations is e.g. less than the Framingham risk score.[28] The merit of MS is that it focuses on a clustering of metabolic risk factors that are thought to cluster more often than by chance alone. Also, MS might perhaps convey more than the sum of its separate parts. It is derived on the basis of a putative pathophysiological understanding rather than prospective cohort analysis such as the Framingham risk score, which incorporates powerful predictors such as age and smoking. To demonstrate that MS is associated with transplant outcomes serves the transplant community to understand the pathophysiological factors involved in limiting long-term success of renal transplantation. Of note, the Framingham Heart Study model seems to underestimate the cardiovascular risk in renal transplant recipients.[29]

Chapter 7 investigates the impact of cardiovascular risk factors such as N-terminal pro-BNP and those associated with the metabolic syndrome on renal transplant recipient mortality in comparison with the general population. We found that the hazard ratio for overall mortality in RTR compared with the general population was 6. NT-pro BNP, renal function,

and use of hypertensive medication explained the majority of this excess hazard.

Even though MS component criteria were more prevalent in the renal transplant population, they did not explain the excess hazard for mortality in comparison with the general population. Although it was difficult to ascertain the exact cause of cardiovascular death in our study (ischemic vs. heart failure) retrospectively, our data suggest that transplant recipients may suffer more from an accelerated state of heart failure than an accelerated state of atherosclerosis. This is not unlike the dialysis population, and might explain to a certain extent the difficulty of getting positive results for statin treatment in the ALERT cohort.[30]

Data from the ALERT trial also corroborate our finding that renal function impairment is an important risk factor for all cause and cardiovascular mortality in the renal transplant population,[31] suggesting a cardiorenal interaction. Renal function has been found to be a stronger determinant of BNP than ventricular function in chronic kidney disease over the spectrum of renal insufficiency.[32] Furthermore, Jardine and co-workers[33] found that renal transplantation is not associated with regression of left ventricular hypertrophy (using MRI) in comparison with patients who remained on dialysis. A total of 86% of our cohort were former dialysis patients who had received a postmortem kidney. The increasing prevalence of pre-emptive living kidney donation might change the cardiovascular epidemiology in current transplant cohorts over coming years by increasing the ratio of atherosclerotic to heart failure incidents.

Even though our results suggest that heart failure might explain the excess mortality compared with the general population, RTR still experience ischemic events. Presence of the metabolic syndrome in renal transplant recipients has been correlated with coronary artery calcification.[35] Furthermore, transplant recipients with the metabolic syndrome at 1 year posttransplant were 3-4-fold more likely to have an atherosclerotic event.[36] Faenza et al[37] found a positive association between number of MS component criteria and incidence of cardiovascular events in transplant recipients over a 4-year follow up period. We found MS to predict cardiovascular and all-cause mortality in RTR.[38] MS predicted all cause mortality independent from its component criteria and other risk factors (HR 1.8 [1.0-3.3], suggesting that MS might constitute more than the sum of its components in our transplant cohort. A similar finding has been shown by Gami et al[39] in a systematic review and meta-analysis of 37 longitudinal studies assessing the association between MS and cardiovascular risk. In their pooled analysis, adjusting for MS and its component criteria in multivariate models, they demonstrated that MS conferred cardiovascular risk beyond that of its component criteria (RR 1.54 [1.32-1.79]. Interestingly, the ALERT researchers found that metabolic syndrome identified those transplant recipients who benefited from statin treatment.[34]

We have not yet investigated the impact of MS on renal endpoints, but Porrini et al[15] found that MS impacted graft survival negatively. Data on

the impact of MS on renal outcome from the ALERT trial is expected to be reported soon.[34]

In **Chapter 8** we investigated the impact of CMV serology determined beyond one year after transplantation on graft failure and mortality as well as associations with cardiovascular risk factors. We found CMV serostatus to be significantly associated with recipient age, BMI, waist circumference, blood pressure, prior history of myocardial infarction, renal function, triglycerides, and use of calcineurin inhibitors. Interestingly, a recent cross-sectional population-based study found association between metabolic syndrome and CMV seropositivity.[40] We did not find association between CMV seropositivity and high sensitivity CRP, a marker of inflammation. Unfortunately, we did not take endothelial parameters and insulin resistance into consideration. In multivariate analyses, the risk of CMV seropositivity appeared to be an independent risk factor for graft failure, but not for mortality. The majority of our cohort was on a calcineurin inhibitor. Our data might not be applicable to patients on mTOR-inhibitors as these newer immunosuppressive drugs might inhibit viral cascades.[41] Naturally, CMV is never easily defeated and seems to be able to activate downstream mTOR pathways independent of mTOR inhibition.[42]

