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ORIGINAL ARTICLE

Immunosuppressive medications, clinical and metabolic parameters in new-onset diabetes mellitus after kidney transplantation

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Summary

New-onset diabetes after transplantation (NODAT) is a growing concern in transplantation. All modifiable risk factors are not yet identified. We assessed the relationship between baseline clinical and biochemical parameters and NODAT. Eight-hundred and fifty-seven in-Caucasian renal transplant recipients were included. Charts were individually reviewed. The follow-up was 5.3 years (ranges: 0.25–20.8; 5613 patient-years). The incidence of NODAT was 15.0%, 18.4% and 22.0% at 10, 15 and 20 years following transplantation. Age, body mass index (BMI), glucose (all $P < 0.0001$) and triglycerides [hazard ratio (HR) per 1 mmol/l: 1.44 [1.17–1.77], $P = 0.0006$] were potent risk factors whereas steroid withdrawal (HR: 0.69 [0.47–1.01], $P = 0.0601$) reduced the risk. As compared to cyclosporine, sirolimus (HR: 3.26 [1.63–6.49], $P = 0.0008$) and tacrolimus (HR: 3.04 [2.02–4.59], $P < 0.0001$) were risk factors for NODAT. The risk of NODAT was comparable for sirolimus (HR: 2.35 [1.06–5.19], $P = 0.0350$) and tacrolimus (HR: 2.34 [1.46–3.75], $P = 0.0004$) after adjustments on age, BMI, glucose and steroid withdrawal; however, unlike sirolimus, tacrolimus remained significant after adjustment on triglycerides. The risk of NODAT appeared similar, but its pathophysiology seemed different in sirolimus- and tacrolimus-treated patients; this observation needs confirmation. However, main independent risk factors were age, BMI, initial glucose and triglycerides.

Introduction

New-onset diabetes after transplantation (NODAT) is one of the most serious complications in solid-organ transplantation [1]. NODAT is associated with reduced graft function, increased graft loss and reduced patient survival [2]. Its incidence varies widely, depending of many parameters including duration of follow-up, age, body mass index (BMI), ethnicity, and definitions of NODAT [1]. To unify the definition of NODAT, the proceedings of the International Expert Panel Meeting were published in 2003, and adaptation of current American Diabetes Association (ADA) criteria was recommended [3].

Detection of subjects at risk for NODAT requires early identification of modifiable risk factors. Immunosuppressive regimen may account to a large extent for the increased risk of diabetes mellitus in renal transplant recipients. The association between steroid use and development of NODAT is established [4,5]. Tacrolimus was also associated with NODAT in kidney transplant recipients [6,7].

Interestingly, sirolimus is one of the most recent immunosuppressive drugs used in solid-organ transplantation. Sirolimus inhibits the mammalian target of rapamycin (mTOR). Early *in vitro* studies supported the view that inhibition of mTOR may be associated with

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improved insulin sensitivity. Consistenly, sirolimus has been proposed as the immunosuppressive medication of choice in islet transplantation for type 1 diabetic patients [8]. However, several pieces of evidence now suggest that sirolimus may result in impaired glucose tolerance: (i) sirolimus decreases islet cell viability and reduces insulin secretion in experimental studies [9]; (ii) sirolimus induces a marked increase in triglycerides levels, which may be associated with type 2 diabetes mellitus [10]; (iii) a higher incidence of NODAT in patients treated with the sirolimus-cyclosporine combination as compared to those treated with cyclosporine only was observed in a recent retrospective, although low-powered study [11]; and (iv) conversion to sirolimus was associated with impaired glucose tolerance and with diabetes in four patients in a recent study [12].

Short-term clinical trials focused on sirolimus were not designed to assess the risk of NODAT [13,14], so that the association between sirolimus use and NODAT remains controversial.

In the present retrospective study we investigate the impact of clinical and biochemical parameters and drugs including sirolimus and tacrolimus on NODAT in a large cohort of Caucasian renal transplant recipients, and we assessed the role of triglycerides on the relationship between NODAT and sirolimus and tacrolimus, respectively.

