Occurrence of Diabetic Nephropathy After Renal Transplantation Despite Intensive Glycemic Control: An Observational Cohort Study

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OBJECTIVE

The kinetics and risk factors of diabetic nephropathy after kidney transplantation remain unclear. This study investigated the posttransplant occurrence of diabetic nephropathy and the contribution of posttransplant glycemic control.

RESEARCH DESIGN AND METHODS

We performed a single-center prospective cohort study of 953 renal allograft recipients and 3,458 protocol-specified renal allograft biopsy specimens up to 5 years after transplantation. The effects of pretransplant diabetes and glycemic control (glycated hemoglobin levels) on the posttransplant histology were studied.

RESULTS

Before transplantation, diabetes was present in 164 (17.2%) renal allograft recipients, primarily type 2 (*n* = 146 [89.0%]). Despite intensive glycemic control (glycated hemoglobin 7.00 ± 1.34% [53 ± 14.6 mmol/mol], 6.90 ± 1.22% [52 ± 13.3 mmol/mol], and 7.10 ± 1.13% [54 ± 12.4 mmol/mol], at 1, 2, and 5 years after transplantation), mesangial matrix expansion reached a cumulative incidence of 47.7% by 5 years in the pretransplant diabetes group versus 27.1% in patients without diabetes, corresponding to a hazard ratio of 1.55 (95% CI 1.07–2.26; *P* = 0.005). Mesangial matrix expansion was not specific for diabetic nephropathy and associated independently with increasing age. Pretransplant diabetes was associated with posttransplant proteinuria but not with estimated glomerular filtration rate, graft failure, or any other structural changes of the glomerular, vascular, or tubulointerstitial renal compartments. The occurrence of diabetic nephropathy was independent of posttransplant glycated hemoglobin levels.

CONCLUSIONS

Mesangial matrix expansion, an early indicator of diabetic nephropathy, can occur rapidly in patients with diabetes before transplantation, despite intensive glycemic control. Prevention of diabetic nephropathy requires more than pursuing low levels of glycated hemoglobin.

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Diabetic nephropathy is the leading cause of chronic kidney disease (1). It is characterized by functional changes such as glomerular hyperfiltration and proteinuria and structural changes such as thickening of the glomerular basement membrane, mesangial matrix expansion, glomerulosclerosis, and arteriolar hyalinosis (2–4). The kinetics of the structural changes of diabetic nephropathy in native kidneys are not well documented because the few histological studies that were performed in native kidneys included patients with a variable duration of diabetes (5,6). Improved glycemic control has been shown to reduce the occurrence of early diabetic nephropathy, but evidence is lacking about whether intensive glycemic control reduces the risk of severe clinical renal outcomes, such as doubling of the serum creatinine level or end-stage renal disease (7).

Kidney transplantation is the first-choice treatment for these patients, as it is for patients with other causes of kidney failure (8,9). The occurrence of diabetic nephropathy in renal allografts has been reported both in patients with pretransplant diabetes and in patients with new-onset diabetes after transplantation (10–15). Most studies were performed in patients with type 1 diabetes or in small data sets or were restricted to clinically indicated biopsies performed at the time of graft dysfunction in a selected subset of patients, which obviates assessing the natural evolution of diabetic nephropathy.

Performing protocol-specified biopsies over time after transplantation on nondiabetic kidneys in patients with pretransplant diabetes represents a unique opportunity to study the natural evolution of diabetic nephropathy lesions because systematic renal biopsies are not routinely performed in native kidneys. A recent study on protocol-specified biopsies illustrated that 52% of patients with pretransplant diabetes had developed mesangial sclerosis at 10 years after transplantation versus 22% in patients without pretransplant diabetes (16), but the presentation and kinetics of diabetic nephropathy and its risk factors remain unclear. In addition, it is not known whether the earlier conclusion that the risk of diabetic nephropathy after kidney transplantation in patients with type 1 diabetes can be decreased by more intensive glycemic control (12) is still valid in the current era of intensive glycemic control and in patients with other types of diabetes. We performed a prospective cohort study on protocol-specified renal allograft biopsy specimens to examine the impact of pretransplant diabetes on the presentation and kinetics of diabetic nephropathy in the first 5 years after kidney transplantation and to evaluate the contribution of posttransplant glycemic control to diabetic nephropathy occurrence.

RESEARCH DESIGN AND METHODS

Patients and Biopsies

All patients who received a single kidney transplant at the University Hospitals Leuven between March 2004 and February 2013 were eligible (n = 1,137) (Supplementary Fig. 1). Recipients of kidney allografts combined with other organ types were excluded (n = 113), as were those who had a history of organ transplantation other than the kidney (n = 24). Full clinical data were required on the following variables: donor/recipient sex, donor/recipient age, donor type (donation after brain death, donation after circulatory death, living donor), transplantation rank (first vs. repeat transplantation), primary kidney disease, donor-specific antibodies, number of HLA-A/B/DR mismatches, presence and type of pretransplant diabetes (type 1, type 2, or secondary diabetes), and glycated hemoglobin levels on the day of transplantation. Protocol kidney transplant biopsies were performed at the time of transplantation and at 3, 12, and 24 months after transplantation. In addition, patients who received transplants before October 2005 were invited for a protocol biopsy at 36, 48, and 60 months after transplantation; patients transplanted between October 2005 and November 2008 for a protocol biopsy at 36 and 60 months; and patients transplanted between November 2008 and January 2010 for a protocol biopsy at 60 months. Patients who did not enroll in the protocol biopsy program were excluded (n = 34).

Data Collection

All data were prospectively collected during routine clinical follow-up in electronic medical records, which were used for clinical patient management and directly linked to the SAS database (SAS Institute, Cary, NC) used in this study. Blood pressure, estimated glomerular filtration rate (GFR), cholesterol values, and proteinuria (total proteinuria/24 h) were assessed routinely at the outpatient clinic visits and per protocol at the biopsy time points. The GFR was estimated using the four-variable MDRD equation (17). Glycated hemoglobin levels were evaluated routinely and per protocol at the biopsy time points also in patients without diabetes. Patients with diabetes received usual diabetes care. HLA antibodies were screened using a Luminex GEN-PROBE LIFECODES Deluxe kit, and in case of positive screening, the donor specificity was assessed using a Luminex GEN-PROBE LIFECODES Single Antigen Bead kit. Because only ELISA data on donor-specific HLA antibodies were available for patients who received transplants before 2008, routinely biobanked serum samples of all patients receiving transplants before this date were retested for the presence of circulating donor-specific HLA antibodies using the same platform and kits.

