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Impact of glucose metabolism abnormalities on histopathological changes in kidney transplant protocol biopsies

Ilkka Helanterä,[1] Fernanda Ortiz,[1] Anne Räisänen-Sokolowski[2] and Petri Koskinen[1]

1 Department of Medicine, Division of Nephrology, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland
2 Department of Pathology, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland

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Correspondence
Ilkka Helanterä MD, PhD, Department of Medicine, Division of Nephrology, Helsinki University Hospital, Kasarmikatu 11–13, PO Box 263, FIN-00029 HUS, Helsinki, Finland.
Tel.: +358 9 47188203; fax: +358 9 47188400; e-mail: ilkka.helantera@helsinki.fi

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Summary

The impact of post-transplant diabetes (PTDM) on kidney transplant histopathology has been poorly described. We examined the association of glucose metabolism abnormalities on the progression of histopathological changes in serial protocol biopsies. Helsinki University Hospital kidney transplant recipients during 2004–2006 were followed up. Patients with pre-existing diabetes or 2-h oral glucose tolerance test (OGTT) performed at 3 months, and protocol biopsies taken at 0 and 12 months were analyzed ($n = 76$). Diabetes was defined according to WHO/ADA. Histology was analyzed with chronic allograft damage index (CADI). Altogether 32 patients had pre-existing diabetes. In OGTT at 3 months, four showed PTDM, eight impaired glucose tolerance (IGT), two impaired fasting glucose, and 30 normal glucose tolerance. Patients with impaired glucose metabolism were older ($P = 0.005$), received grafts from older donors ($P = 0.04$), and had reduced renal function at 12 months ($P = 0.003$). In patients with IGT or PTDM, 2-h postload glucose values in OGTT correlated with CADI at 12 months ($R = 0.84$, $P = 0.001$) and with the change in CADI score between 0 and 12 months ($R = 0.67$, $P = 0.025$). Graft survival was reduced in patients with pre-existing diabetes ($P = 0.01$). Glucose abnormalities were associated with the progression of histopathological changes, especially in patients with already compromised kidneys, supporting the harmful role of PTDM to the kidney allograft.

Introduction

As both short- and long-term outcomes of kidney transplantations have dramatically improved over the past decades [1], the long-term side effects of immunosuppressive therapy and co-morbidities have achieved an increasing importance in the long-term follow-up of kidney transplant recipients.

Approximately 25% of kidney transplantations in Finland are performed on recipients with diabetic nephropathy as the underlying renal disease [1]. Diabetes mellitus is associated with increased risk of cardiovascular complications after transplantation and poorer patient and transplant outcome [2–4]. Post-transplant diabetes mellitus (PTDM) developing after transplantation is similarly a complication associated with increased cardiovascular morbidity and reduced patient and graft survival in kidney transplant recipients [5,6]. The reported incidence of PTDM is between 7% and 24%, varying between different populations and studies using different definitions of diabetes and varying length of follow-up [5,7–9]. Several risk factors for the development of PTDM have been identified such as increased age, increased body mass index (BMI), hepatitis C infection, cytomegalovirus (CMV) infection, and tacrolimus and corticosteroid therapies [5,7–10].

Long-term allograft failure because of chronic allograft nephropathy (CAN), or chronic rejection or interstitial fibrosis/tubular atrophy (IF/TA) of undefined cause [11] is one of the most important reasons for kidney graft loss together with premature patient death with a functioning graft [12]. Although the histopathological progression of

these chronic changes has been well described [13–15], the pathogenesis of this condition is still incompletely understood [16]. Both immunological and non-immunological factors are thought to play a role in the progression of histopathological changes in the graft [12]. Changes attributable to diabetic nephropathy commonly occur in the kidney grafts years after transplantation in patients with diabetic nephropathy as the underlying cause of transplantation [17–21], and PTDM has also been associated with *de novo* diabetic nephropathy after transplantation [21,22]. However, the impact of either pre-existing diabetes, post-transplant diabetes, or other glucose metabolism abnormalities developing after transplantation on the progression of histopathological changes attributable to chronic rejection in serial protocol biopsies during the first year after transplantation have not been described in detail.

The aim of this follow-up study was to describe the impact of glucose metabolism abnormalities on the progression of chronic histopathological changes in a Finnish protocol biopsy material.