Patients and methods

Selection of the population

Nine-hundred ninety-three patients received a renal transplant between October 1985 and October 2006 in our center. Among these 993 transplant recipients, we excluded 136 patients from the present analysis for the following reasons: known diabetes mellitus before transplantation ($n = 82$), graft loss or death <3 months after allograft ($n = 36$), no data on diabetes mellitus at the 3-month visit ($n = 15$) and recipient age <16 years ($n = 3$).

Finally, 857 nondiabetic patients were included. Duration of follow-up was 6.5 ± 5.3 year (median: 5.3 years; ranges: 0.25–20.8 years; total observation period: 5613 patient-years).

Initial immunosuppression therapy included methylprednisolone: 250 mg pre- and postoperatively, antithymocyte antibodies (Thymoglobulin®; Imtix-Sangsat, Lyon, France) during 5 days or anti-IL-2 receptor antibodies (Basiliximab, Simulect®; Novartis, Rueil-Malmaison, France) at day 0 and day 4.

Maintenance immunosuppressive regimen included prednisone with a gradual taper andmycophenolate mofetil (MMF) or azathioprine. Patients received either cyclosporine, tacrolimus or sirolimus. Target trough levels at 3 months were: cyclosporine: 150–250 ng/ml; tacrolimus: 8–12 ng/ml; sirolimus: 4–12 ng/ml.

Patients with acute rejection episodes were treated with methylprednisolone (8, 6, 4, 3 and 2 mg/kg for five consecutives days) followed by oral prednisone [15].

Visits in our ward were organized as followed: three visits per week during the first 2 weeks; two visits per week until day 60; weekly visits until day 240; monthly visits during the first year; one visit every other month during the second year; three visits per year thereafter until death, ESRD or re-transplantation.

Parameters studied

At the time of transplantation, the following variables were recorded: type of donor (living or cadaveric), donor age, age and gender of the recipient, smoking, cause of renal failure, immunosuppressive induction treatment, cold ischemia time, delayed graft function, hepatitis C virus (HCV).

At the 3-month visit after transplantation, the following parameters were recorded: body weight and BMI, systolic and diastolic arterial pressure, acute rejection episodes, biochemical parameters including fasting glucose, serum cholesterol and triglycerides, serum creatinine, estimated creatinine clearance (Cockcroft formula) [16], immunosuppressive regimen and cytomegalovirus (CMV) infection (defined as positive pp65 antigenemia).

Definition of NODAT

All 857 charts were individually reviewed. NODAT was assessed at each visit in our center. NODAT was defined according to the ADA: symptoms of diabetes plus casual plasma glucose concentration ≥11.1 mmol/l, casual being defined as any time of day without regard to time since last meal, or fasting glucose ≥7.0 mmol/l, fasting being defined as no caloric intake for at least 8 h (oral glucose tolerance tests were not usually run in our center, as this is not recommended as a routine practice) [17]. These criteria were confirmed by repeat testing on a different day [18]. Patients with transient elevations of fasting glucose (i.e., during high steroid administration) were not classified as having NODAT.

Statistical analyses

Results were expressed as percentages or as mean ± standard deviation. Median was also presented when the distribution of the parameters was not normal.

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Cox models were used in univariate and multivariate analyses to assess the association between clinical and biochemical recipient parameters and the risk of NODAT during follow-up. The results were expressed as hazard ratios (HR), 95% confidence intervals (CI) and *P*-values. Patients were censored at their date of death, graft loss or date of last visit. Parameters selected for the multivariate models were those found significant in the univariate models (P-value < 0.05). Several models were used in the multivariate analyses; adjustments on age, BMI, plasma fasting glucose were systematically used.