We included all renal allograft protocol-specified biopsies performed in this cohort. Slides were stained with hematoxylin-eosin, periodic acid Schiff, and methenamine silver (Jones' stain). One pathologist reviewed all biopsy specimens included in this study. The severity of chronic histological lesions was semiquantitatively scored according to the Banff categories for mesangial matrix expansion, tubular atrophy, vascular intimal thickening/arteriosclerosis, interstitial fibrosis, arteriolar hyalinosis, and transplant glomerulopathy (18,19). In addition, the number of sclerosed glomeruli was scored separately (0 = 0%; 1 = 1–25%; 2 = 25–50%; 3 >50% glomerulosclerosis). Immunohistochemistry for C3d, C4/C4d, and IgA was routinely performed on all biopsy specimens. In case of clinical or histological suspicion for de novo or recurrent glomerular disease, electron microscopy was performed.

Statistical Analysis

To assess the temporal evolution of renal allograft histology in patients with versus without pretransplant diabetes, we estimated cumulative incidences of the histological lesions using an interval-censored algorithm (20). Patients who had no biopsies (since the day of transplantation) before the first occurrence of an event were left-censored. If no lesion occurred before the end of follow-up, patients were right-censored

at the date of the last biopsy. The clinical determinants of this histological evolution were assessed using univariate proportional hazards models with Weibull-distributed failure times, again taking interval censoring into account. For each histological lesion, the effect of diabetes was assessed on the hazard of first-time occurrence of each lesion (mesangial matrix expansion >0, tubular atrophy >1, vascular intimal thickening/arteriosclerosis >1, interstitial fibrosis >1, arteriolar hyalinosis >1, transplant glomerulopathy >1, and glomerulosclerosis $>25\%$). These lesions were considered chronic (nonregressing) and were dichotomized to gain statistical power. In subsequent multivariate proportional hazards models, we evaluated the effects of pretransplant diabetes and glycated hemoglobin levels on the hazard of mesangial matrix expansion corrected for donor and recipient age and sex, recipient BMI, pretransplant donor-specific antibodies, number of HLA-A/B/DR mismatches, repeat transplantation, IgA nephropathy as primary cause of end-stage renal disease, and donor type. We determined the evolutions of glycated hemoglobin, estimated GFR, and log-transformed proteinuria after transplantation using a linear mixed model with pretransplant diabetes state and time after transplantation as fixed (spline) effects, random intercepts, and quadratic random slopes of time after transplantation while controlling for the same covariates as in the multivariate survival model. Subsequently, we used the linear mixed model for glycated hemoglobin evolution to estimate subject-specific glycated hemoglobin trajectories, which were used in a joint longitudinal-survival model to study the relation between longitudinal glycated hemoglobin trends and the hazard of developing mesangial matrix expansion. The area under the glycated hemoglobin curve, reflecting its cumulative effect over time, was included as a predictor in a multivariate, interval-censored, proportional hazards model. We also evaluated the cumulative effect of glycated hemoglobin conditional on pretransplant diabetes status. A sensitivity analysis was performed where we right-censored patients at the time of the occurrence of new-onset diabetes after transplantation to exclude potential bias caused by this group. New-onset diabetes exempts patients from being at risk in the no diabetes group. Nonetheless, inferences about baseline covariates are not affected by this patient group. Graft failure, censored for patient death with a functioning graft, was analyzed using the Cox model. To compare groups, we used ANOVA for continuous variables and the χ^2 test for categorical variables. Associations between continuous variables and histological lesions were assessed using Spearman correlation analysis. We used SAS 9.4 software (SAS Institute) for statistical analysis and the JMbayes package in R for the fitting of the joint model (21). We used two-sided hypothesis tests with a significance level of 5%.

RESULTS

Patients and Biopsies

Of the 953 patients included in the study, 164 (17.2%) had diabetes before transplantation. The characteristics of the patients are described in Table 1. Patients with pretransplant diabetes were significantly older, received older donor kidneys, and had a higher BMI. Within the diabetes group, 15 patients had type 1 diabetes (9.2%), 146 patients had type 2 diabetes (89.0%), and 3 patients had other specific types of diabetes (trauma, pancreatitis, and cystinosis) (1.8%). In total, 3,458 protocol-specified biopsies, with a median follow-up time of 2 years, were included in the analyses (Supplementary Fig. 1): 707 at the time of transplantation and 823, 762, 639, 212, 23, and 292 at 3, 12, 24, 36, 48, and 60 months, respectively, after transplantation. The median number of biopsies per patient was four. By 5 years after transplantation, there was a high incidence of Banff grade 1 lesions of tubular atrophy (94.9%), vascular intimal thickening (63.0%), interstitial fibrosis (68.5%), arteriolar hyalinosis (63.7%), and glomerulosclerosis (87.7%) in protocol-specified biopsy specimens (Supplementary Table 1). Therefore, we modeled severity grades of ≤ 2 for these lesions. Mesangial matrix expansion and glomerulopathy were less prevalent at 5 years after transplantation (12.0% and 3.4%, respectively) and were modeled at grade ≥ 1. Supplementary Table 2 provides information on the causes of renal failure by categories of primary renal diagnosis and glomerular diseases.

Diabetes and Posttransplant Histological Evolution

Pretransplant diabetes was associated with a significantly increased cumulative incidence of mesangial matrix expansion (Fig. 1*A*). Mesangial matrix expansion reached a cumulative incidence of 47.7% by 5 years after transplantation in the patients with pretransplant diabetes versus 27.1% in the absence of diabetes ($P = 0.005$). The graph for mesangial matrix expansion started differentiating as early as 2 years after transplantation. Also in univariate proportional hazards analyses (Supplementary Table 3), patients with pretransplant diabetes were more prone to develop mesangial matrix expansion after transplantation ($P = 0.001$), whereas there was no difference in the development of tubular atrophy ($P = 0.74$), vascular intimal thickening ($P = 0.44$), interstitial fibrosis ($P = 0.28$), arteriolar hyalinosis ($P = 0.38$), transplant glomerulopathy ($P = 0.99$), and glomerulosclerosis ($P = 0.75$). When we categorized patients with diabetes into insulin-dependent ($n = 92$) and non–insulin-dependent ($n = 72$) subgroups, we observed that insulin-dependent patients had a higher cumulative incidence of mesangial matrix expansion (64.9% by 5 years) both compared with patients without diabetes (27.1% by 5 years) ($P < 0.001$) and compared with patients with non–insulin-dependent diabetes (27.7% by 5 years; $P = 0.002$) (Fig. 1*B*). Diabetes did not associate with any other histological lesion (Fig. 1*C*).

