TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Vitamin D deficiency is an independent risk factor for PTDM after kidney transplantation

Awena Le Fur,[1,2] Marie-Cécile Fournier,[2,3] Florence Gillaizeau,[1,2,3] Damien Masson,[4] Magali Giral,[1,2,3] Bertrand Cariou,[5,6] Diego Cantarovich[1,2] and Jacques Dantal[1,2]

1 Institute of Transplantation, Urology and Nephrology (ITUN), Nantes University Hospital, Nantes, France
2 INSERM UMR 1064, Nantes University Hospital, Nantes, France
3 EA 4275 – SPHERE Biostatistics, Pharmacoepidemiology, and Human Sciences Research, Nantes University Hospital, Nantes, France
4 Biochemistry Laboratory, Nantes, France
5 Endocrinology Clinic, Nantes University Hospital, Nantes, France
6 INSERM UMR 1087, CNRS UMR 6291, Thorax Institute, Nantes University Hospital, Nantes, France

Keywords
kidney transplantation, post-transplant diabetes mellitus, vitamin D.

Correspondence
Awena Le Fur MD, ITUN, 30 boulevard Jean Monnet, 44093 Nantes, France.
Tel.: 0240087453;
fax: 0240084660;
e-mail: awena.lefur@chu-nantes.fr

Conflicts of interest
The authors have declared no conflicts of interest.

Received: 16 March 2015
Revision requested: 14 April 2015
Accepted: 25 September 2015
Published online: 29 October 2015

doi:10.1111/tri.12697

Summary

An association between 25 hydroxyvitamin D [25(OH)D] deficiency and type 2 diabetes was observed in the general population. Such association was not investigated in kidney transplant recipients. We prospectively evaluated 444 patients following primary kidney transplantation between 2000 and 2010. The 25(OH)D level at transplantation was classified into three grades: deficiency (< 10 ng/ml), insufficiency (≥ 10 and < 30 ng/ml), and normal range (≥ 30 ng/ml). Time to Post-Transplant Diabetes Mellitus (PTDM) was defined according to the day of first prescription of hypoglycemic treatment. The 25(OH)D level at transplantation was deficient in 88 patients, insufficient in 264 patients, and normal in 92 patients. At 1 year post-transplantation, cumulative incidence of PTDM was 13.2%. Cox multivariate analysis indicated that 25(OH)D deficiency (≤ 10 ng/ml) at the time of transplantation was an independent risk factor for PTDM within the first year post-transplantation (HR = 2.41, 95% CI 1.01–5.75, $P = 0.048$), whereas insufficiency tended to increase this risk, although not significantly. 25(OH)D deficiency is a new independent risk factor for PTDM within the first year after kidney transplantation. Our study suggests that 25(OH)D may be a marker of general health in kidney transplant recipients and could alert clinicians for PTDM risk.

Introduction

Post-transplant diabetes mellitus (PTDM) is a frequent metabolic complication following solid organ transplantation, affecting 10–45% of kidney transplant recipients. PTDM is commonly associated with unfavorable outcomes [1–4] such as an impaired patient and graft survival and an increased rate of cardiovascular events [5,6]. These poor outcomes are associated with an increased healthcare cost [7], making the early diagnosis and management of PTDM a major clinical challenge.

Common risk factors for PTDM are older age, high Body Mass Index (BMI), family history of type 2 diabetes, ethnicity, insulin resistance, increased plasma triglyceride level, and chronic hepatitis C infection [1,8,9]. The type of immunosuppression is well known to be a major contributor for PTDM (calcineurin inhibitors [CNIs] and corticosteroids), impairing both the beta cells' function and insulin sensitivity [3]. The occurrence of PTDM in the early post-transplant period suggests that the risk factors already exist or develop at the time or even before the transplantation. For example, polycystic kidney disease, number of Human Leucocyte Antigen (HLA) mismatches, occurrence of CMV infection, hypomagnesaemia, and surgical stress at the time of transplantation have been mentioned to increase beta cell dysfunction [10].

In order to prevent PTDM, identification of modifiable risk factors at the time of transplantation may be useful to develop primary preventive therapeutic strategies for high-risk patients. For example, a strategy to protect beta cells by an early preventive intervention with exogenous insulin therapy was recently proposed [11–13].

25 hydroxyvitamin D [25(OH)D] is well known for its essential role in calcium and bone homeostasis [14]. Large number of studies, both *in vitro* and *in vivo*, suggested extraskeletal effects [15,16]. The almost universal presence of vitamin D receptors (VDR) on large number of cell types, including Langerhans's islets, explain why 25(OH)D deficiency is associated with an increased risk of human diseases such as cardiovascular disease [17], cancer [18], multiple sclerosis [19], type 1 diabetes [20], and insulin resistance [21]. In the general population, observational studies suggest an association between plasma 25(OH)D level, which is the surrogate of vitamin D status, and the onset of type 2 diabetes. A recent meta-analysis reported a 4% lower risk of type 2 diabetes for each 4 ng/ml increment in 25(OH)D level [22]. Additionally, some interventional studies demonstrated that 25(OH)D substitution improves beta cell function [23]. Low 25(OH)D level is prevalent in many populations, but reduced plasma concentrations of 25(OH)D (< 30 ng/ml) are common in patients with chronic kidney disease (CKD). In the NHANES III cohort study, the adjusted odds for ratio 25(OH)D deficiency was 32% higher in patients with chronic kidney disease [25] and related to a lower rate of sunshine exposition, a decrease in the skin production and food intakes, and a lower activity of 25-OH-hydroxylase. After kidney transplantation, half of the recipients remained 25(OH)D deficient despite the high rate of 25(OH)D substitution [26] and 25(OH)D status at 3 months being indeed associated with lower glomerular filtration rate and increased interstitial fibrosis [27].