Future studies

The studies performed during my PhD-period made me aware about the impact that the emerging epidemic of obesity may have for the incidence and prevalence of chronic kidney disease and end-stage renal disease in the general population. In the future, I would like to investigate the potential role of lipid nephrotoxicity on this relationship in the general population. Reasons and background are the following:

The worldwide epidemic of obesity is paralleled by an increase in chronic kidney disease (CKD).[43] CKD affects 10% of population in Western nations and is most often due to renovascular and type-2 diabetic nephropathy.[44] Obesity is not only the driving force behind renovascular risk factors such as hypertension, dyslipidemia, and diabetes; it is thought to be an independent progression factor of primary and secondary nephropathies as well.[43,45] Obese men and women have a weight-dependent 2 to 7-fold increased risk in progression of CKD irrespective of the underlying cause.[46-48] A meta-analysis of weight loss interventions in obese patients showed normalization of renal function and microalbuminuria; strikingly, it seemed to prevent further decline in renal function.[49] Still surprisingly little is known on how obesity may initiate renal disease. Since the description of Kimmelstiel and Wilson of nodular glomerulosclerosis and presence of lipid deposits in diabetic kidney,[50] there is growing evidence that lipid accumulation may play a role in the etiology of glomerulosclerosis.[51]

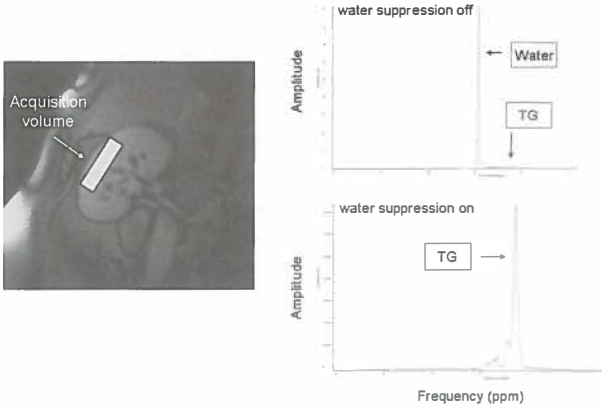
Obesity often precedes type-2 diabetes, and renal biopsy specimens of obese but otherwise healthy non-diabetic, non-hypertensive kidney donors already show glomerulomegaly and microalbuminuria compared to non-obese donors.[52, 53] This suggests that obesity may initiate a cascade of events that can lead to glomerulosclerosis already before onset of diabetes. Obesity-induced lipid nephrotoxicity might constitute the pathophysiological link, analogous to atherosclerosis.[54, 55]

Insights on how obesity leads to disease are just emerging.[56-59] It is thought that abdominal adipose tissue generates high levels of circulating nonesterified fatty acids (NEFA).[60] NEFA overload stimulates hepatic synthesis of VLDL cholesterol, which delivers increased amounts of triglycerides (TG) and cholesterol (CE) to various nonadipose peripheral tissue such as muscle, cardiomyocytes, and macrophages.[60] Here, lipoprotein lipase (LPL)-mediated hydrolysis of VLDL-TG may result in direct cellular uptake of NEFA, followed by intracellular re-esterification into lipids.[61, 62] As a result, VLDL is metabolized to small dense LDL and oxidized LDL (oxLDL) cholesterol.[63] Uptake of (ox)LDL via LDL- and scavenger receptors by e.g. macrophages may lead to intracellular accumulation of cholesteryl esters.[63] Both TG and CE are stored intracellularly in lipid droplets, which are spherical organelles covered by a phospholipid monolayer and coated with regulatory proteins.[64] Lipid droplets have been reported to play active and diverse roles in cellular life[65] and are e.g. involved in foam cell transformation of macrophages.[66] Intracellular lipid is not considered inert.[67] Toxic lipid metabolites (fatty-acyl CoA's, diacylglycerol, and ceramides) may change the cellular redox environment to a more oxidized state; reduce mitochondrial function and subsequently the capacity to oxidize NEFA leading to further accumulation of fat and to insulin resistance in e.g. skeletal muscle and liver.[68-70]