The univariate estimates of potential confounders and the (significant) multivariate estimates of the hazard for the development of mesangial matrix expansion over time after transplantation are shown in Table 2. In the multivariate model (model 1), pretransplant diabetes significantly increased the hazard of developing mesangial matrix expansion (hazard ratio 1.55 [95% CI 1.07–2.26]; $P = 0.02$), as did recipient age (hazard ratio 1.20 per 10 years [95% CI, 1.05–1.38]; $P = 0.008$). Diabetes in the kidney donors, IgA nephropathy as original disease, and donor-specific HLA antibodies were not associated with an increased risk of mesangial matrix expansion. Older donor age was significantly associated with mesangial matrix expansion in univariate analysis but was not significant in the

Table 1—Demographic and clinical characteristics of renal transplant donors and recipients at time of transplantation, according to pretransplant diabetes state

	Total (n = 953)	No pretransplant diabetes (n = 789)	Pretransplant diabetes (n = 164)	P value[e]
Recipient demographics				
Female sex	373 (39.1)	315 (39.9)	58 (35.4)	0.28
Age (years)	53.6 ± 13.1	52.6 ± 13.5	58.7 ± 9.53	<0.001
Repeat transplant	146 (15.3)	124 (15.7)	22 (13.4)	0.46
Weight (kg)	72.8 ± 14.7	71.4 ± 14.0	79.9 ± 16.0	<0.001
BMI (kg/m^2)	25.35 ± 4.48	24.80 ± 4.23	28.03 ± 4.69	<0.001
Normal (<25)	485 (50.9)	442 (56.0)	43 (26.2)	<0.001
Overweight (25–30)	318 (33.4)	252 (31.9)	66 (40.2)	0.04
Obesity (>30)	150 (15.7)	95 (12.0)	55 (33.5)	<0.001
IgA nephropathy	97 (10.2)	86 (10.9)	11 (6.71)	0.11
Pretransplant donor-specific HLA antibodies	116 (12.2)	96 (12.2)	20 (12.2)	0.99
HLA-A/B/DR mismatches	2.73 (1.31)	2.72 (1.29)	2.77 (1.40)	0.65
Glycated hemoglobin (% [mmol/mol])	5.46 ± 0.72 [36 ± 7.9]	5.26 ± 0.36 [34 ± 3.9]	6.42 ± 1.12 [47 ± 12.2]	<0.001
Hemoglobin (g/dL)	11.18 ± 1.77	11.18 ± 1.79	11.21 ± 1.69	0.82
Type 1 diabetes	—	—	15 (9.15)	—
Type 2 diabetes	—	—	146 (89.0)	—
Other specific diabetes type	—	—	3 (1.83)	—
Insulin use	—	—	92 (56.1)	—
Donor demographics				
Female sex	445 (46.7)	361 (45.8)	84 (51.2)	0.20
Age (years)	47.8 ± 14.8	47.4 ± 14.8	49.9 ± 14.9	0.04
Living donors	53 (5.56)	52 (6.59)	1 (0.61)	0.002
Donation after brain death	743 (78.0)	607 (76.9)	136 (82.9)	0.09
Donation after cardiac death	157 (16.5)	130 (16.5)	27 (16.5)	1.00
Donor diabetes[a]	25 (3.39)	19 (3.13)	6 (4.62)	0.39
Cold ischemia time (h)	14.3 ± 5.56	14.1 ± 5.67	15.3 ± 4.93	0.008
Extended criteria donor[b]	261 (29.6)	209 (28.7)	52 (33.8)	0.21
Delayed graft function	179 (18.8)	132 (16.7)	47 (28.7)	<0.001
Posttransplant data				
Estimated GFR (mL/min/1.73 m^2)				
At 1 year	51.7 ± 17.7	51.6 ± 17.7	52.3 ± 17.8	0.68
At 2 years	51.4 ± 17.9	51.5 ± 17.7	50.9 ± 19.2	0.71
At 5 years	50.5 ± 18.3	50.7 ± 18.1	49.0 ± 19.4	0.43
Proteinuria (g/24 h)				
At 1 year	0.27 ± 0.72	0.25 ± 0.69	0.38 ± 0.86	0.004
At 2 years	0.26 ± 0.48	0.24 ± 0.44	0.37 ± 0.63	0.001
At 5 years	0.24 ± 0.32	0.24 ± 0.32	0.26 ± 0.27	0.88
Systolic blood pressure (mmHg)				
At 1 year	135 ± 16.8	134 ± 16.1	142 ± 18.5	<0.001
At 2 years	137 ± 17.4	136 ± 16.8	142 ± 19.4	0.003
At 5 years	138 ± 18.1	138 ± 17.2	142 ± 22.5	0.166
Diastolic blood pressure (mmHg)				
At 1 year	76.6 ± 10.0	77.0 ± 9.87	75.0 ± 10.5	0.036
At 2 years	78.3 ± 11.1	78.8 ± 10.8	75.4 ± 12.3	0.003
At 5 years	79.0 ± 11.9	79.7 ± 11.7	75.0 ± 12.6	0.004
Glycated hemoglobin (%)				
At 1 year	5.96 ± 0.88	5.78 ± 0.61	7.00 ± 1.34	<0.001
At 2 years	5.92 ± 0.84	5.75 ± 0.60	6.90 ± 1.22	<0.001
At 5 years	5.94 ± 0.85	5.75 ± 0.62	7.10 ± 1.13	<0.001
Total cholesterol (mg/dL)				
At 1 year	177 ± 36.8	179 ± 35.85	165 ± 39.3	<0.001
At 2 years	178 ± 37.8	180 ± 38.06	167 ± 34.5	<0.001
At 5 years	174 ± 38.3	176 ± 37.65	157 ± 38.9	<0.001
Triglycerides (mg/dL)				
At 1 year	139 ± 79.9	137 ± 71.4	154 ± 84.3	0.026
At 2 years	140 ± 73.3	137 ± 72.0	153 ± 78.8	0.032
At 5 years	138 ± 85.5	137 ± 85.9	152 ± 81.9	0.255
Overall graft survival[c]				
At 1 year	94.8	95.7	90.2	0.004
At 2 years	91.9	93.4	84.8	<0.001
At 5 years	82.1	84.7	69.5	<0.001

Continued on p. 629

Table 1—Continued

	Total (n = 953)	No pretransplant diabetes (n = 789)	Pretransplant diabetes (n = 164)	P value[e]
Death-censored graft survival[d]				
At 1 year	96.6	97.1	94.4	0.09
At 2 years	95.4	95.9	93.1	0.12
At 5 years	91.0	91.4	89.2	0.33

Data are n (%) or mean ± SD. [a]Frequency is based on 738 donors for whom these data were available. [b]Frequency is based on 882 donors for whom these data were available. [c]Overall graft survival = composite of graft failure and recipient death. [d]Death-censored graft survival = graft failure censored at time of recipient death with a functioning graft. [e]P values are based on χ^2 test for the categorical variables, on two-sample t test for continuous variables, and on the log-rank test for survival estimates. Proteinuria was log-transformed.

multivariate model as a result of collinearity with recipient age (correlation coefficient 0.29). We found no differences between patients with type 1 and type 2 diabetes in the hazard of developing mesangial matrix expansion after transplantation (P = 0.80 in the multivariate model).

