Supplementary material

Methods

Study patients, ethical statements, and exposure

We performed a retrospective observational study among all consecutive adult recipients who received a kidney transplant at Turin University Renal Transplant Center "A. Vercellone" from January 2003 to December 2013. The local Ethical Committee approved this study (*Comitato Etico Interaziendale A.O.U. Città Della Salute e Della Scienza di Torino - A.O. Ordine Mauriziano - A.S.L. Città di Torino*, resolution number 1449/2019 on 11/08/2019). This study is conducted according to the principles of the Helsinki Declaration. All participants provided written informed consent after a detailed explanation of the study procedures.

NODAT and T2DM were defined according to the American Diabetes Association criteria (HbA1c level ≥ 6.5% or fasting blood glucose level ≥ 126 mg/dl or blood glucose level ≥ 200 mg/dl during glucose tolerance testing or random blood glucose level ≥ 200 mg/dl in the presence of typical diabetes symptoms, whereas, in the absence of unequivocal hyperglycemia, diagnosis requires two abnormal test results from the same sample or in 2 separate test samples)[S1] and diabetic nephropathy according to 2012 KDOQI[S2] (macroalbuminuria or microalbuminuria in the presence of diabetic nephropathy).

All patients received only for cause biopsy, and we considered recurrent patients only subjects with clinical alterations and histological evidence of diabetic nephropathy. Patients without clinical signs and/or negative histology were classified as negative. The same nephropathologist team analyzed the biopsies, defining the recurrence of diabetic nephropathy in the case of typical nodular glomerulosclerosis, expansion of mesangial matrix, chronic interstitial inflammatory infiltrate, or

severe interstitial fibrosis. For the sub-analysis of recurrence, patients with T1DM or NODAT were excluded.

Acute transient hyperglycemia after steroid induction at transplant was defined according to the last guidelines[S3]: briefly, pre-prandial capillary plasma glucose > 130 mg/dl and peak post-prandial capillary plasma glucose (made 1–2 h after the beginning of the meal) > 180 mg/dl.

Simultaneously with a progressive reduction of steroid posology (day 0 500 mg i.v., day 1 200 mg i.v., day 2 50 mg i.v., from day 4 20 mg orally, to tape gradually), strict glycemic monitoring was performed; according to adopted protocol, the pre-prandial glycemic target was < 140 mg/dl, while the post-prandial one was < 180 mg/dl. In case of two consecutive pre-prandial values ≥ 180 mg/dl or post-prandial blood glucose values ≥ 220 mg/dl, insulin lispro was administered and, in case of basal blood glucose > 140 mg/dl, insulin glargine was added. The diabetes specialist was consulted if two blood glucose measurements were > 250 mg/dl or if intensification of insulin therapy was required three times.

Posttransplant management and data collection

All patients were initially managed by the Renal Transplant Center (Hub center) and received induction therapy (steroids and basiliximab/anti-thymocyte globulin [ATG] according to donor type and immune risk) and maintenance immunosuppression mainly composed of Tacrolimus (10–15 ng/ml for the first three months and of 6–8 ng/ml thereafter), mycophenolate mofetil/mycophenolic acid, and/or steroids (progressively tapered to 5 mg/day or withdrawn according to patients characteristics and immunological risk).

After discharge, post-transplant care followed a standardized schedule, and every recipient was followed by the transplant center (Hub center) with at least one annual visit and by the local

nephrologist (eleven peripherical centers covering most of the Piedmont region) for their periodical follow-up.

All clinical and medical information was collected from patients' charts, including donors' data, immunosuppressive medications, demographic and clinical characteristics such as sex, Body Mass Index (BMI), age at transplant, age at T2DM diagnosis, pre-transplant arterial hypertension, and cardiovascular disease, pre-transplant and transplant vascular complications, post-transplant infectious complications; additionally, in the sub-analysis among patients with T2DM smoking abit and pre-transplant glycolipid profile (the last available before graft) were recorded. Renal allograft function (eGFR), when available, was estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Serum creatinine, eGFR, and proteinuria were analyzed at discharge, at 3 and 6 months, and 1, 2, 5, and 10 years after transplant.