The aim of our study was to assess the association between 25(OH)D concentration at the time of kidney transplantation and the risk of developing PTDM within the first year following transplantation. Additionally, we evaluated the possible association between 25(OH)D level at transplantation and nonmelanoma skin cancer (NMSC), acute rejection episode, and patient–graft survival.

Methods

Patient population and data collection

We included adult patients (older than 18 years old) who underwent primary kidney transplantation without other simultaneous organ, without history of diabetes (all types) or cancer (including skin cancer) at Nantes University Hospital (France) between January 2000 and December 2011. Patients without a measurement of 25(OH)D level at the time of transplantation were not included. Data were prospectively collected and extracted from the French clinical database DIVAT (http://www.divat.fr/en; Information Commissioner's Office data privacy declaration in France no. 891735 CNIL agreement). Codes were used to assure the anonymity in accordance with the hospital ethical committee requirements, and the patient's written consent was required for data collection.

25(OH)D measurements

Plasma 25(OH)D concentration was measured by radioimmunoassay (RIA DiaSorin and LIAISON DiaSorin, Stillwater, MN). All samples were collected at the time of transplantation. About 198 measurements were performed at the day of transplantation, whereas 248 measurements were performed on sera collected at the time of transplantation and stored in our bio collection at −80 °C. We compared three groups of patients according to the 25(OH)D level: 25(OH)D < 10 ng/ml (deficiency), 10–30 ng/ml (insufficiency), or ≥ 30 ng/ml (within the normal range) [28]. These clinical thresholds seemed to be consistent with smoothing plots of the confounder-adjusted effect of 25 (OH)D for PTDM analysis (data not shown) [29].

Definition of PTDM

Onset of PTDM was considered the day of first prescription of hypoglycemic treatment (either insulin or oral antidiabetic drugs). According to the local standards, antidiabetic medication was prescribed by physicians in all patients with at least 2 consecutive measurements of fasting plasma glucose levels > 7.0 mmol/l or at any time > 11.1 mmol/l. Physicians were not aware of 25(OH)D concentration at any time during the study period.

Statistical analysis

Characteristics of the donor, recipient, and graft between the three groups according to 25(OH)D status at transplantation were compared using chi-squared test for qualitative variables and using ANOVA for quantitative variables. The cumulative incidence function for PTDM occurrence was estimated by taking into account the competition risk between return to dialysis and death with a functioning graft [24]. Patient–graft survival analysis was based on the time between transplantation and the first event among return to dialysis and death with functioning graft. Survival curve was estimated by the Kaplan–Meier method. Cox proportional hazard regression models were used to assess the association between the 25(OH)D status at transplantation and the kidney transplant recipients' outcomes: PTDM (censored at 1 year post-transplant for clinical relevance),

NMSC, acute rejection episode (for all, return to dialysis and death with functioning graft were censored), and patient–graft survival. The proportional assumption for 25(OH)D status was examined on log–log survival plots and Schoenfeld residuals. We tested in univariate analysis variables clinically relevant or that were significantly differently distributed according to 25(OH)D level. Possible risk factors studied in univariate analysis were as follows: type of donor (deceased versus living); recipient characteristics such as age at transplantation (> 55 vs. ≤ 55 years old), gender, ethnicity (Causasian versus not Caucasian), BMI, history of cardiovascular diseases, polycystic kidney disease, type and duration of dialysis before transplantation, and hepatitis C antibody status at transplantation; and graft characteristics such as season of transplantation (winter October to April versus summer June to September), number of HLA mismatches (> 4 vs. ≤ 4), type of immunosuppressive induction (T-cell depletion versus blocking antibody), and type of maintenance therapy (tacrolimus, ciclosporine, imTOR, MMF, azathioprine, cosrticosteroids). We introduced in multivariate analysis all variables that reached a *P*-value < 0.20 in univariate analysis and forced the well-known risk factors for PTDM (age, gender, BMI, tacrolimus, corticosteroids, and ethnicity). We then performed a backward selection. Interactions were tested between the 25(OH)D status at transplantation and the parameters selected in the final multivariate model, and the linearity assumption for continuous variables was checked. Statistical significance was considered at the 5% level, and adjusted hazard ratios were presented with their 95% confidence intervals (95% CI). Statistical analyses were performed using R software, version 2.14.1 (R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org).

Results

Study population

Between January 2000 and December 2011, 1083 primary kidney transplantations were performed in recipients over 18 years of age without history of diabetes or cancer in our center. Among them, 444 recipients had a measure of 25(OH)D concentration at the day of transplantation (whose 246 were performed on stored sera). The median time of follow-up was 4 years [min–max: 0–11 years]. Characteristics of the 444 recipients are described in Table 1. Mean age at transplantation was 50.9 (± 13.7) years, 60.6% were men, 89.2% were Caucasian, and 2.5% had a positive hepatitis C serology. Main causes of end-stage renal diseases (ESRD) were polycystic kidney disease (18.2%), hyperten-

Table 1. Characteristics of the 444 kidney transplant recipients according to 25(OH)D level at transplantation.