To assess the impact of cyclosporine, tacrolimus and sirolimus on NODAT, we considered the cyclosporine-treated patients as the reference group: we thus expressed the risk of NODAT in sirolimus- and tacrolimus-treated patients as compared to the group of cyclosporine-treated patients. Moreover, steroid use at the 3-month visit and steroid withdrawal during follow-up were forced into the models because these two parameters are usually associated with NODAT in the literature [1]. Last, adjustment on triglyceride levels was used in order to explore the relationship between the development of NODAT and the use of sirolimus and tacrolimus, respectively. Analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). A P-value <0.05 was considered significant.

Results

Patient characteristics

Donor and recipient characteristics at the time of transplantation and at the 3-month visit are shown in Table 1. Steroids were withdrawn in 42% of patients (most of them in the first year after transplantation). Tacrolimus and MMF are used since 1996 and sirolimus is used since 1998 in our center.

At the 3-month visit, median cyclosporine level was 437 ng/ml before 1990 and was drastically reduced, and has remained stable ever since (160–165 ng/ml); median tacrolimus trough levels was stable (9.0–9.5 ng/ml); sirolimus median trough levels were 12.6 ng/ml before 2004, and were reduced at 6.8 ng/ml thereafter. The median doses of immunosuppressive medications at the 3-month visit were: cyclosporine: 320 mg/day before 1990 and were reduced to 245 mg/day after 2004; tacrolimus: 6 mg/day before 2000, 5 mg/day ever since; MMF: 2000 mg/day; azathioprine: 100 mg/day; steroids: 10 mg/day (of note, the median doses of steroid, azathioprine and MMF have not changed with time in our center); sirolimus: 5 mg/day before 2004, 4 mg/day ever since.

Of note, 84.6% of HCV patients were treated with cyclosporine; only 14.6% were treated with tacrolimus; none with sirolimus.

Table 1. Patient characteristics.

No. patients	857
Donor characteristics	
Living donor (%)	0.9
Donor age (years)	41.6 ± 15.4
Recipient characteristics	
Clinical characteristics at the time of transplantation	
Second/third graft (%)	10.5/0.8
Males (%)	59.9
Age (years)	45.3 ± 13.5
Panel reactive antibodies (PRA) >75% (%)	5.4
Cold ischemia (min)	1298 ± 501
Delayed graft function (%)	19.4
BMI (kg/m^2)	23.9 ± 3.9
Smokers (%)	15.1
Acute rejection (%)	28
CMV infection (%)	21
Hepatitis C virus (%)	7.8
Cause of renal failure	
Glomerulonephritis (%)	33.6
Autosomal-dominant polycystic kidney disease (%)	16.4
Renal vascular disease (%)	5.7
Hereditary kidney disease (non-ADPKD) (%)	8.5
Uropathy (%)	6.2
Unknown nephropathy (%)	29.5
Biochemical parameters at the 3-month visit	
Glucose (mmol/l)	5.34 ± 0.89
Cholesterol (mmol/l)	5.77 ± 1.46
Triglycerides (mmol/l)	1.87 ± 0.97
Creatinine (μmol/l)	131 ± 44
Estimated creatinine clearance (ml/min)	59.4 ± 18.1
Hemoglobin (g/l)	118 ± 18
Immunosuppressive drugs at the 3-month visit	
Anti-IL-2 receptor antibody induction (%)	24
Antithymocyte antibody induction (%)	70
Corticosteroids (%)	95
Cyclosporine (%)	76
Tacrolimus (%)	20
MMF (%)	59
Azathioprine (%)	36
Sirolimus (%)	4

Risk factors for NODAT: univariate analysis

New-onset diabetes after transplantation occurred in 112 patients during the follow-up (2 per 100 patient-years). The incidence of NODAT was 8.2% in the first year, 10.3% at 3 years, 11.5% at 5 years and 15.0% at 10 years after transplantation. It rose to 18.4% and 22.0% at 15 and 20 years, respectively.