Outcomes

The primary endpoint of this study was to identify and compare the significant risk factors for NODAT in the overall population without pre-transplant T2DM and for diabetic nephropathy recurrence among patients with pre-transplant T2DM. Secondary endpoints are the potential impact of these variables and the recurrence by itself on death-censored graft survival rates and graft function.

Statistical methods

Continuous variables were described as the median and interquartile range (IQR) according to their non-normal distribution. The Mann-Whitney test was used to compare independent groups, and the Wilcoxon signed-rank test was used to compare related variables. Categorical variables were presented as a fraction, and Pearson's 2c or, for small samples, Fisher's exact test was employed to

compare groups. The odds ratios (OR) with a 95% Confidence Interval (CI) were used to measure relative risk. Some cut-offs of continuous variables were selected with ROC curves. Kaplan-Meier (KM) curves analyzed cumulative survival. Relevant variables were checked first in univariate analysis and then, if p<0.1, included in a logistic regression model and Cox regression analysis. The significance level for all tests was set at α<0.05. All statistical analyses were performed using Spss (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.).

Population characteristics

Among the 1127 consecutive transplants performed during the analyzed period, we included only patients with available records (n=1061). The demographic and clinical characteristics of the overall population of KTRs without pre-transplant T2DM stratified for the occurrence of NODAT are summarized in Table S1: no differences were noted among groups for sex, weight at transplant, blood group, Rh factor, pre-transplant vascular complications and post-transplant infectious complications. Also, donors' characteristics, including the Karpinski pre-transplant histological score on renal biopsy, and the percentage of donors with a history of diabetes or hypertension were similar among groups. Some conditions previously related in literature to NODAT were also noted in our population: higher age at transplant (53.5 years (44.6-62.7) in NODAT group vs. 57.3 (48.8-63.3) in no NODAT, p=0.005), number of patients treated with Tacrolimus at discharge (97.4% in NODAT vs. 93.3% in no NODAT, p=0.049), pre-transplant history of cardiovascular disease and hypertension (p=0.039 e p=0.022). However, acute transient hyperglycemia after steroid induction seems to be strongly correlated to NODAT (p<0.001, OR 10.05 [7.05-14.32]). Patients in both groups received Basiliximab in 90.5% of cases (9.5% received Thymoglobulines alongside Basiliximab or as an exclusive therapy). After the

transplant, as expected, steroid withdrawal was more frequent in the NODAT group (p=0.038 at one year and p=0.003 at five years vs. no NODAT).

For the sub-analysis in KTRs with pre-existing T2DM stratifying for the recurrence of diabetic nephropathy (Table S2), no differences were noted among groups for sex, BMI, age at T2DM diagnosis, blood group, Rh factor, smoking, pre-transplant arterial hypertension and cardiovascular disease, pre-transplant, and vascular transplant complications and post-transplant infectious complications. Also, donors' characteristics, including the Karpinski pre-transplant histological score on renal biopsy, were similar between patients with or without recurrence. Induction included the administration of Basiliximab in 93% of cases (only 7% received Thymoglobulines alongside Basiliximab or as an exclusive therapy). Maintenance treatment was composed of the triple standard treatment (Tacrolimus, Mycophenolate Mofetil [MMF], or Mycophenolic Acid [MPA] and steroid) on 53.6% of patients, while 15.5% of them received Tacrolimus and steroid, 2.8% Tacrolimus and MMF/MPA, 1.4% Tacrolimus, Azathioprine and steroid, 1.4% Tacrolimus, Sirolimus, and steroid, 23.9% Cyclosporine instead of Tacrolimus, 1.4% Ciclosporin and steroid. The limited number of KTRs with early steroid withdrawal does not differ among patients with or without recurrence. There wasn't statistical significance about rejection treatment with steroid boluses, antiproteinuric therapy with Angiotensin Converter Enzyme inhibitors, or Angiotensin Receptor Blockers between transplant and recurrence, as the use of statins at the moment of transplant and delayed graft function.