Diabetes, Posttransplant Graft Function, and Graft Failure

In Fig. 2*A* and *B*, the posttransplantation trends of estimated GFR and concomitant 24-h proteinuria are shown at the protocol biopsy time points. Mesangial matrix expansion did not significantly correlate with estimated GFR at any given posttransplant time point, whereas proteinuria was positively correlated at 24 and 36 months (P = 0.02 and 0.04, respectively) but not at 5 years. On the basis of a multivariate linear mixed model with 73,679 values for estimated GFR and 43,516 values for 24-h proteinuria collected over time after transplantation and corrected for baseline donor and recipient characteristics, pretransplant diabetes was associated with proteinuria ($P < 0.001$) but not with estimated GFR (P = 0.30). The association with proteinuria was significant early after transplantation but disappeared over time. Graft failure occurred in 79 (8.2%) of 953 patients: 16 (9.8%) of 164 (9.8%) patients with pretransplant diabetes and 63 (8.0%) of 789 patients without diabetes before transplantation, which was not significantly different (log-rank test P = 0.33). During this period, 82 (8.6%) of 953 patients died with a functioning graft: 31 (18.9%) of 164 patients with pretransplant diabetes and 51 (6.5%) of 789 patients without diabetes before transplantation, which was significantly different (log-rank test $P < 0.001$).

Glycemic Control and Mesangial Matrix Expansion

For the total cohort, 8,750 glycated hemoglobin values were available. At the time of transplantation, mean glycated hemoglobin in patients with diabetes was 6.42 ± 1.12% (47 ± 12.2 mmol/mol) vs. 5.26 ± 0.36% (34 ± 3.9 mmol/mol) in patients without diabetes ($P < 0.001$) (Table 1). Supplementary Figure 2 shows a sample of 80 patients' glycated hemoglobin profiles by time after transplantation, categorized by pretransplant diabetes state. Patients with pretransplant diabetes had higher glycated hemoglobin levels, but the glycated hemoglobin trajectories were highly variable and partly overlapped with those of patients without pretransplant diabetes (Fig. 2*C*). Immediately posttransplantation, average glycated hemoglobin levels increased in both groups and then remained stable.

An increase in pretransplant glycated hemoglobin levels increased the hazard of developing mesangial matrix expansion after transplantation (hazard ratio 1.23 [95% CI 1.02–1.48]; P = 0.03) (Table 2 and Supplementary Fig. 3). The cumulative effect of glycated hemoglobin levels (until event time) on the hazard of the first occurrence of mesangial matrix expansion was not significant (P = 0.61) (Supplementary Table 4 and Supplementary Fig. 3). Also conditionally on the diabetes group effect, no cumulative effect of glycated hemoglobin levels was found (P = 0.80). Although pretransplant diabetes increased the hazard of mesangial matrix expansion after transplantation, this effect could not be explained by the patients' glycated hemoglobin levels after transplantation.

Sensitivity Analysis

A sensitivity analysis in which patients with new-onset diabetes after transplantation (n = 249) were right-censored at the time of diabetes onset confirmed our findings. Also in this analysis, patients with pretransplant diabetes were more prone to develop mesangial matrix expansion after transplantation (hazard ratio 1.61 [95% CI 1.07–2.42]; P = 0.02), as were patients with higher glycated hemoglobin levels at the time of transplantation (hazard ratio 1.24 [95% CI 1.02–1.51]; P = 0.03). Posttransplant glycemic control, as measured by the area under the glycated hemoglobin curve, was not associated with mesangial matrix expansion (hazard ratio 1.04 [95% CI 0.89–1.21]; P = 0.61). A second sensitivity analysis was performed in which we expanded the joint model with the (modeled) patient-specific BMI and hemoglobin trajectories. Time-dependent box plots for these parameters are shown in Supplementary Fig. 4. Also in this sensitivity analysis, the cumulative effect of glycated hemoglobin on the risk for developing mesangial matrix expansion remained insignificant (P = 0.77). Hence, we concluded that the relation between glycated hemoglobin and mesangial matrix expansion was not influenced by BMI or hemoglobin levels.

Because of its time-varying and patient-specific nature, medication use after transplantation was not included in the multivariate models. Supplementary Table 5 shows medication use at the biopsy time points and thereby gives a general idea about the patients' therapies. Except for insulin use, no notable differences in drug use were observed between patients with and without pretransplant diabetes.

CONCLUSIONS

In our analysis of systematic, protocol-specified, renal allograft biopsy specimens in a large patient cohort, pretransplant diabetes was associated with the