Impact of age, BMI and initial fasting plasma glucose

As illustrated in Fig. 1, the incidence of NODAT was highly dependent on recipient age, BMI and initial fasting glucose : the incidence was 0.6 per 100 patient-years in patients <40, but rose to 1.5, 3.8 and 4.3 in patients aged

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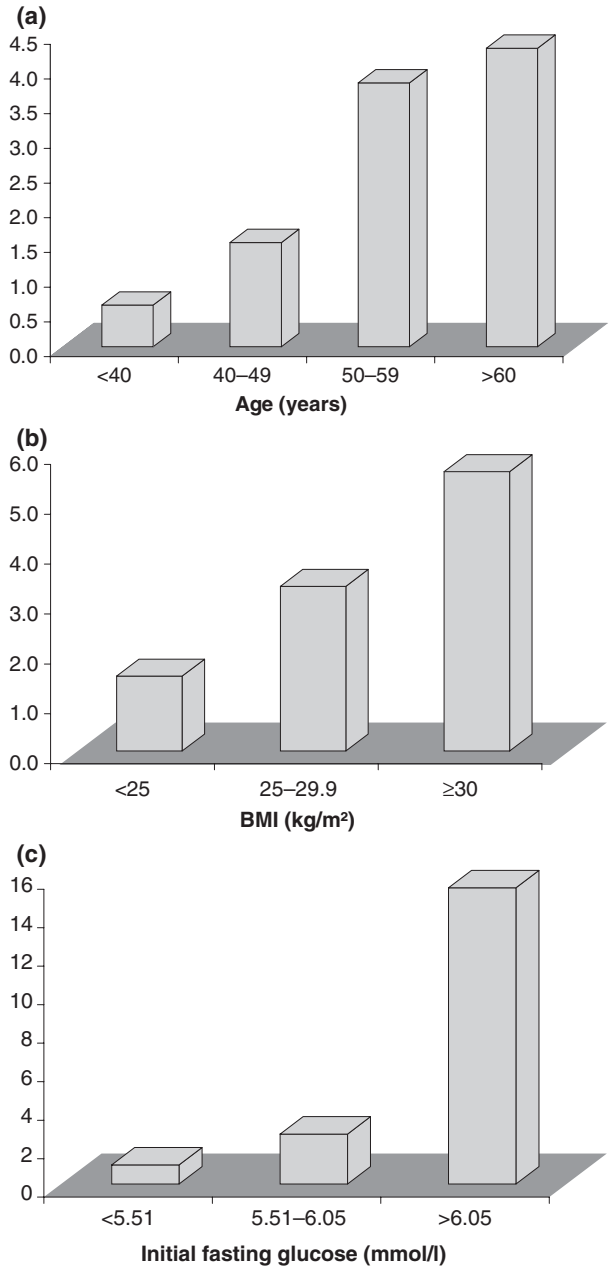

Figure 1 Impact of age (a), body mass index (BMI) (b), and fasting glucose (c) at the 3-month visit on the incidence of NODAT expressed per 100 patient-years. Cox analysis indicated that there was a significant relationship between age, BMI, and fasting glucose, respectively (all $P < 0.0001$) and the development of NODAT.

40–49, 50–59 and ≥60, respectively ($P < 0.0001$, Fig. 1a); the incidence was 1.5 per 100 patient-years in patients <25 kg/m^2, 3.3 in patients with BMI 25–29.9 kg/m^2 and 5.6 per 100 patient-years in those with BMI ≥30 kg/m^2 ($P < 0.0001$, Fig. 1b); the incidence was 1 per 100 patient-years when initial fasting glucose <5.5 mmol/l, and rose to 2.6 and 15.4 when fasting glucose was 5.5–6.04 mmol/l and ≥6.05 mmol/l (i.e., impaired fasting glucose), respectively ($P < 0.0001$, Fig. 1c).

Impact of other parameters

In addition to recipient age, BMI and initial fasting glucose, univariate analysis indicated that serum triglycerides, hemoglobin, polycystic kidney disease, antithymocyte antibodies and MMF (and to a lesser extent smoking) were also associated with NODAT (Table 2, univariate analysis).