Considering patients with pre-transplant T2DM according to the recurrence of dianetic nephropathy after transplant, in univariate analysis, a pre-transplant LDL-cholesterol ≥100 mg/dl, calculated by ROC curves, was most frequently observed in KTRs who developed a recurrence (68.4% vs. 36.5% in KTRs without recurrence, p=0.03, OR 3.76, 95% CI [1.23-11.53]). In contrast, pre-transplant Hb1Ac or triglycerides appear not to influence the risk of recurrence (p>0.05). Most KTRs with pre-transplant

T2DM were already treated with insulin before the transplant (68.6%), and only a trend without statistical significance for increased pre-transplant and post-transplant insulin use was noted (Table S5). A pre-transplant HDL-cholesterol ≤40 mg/dl showed a trend to an increased occurrence in patients with recurrence without significance (68.4% vs. 42.3%, p=0.06) (Table S5).

Supplementary Table S1. Demographic and clinical characteristics of kidney-transplant patients according to NODAT occurrence. Data are expressed as percentages or medians with IQR according to their distribution.

	No NODAT (n=800)	NODAT (n=190)	p	OR [95% CI]
Men, n (%)	513 (64.1)	107 (56.3)	0.055	
Age at transplant, median (25-75 percentile)	53.5 (44.6-62.7)	57.3 (48.8-63.3)	0.005	
Hemodialysis before transplant, n (%)	636 (79.5)	146 (76.8)	0.317	
HCV positive before transplant, n (%)	69 (8.6)	13 (6.8)	0.586	
Pre-transplant arterial hypertension, n (%)	658 (82.3)	168 (88.4)	0.039	1.66 [1.03-2.68]
Acute transient hyperglicemia after transplant, n (%)	126 (15.8)	124 (65.3)	<0.001	10.05 [7.05-14.32]
Pre-transplant cardiovascular disease, n (%)	228 (28.5)	71 (37.4)	0.022	1.48 [1.06-2.06]
Pre-transplant and transplant vascular complications, n (%)	210 (26.3)	54 (28.4)	0.585	
Post-transplant infectious complications, n (%)	532 (66.5)	134 (70.5)	0.385	
Post-transplant vascular complications, n (%)	194 (24.3)	72 (37.9)	<0.001	1.86 [1.33-2.6]
Donor history of diabetes, n (%)	58 (7.3)	14 (7.4)	0.875	
Donor history of hypertension, n (%)	362 (45.3)	82 (43.2)	0.738	
Delayed graft function after transplant, n (%)	206 (25.8)	51 (26.8)	0.118	
Weight at transplant, median (25-75 percentile)	65 (58-75)	67 (58-76)	0.192	
Weight one year after transplant (25-75 percentile)	65 (57-75)	68 (58-77)	0.153	
Weight five years after transplant (25-75 percentile)	66 (59-76)	70 (60-78)	0.086	
Tacrolimus at discharge, n (%)	746 (93.3)	185 (97.4)	0.049	2.480 [0.98-6.31]
Tacrolimus one year after transplant, n (%)	714 (94.7)*	184 (96.8)*	0.261	
Steroids one year after transplant, n (%)	701 (93)*	167 (87.9)*	0.038	
Tacrolimus five years after transplant, n (%)	610 (92.7)*	162 (93.6)*	0.118	
Steroids five years after	431 (65.5)*	91 (52.6)*	0.003	0.596 [0.42-

transplant, n (%)			0.84]

*Percentage calculated on functioning graft at the f/up time

Supplementary Table S2. Demographic and clinical characteristics of kidney-transplant patients with pre-existing T2DM with or without clinically relevant and biopsy-proven recurrence of diabetic nephropathy. Data are expressed as percentages or medians with IQR according to their distribution.