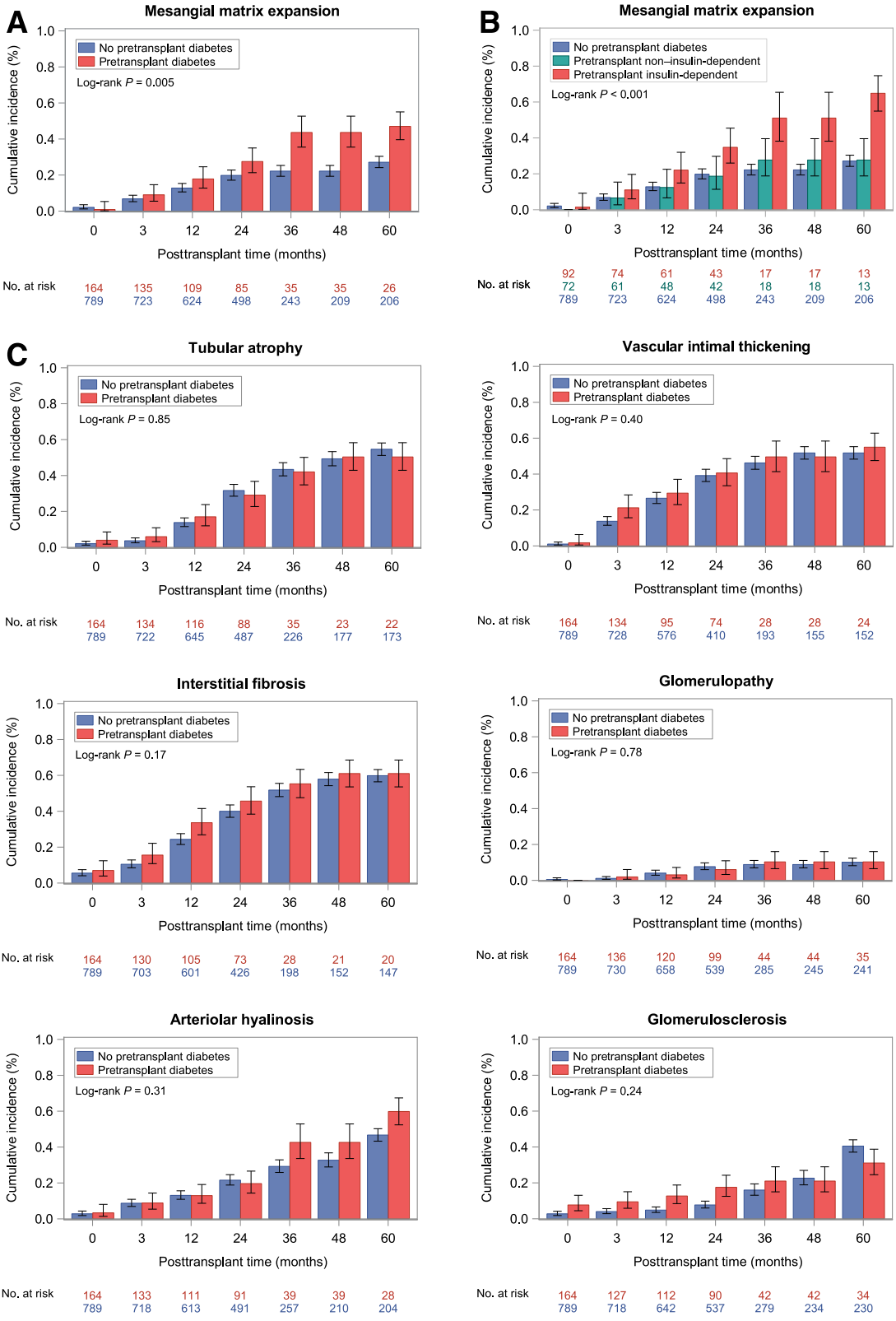

Figure 1—Cumulative incidence of kidney allograft histology over time after transplantation, according to pretransplant diabetes state and insulin dependency. *A*: Cumulative incidence plot for first detection of mesangial matrix expansion (grade ≥1) according to pretransplant diabetes state. *B*: Cumulative incidence plot for first detection of mesangial matrix expansion (grade ≥1) according to pretransplant insulin dependency. *C*: Cumulative incidence plots are shown for first detection of tubular atrophy (grade ≥2), vascular intimal thickening (grade ≥2), interstitial fibrosis (grade ≥2), glomerulopathy (grade ≥1), arteriolar hyalinosis (grade ≥2), and glomerulosclerosis (>25%) according to pretransplant diabetes state. The estimates are based on combining the expectation-maximization algorithm and the (modified) iterative convex minorant algorithm (to account for interval censoring), and the numbers at risk are based on the Kaplan-Meier method, considering biopsy time as event or right-censored time.

Table 2—Hazard ratios for occurrence of mesangial matrix expansion for donor and recipient characteristics, including pretransplant diabetes state in univariate and multivariate analyses

Parameter	Hazard ratio (95% CI)	*P* value
Univariate analysis		
Pretransplant diabetes	1.80 (1.27–2.55)	0.001
Type 1 diabetes (vs. type 2)	0.53 (0.13–2.20)	0.38
Insulin-dependent diabetes (vs. no diabetes or non–insulin-dependent diabetes)	2.44 (1.64–3.64)	<0.001
Glycated hemoglobin at time of transplantation (per %)	1.31 (1.12–1.54)	0.001
Recipient age (per 10 years)	1.25 (1.10–1.41)	<0.001
Recipient weight (per kg)	1.01 (1.00–1.02)	0.11
Recipient male (vs. female)	1.07 (0.79–1.45)	0.65
Recipient BMI (per 1 kg/m^2)	1.03 (1.00–1.06)	0.11
IgA nephropathy (vs. other cause of end-stage renal disease)	0.85 (0.52–1.41)	0.53
Glomerulonephritis as primary renal disease	0.89 (0.63–1.24)	0.49
Repeat transplant (vs. first transplant)	1.04 (0.67–1.59)	0.87
Pretransplant donor-specific HLA antibodies (vs. no pretransplant donor-specific antibodies)	0.98 (0.61–1.57)	0.93
HLA-A/B/DR mismatches (per mismatch)	1.10 (0.98–1.23)	0.10
Cold ischemia time (per hour)	1.02 (0.99–1.05)	0.17
Donor age (per 10 years)	1.14 (1.03–1.27)	0.01
Donation after cardiac death	0.99 (0.72–1.37)	0.97
Living donor	0.98 (0.65–1.49)	0.93
Male donor (vs. female)	0.90 (0.67–1.20)	0.46
Donor diabetes (vs. no diabetes)	0.45 (0.11–1.82)	0.26
Multivariate analysis[a]		
Model 1		
Pretransplant diabetes (vs. no pretransplant diabetes)	1.55 (1.07–2.26)	0.02
Recipient age (per 10 years)	1.20 (1.05–1.38)	0.008
Model 2		
Glycated hemoglobin at time of transplantation (per %)	1.23 (1.02–1.48)	0.03
Recipient age (per 10 years)	1.19 (1.04–1.37)	0.01

All analyses were based on the Weibull survival model for mesangial matrix expansion as end point, taking interval censoring into account. Model 1 is the multivariate model that included the covariate pretransplant diabetes state. Model 2 is the multivariate model in which pretransplant diabetes state was replaced by glycated hemoglobin levels at time of transplantation. [a]Both multivariate models were corrected for donor and recipient sex, donor and recipient age, recipient BMI, donor type, repeat transplantation, IgA nephropathy as original kidney disease, presence of donor-specific antibodies, and number of HLA mismatches.

development of mesangial matrix expansion, the earliest structural sign of diabetic nephropathy. This effect was noted already by 2 years after transplantation. Diabetic nephropathy developed despite good glycemic control. Pretransplant diabetes associated with posttransplant proteinuria but not with estimated GFR or graft failure. Mesangial matrix expansion was not specific for diabetic nephropathy and associated with increasing age also in patients without diabetes.