	All patients (*n* = 444)	< 10 ng/ml (*n* = 88)	10–30 ng/ml (*n* = 264)	≥ 30 ng/ml (*n* = 92)	*P*-value
Recipient characteristics					
Age (years)	50.9 ± 13.7	50.8 ± 13.0	51.3 ± 13.8	50.0 ± 14.1	0.7326
Male (%)	269 (60.6)	46 (52.3)	162 (61.4)	61 (66.3)	0.1442
Body mass index (kg/m²)	24.3 ± 4.4	24.0 ± 5.0	24.4 ± 4.2	24.0 ± 4.1	0.6996
History of hepatitis C virus (%)	11 (2.5)	3 (3.4)	8 (3.0)	0 (0.0)	0.2030
History of cardiovascular disease (%)	159 (35.8)	13 (14.8)	31 (11.7)	10 (10.9)	0.6877
Time of dialysis prior transplantation (years)	2.8 ± 3.2	3.6 ± 4.1	2.7 ± 3.1	1.9 ± 1.9	0.0040
Caucasian (%)	396 (89.4)	76 (87.4)	235 (89.0)	85 (92.4)	0.5241
Polycystic kidney disease (%)	81 (18.2)	8 (9.1)	49 (18.6)	24 (26.1)	0.0126
Graft characteristics					
Pre-emptive graft (%)	90 (20.3)	13 (14.8)	55 (20.8)	22 (23.9)	0.2933
Deceased donor (%)	394 (88.7)	85 (96.6)	225 (85.2)	84 (91.3)	0.0096
Anticlass I PRA > 20% (%)	87 (19.6)	19 (21.6)	44 (16.7)	24 (26.1)	0.1274
Anticlass II PRA > 20% (%)	62 (14.0)	19 (21.6)	30 (11.4)	13 (14.1)	0.0565
HLA ABDR mismatches > 4 (%)	91 (20.5)	21 (23.9)	47 (17.8)	23 (25.0)	0.2308
HLA B27 and/or B42 (%)	41 (9.2)	12 (13.6)	24 (9.1)	5 (5.4)	0.1632
Induction with T-cell depletion (%)	120 (27.0)	31 (35.2)	59 (22.4)	30 (32.6)	0.0249
Treatment with CNIs (%)	432 (97.3)	84 (95.5)	258 (97.7)	90 (97.8)	0.4636
Treatment with cyclosporine (%)	103 (23.2)	32 (36.4)	50 (18.9)	21 (22.8)	0.0036
Treatment with tacrolimus (%)	331 (74.5)	52 (59.1)	210 (79.6)	69 (75.0)	0.0007
Treatment with imTOR (%)	7 (1.6)	2 (2.3)	3 (1.1)	2 (2.2)	0.6491
Treatment with MMF (%)	425 (95.7)	84 (95.5)	254 (96.2)	87 (94.6)	0.7903
Treatment with azathioprine (%)	4 (0.9)	0 (0.0)	1 (0.4)	3 (3.3)	0.0654
Treatment with corticosteroids (%)	278 (62.6)	63 (71.6)	164 (62.1)	51 (55.4)	0.0788
Transplanted in winter (%)	195 (43.9)	55 (62.5)	108 (40.9)	32 (34.8)	0.0003

sive disease (10.5%), and glomerulonephritis (32.8%). About 20.3% of patients received a pre-emptive transplantation, and the duration of median dialysis duration before transplantation was 1.8 years [min–max: 0.0 – 32.8]. Most of the transplanted kidneys were from deceased donors (88.7%), and 92% were considered as of low immunological risk (PRA < 20%). About 96.4% of patients received an induction treatment with anti-IL2 receptor monoclonal antibody. Maintenance immunosuppression was mainly based on CNIs (97.3%) and MMF (95.7%), and 62.6% received corticosteroids after transplantation. Finally, our standard protocol included corticosteroids maintenance for 3 months following the transplantation, but 37.4% of recipients did not receive maintenance corticosteroids therapy according to the clinical conditions (steroids were stopped in cases of septic complication or metabolic disorders, for example, and prolonged if immunological risk was high or if MMF was stopped). Plasma level for tacrolimus ranged between 7 and 10 ng/ml and for cyclosporine between 100 and 150 ng/L within the first years following transplantation. There were differences between the study population (n = 444) and patients excluded because of no 25(OH)D measurement at transplantation (n = 639) on the following characteristics: polycystic kidney disease (18.2% vs. 10.9%, $P < 0.001$), pre-emptive graft (20.3% vs. 11.9% $P < 0.001$), tacrolimus therapy (74.5% vs. 65.5%, $P = 0.002$), and corticosteroids (62.6% vs. 75.1%, $P < 0.001$). These differences can be partly explained by the period of transplantation in both groups: More than half of patients included in the study were transplanted after 2006 (61.3%), the year from which 25(OH)D measurement was routinely performed, whereas 29.1% were transplanted after this date among the 885 patients excluded.

High prevalence of 25(OH)D deficiency and insufficiency

At the time of transplantation, median 25(OH)D concentration was 19.4 ng/ml (range 3.4–160.0 ng/ml). A total of 88 patients (19.8%) were considered 25(OH)D deficient (< 10 ng/ml), 264 (59.5%) insufficient (≥ 10 and < 30 ng/ml), and 92 (20.7%) had 25(OH)D within normal range (≥ 30 ng/ml). Characteristics of the 444 patients according to 25(OH)D level are described in Table 1.