	No recurrence of diabetic nephropathy (n=52)	Recurrence of diabetic nephropathy (n=19)	p	OR [95% CI]
Men, n (%)	43 (82.7)	11 (57.9)	0.06	0.29 [0.09-0.92]
Age at T2DM diagnosis, median (25-75 percentile)	35 (32-43)	41 (32-48)	0.21	
BMI on waiting list, median (25-75 percentile)	25.85 (23.5-28.7)	26.2 (24.35-29.5)	0.58	
Sub-Saharan heritage, n (%)	2 (3.8)	3 (15.7)	0.12	
Smoking, n (%)	29 (55.8)	8 (42.1)	0.42	0.58 [0.2-1.67]
Pre-transplant arterial hypertension, n (%)	52 (100)	19 (100)		
Pre-transplant cardiovascular disease, n (%)	26 (50)	11 (57.9)	0.6	1.37 [0.48-3.97]
Pre-transplant and transplant vascular complications, n (%)	31 (59.6)	7 (36.8)	0.1	0.36 [0.12-1.07]
Donor history of diabetes, n (%)	2 (3.8)	0 (0)	0.34	
Post-transplant infectious complications, n (%)	43 (82.7)	16 (84.2)	0.99	1.11 [0.27-4.65]
Steroid withdrawal at three months, n (%)	7 (13.5)	3 (15.8)	0.52	
Post-transplant BMI at five years, median (25-75 percentile)	26.8 (24.8-28.5)	26.7 (24.8-28.5)	0.4	
Acute rejection episodes, n (%)	5 (9.6)	3 (15.7)	0.68	

Supplementary Table S3. Multivariate analysis of clinical determinants of clinically relevant and biopsy-proven recurrence of diabetic nephropathy.

|	p	OR [95% CI]
Glycemic decompensation after steroid induction at transplant	<0.001	57.51 [7.68-430.96]
Post-transplant insulin	0.97	0.96 [0.07-12.31]
Pre-transplant LDL-cholesterol ≥100 mg/dl	0.93	1.08 [0.21-5.63]
Pre-transplant HDL-cholesterol ≤40 mg/dl	0.06	5.64 [0.9-35.25]

Supplementary Table S4. Risk factors for glycemic decompensation after steroid induction at transplant. Data are expressed as percentages or medians with IQR according to their distribution.

|	No glycemic decompensation after steroid induction at transplant (n=49)	Glycemic decompensation after steroid induction at transplant (n=22)	p	OR [95% CI]
Men, n (%)	42 (85.7)	12 (54.5)	0.005	0.17 [0.05-0.57]
BMI on waiting list, median (25-75 percentile)	26.7 (23.7-29.4)	25 (23.8-27.6)	0.23	
Age at T2DM diagnosis median (25-75 percentile)	37 (33-43.75)	36.5 (30-43.5)	0.54	
Smoking, n (%)	26 (53)	11 (50)	0.8	0.85 [0.31-2.32]
Pre-transplant arterial hypertension, n (%)	49 (100)	22 (100)	/	/
Pre-transplant cardiovascular disease, n (%)	27 (55.1)	10 (45.5)	0.447	0.65 [0.24-1.79]
Pre-transplant and transplant vascular complications, n (%)	13 (26.5)	8 (36.4)	0.58	1.54 [0.52-4.52]
Post-transplant infectious complications, n (%)	39 (79.6)	19 (86.4)	0.74	1.46 [0.35-6.03]
Pre-transplant insulin, n (%)	36 (73.5)	18 (81.8)	0.76	1.5 [0.42-5.32]
Pre-transplant Hb1Ac, median (25-75 percentile)	6 (5.2-7)	6 (5.3-7)	0.53	
Pre-transplant total cholesterol, median (25-75 percentile)	150 (145/188.5)	196.5 (148.75-205)	0.013	
Pre-transplant triglycerides, median (25-75 percentile)	110 (107/197.5)	179 (121.25-206.5)	0.05	

Supplementary Table S5. Pre-transplant and post-transplant insulin therapy, glycemic decompensation after steroid induction at transplant, and pre-transplant glycolipid profiles.

|	No recurrence of diabetic nephropathy (n=52)	Recurrence of diabetic nephropathy (n=19)	p	OR [95% CI]
Pre-transplant insulin, n (%)	33 (63.5)	15 (79)	0.26	
Glycemic decompensation after steroid induction at transplant, n (%)	6 (11.5)	16 (84.2)	<0.001	40 [8.93-179.04]
Post-transplant insulin, n (%)	32 (61.5)	18 (94.7)	0.009	
Pre-transplant Hb1Ac ≥6%, n (%)	25 (48.1)	13 (68.4)	0.18	2.34 [0.77-7.1]
Pre-transplant LDL-cholesterol ≥100 mg/dl, n (%)	19 (36.5)	13 (68.4)	0.03	3.76 [1.23-11.53]
Pre-transplant HDL-cholesterol ≤40 mg/dl, n (%)	22 (42.3)	13 (68.4)	0.06	2.95 [0.97-8.99]
Pre-transplant triglycerides ≥147 mg/dl, n (%)	20 (38.5)	12 (63.2)	0.1	2.74 [0.92-8.13]