Mesangial cells represent a specialized form of microvascular pericytes that support glomerular capillary loops and regulate capillary flow.[71] Like any pericyte, mesangial cells can accumulate CE via LDL- and scavenger receptors,[72] especially in inflammatory conditions;[73] and TG via LPL.[74, 75] Mesangial cells can transform to lipid-laden foam cells with loss of contractile function in vitro.[76, 77] It is hypothesized that this transformation results in capillary rarefaction, glomerulomegaly, and microalbuminuria, thereby initiating a cascade of events that may lead to glomerulosclerosis.[71, 78]

I am currently preparing for studies on this topic using the new technique of proton spectroscopy. Proton-magnetic resonance spectroscopy (1H-MRS) has evolved over past years as a valid[79] noninvasive in vivo technique to study lipid content in tissue such as liver,[64] muscle,[65] and heart.[66] MRI is a powerful tool to study disease models because it provides (non-invasively) multi-parametric information (anatomy, perfusion, metabolic data) in one examination in vivo. A direct measure of ectopic fat

accumulation in organs is likely to be more sensitive and specific than general anthropomorphic measures of obesity to identify patients at risk for obesity-induced organ damage. Even in obesity, a metabolically benign fat distribution phenotype may exist.[80] 1H-MRS of intramyocellular lipid accumulation has been found to correlate more closely with insulin resistance than any commonly measured indices including body mass index, waist-to-hip ratio, or total body fat.[70] If our hypothesis is true, 1H-MRS might give in vivo and timely insight into lipid nephrotoxicity. It may theoretically identify patients at risk even before onset of microalbuminuria. For these reasons, 1H-MRS of kidney will likely proof to be a valuable tool for identifying high-risk obese patients and an excellent intermediate endpoint for development of interventions targeted at preventing or slowing obesity-induced renal damage. As lipid accumulation is thought to be involved in the development of diabetic nephropathy as well,[51] it may also proof to be a valuable tool in this line of research. Finally, other magnetic resonance techniques such as 13-C(arbon)-MRS (glucose metabolism) and 31-P(hosphate)-MRS (ATP; mitochondrial oxidation) are emerging to explore renal metabolic imaging in vivo.
We already performed some explorative studies with this tool in healthy volunteers (Figure 1) and in an animal model of lipotoxicity (Figure 2 and Picture 1a and b): ApoE3.Leiden.CETP transgenic mice. We were able to demonstrate feasibility of this technique and aim to start further studies soon.



Left figure shows plan scan in healthy volunteer for spectroscopic volume (8 ml), positioned on the renal cortex. Upper right panel shows non-suppressed 1H-MR spectrum of the volume shown on the left. Main signal originates from water (4.7 ppm), a small resonance signal is visible from triglycerides (TG, 1.3 ppm). To increase the relative signal of TG, water suppression was performed, as shown in the right lower panel. The 1H-MR spectrum clearly shows the details of the TH resonance peaks centered around 1.3 ppm. By using both spectra, the indexed fat/water content can be calculated, in this subject around 1.5%.

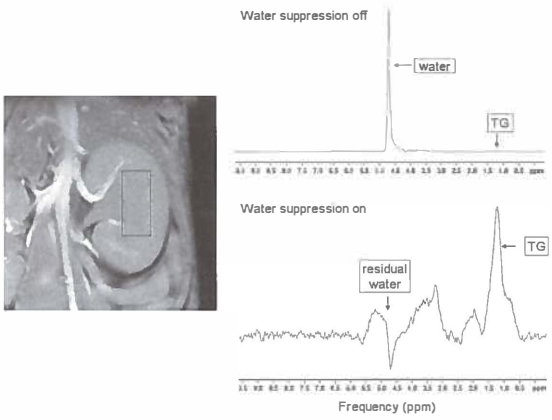
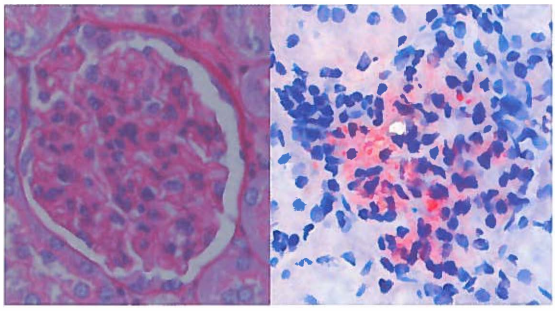


Figure 2: Steatosis renum in ApoELeiden.CETP mouse

Left figure shows plan scan in ApoE3Leiden.CETP mouse for spectroscopic volume (10 microliter), positioned on the renal cortex. Upper right panel shows non-suppressed 1H-MR spectrum of the volume shown on the left. Main signal originates from water (4.7 ppm), a very small resonance signal is visible from triglycerides (TG, 1.3 ppm). To increase the relative signal of TG, water suppression was performed, as shown in the right lower panel. The TH resonance peaks can be observed around 1.3 ppm.