25(OH)D deficiency is independently associated with an increased risk of PTDM

A total of 58 of 444 (13%) patients developed PTDM within the first year post-transplantation. For the others, the mean duration of hypoglycemic treatment was 6 years (± 2.6). The unadjusted cumulative incidences for PTDM at 3 and 12 months were estimated to be, respectively, 11.8% (95% CI 9.1–15.1%) and 13.2% (95% CI 10.3–16.7%). The cumulative incidence of PTDM was comparable in the 639 patients not included in the study (11.1% [95% CI 8.9–13.8%] at 3 months and 12.8% [95% CI 10.5–15.7%] at 1 year). Patients with 25(OH)D deficiency had the highest incidence of PTDM, while patients with normal ranges of 25(OH)D had the lowest (Fig. 1). Cumulative incidence of PTDM at 3 and 12 months were, respectively, 15.9% (95% CI 9.7–25.4%) and 18.2% (95% CI 11.6–28.0%) in patients with 25(OH)D deficiency; 11.8% (95% CI 8.5–16.4%) and 13.0% (95% CI 9.5% to 17.7%) in patients with 25(OH)D insufficiency; and 7.6% (95% CI 3.7–15.3%) and 8.7% (95% CI 4.5–16.7%) in patients with normal ranges of 25(OH)D. Multivariate Cox analysis (Table 2, n = 443) indicated an increased risk of PTDM in the first year post-transplantation in patients with low 25(OH)D concentration (< 30 ng/ml) at transplantation (global P-value = 0.073). 25(OH)D-deficient patient at transplantation had more than two times risk for PTDM than patients with normal concentration (HR = 2.41, 95% CI 1.01–5.75, $P = 0.048$). Patients with 25(OH)D insufficiency tended to have an increased risk of PTDM within the first year post-transplantation although the difference was not statistically significant (HR = 1.22, 95% CI 0.56–2.66, $P = 0.61$). Other independent risk factors of PTDM were recipient age (HR = 2.21 for ≥ 55 years, 95% CI 1.28–3.80), BMI (HR = 1.72 for each 5 kg/m^2 increase; 95% CI 1.29–2.28), tacrolimus therapy (HR = 4.73, 95% CI 1.86–12.04), and maintenance corticosteroids therapy (HR = 1.99, 95% CI 1.10–3.62) (Table 2). In our study, a 25(OH)D substitution was prescribed to 19.1% of the patients at any time after the transplantation, but only 6.8% of patients received 25(OH)D substitution within the

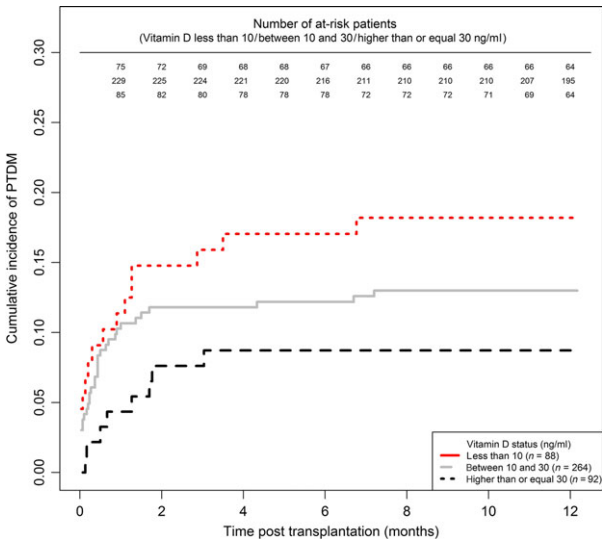

Figure 1 Cumulative incidence of PTDM in the first year post-transplantation according to 25(OH)D status at transplantation.

Table 2. Cox survival analysis: relationship between 25(OH)D at transplantation and the time to develop PTDM in the first year post-transplantation.

	Multivariate analysis (n = 443)		
	HR	95% CI	*P*-value
25(OH)D status (ng/ml)			
10–30 vs. ≥ 30	1.22	0.56–2.66	0.614
< 10 vs. ≥ 30	2.41	1.01–5.75	0.048
Recipient age (≥ 55 vs. < 55 years)	2.21	1.28–3.80	0.004
Treatment with corticosteroids maintenance (yes vs. no)	1.99	1.10–3.62	0.024
Treatment with tacrolimus (yes vs. no)	4.73	1.86–12.04	0.001
Body mass index, for a 5 kg/m² increase	1.72	1.29–2.28	<0.001
Gender recipient (man vs. woman)	1.06	0.62–1.81	0.844
Ethnicity (caucasian vs. no caucasian)	0.60	0.28–1.29	0.190

P-value in univariate analysis for 25(OH)D: 0.158; *P*-value in final model P = 0.073. The proportional assumption was checked for the final model (Schoenfeld residuals = 0.589).

first year post-transplantation. Additionally, multivariate Cox analysis remains unchanged when patients treated with 25(OH)D substitution during the first post-transplant year were excluded (data not shown). As the association between steroid exposure and onset of diabetes has been already established, as well as an increased sensitivity to steroids with 25(OH)D [30], we assessed whether adjustment on corticosteroid pulses given in case of acute rejection could change the estimations of HR for 25(OH)D in the final multivariate model of PTDM. Results were roughly stable: HR for 25(OH)D < 10 ng/ml 2.12 with adjustment vs. 2.41 without adjustment.