Not surprisingly, steroid use at the 3-month visit was not associated with diabetes as 95% of them were treated with steroids at this time of transplantation. Of note, patients in whom steroids were withdrawn were older than those who continued steroids (46.4 ± 13.6 vs. 44.2 ± 13.6, $P = 0.0114$). After adjustment on age, the risk of NODAT was reduced by 31% in patients in whom steroids were withdrawn (HR: 0.69 [0.47–1.01], $P = 0.0601$) (Table 2, univariate analysis).

In univariate analysis, the year of graft was associated with NODAT (HR for 5-year increment: 1.58 [1.31–1.91], $P < 0.0001$). However, when age, BMI and fasting glucose were entered into the models, the association was no longer significant (HR: 1.23 [0.95–1.59], $P = 0.1201$).

Independent risk factors for NODAT: multivariate analysis

Parameters entered into the multivariate models were those significantly associated with NODAT in the univariate analysis. After adjustments on recipient age, BMI and initial fasting glucose, triglycerides remained significantly associated with NODAT, but not hemoglobin, polycystic kidney disease, antithymocyte antibodies or MMF (Table 2, multivariate analysis).

Of note, after adjustment on the type of CNI used, MMF was not associated with NODAT (HR: 1.13 [0.65–1.96], $P = 0.66$).

Sirolimus and tacrolimus as risk factors for NODAT

For this analysis, we considered the cyclosporine-treated patients as the reference group: we thus expressed the risk of NODAT in sirolimus- and tacrolimus-treated patients as compared to the group of cyclosporine-treated patients.

Patients who developed NODAT had greater fasting glucose levels at the 3-month visit than those who did not in sirolimus-treated patients (6.49 ± 0.94 vs. 5.24 ± 0.41 mmol/l, $P = 0.0029$), in cyclosporine-treated patients (6.10 ± 1.09 vs. 5.16 ± 0.75 mmol/l, $P < 0.0001$) and in tacrolimus-treated patients (6.30 ± 1.07 vs. 5.39 ± 0.88 mmol/l, $P < 0.0001$). Among sirolimus-treated patients, the sirolimus dose (4.6 ± 1.6 vs.

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Table 2. Risk factors for NODAT.

	Univariate analysis			Multivariate analysis (adjustment on age, BMI, and initial fasting glucose)		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Clinical parameters						
Age (per 1 year)	1.06	1.04–1.07	<0.0001	_		
Smoking (yes versus no)	1.60	0.98–2.60	0.0594	_		
Polycystic kidney disease (yes versus no)	1.96	1.29–2.98	0.0016	1.46	0.91–2.34	0.1175
Body mass index (per 1 kg/m^2)	1.13	1.08–1.18	<0.0001	_		
Acute rejection (yes versus no)	1.07	0.70–1.63	0.7600	_		
CMV infection (yes versus no)	1.03	0.64–1.65	0.9061	_		
Hepatitis C virus (yes versus no)	0.55	0.19–1.58	0.2647	_		
Biochemical parameters						
Glucose (per 1 mmol/l)	2.22	1.96–2.52	<0.0001	_		
Cholesterol (per 1 mmol/l)	0.98	0.82–1.16	0.7900	_		
Triglycerides (per 1 mmol/l)	1.58	1.31–1.90	<0.0001	1.44	1.17–1.77	0.0006
Creatinine (per 1 μmol/l)	1.00	1.00–1.00	0.1000	_		
Estimated creatinine clearance (ml/min)	0.99	0.98–1.00	0.1500	_		
Hemoglobin (per 1 g/l)	0.98	0.97–1.00	0.0121	0.99	0.97–1.00	0.1252
Immunosuppressive medications						
Anti-IL-2 receptor antibody (yes versus no)	1.56	0.86–2.83	0.1402	_		
Antithymocyte antibody (yes versus no)	0.47	0.28–0.77	0.0027	0.71	0.39–1.29	0.2642
Azathioprine (yes versus no)	0.67	0.41–1.10	0.1118	_		
MMF (yes versus no)	2.24	1.44–3.49	0.0003	1.39	0.82–2.36	0.2232
Steroid at the 3-month visit (yes versus no)	0.97	0.45–2.11	0.9476	_		
Steroid withdrawal* (yes versus no)	0.69	0.47–1.01	0.0601	_		

*After adjustment on age.