Picture 1a + b
A glomerulus of a ApoE3Leiden-CETP mouse after 28 weeks on a high fat diet. In the PAS-staining (1a), mesangial expansion is apparent, while Oil-red-O staining (1b) demonstrates the presence of lipid in mesangial cells (in red).

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Samenvatting in het Nederlands (in lekentermen).

Alle ontwikkelingen in de transplantatiegeneeskunde in de afgelopen 30 jaar ten spijt, verliest nog steeds ongeveer de helft van de getransplanteerden de functie van hun transplantaat binnen 10 tot 12 jaar na transplantatie. Dit transplantaatfalen komt enerzijds doordat patienten zogenaamde 'chronische transplantaatdysfunctie' (CTD) ontwikkelen, en anderzijds doordat zij vroegtijdig komen te overlijden aan voornamelijk hart- en vaatzieken, waardoor de transplantaatfunctie op indirecte manier verloren gaat. CTD is een soort versnelde slijtage van het niertransplantaat, dat parallellen vertoont met vaatziekte (atherosclerose) zonder dat altijd een eenduidige oorzaak valt aan te wijzen.

In hoofdstuk 2 wordt de hypothese besproken dat insulineresistentie ten grondslag ligt aan veel niet-immunologische risicofactoren voor CTD, zoals overgewicht, hoge bloeddruk, hoog triglyceriden, laag HDL-cholesterol en (post-transplantatie) suikerziekte. Deze hypothese is moelijk te toetsen omdat insulineresistentie gemeten dient te worden met de hyperinsulinemische euglycemische clamp techniek. Deze gouden standaard is echter te bewerkelijk en te tijdrovend om gebruikt te worden in grote populatiestudies.

In hoofdstuk 3 valideerden we daarom in een groep niertransplantatiepatienten drie bekende surrogaat markers voor insulineresistentie tegen de gouden standaard, de zgn. hyperinsulinemische euglycemische clamp. Het bleek dat de McAuley index, een simpele maat die berust op het alleen meten van nuchter glucose, insuline, en triglyceriden in het bloed, het beste overeenkwam met de gouden standaard van insulineresistentie, maar dat twee andere veel gebruikte maten, gebaseerd op alleen nuchter insuline en/of glucose ook valide waren.

In hoofdstuk 4 gebruikten we de gevalideerde McAuley index om na te gaan welke traditionele (o.a. overgewicht, weinig bewegen) en welke meer transplantatie-gebonden factoren (bijv. afweeronderdrukkende medicatie en opportunistische infecties met virussen zoals het cytomegalovirus (CMV)) in stabiele transplantatiepatienten geassocieerd zijn met insulineresistentie. We vonden dat overgewicht (m.n. de zgn. appelvorm) en het afweeronderdrukkende medicijn prednisolon de belangrijkste determinanten van insulineresistentie zijn langer dan 1 jaar na niertransplantatie. Insulineresistentie wordt gedacht centraal te staan in de ontwikkeling van het metabool syndroom. Een persoon heeft het metabool syndroom als 3 of meer van de volgende risicofactoren voor hart- en vaatziekten in een persoon clusteren: overgewicht, hoge bloeddruk, hoog triglyceriden, laag HDL-cholesterol en (post-transplantatie) suikerziekte.

Uit hoofdstuk 5 blijkt dat een meerderheid van de onderzochte niertransplantatie-patienten het metabool syndroom had en dat dit geassocieerd was met insulineresistentie en een verminderde

transplantaatfunctie. Binnen het metabool syndroom waren m.n. hoge bloeddruk en hoge triglyceridenconcentraties debet aan deze associatie. Opvallend was dat vrouwen relatief vaker posttransplantatie gewichtstoename en metabool syndroom hadden dan mannen.