25(OH)D deficiency was not significantly associated with patient–graft survival, acute rejection episode, and NMSC

In the total cohort, 21 (4.7%) patients died with a functioning graft and 47 (10.6%) lost their graft during follow-up. The unadjusted cumulative incidence of graft failure (return to dialysis or death with functioning graft) at 5 years was estimated to be 14.2% (95% CI 10.1–18.1%). In patients with 25(OH)D deficiency, cumulative incidence of graft failure at 5 years was 16.4% (95% CI 7.7–24.2%), while in patients with normal range of 25(OH)D, the cumulative incidence was 20.2% (95% CI 7.9–30.9%) (Fig. 2). Cox multivariate regression analysis did not reveal any significant relationship between 25(OH)D deficiency and patient–graft survival (Table 3). During follow-up, 52 acute rejection episodes were observed and no significant relationship between 25(OH)D deficiency and this outcome was found (Table 4). There was in addition, no significant association between 25(OH)D and the risk of nonmelanoma skin cancer (n = 25, data not shown).

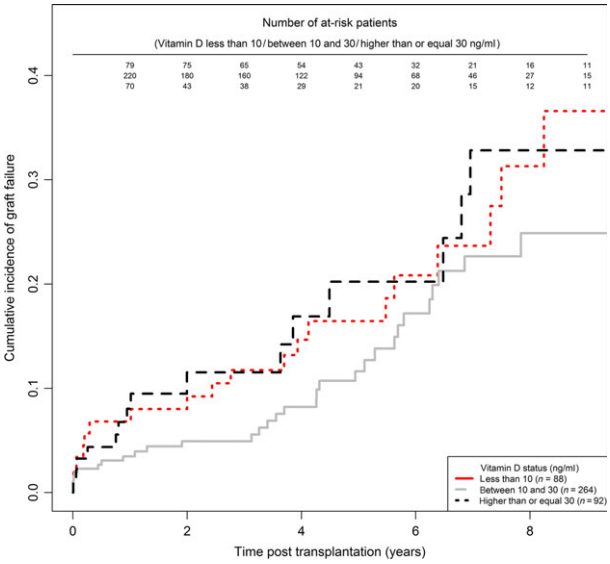

Figure 2 Cumulative incidence of graft failure (first event among return to dialysis or death with a functioning graft) according to 25(OH)D status at transplantation.

Discussion

To the best of our knowledge, our study identified for the first time 25(OH)D deficiency as an independent risk factor for PTDM within the first year after kidney transplantation. However, we found no significant relationship between 25(OH)D and acute rejection episodes, NMSC, and patient–graft survival. In all patients, the use of tacrolimus, recipient age ≥ 55 years old, high BMI, and the use of maintenance corticosteroids were also confirmed as independent risk factors of PTDM in our cohort. Diabetogenic effect of 25(OH)D deficiency results from insulin secretion impairment, which plays a key role in the early development of PTDM. Surgical stress impairs beta cell function because of catecholamine's toxicity, and insulin secretion is additionally decreased by CNIs and corticosteroids [34]. Moreover, a new management strategy with the goal of protecting or rescuing beta cell function early after transplantation by intensive exogenous insulin therapy has been proposed [13]. Human's pancreatic beta cells express vitamin D receptor (VDR) and 1-alpha hydroxylase [31] and are also able to convert *in situ* 25(OH)D into calcitriol (1,25 dihydroxyvitamin D), which is the active form of 25 (OH)D. Calcitriol improves insulin secretion by regulating insulin gene transcription and beta cell intra-cellular calcium flux [32]. Recently, an *in vitro* protective effect of calcitriol on beta cell function and beta cell survival was reported in human and mouse islets when exposed to pro-inflammatory cytokines [33]. So far, 25(OH)D-deficient kidney transplant recipients may have an increased predisposition to beta cell dysfunction.

Table 3. Cox survival analysis: relationship between 25(OH)D at transplantation and patient–graft survival.

	Multivariate analysis (n = 443)		
	HR	95% CI	*P*-value
25(OH)D status (ng/ml)			
10–30 vs. ≥ 30	0.63	0.34–1.17	0.143
< 10 vs. ≥ 30	0.82	0.40–1.65	0.571
Recipient age (≥ 55 vs. < 55 years)	1.08	0.64–1.81	0.783
Gender recipient (man vs. woman)	0.78	0.46–1.34	0.369
Body mass index, for a 5 kg/m² increase	1.02	0.75–1.37	0.921
HLA ABDR mismatches(> 4 vs. ≤ 4)	1.25	0.72–2.17	0.438
History of cardiovascular disease (yes vs. no)	1.31	0.79–2.20	0.295
Time of dialysis prior transplantation for a 1-year increase	1.05	0.98–1.14	0.158
Treatment with tacrolimus (yes vs. no)	0.60	0.36–1.02	0.061

P-value in univariate analysis for 25(OH)D level: 0.162; *P*-value in final model: 0.303.
The proportional hazards assumption was checked for the final model with Schoenfeld residuals (P = 0.845).

Table 4. Cox survival analysis: relationship between 25(OH)D at transplantation and risk of acute rejection episode.