Supplementary Figure S1. Disease-free survival (DFS) for NODAT in the overall population without T2DM, according to acute steroid-induced hyperglycemia occurrence.
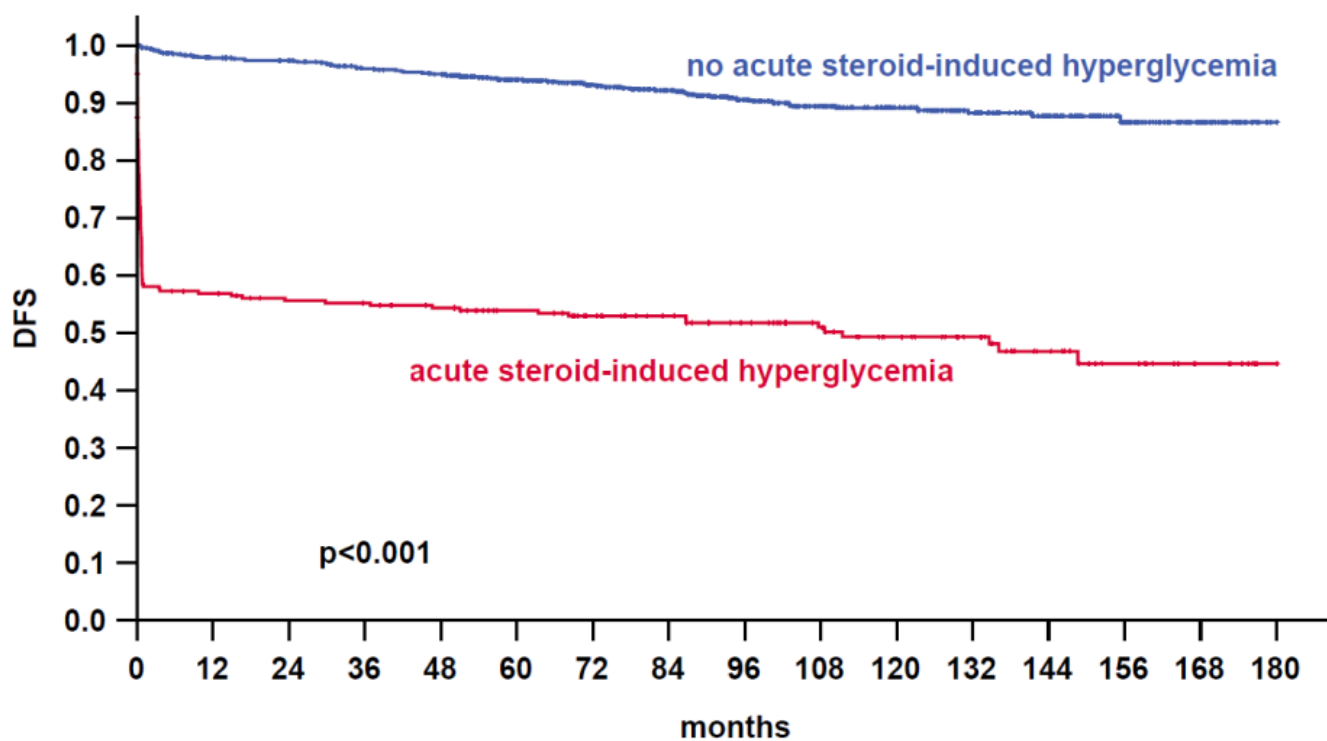
Supplementary Figure S2. Changes in HbA1c at five years in patients with/without recurrence.