4.4 ± 2.2 mg/day, $P = 0.5152$) and trough levels (11.9 ± 3.9 vs. 11.3 ± 5.7 ng/ml, $P = 0.5976$) were numerically greater in those who developed NODAT, but the difference was not significant.

Mean age (45.6 ± 14.0, 44.6 ± 13.2, and 51.6 ± 12.8, respectively), BMI (23.8 ± 3.7, 24.2 ± 4.3, and 24.2 ± 4.3 kg/m^2, respectively) and fasting glucose (5.26 ± 0.84, 5.58 ± 0.99, and 5.61 ± 0.83 mmol/l, respectively) at the 3-month visit were different in cyclosporine-, tacrolimus- and sirolimus-treated patients. To ensure that sirolimus and tacrolimus were independent predictors of NODAT, we used several models (Table 3). We found that sirolimus and tacrolimus were risk markers of NODAT and the association was independent of age, BMI and initial fasting glucose (Table 3). Further adjustments did not alter the relationship between NODAT and sirolimus and tacrolimus, respectively.

The risk of NODAT remained qualitatively unchanged regardless of whether steroids were withdrawn (HR for sirolimus-treated patients: 4.84 [1.48–15.77], $P = 0.0089$; HR for tacrolimus-treated patients: 5.65 [2.90–11.00], $P < 0.0001$) or continued (HR for sirolimus-treated patients: 2.58 [1.10–6.05], $P = 0.0296$; HR for tacrolimus-treated patients: 2.28 [1.32–3.94], $P = 0.0031$) during follow-up; this remained true even after further adjustment on age, BMI and initial glucose (Table 3). The risk of NODAT associated with sirolimus and tacrolimus remained unchanged after adjustment on PRA (Table 3). The risk of NODAT associated with sirolimus and tacroli-

Table 3. Sirolimus and tacrolimus as risk factors for NODAT.

	Sirolimus			Tacrolimus		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Univariate analysis						
Yes versus no	3.26	1.63–6.49	0.0008	3.04	2.02–4.59	<0.0001
Multivariate analysis						
Model 1	2.52	1.15–5.52	0.0213	2.41	1.54–3.76	0.0001
Model 2	2.34	1.06–5.20	0.0362	2.35	1.50–3.68	0.0002
Model 3	2.52	1.15–5.52	0.0212	2.42	1.55–3.78	0.0001
Model 4	2.35	1.06–5.19	0.0350	2.34	1.46–3.75	0.0004
Model 5	5.45	2.57–11.54	<0.0001	2.57	1.49–4.46	0.0007
Model 6	5.33	2.51–11.28	<0.0001	2.61	1.50–4.55	0.0007

Model 1: adjustment on age, BMI, and glucose.
Model 2: Model 1 + adjustment on MMF.
Model 3: Model 1 + adjustment on steroid use at 3 months.
Model 4: Model 1 + adjustment on steroid withdrawal during follow-up.
Model 5: adjustment on PRA (>75%).
Model 6: adjustment on graft rank.

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mus was unchanged after adjustment on the number of grafts (i.e., first/second graft) (Table 3).

When the analysis was restricted to patients with at least 6 months of follow-up, the results were unchanged for sirolimus (HR: 5.36 [2.27–12.65], $P = 0.0001$) and tacrolimus (HR: 2.34 [1.27–4.30], $P = 0.0065$). Finally, 25% of the patients who were initially treated with sirolimus stopped this treatment because of the side-effects during follow-up. No significant association was found between sirolimus withdrawal and NODAT: the relationship remained significant after adjustment on sirolimus withdrawal (HR: 4.96 [1.97–12.50], $P = 0.0007$), and when the analysis was restricted to the patients who continued sirolimus during follow-up (HR: 5.04 [2.00–12.71], $P = 0.0006$).