Also in previous studies, diabetic nephropathy was observed within the first 2 years after transplantation (10,13, 22–24), but this phenomenon was noted in an era with less-intensive glycemic control than is possible today and than was achieved in the current cohort. Glycated hemoglobin levels increased immediately after transplantation in our cohort both in patients with pretransplant diabetes and in patients without diabetes, which can be explained by the universal use of calcineurin inhibitors and steroids and by the occurrence of new-onset diabetes after transplantation, as was also reported previously by others (25). Posttransplant glycated hemoglobin levels, however, did not predict the occurrence of mesangial matrix expansion in our study, whereas pretransplant diabetes status remained associated, even after adjustment for other demographic factors like BMI, age, and sex. This is in contrast with an older study where better glycemic control was associated with a lower risk of mesangial matrix expansion in renal allografts (12). The results of this historical study do not necessarily correspond to current clinical practice with the advent of novel glucose-lowering agents and new insulin analogs. Our cohort showed average posttransplant glycated hemoglobin levels of ~7% (53 mmol/mol) in patients with pretransplant diabetes compared with 12% (108 mmol/mol) in the control arm of the previous study (12). The better glycemic control in our patients thus might partially explain why no cumulative effect of glycated hemoglobin levels after transplantation was found. In addition, most patients included in our study had type 2 diabetes, which is an important difference with the earlier study that was restricted to patients with type 1 diabetes. The finding that diabetic nephropathy occurs independently of glycated hemoglobin levels could be explained by effects of diabetes on hemodynamics, inflammation, advanced glycation end product formation, and oxidative stress that contribute to mesangial matrix expansion (26–28).

These other metabolic factors also might have contributed to the development of mesangial matrix expansion in our study but could not be tested. Other residual metabolic factors like dyslipidemia, hemodynamic factors, and other features of the metabolic syndrome

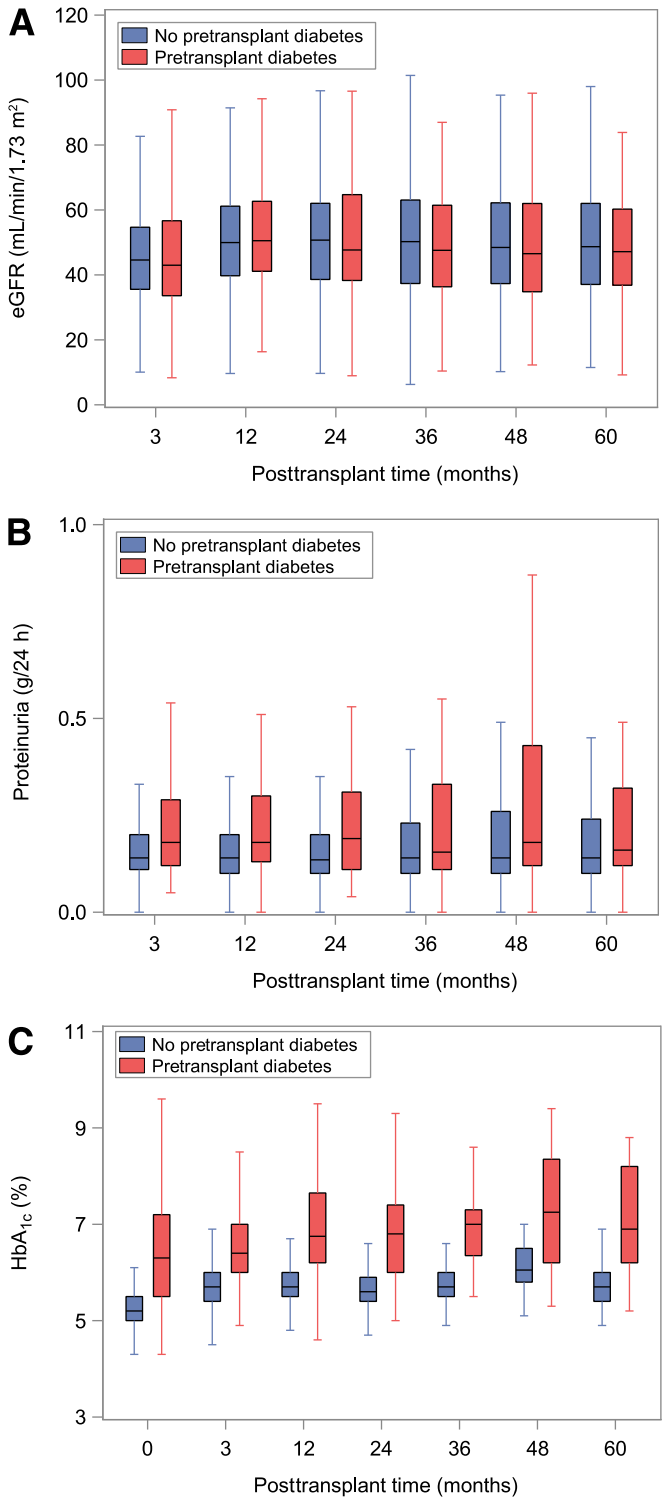

Figure 2—Evolution of estimated GFR (eGFR), proteinuria, and glycated hemoglobin levels over time after transplantation according to pretransplant diabetes state. Time-dependent box plots, with whiskers maximally 1.5 times the interquartile range, are shown for eGFR (*A*), proteinuria (*B*), and glycated hemoglobin (HbA_{1c}) (*C*). Plots are based on all available data at 3, 12, 24, 36, 48, and 60 months after transplantation. For HbA_{1c}, we also included the box plot at day of transplantation. On the basis of a linear mixed model (see text), it was shown that patients with pretransplant diabetes had a significantly different posttransplant evolution of proteinuria ($P < 0.001$) and HbA_{1c} ($P < 0.001$) but not of eGFR ($P = 0.30$).

might contribute to the development of mesangial matrix expansion. However, patient-specific estimated BMI did not show a significant association with the hazard for developing mesangial matrix expansion in this study. The higher cumulative incidence of mesangial matrix expansion in insulin-dependent patients with diabetes might reflect an effect of insulin treatment itself, with more hypoglycemic hyperinsulinemic episodes, a trophic effect of insulin, and more fluctuating glucose profiles. Another explanation for the apparent independence of glycemic control in developing mesangial matrix expansion might be hyperglycemia-induced oxidative stress and inflammation (29). This is illustrated by experimental studies where use of glucose-sodium transporter blockade with SGLT2 inhibitors prevented or reversed diabetic nephropathy (30,31), also independently of its glucose-lowering effect (32,33). In addition, GLP-1 agonists in clinical trials and more novel therapeutics targeting podocyte histone modification and apoptosis in preclinical trials have shown promising nephroprotective effects in diabetic kidney disease (34). Whether more systematic use of drugs like SGLT2 inhibitors in the posttransplant setting would be able to lower the risk of rapid-onset diabetic nephropathy needs further study.