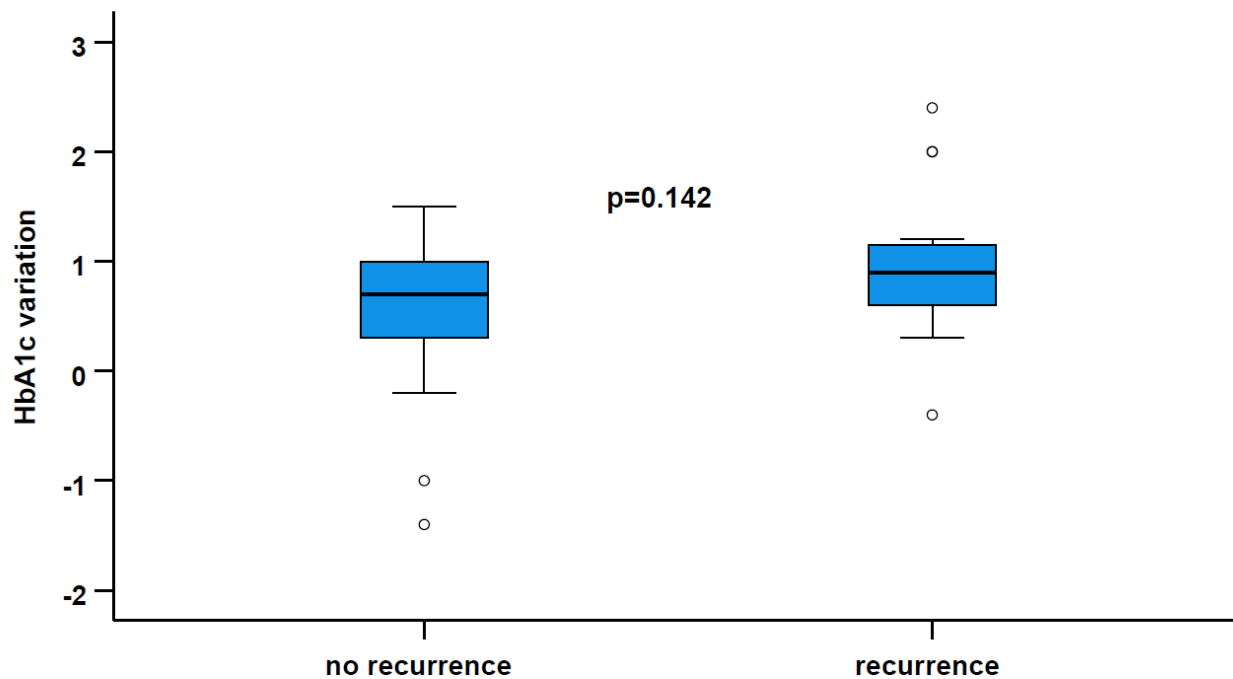
Supplementary Figure S3. Death-censored graft survival in the overall cohort of kidney transplanted patients and in patients with pre-existing diabetes mellitus with/without recurrence of diabetic nephropathy.