In hoofdstuk 6 onderzochten we in hoeverre de functie van het transplantaat, eiwitverlies in de urine (als maat voor nierschade) en een maat voor vaatweerstand (als indirecte maat voor vaatziekte) in de transplantatienier het ontwikkelen van transplantaatfalen konden voorspellen. Het bleek dat alle drie redelijk goede voorspellers waren van transplantaatfalen. Het meten van de vaatweerstand was echter niet beduidend beter dan het meten van nierfunctie en eiwitverlies in de urine. Omdat transplantaatfalen ook indirect plaats kan vinden door het overlijden van patiënten, onderzochten we ook of vaatweerstand in de nier vroegtijdige sterfte van de niertransplantatiepatiënt zou kunnen voorspellen. Dit bleek inderdaad het geval, maar deze associatie bleek voornamelijk te berusten op een parallelle associatie met verhoogde bloeddruk.

In hoofdstuk 7 onderzochten we in hoeverre niertransplantatie-patienten vaker kwamen te overlijden door het metabool syndroom dan mensen in de algemene bevolking (PREVEND populatie). Het bleek dat niertransplantatiepatienten in vergelijking met niet-getransplanteerde mensen van hetzelfde geslacht en leeftijd een 6-7x verhoogd risico hebben om voortijdig te overlijden. Een maat voor hartfalen (NT-proBNP), de nierfunctie, en het gebruik van bloeddrukverlagende middelen verklaarden dit verhoogde risico op versneld overlijden meer dan de toegenomen aanwezigheid van het metabool syndroom.

In hoofdstuk 8 vonden we tot slot dat onderdelen van het metabool syndroom geassocieerd waren met latente infectie van het cytomegalovirus (CMV); CMV bleek ook een onafhankelijke risicofactor voor transplantaatverlies. De preciese samenhang van het metabool syndroom met CMV blijft vooralsnog onduidelijk.

Een gezonde levenstijl (gezond eten, veel bewegen), alsook het zoveel mogelijk reduceren en op maat toesnijden van de afweeronderdrukkend medicijnen, zullen waarschijnlijk het ontstaan van het metabool syndroom na transplantatie kunnen vertragen.

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En wat is de Kidney Alley zonder de nefro-nerds? Drie hadden evident de XX-factor. Lieve Mieneke, nog steeds een plaatje!; Titia, een ACE in alles, dank voor het overnemen van de Journal Club!, en Inge: jullie creatieve inborst heeft menig promotie tot een fantastisch feest gemaakt. Ook Femke, Folkert, Martin, Menno, Sasha, Wynand, Mirjan, Andrea, Eelke, Peter, Arnold, Goos, Jacobien, Jan-Stephan, Erik en nog vele andere van de oude en nieuwe garde wier naam mij nu ontschiet: dank voor jullie gezelligheid, flauwe grappen, Feithuis borrels, etentjes in San Diego, Philly, Boston, of Sydney en de broodnodige ontspanning in de Alley. Niet te vergeten valt de stille doch cruciale kracht op de achtergrond: Rieta, Winie, en Joke, bedankt!

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Dear Luis, you're the best that has happened to me over past years! I'm so glad to share my life with you. Te quiero mucho!

Curriculum Vitae

Aiko de Vries (Groningen,1976) groeide op in Vught en doorliep het R.K. Gymnasium Beekviet te St. Michielsgestel. In 2001 studeerde hij cum laude af in de geneeskunde aan de Rijksuniversiteit Groningen, waarbij hij tevens een jaar volbracht aan de University of Pennsylvania te Philadelphia. Als 100e Agiko van NWO combineerde hij zijn promotie-onderzoek binnen de afdelingen algemene interne geneeskunde en nefrologie met zijn specialisatie tot internist aan het Universitair Medisch Centrum te Groningen (opleider: prof. dr. R.O.B. Gans, internist). Zijn perifere opleiding volgde hij in 2006/2007 aan het St. Elisabeth Ziekenhuis te Curaçao (opleider: dr. K. Berend, internist). In 2008 verhuisde hij naar Amsterdam waar hij zijn opleiding tot nefroloog voortzette aan het Leids Universitair Medisch Centrum onder het opleiderschap van Prof. dr. T.J. Rabelink en Prof. dr. J.W. de Fijter. Thans werkt hij als staflid aan de afdeling nierziekte en transplantatiegeneeskunde van het Leids Universitair Medisch Centrum.

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