	Multivariate analysis (n = 443)		
	HR	95% CI	*P*-value
25(OH)D status (ng/ml)			
10–30 vs. ≥ 30	1.31	0.59–2.90	0.513
< 10 vs. ≥ 30	1.48	0.61–3.60	0.390
HLA ABDR mismatches(> 4 vs. ≤ 4)	1.90	1.03–3.52	0.041
History of hypertension (yes vs. no)	0.47	0.21–1.05	0.066
History of dyslipidemia (yes vs. no)	0.56	0.30–1.04	0.067
Treatment of induction	0.36	0.13–1.03	0.057
Anticlass I PRA > 20%	1.78	0.93–3.41	0.081

P-value, univariate analysis for 25(OH)D level: 0. 641; in final model: P = 0. 679.
The proportional hazards assumption was checked for the final model with Schoenfeld residuals (P = 0. 843).

Renal transplant recipients are more insulin resistant than the general population, particularly because of obesity, waist to hip ratio, and corticosteroids [35]. The role of insulin resistance might occur later after transplantation than beta cell dysfunction.

In 2003, an International Expert Panel Meeting, based on American Diabetes Association (ADA) [1], published PTDM diagnosis criteria. In these first recommendations, the diagnosis of PTDM was defined according to fasting plasma glucose (FPG) > 126 mg/dl (7.0 mmol/l), or to nonfasting plasma glucose > 200 mg/dl (11 mmol/l) or to a 2 h glucose level of an oral glucose tolerance test (OGTT) > 200 mg/dl. In 2010, the level of standardized hemoglobin A1c has been added by an international expert committee (i.e. standardized HbA1c > 6.5%) [36,37]. More recently, a new screening strategy has been proposed, based on afternoon glucose testing [38]. This glucose measurement seems more sensitive than FPG and OGTT in the first 3 months following the transplantation, whereas after 3 months, HbA1c and OGTT had comparable sensitivity [38]. Nevertheless, several authors suggest that these criteria are too sensitive for clinical studies in transplant population [39]. Another limitation of these ADA's diagnostic criteria is the heterogeneity of glucose disorders, particularly for clinical studies. Some patients develop transient PTDM, whereas patients develop persistent diabetes. Particularly, several renal transplant recipients develop very early and transient postoperative hyperglycemia [40], which is probably a different pathology but may herald persistent PTDM. Whether resolution of PTDM is associated with reduced adverse outcomes remains unknown. A recent international consensus has proposed to exclude transient post-transplant hyperglycemia from the PTDM diagnosis definition [41].

Our cohort study was limited by some difficulties resulting from its observational nature, mainly the lack of systematic collection of some important variables for that type of research. For example, some relevant risk factors for diabetes such as pretransplantation glucose intolerance, family history of diabetes, and history of gestational diabetes were not evaluated. HbA1c was not routinely performed in our center between 2000 and 2011, and plasma glucose level, although measured, was not always fasting and mostly influenced by the corticosteroid pulse (500 mg of methylprednisolone) given pretransplant. So far, we may have included some patients with pretransplant glycaemia disorders, as glucose intolerance or even diabetes.

Hypoglycemic treatment as diagnostic criterion was another limitation in our study. We focused on patients requiring antidiabetic medication in the first year post-transplant (insulin and/or oral hypoglycemic drug), and among them, 28 (48%) still received antidiabetic medication at the last follow-up. Patients requiring antidiabetic medication are probably those with the poorer expected outcomes and the higher healthcare costs [7]. We have probably under-estimated the global incidence rate of PTDM, especially in the patients transplanted 10 years ago, when plasma glucose level was not routinely screened after transplantation. Nevertheless, the same diagnostic criteria and medical management were applied among the three groups of patients during the study period.

Due to the high number of studied variables and the few number of encountered endpoints [i.e. PTDM, acute rejection, survivals), some over-adjustments can be observed, leading to some bias [42]. In this study, we focused on a biologic parameter (25(OH)D level)] in order to provide preliminary data to further analyze vitamin D substitution

if necessary and doses needed in these patients developing PTDM. Concerning the characteristics of our population, the great majority of patients were Caucasian and received tacrolimus-based immunosuppression.

There were some differences between our study population and the excluded patients regarding immunosuppressive regimen, initial kidney disease, and proportion of preemptive grafts, what could be seen as a selection bias. However, as excluded patients were mainly transplanted before 2006 (the year from which 25(OH)D measurement was routinely performed), our study sample is probably more representative of the patients recently transplanted. The incidence of PTDM was 13.2% at 1 year and comparable to the incidence rate of PTDM reported by other European authors. A French multicenter observational study using ADA's biological diagnosis criteria reported 7% of PTDM at 1 year (17.4% and 5.5% under tacrolimus and cyclosporine, respectively) [43]. In the observational cohort study of Medical University of Vienna, 11% of kidney transplant recipients had history of PTDM and 43% of the patients without diabetes history had abnormal OGTT results [44]. The incidence rate of PTDM increased in US population up to 30–37% at 1 year using four biological parameters including glycated hemoglobin level [39].

The most recent Kidney Disease Global Outcomes guidelines for the management of mineral bone disorders in patients with chronic kidney disease suggest periodic measurement of 25(OH)D concentration and a substitution, in the case of deficiency or insufficiency, using the strategies recommended for the general population to maintain 25 (OH)D concentrations above level desirable for fracture prevention (i.e. > 30 ng/ml) [40]. In our study, conducted between 2000 and 2011, 19.8% of renal transplant recipients were 25(OH)D deficient (< 10 ng/ml) at the time of transplantation. Indeed, since few years, vitamin substitution is widely used in dialysis centers. However, in our center, in the past 2 years, 60% of kidney transplant recipients remained 25(OH)D insufficient (≤ 30 ng/ml), and among them, 9% were 25(OH)D deficient (< 10 ng/ml) at the time of transplantation.