Patients and methods

All Helsinki University Hospital district adult kidney transplant recipients who received a graft between 2004 and 2006 ($n = 163$) were prospectively followed up. Patients who had a protocol biopsy taken at 12 months and had either pre-existing diabetes, an oral glucose tolerance test (OGTT) performed at 3 months according to the policy of our clinic, or who developed PTDM requiring treatment during the first 3 months after transplantation were included in a retrospective follow-up study ($n = 76$). A protocol biopsy at 12 months was taken from altogether 87 patients. Reasons for not taking or delaying protocol biopsies in the remaining 76 patients were: complications after transplant operation (such as wound healing problems, lymphocele, ureteral stricture, repeated acute rejections) and modified follow-up protocol ($n = 26$), increased risk of bleeding because of anticoagulation medication or other reasons ($n = 14$), infection or other acute illness and hospitalization at the time of biopsy ($n = 9$), non-functioning graft ($n = 8$), logistic reasons ($n = 8$), patient refusal ($n = 7$), and living donor transplantation ($n = 4$). Eleven patients with no pre-existing diabetes and 12 months protocol taken had no oral glucose tolerance performed because of logistic reasons and were not included in the study. These 87 patients were not studied, and follow-up data were not collected. One patient was diagnosed with PTDM 2 months after transplantation and required treatment with subcutaneous insulin and was included in the study. Other data from the same patient population have been published previously [23]. The selection of patients to this study is depicted in Fig. 1.

Baseline immunosuppression was usually a triple-drug regimen with Cyclosporine A, mycophenolate mofetil and steroid. In immunologically high-risk patients (long waiting time, poor match, re-transplantation), cyclosporine was replaced by tacrolimus, and/or induction therapy with basiliximab was administered. In patients with stable graft function and especially in patients with problems in glycemic control or osteoporosis, steroids were withdrawn slowly during the second post-transplant year. Biopsy-proven acute rejections of grade I–II [24] were treated with high-dose intravenous corticosteroids, and/or conversion of cyclosporine to tacrolimus.

Protocol biopsies at 3 and 12 months were performed under ultrasound guidance with either Bard Magnum® or Bard Biopty® devices and 18 gauge Biopty-cut® needles (Bard Biopsy Systems, Tempe, AZ, USA). Two biopsy cores were obtained. For light microscopy, serial tissue sections were stained with hematoxylin and eosin, PAS, methenamine silver, and Masson's trichrome. A day-zero biopsy was obtained from donor kidney during the donor

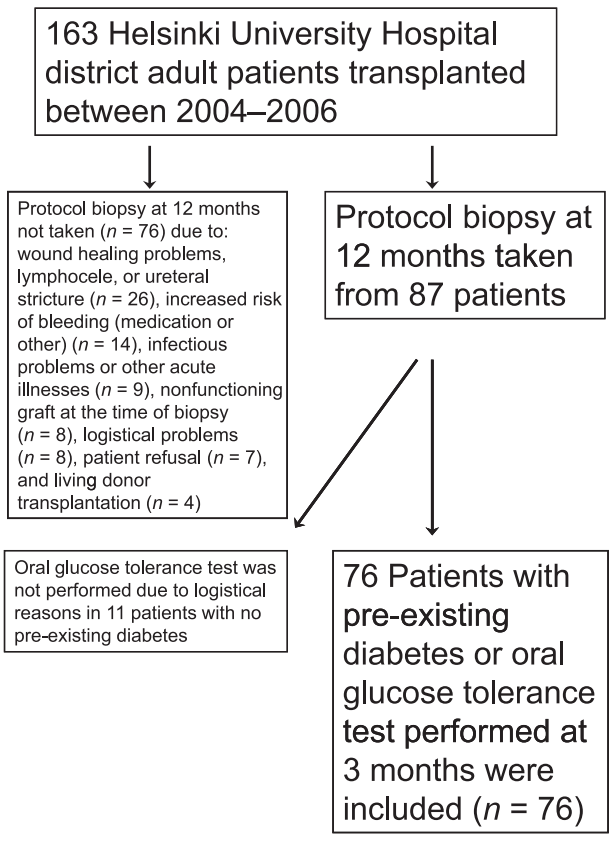

Figure 1 Flow-chart describing the selection of the patients in the study.