Our analysis did not show associations of diabetes with other chronic histologic lesions after transplantation. This is in concordance with a recent study where occurrence of arteriolar hyalinosis and interstitial fibrosis at 10 years posttransplantation were not significantly associated with pretransplant diabetes (16). These findings suggest that in clinical routine, vascular intimal thickening and arteriolar hyalinosis can no longer be interpreted as a function of diabetes state. We also described that mesangial matrix expansion is not specific for diabetic nephropathy and associates independently with increasing age. This finding confirms previous suggestions in experimental settings that mesangial matrix expansion is a characteristic of renal aging, which could ultimately contribute to age-associated glomerulosclerosis (35,36). The suggestion that senescence is a main mechanism by which kidneys are damaged in type 2 diabetic nephropathy thus could further explain our findings and merits additional study (37,38).

In addition to the evaluation of the effect of diabetes on graft structural changes, we assessed the effect of diabetes on graft function and proteinuria. The early effects of hyperglycemia during the clinically silent phase of diabetic nephropathy are dominated by hemodynamic factors, including glomerular hyperfiltration as a consequence of elevated intraglomerular pressure and resulting in glomerular injury, only later to be followed by microalbuminuria and progressive decline of GFR (39–45). We did not observe such hyperfiltration or progressive decline of glomerular filtration. In addition to controversy around hyperfiltration in the pathogenesis of diabetic nephropathy, especially in type 2 diabetes, hyperfiltration remains difficult to detect clinically with current equations for estimating glomerular filtration (46,47). Moreover, follow-up time in our study may be have been too short to detect these functional changes. Finally, other confounding factors might abort hyperfiltration in transplant recipients, such as the use of calcineurin inhibitors, renal denervation, and frequent use of other drugs that directly affect renal hemodynamics, including ACE inhibitors and angiotensin receptor blockers. The association between pretransplant diabetes and proteinuria was significant early after transplantation but disappeared over time. Although our data cannot prove causality, this time dependency of the association suggests that proteinuria is not the reflection of progressive diabetic nephropathy in the transplanted kidney. This finding could be explained by early proteinuria originating from the native kidneys in patients with pretransplant diabetes and loss of residual native kidney function over time after transplantation.

This study has some limitations. All posttransplant surveillance biopsy specimens were scored according to the Banff semiquantitative biopsy assessment (18,19), which is not specifically developed for scoring diabetic nephropathy. We did not perform electron microscopy to evaluate glomerular basement membrane thickening or more subtle grades of mesangial matrix expansion that could have been missed in our study. We also did not use computerized imaging for measurement of mesangial matrix volume, as was used in earlier studies on diabetic nephropathy (6). We analyzed the time to first occurrence of lesions, thereby not looking at injury progression. Data on microalbuminuria, macroalbuminuria, and glucose-level variability were lacking, and glycated hemoglobin levels could be affected by patient-specific factors such as red cell survival, hemolysis, use of erythropoietin, and renal impairment itself. Fewer biopsies were performed late after transplantation compared with the early phase, which is inherent to the protocol biopsy program. By right-censoring the patients at time of last follow-up, we assumed that the observed patients are representative of those who dropped out. There is a possibility that information is conveyed within the dropout mechanism. However, because diabetic nephropathy develops over a long period without immediate clinical impact, it is unlikely that patients dropped out as a result of diabetic nephropathy within the first 5 years after transplantation. It is unlikely that mesangial matrix expansion influenced the patients' or physicians' decisions to not participate in the protocol biopsy program. In addition, we controlled our multivariate models for related confounders, like donor and recipient age, which also partly captures the dropout mechanism. Given this and the prospective nature of the protocol biopsies, we believe that the statistical bias of our results is minimized. With regard to the lack of association between pretransplant diabetes and posttransplant estimated GFR or graft failure, it needs to be emphasized that follow-up might be too short to observe such associations and that studies with even longer follow-up are needed to study such effects. Finally, our study design did not allow the drawing of conclusions about the renal impact of new-onset diabetes after transplantation.

In conclusion, our analysis of systematic, protocol-specified renal allograft biopsy specimens obtained over the first 5 years after transplantation indicate that the structural changes of diabetic nephropathy can occur very rapidly, despite intensive glycemic control and irrespective of the type of diabetes, which reinforces the need for research on better treatment options for the prevention of diabetic nephropathy also after kidney transplantation. The effect of pretransplant diabetes on mesangial matrix expansion was independent of posttransplant glycemic control, hallmarked by glycated hemoglobin levels, which suggests that other mechanisms also underlie the rapid development of mesangial matrix expansion in patients with diabetes. Prevention of diabetic nephropathy requires more than pursuing low levels of glycated hemoglobin.

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References

1. Webster AC, Nagler EV, Morton RL, Masson P. Chronic kidney disease. Lancet 2017;389:1238–1252
2. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. J Clin Invest 1984;74:1143–1155
3. Adler S. Diabetic nephropathy: linking histology, cell biology, and genetics. Kidney Int 2004;66:2095–2106
4. Tonneijck L, Muskiet MH, Smits MM, et al. Glomerular hyperfiltration in diabetes: mechanisms, clinical significance, and treatment. J Am Soc Nephrol 2017;28:1023–1039
5. Steinke JM, Sinaiko AR, Kramer MS, Suissa S, Chavers BM, Mauer M; International Diabetic Nephropathy Study Group. The early natural history of nephropathy in type 1 diabetes: III. Predictors of 5-year urinary albumin excretion rate patterns in initially normoalbuminuric patients. Diabetes 2005;54:2164–2171
6. Mauer M, Zinman B, Gardiner R, et al. Renal and retinal effects of enalapril and losartan in type 1 diabetes. N Engl J Med 2009;361:40–51
7. Coca SG, Ismail-Beigi F, Haq N, Krumholz HM, Parikh CR. Role of intensive glucose control in development of renal end points in type 2 diabetes mellitus: systematic review and meta-analysis intensive glucose control in type 2 diabetes. Arch Intern Med 2012;172:761–769