graft survival (death censored)

no recurrence vs recurrence: p<0.001

no recurrence vs over all: p<0.001
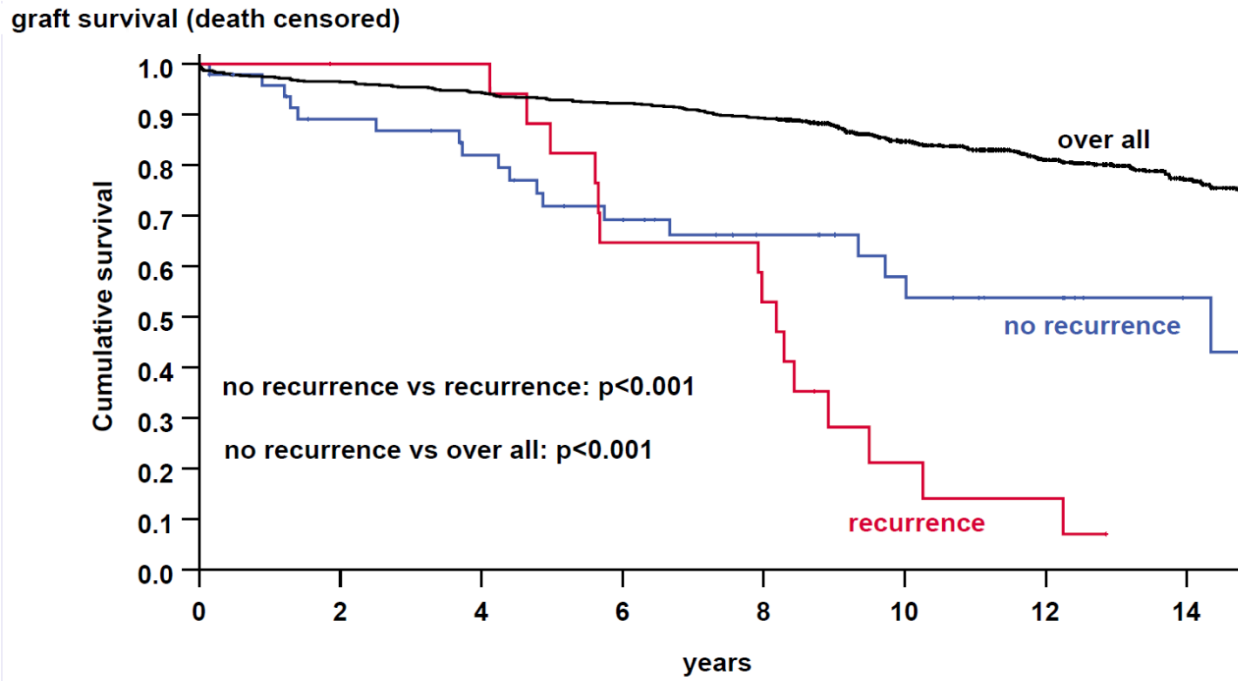
n at risk								
no recurrence	48	39	33	26	19	14	9	5
recurrence	18	17	17	11	9	3	2	0
over all	787	759	743	726	703	530	385	259

Supplementary Figure S4. Patient survival in kidney transplanted patients with pre-existing diabetes mellitus with/without recurrence of diabetic nephropathy.
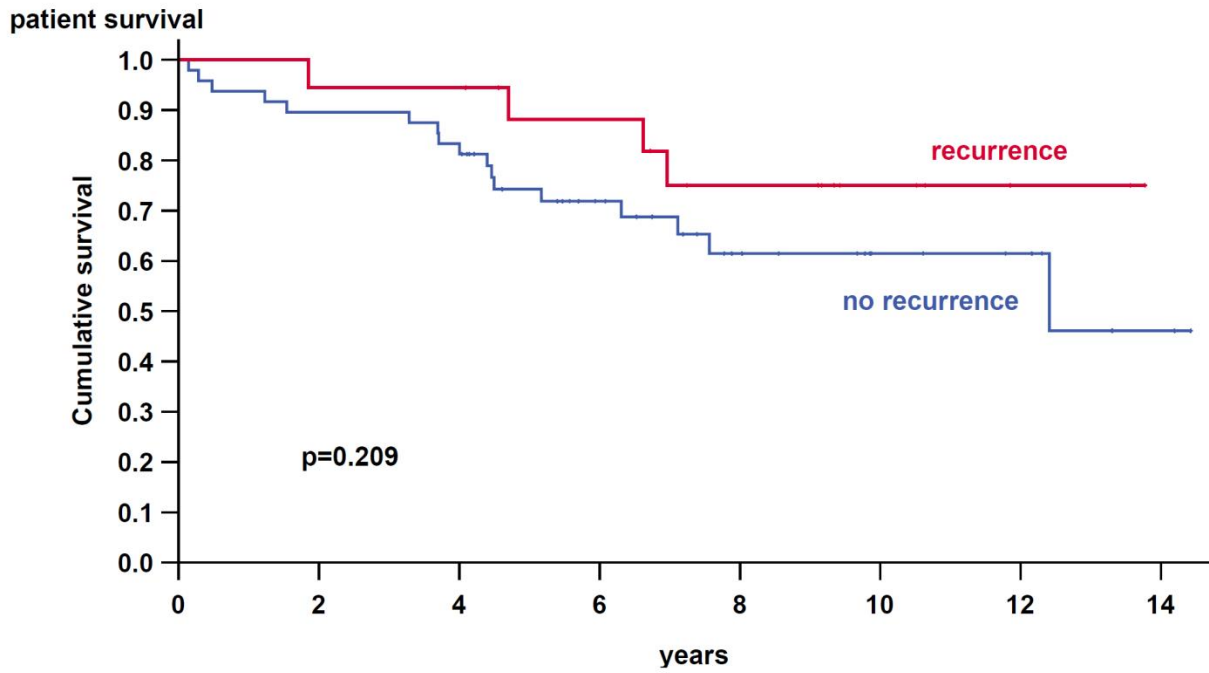
n at risk								
no recurrence	48	43	40	24	14	8	6	2
recurrence	18	17	17	14	10	6	2	0