To understand the diabetogenic effect of sirolimus and tacrolimus in a better manner, adjustment on triglycerides was used: although the risk of NODAT associated with tacrolimus and sirolimus was comparable in univariate and multivariate analyses (Table 3), the relationship between sirolimus and NODAT was no longer significant (HR: 1.50 [0.45–5.10], $P = 0.5100$) whereas the relationship between tacrolimus and NODAT remained unchanged (HR: 3.11 [1.78–5.43], $P < 0.0001$) after adjustment on triglycerides.

We found the association between tacrolimus and NODAT was qualitatively similar in patients with triglycerides levels ≥200 mg/dl and in those with lower levels (HR: 3.52 vs. 2.51).

Discussion

In the present study, we found that major independent risk factors for NODAT were age, BMI, and initial fasting glucose. We also observed that initial triglycerides and use of sirolimus and tacrolimus were independent risk factors. The association between sirolimus and NODAT was independent of age, BMI, glucose levels, steroid use, and persisted after exclusion of patients who discontinued sirolimus during follow-up.

The incidence of NODAT was lower in our cohort of Caucasian patients than in several studies [18,19]. This can be explained by many factors including the fact that our cohort includes Caucasian patients with a low rate of obesity as compared to other populations [18,19].

In the present analysis, we observed that sirolimus use was associated with NODAT, even after multiple adjustments on age, BMI, glucose. Obviously, this observation needs to be confirmed in prospective studies; however, if true, this finding needs to be discussed. First, our patients were all Caucasians; the association may be different in African-Americans or Hispanics [20]. Of note, the effect of sirolimus remained significant, regardless of steroid use or withdrawal. In fact, many arguments support the view that sirolimus may be a risk factor for NODAT. A novel inhibitor of mTOR, temsirolimus resulted in a 20% incidence of hyperglycemia in a recent phase II study [21,22]. Moreover, conversion to sirolimus reduced insulin sensitivity, glucose clearance and impaired compensatory β-pancreatic cell response [12]. In addition, median time to NODAT was shorter (27 vs. 104 days) with the sirolimus–tacrolimus combination than with tacrolimus alone in another study [23]. Finally, the incidence of NODAT was significantly greater in patients receiving both sirolimus and cyclosporine than in those receiving cyclosporine alone (31.6% vs. 10.4%) in a study from an Italian group [11]. Other studies did not reveal any association between sirolimus and NODAT. However, they were not usually designed to assess the risk of NODAT [24]. A recent study did not find a relationship between sirolimus and NODAT, although NODAT defined as insulin use was numerically greater in sirolimus- than in cyclosporine-treated patients; however, the results were not adjusted on concomitant immunosuppressive use, BMI, age and glucose, and the follow-up was shorter than in our study [25].

We found that the relationship between sirolimus and NODAT was no longer significant, whereas the relationship between tacrolimus and NODAT remained unchanged after adjustment on triglycerides. Hypertriglyceridemia is a known side-effect of sirolimus [26,27]; abnormalities of triglycerides storage have been shown to lead to impaired pancreatic β-cell function [28], and hypertriglyceridemia is a known risk factor for type 2 diabetes mellitus in nontransplanted populations [29]. In a recent study, changes in serum triglyceride after conversion to sirolimus strongly correlated with reduced insulin sensitivity [12]. Our own observation supports the view that sirolimus and tacrolimus may act differently to induce NODAT. The fact that another mTOR inhibitor (temsirolimus) is associated with a marked risk of hyperglycemia supports the view that the diabetogenic effect of sirolimus is a class effect [21]. Sirolimus inhibits a serine-threonine kinase: the mTOR. Sirolimus binds FKBP12 and this complex inhibits a specific cell cycle regulatory kinase (mTOR). The inhibition of mTOR results in suppression of T-cell proliferation, inhibiting the progression from G1 to the S phase of the cell cycle [30]. It has been shown that mTOR pathway is a chronic modulator of insulin-mediated glucose metabolism, and this pathway participates to the desensitization of insulin action induced by chronic exposure toplatelet-derived growth factor (PDGF), insulin, TNF-α and amino acids [31]. Recently, it has been demonstrated that mTOR regulates skeletal muscle glucose uptake *in vivo* in humans [32]. However, chronic administration of sirolimus resulted in