8. EBPG (European Expert Group on Renal Transplantation); European Renal Association (ERA-EDTA); European Society for Organ Transplantation (ESOT). European best practice guidelines for renal transplantation (part 1). Nephrol Dial Transplant 2000;15(Suppl. 7):1–85
9. Nieto T, Inston N, Cockwell P. Renal transplantation in adults. BMJ 2016;355:i6158
10. Mauer SM, Steffes MW, Connett J, Najarian JS, Sutherland DE, Barbosa J. The development of lesions in the glomerular basement membrane and mesangium after transplantation of normal kidneys to diabetic patients. Diabetes 1983;32:948–952
11. Osterby R, Nyberg G, Hedman L, Karlberg I, Persson H, Svalander C. Kidney transplantation in type 1 (insulin-dependent) diabetic patients. Early glomerulopathy. Diabetologia 1991;34:668–674
12. Barbosa J, Steffes MW, Sutherland DE, Connett JE, Rao KV, Mauer SM. Effect of glycemic control on early diabetic renal lesions. A 5-year randomized controlled clinical trial of insulin-dependent diabetic kidney transplant recipients. JAMA 1994;272:600–606
13. Bohman SO, Wilczek H, Tydén G, Jaremko G, Lundgren G, Groth CG. Recurrent diabetic nephropathy in renal allografts placed in diabetic patients and protective effect of simultaneous pancreatic transplantation. Transplant Proc 1987;19:2290–2293
14. Mauer SM, Goetz FC, McHugh LE, et al. Long-term study of normal kidneys transplanted into patients with type I diabetes. Diabetes 1989;38:516–523
15. Bhalla V, Nast CC, Stollenwerk N, et al. Recurrent and de novo diabetic nephropathy in renal allografts. Transplantation 2003;75:66–71
16. Stegall MD, Cornell LD, Park WD, Smith BH, Cosio FG. Renal allograft histology at 10 years after transplantation in the tacrolimus era: evidence of pervasive chronic injury. Am J Transplant 2018;18:180–188
17. Levey AS, Greene T, Kusek J, Beck GJ, Group MS. A simplified equation to predict glomerular filtration rate from serum creatinine (Abstract). J Am Soc Nephrol 2000;11:A0828
18. Loupy A, Haas M, Solez K, et al. The Banff 2015 kidney meeting report: current challenges in rejection classification and prospects for adopting molecular pathology. Am J Transplant 2017;17:28–41
19. Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 kidney meeting report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. Am J Transplant 2018;18:293–307
20. Wellner JA, Zhan YH. A hybrid algorithm for computation of the nonparametric maximum likelihood estimator from censored data. J Am Stat Assoc 1997;92:945–959
21. Rizopoulos D. The R package JMbayes for fitting joint models for longitudinal and time-to-event data using MCMC. J Stat Softw 2016;72:1–46
22. Hariharan S, Smith RD, Viero R, First MR. Diabetic nephropathy after renal transplantation. Clinical and pathologic features. Transplantation 1996;62:632–635
23. Najarian JS, Kaufman DB, Fryd DS, et al. Long-term survival following kidney transplantation in 100 type I diabetic patients. Transplantation 1989;47:106–113
24. Mauer SM, Barbosa J, Vernier RL, et al. Development of diabetic vascular lesions in normal kidneys transplanted into patients with diabetes mellitus. N Engl J Med 1976;295:916–920
25. Hecking M, Werzowa J, Haidinger M, et al.; European-New-Onset Diabetes After Transplantation Working Group. Novel views on new-onset diabetes after transplantation: development, prevention and treatment. Nephrol Dial Transplant 2013;28:550–566
26. Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. Nat Clin Pract Endocrinol Metab 2008;4:444–452
27. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–1625
28. Valencia WM, Florez H. How to prevent the microvascular complications of type 2 diabetes beyond glucose control [published correction appears in BMJ 2017;356:j1018]. BMJ 2017;356:i6505
29. Donegan D, Bale LK, Conover CA. PAPP-A in normal human mesangial cells: effect of inflammation and factors related to diabetic nephropathy. J Endocrinol 2016;231:71–80
30. Terami N, Ogawa D, Tachibana H, et al. Long-term treatment with the sodium glucose cotransporter 2 inhibitor, dapagliflozin, ameliorates glucose homeostasis and diabetic nephropathy in db/db mice. PLoS One 2014;9:e100777
31. Vallon V, Gerasimova M, Rose MA, et al. SGLT2 inhibitor empagliflozin reduces renal growth and albuminuria in proportion to hyperglycemia and prevents glomerular hyperfiltration in diabetic Akita mice. Am J Physiol Renal Physiol 2014;306:F194–F204
32. De Nicola L, Gabbai FB, Liberti ME, Sagliocca A, Conte G, Minutolo R. Sodium/glucose cotransporter 2 inhibitors and prevention of diabetic nephropathy: targeting the renal tubule in diabetes. Am J Kidney Dis 2014;64:16–24
33. Hatanaka T, Ogawa D, Tachibana H, et al. Inhibition of SGLT2 alleviates diabetic nephropathy by suppressing high glucose-induced oxidative stress in type 1 diabetic mice. Pharmacol Res Perspect 2016;4:e00239
34. Cooper M, Warren AM. A promising outlook for diabetic kidney disease. Nat Rev Nephrol 2019;15:68–70
35. Zhou XJ, Rakheja D, Yu X, Saxena R, Vaziri ND, Silva FG. The aging kidney. Kidney Int 2008;74:710–720
36. Hill GS, Heudes D, Bariéty J. Morphometric study of arterioles and glomeruli in the aging kidney suggests focal loss of autoregulation. Kidney Int 2003;63:1027–1036
37. Verzola D, Gandolfo MT, Gaetani G, et al. Accelerated senescence in the kidneys of patients with type 2 diabetic nephropathy. Am J Physiol Renal Physiol 2008;295:F1563–F1573
38. Palmer AK, Tchkonia T, LeBrasseur NK, Chini EN, Xu M, Kirkland JL. Cellular senescence in type 2 diabetes: a therapeutic opportunity. Diabetes 2015;64:2289–2298
39. Christiansen JS, Gammelgaard J, Frandsen M, Parving HH. Increased kidney size, glomerular filtration rate and renal plasma flow in short-term insulin-dependent diabetics. Diabetologia 1981;20:451–456
40. Hostetter TH, Rennke HG, Brenner BM. The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. Am J Med 1982;72:375–380
41. Zatz R, Meyer TW, Rennke HG, Brenner BM. Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. Proc Natl Acad Sci U S A 1985;82:5963–5967
42. Nelson RG, Bennett PH, Beck GJ, et al.; Diabetic Renal Disease Study Group. Development and progression of renal disease in Pima Indians with non-insulin-dependent diabetes mellitus. N Engl J Med 1996;335:1636–1642
43. Amin R, Turner C, van Aken S, et al. The relationship between microalbuminuria and glomerular filtration rate in young type 1 diabetic subjects: the Oxford Regional Prospective Study. Kidney Int 2005;68:1740–1749
44. Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG. Is hyperfiltration associated with the future risk of developing diabetic nephropathy? A meta-analysis. Diabetologia 2009;52:691–697
45. Ruggenenti P, Porrini EL, Gaspari F, et al.; GFR Study Investigators. Glomerular hyperfiltration and renal disease progression in type 2 diabetes. Diabetes Care 2012;35:2061–2068
46. Bjornstad P, Cherney D, Maahs DM. Early diabetic nephropathy in type 1 diabetes: new insights. Curr Opin Endocrinol Diabetes Obes 2014;21:279–286
47. Gaspari F, Ruggenenti P, Porrini E, et al.; GFR Study Investigators. The GFR and GFR decline cannot be accurately estimated in type 2 diabetics. Kidney Int 2013;84:164–173