In summary, in this single center observational study, we identified 25(OH)D deficiency at the time of transplantation as an independent risk factor of PTDM within the first year after transplantation. 25(OH)D deficiency at the time of transplantation may be a strong biological marker of general health and can alert clinicians about the possible risk of PTDM. This study reported an association between 25(OH)D deficiency and PTDM risk, but could not provide evidence that 25(OH)D deficiency is causative of PTDM. Evidence for the benefit with substitution is limited in general population [45] but was not yet evaluated in kidney transplant recipients. Our study could represent a step toward the design of an interventional randomized placebo controlled trial. It could be hypothesized that a sufficient 25(OH)D substitution at the time of transplantation should be able to preserve, maintain, and/or improve beta cell function after kidney transplantation. A such controlled trial may be conducted either before or at the time of transplantation. PTDM often occurred within the first 3 months after transplantation, suggesting that 25(OH)D prescription may be useful in this early post-transplant period. 25 (OH)D repletion, which is a nonexpensive and nontoxic medication, could then decrease the healthcare costs generated by antidiabetic medication and cardiovascular events.

Authorship

ALF: participated in research design, in the writing of the paper, in the performance of the research, and in the data analysis. MCF: participated in the research design, in the writing of the paper, in the performance of the research, and in the analytic tools. FG: participated in the research design, in the performance of the research, and in the analytic tools. DM: participated in the performance of the research. MG: participated in the performance of the research. DC: participated in the writing of the paper and in the data analysis. BC: participated in the data analysis. JD: participated in research design, in the writing of the paper, in the performance of the research, and in the data analysis.

Funding

The authors have declared no funding.

Acknowledgements

The authors thank Roche Pharma for supporting DIVAT cohort. This project was supported by AMGEN.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. spline based approach to study the appropriateness of clinical threshold for the 25(OH)D on the risk of PTDM (adjusted on the others risk factors).

References

1. Davidson J, Wilkinson A, Dantal J, *et al.* New-onset diabetes after transplantation: 2003 International consensus guidelines. Proceedings of an international expert panel meeting. *Transplantation* 2003; **27**: SS3.
2. Cole EH, Johnston O, Rose CL, Gill JS. Impact of acute rejection and new-onset diabetes on long-term transplant