Supplemental Discussion

In this highly detailed retrospective analysis of consecutive first single KTRs from deceased donors with homogeneous characteristics regarding immunosuppression therapy, clinical management, and interpretation of histological results, 190 KTRs developed NODAT, and 19 out of the 71 KTRs with pre-transplant T2DM associated with diabetic nephropathy developed a clinically significant and biopsy-proven recurrence. Both NODAT and recurrence of diabetes nephropathy showed a strong correlation with acute steroid-induced transient hyperglycemia after steroid induction.

Many studies focused on potential therapies to avoid or reduce hyperglycemia in the early post-transplant phase to reduce complications and increase graft survival, including steroid-free regimens or CNI abrogation using belatacept[S4,5]. For example, Rostaing et al. reported that patient/graft survival and renal function at 12 months were numerically higher with belatacept versus CyA but not statistically significant[S6].

The impact of acute steroid-induced transient hyperglycemia at transplant may suggest further studies to assess the proper immunosuppression characterized by a less metabolic effect.

Supplementary References

S1.   American Diabetes Association. 2. Classification and diagnosis of diabetes: Standards of

Medical Care in Diabetes--2018. Diabetes Care 2018;41(Suppl. 1):S13–S27

S2.   Rocco M V., Berns JS. KDOQI clinical practice guideline for diabetes and CKD: 2012 update. *Am J Kidney Dis*. 2012;60(5):850-886. doi:10.1053/j.ajkd.2012.07.005

S3.   American Diabetes Association. 6. Glycemic targets: Standards of Medical Care in Diabetes--

2021. Diabetes Care 2021; 44(Suppl. 1):S73–S84

S4.   Balducci S, Sacchetti M, Haxhi J, et al. Physical Exercise as therapy for type II diabetes. *Diabetes Metab Res Rev*. 2014;32(30):13-23. doi:10.1002/dmrr

S5.   Masson P, Henderson L, Chapman JR, Craig JC, Webster AC. Belatacept for kidney transplant recipients. *Cochrane Database Syst Rev*. 2013;2013(8). doi:10.1002/14651858.CD010699

S6.   Rostaing L, Neumayer HH, Reyes-Acevedo R, et al. Belatacept-versus cyclosporine-based immune suppression in renal transplant recipients with pre-existing diabetes. *Clin J Am Soc Nephrol*. 2011;6(11):2696-2704. doi:10.2215/CJN.00270111

STROBE Statement

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Done
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	N/A
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Done
Objectives	3	State specific objectives, including any prespecified hypotheses	Done
Methods			
Study design	4	Present key elements of study design early in the paper	Done
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Done
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Done
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Done
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Done
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Done
Bias	9	Describe any efforts to address potential sources of bias	Done
Study size	10	Explain how the study size was arrived at	Done
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Done
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Done
		(*b*) Describe any methods used to examine subgroups and interactions	Done
		(*c*) Explain how missing data were addressed	Done
		(*d*) If applicable, explain how loss to follow-up was addressed	Done
		(*e*) Describe any sensitivity analyses	Done
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Done

		(b) Give reasons for non-participation at each stage	Done
		(c) Consider use of a flow diagram	Done
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Done
		(b) Indicate number of participants with missing data for each variable of interest	Done
		(c) Summarise follow-up time (eg, average and total amount)	Done
Outcome data	15*	Report numbers of outcome events or summary measures over time	Done
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Done
		(*b*) Report category boundaries when continuous variables were categorized	Done
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Done
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Done

Discussion

Key results	18	Summarise key results with reference to study objectives	Done
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Done
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Done
Generalisability	21	Discuss the generalisability (external validity) of the study results	Done

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Done