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a decreased insulin-stimulated activation of AKT responsible for many of the metabolic actions of insulin [33]; of interest, the inhibition of AKT activation led to reduced insulin sensitivity [33]. The effects of chronic mTOR inhibition may thus result in insulin resistance and explain our findings. Alternatively, it was demonstrated that sirolimus has deleterious effects on pancreatic islets *in vitro*, i.e., insulin secretion [9]. Both mechanisms may play a role in the development of NODAT in sirolimus-treated patients.

In our study, sirolimus trough levels were initially high, and they were reduced after 2004 in our center. It is therefore possible that our results only apply to high doses of sirolimus which are no longer used.

In univariate analysis, smoking, hemoglobin, polycystic kidney disease, antithymocyte antibodies and MMF were associated with NODAT; however, the association became nominal after adjustment on age, BMI and initial fasting glucose.

This study has many limitations. No information about family history of type 2 diabetes was available in our database. Only a small subset of our patients received sirolimus. However, we believe that increasing the sample size could only lead to improved power. We carefully took into account confounding parameters using multiple models. Our analysis is based on a close follow-up of a large cohort, and charts were reviewed individually. The total duration of observation exceeded 5600 patient-years which is larger than most studies. Finally, we used the ADA definition for NODAT as recommended, to avoid any confusion.

Some patients with early diagnosis of NODAT may have diabetes mellitus for some time before transplantation; however, the association between sirolimus (and tacrolimus) and NODAT is probably not due to the inclusion of diabetic patients that may have been missed at the time of transplantation. In effect, when the analysis was restricted to patients with at least 6 months of follow-up, the results were unchanged.

HCV was not associated with NODAT in our cohort, even after adjustment on immunosuppressive medications used. A higher incidence of NODAT in patients with HCV is often but not always reported [34].

In a recent paper [34], tacrolimus and pretransplant triglycerides were found to be associated with NODAT; in addition, the authors found that tacrolimus was no longer associated with NODAT in patients with pretransplant triglycerides <200 mg/dl. In our own investigation, the association between tacrolimus and NODAT was qualitatively similar in patients with triglycerides levels >200 mg/dl and in those with lower levels (HR: 3.52 vs. 2.51). Of note, in the paper of Porrini *et al.* [34], the authors used pretransplant triglycerides levels, the data on which were not available in our center.

Other parameters may influence the development of NODAT includingethnicity [35,36] and pretransplant fasting glucose [37]; in addition, a careful assessment of glucose metabolism with HbA1C levels may be useful for the management of patients with NODAT [38].

In conclusion, we observed that major independent risk factors for NODAT were age, BMI, initial fasting glucose and triglycerides; in addition, sirolimus and tacrolimus (as compared to cyclosporine) appeared similarly associated with NODAT. We used many multivariate models to account for possible differences between groups (immunosuppressive medications, age, BMI, fasting glucose); our findings remained significant. However, our findings deserve confirmation in large long-term clinical trials. If true, this association may influence the choice of the immunosuppressive regimen in kidney transplant recipients who are already at risk for diabetes (older age, impaired fasting glucose, obesity,…).

Authorship

MR, YL, HN and J-MH wrote the paper. PG, CB, AA-N, VC and J-FM were responsible for data collection. CD and MB were responsible for data analysis.

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