graft and patient survival. *Clin J Am Soc Nephrol* 2008; **3**: 814.
3. Markell M. New-onset diabetes mellitus in transplant patients: pathogenesis, complications, and management. *Am J Kidney Dis* 2004; **43**: 953.
4. Sharif A, Baboolal K. Complications associated with new-onset diabetes after kidney transplantation. *Nat Rev Nephrol* 2011; **8**: 34.
5. Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ. Diabetes mellitus after kidney transplantation in the United States. *Am J Transplant* 2003; **3**: 178.
6. Cosio FG, Pesavento TE, Kim S, Osei K, Henry M, Ferguson RM. Patient survival after renal transplantation: IV. Impact of post-transplant diabetes. *Kidney Int* 2002; **62**: 1440.
7. Woodward RS, Schnitzler MA, Baty J, *et al.* Incidence and cost of new onset diabetes mellitus among U.S. wait-listed and transplanted renal allograft recipients. *Am J Transplant* 2003; **3**: 590.
8. Sarno G, Muscogiuri G, De Rosa P. New-onset diabetes after kidney transplantation: prevalence, risk factors, and management. *Transplantation* 2012; **93**: 1189.
9. Yates CJ, Fourlanos S, Hjelmesaeth J, Colman PG, Cohney SJ. New-onset diabetes after kidney transplantation-changes and challenges. *Am J Transplant* 2012; **12**: 820.
10. Garg N, Weinberg J, Ghai S, *et al.* Lower magnesium level associated with new-onset diabetes and pre-diabetes after kidney transplantation. *J Nephrol* 2014; **27**: 339.
11. Hjelmesaeth J, Jenssen T, Hagen M, Egeland T, Hartmann A. Determinants of insulin secretion after renal transplantation. *Metabolism* 2003; **52**: 573.
12. Nam JH, Mun JI, Kim SI, *et al.* Beta-Cell dysfunction rather than insulin resistance is the main contributing factor for the development of postrenal transplantation diabetes mellitus. *Transplantation* 2001; **71**: 1417.
13. Hecking M, Haidinger M, Döller D, *et al.* Early basal insulin therapy decreases new-onset diabetes after renal transplantation. *J Am Soc Nephrol* 2012; **23**: 739.
14. Cranney A, Weiler HA, O'Donnell S, Puil L. Summary of evidence-based review efficacy and safety in relation to bone health. *Am J Clin Nutr* 2008; **88**: 513.
15. Christakos S, Hewison M, Gardner DG, *et al.* Vitamin D: beyond bone. *Annu N Y Acad Sci* 2013; **1287**: 45.
16. Verstuyf A, Carmeliet G, Bouillon R, Mathieu C. Vitamin D: a pleiotropic hormone. *Kidney Int* 2010; **78**: 140.
17. Wang TJ, Pencina MJ, Booth SL, *et al.* Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008; **117**: 503.
18. Garland CF, Garland FC, Gorham ED, *et al.* The role of vitamin D in cancer prevention. *Am J Public Health* 2006; **96**: 252.
19. Ascherio A, Munger KL, Simon KC. Vitamin D and multiple sclerosis. *Lancet Neurol* 2010; **9**: 599.
20. Mathieu C, Badenhoop K. Vitamin D and type 1 diabetes mellitus: state of the art. *Trends Endocrinol Metab* 2005; **16**: 261.
21. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990–2000. *Diabetes* 2008; **57**: 2619.
22. Song Y, Wang L, Pittas AG, *et al.* Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care* 2013; **36**: 1422.
23. Mitri J, Dawson-Hughes B, Hu FB, Pittas AG. Effects of vitamin D and calcium supplementation on pancreatic β cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. *Am J Clin Nutr* 2011; **94**: 486.
24. Aalen OO, Johansen S. An empirical transition matrix for non-homogeneous Markov chains based on censored observations. *Scand J Stat* 1978; **5**: 141.
25. Mehrotra R, Kermah DA, Salusky IB, *et al.* Chronic kidney disease, hypovitaminosis D, and mortality in the United States. *Kidney Int* 2009; **76**: 977.
26. Beique LC, Kline GA, Dalton B, Duggan K, Yilmaz S. Predicting deficiency of vitamin D in renal transplant recipients in northern climates. *Transplantation* 2013; **95**: 1479.
27. Bienaimé F, Girard D, Anglicheau D, *et al.* Vitamin D status and outcomes after renal transplantation. *J Am Soc Nephrol* 2013; **24**: 831.
28. Norman AW, Bouillon R, Whiting SJ, Vieth R, Lips P. 13th Workshop consensus for vitamin D nutritional guidelines. *J Steroid Biochem Mol Biol* 2007; **103**: 204.
29. Meira-Machado L, Cadarso-Svarez C, Gude F, Araujo A. SmoothHR: an R package for pointwise nonparametric estimation of hazard ratio curves of continuous predictors. *Comput Math Methods Med* 2013; **2013**: 745742.
30. Chambers E, Nanzer A, Richards D, *et al.* Serm 25-dihydroxyvitamin D levels correlate with $CD4^+FoxP3^+$ T-Cell numbers in moderate/severe asthma. *J Allergy Clin Immunol* 2012; **130**: 542.
31. Johnson JA, Grande JP, Roche PC, Kumar R. Immunohistochemical localization of the 1,25(OH)2D3 receptor and calbindin D28k in human and rat pancreas. *Am J Physiol* 1994; **267**: 356.
32. Kajikawa M, Ishida H, Fujimoto S, *et al.* An insulinotropic effect of vitamin D analog with increasing intracellular Ca^{2+} concentration in pancreatic beta-cells through nongenomic signal transduction. *Endocrinology* 1999; **140**: 4706.
33. Wolden-Kirk H, Rondas D, Bugliani M, *et al.* Discovery of molecular pathways mediating 1,25-dihydroxyvitamin D3 protection against cytokine-induced inflammation and damage of human and male mouse islets of Langerhans. *Endocrinology* 2014; **155**: 736.
34. Zelle DM, Corpeleijn E, Deinum J, *et al.* Pancreatic β-cell dysfunction and risk of new-onset diabetes after kidney transplantation. *Diabetes Care* 2013; **36**: 1926.

35. Oterdoom LH, de Vries AP, Gansevoort RT, *et al.* Determinants of insulin resistance in renal transplant recipients. *Transplantation* 2007; **83**: 29.
36. Gillett MJ. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. International Expert Committee. *Diabetes Care* 2009; **32**: 1327.
37. Shabir S, Jham S, Harper L, Ball S, Borrows R, Sharif A. Validity of glycated haemoglobin to diagnose new onset diabetes after transplantation. *Transpl Int* 2013; **26**: 315.
38. Yates CJ, Fourlanos S, Colman PG, Cohney SJ. Screening for new-onset diabetes after kidney transplantation: limitations of fasting glucose and advantages of afternoon glucose and glycated hemoglobin. *Transplantation* 2013; **96**: 726.
39. First MR, Dhadda S, Croy R, Holman J, Fitzsimmons WE. New-onset diabetes after transplantation (NODAT): an evaluation of definitions in clinical trials. *Transplantation* 2013; **96**: 58.
40. Wojtusciszyn A, Mourad G, Bringer J, Renard E. Continuous glucose monitoring after kidney transplantation in non-diabetic patients: early hyperglycaemia is frequent and may herald post-transplantation diabetes mellitus and graft failure. *Diabetes Metab* 2013; **39**: 404.
41. Sharif A, Hecking M, de Vries APJ, *et al.* Proceedings from an international consensus meeting on posttranplantation diabetes mellitus: recommendations and future directions. *Am J Transplant* 2014; **14**: 1992.
42. Sauerbrei W. The use of resampling methods to simplify regression models in medical statistics. *Appl Stat* 1999; **48**: 313.
43. Kamar N, Mariat C, Delahousse M, *et al.* Diabetes mellitus after kidney transplantation: a French multicentre observational study. *Nephrol Dial Transplant* 2007; **22**: 1986.
44. Hecking M, Kainz A, Werzowa J, *et al.* Glucose metabolism after renal transplantation. *Diabetes Care* 2013; **36**: 2763.
45. Theodoratou E, Tzoukaki I, Zgaga L, Loannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ* 2014; **348**: 2035.