operation or in a minority of cases after revascularization in the recipient operation. All biopsies were scored according to the chronic allograft damage index (CADI) [25], with the individual parameters scored from 0 to 3 according to Banff '97 classification [24], except for the percentage of globally sclerosed glomeruli, which is not included in the Banff classification (0, no globally sclerosed glomeruli; 1, <15%; 2, 16–50%; and 3, >50% globally sclerosed glomeruli). Also, arteriolar hyaline thickening was analyzed from the biopsies according to Banff '97, although not included in the CADI. The progression of histopathological changes (ΔCADI) was estimated by calculating the difference between the CADI score in the protocol biopsies and in the donor biopsies. Diabetic nephropathy was histopathologically defined as nodular mesangial expansion caused by increased matrix material, hyalinization of arterioles, and thickening of glomerular and/or peritubular capillary basement membranes. All the biopsies analyzed in this study were taken according to our clinical follow-up protocol, and no extra biopsy or blood samples were taken for the purpose of this study. This study had the approval of the ethics committee of Helsinki University Hospital and a research license from the Helsinki University Hospital research committee was granted before the initiation of this study.

Oral glucose tolerance test was performed 3 months after transplantation in nondiabetic subjects. After measuring fasting plasma glucose after an overnight fast, 75 g anhydrous glucose dissolved in 300 ml water was ingested, and plasma glucose was measured 60 and 120 min after ingestion. PTDM was defined according to criteria of World Health Organization (WHO) and American Diabetes Association (ADA) [26,27]. Impaired fasting glucose (IFG) was defined as fasting plasma glucose between 6.1 and 6.9 mmol/l and 2-h postload glucose in OGTT <7.8 mmol/l; impaired glucose tolerance (IGT) was defined as fasting plasma glucose <7 mmol/l and 2-h postload glucose being between 7.8 and 11.0 mmol/l, and diabetes was defined as fasting plasma glucose ≥7.0 mmol/l, or 2-h postload glucose ≥11.1 mmol/l. Treatment of PTDM was decided by the clinician at the outpatient clinic and included lifestyle changes, weight loss, oral hypoglycaemic drugs (mostly glimepirid), or subcutaneous insulin.

Baseline clinical data at the time of transplantation and clinical follow-up data at 1, 3, 6, 12, 18 and 24 months after transplantation and at the latest follow-up were collected from patient charts and laboratory database. Baseline data included primary renal disease leading to uremia, length and modality of dialysis treatment preceding transplantation, recipient and donor age and gender, cold ischemia time, delayed graft function as defined by the need of dialysis during first post-transplant week, and HLA A-, B-, and DR-mismatch. Follow-up data included kidney function as measured by plasma creatinine and estimated glomerular filtration rate (GFR) using the Cockcroft–Gault equation [28], trough levels of cyclosporine and tacrolimus, steroid dose, blood pressure medication, lipid status, glycosylated hemoglobin (HBA1c), CMV and hepatitis C infections, and BMI.

All data are expressed as mean ± 1 standard deviation, unless otherwise indicated. Difference in the distribution of continuous and ordinal variables was assessed using the nonparametric Mann–Whitney's *U*-test. Comparisons between more than two groups were calculated with the nonparametric Kruskal–Wallis one-way analysis, and significance between groups was assessed with the Dunn test. Relationship between binary variables was calculated using the Fisher's exact test. Correlations between variables were analyzed using linear regression, and possible confounding factors were analyzed using multiple linear regression. Graft survival probabilities were estimated using the Kaplan–Meier method, and differences between two or more groups were determined using the log rank test. The calculations were performed using SPSS statistical software (version 16.0, SPSS Inc, Chicago, IL, USA). *P* -values of <0.05 were considered significant.

Results

Of the 76 patients included in the analysis, one patient died 13 months after transplantation, and four patients lost their grafts. Two patients lost their grafts because of CAN at 53 and 36 months after transplantation, one patient returned to dialysis because of treatment-resistant tubulointerstitial nephritis at 38 months after transplantation, and one graft was lost to acute irreversible renal failure because of pyelonephritis 28 months after transplantation. Other grafts were functioning at the end of follow-up of mean 38.9 months (range 24–60 months). All the patients who lost their grafts during the follow-up or died had pre-existing diabetes before transplantation. Mean estimated GFR at the end of follow-up was 61.15 ± 22.66 ml/min. Acute rejection developed in 15 patients during the follow-up. Of the acute rejections, three were grade II rejections, and 12 were grade I rejections. Of the grade I rejections, three were treated with high-dose i.v. steroids, three with conversion from cyclosporine to tacrolimus, five with both high-dose i.v. steroids and conversion to tacrolimus, and one clinically mild rejection resolved spontaneously in control biopsy without treatment. Grade II rejections were treated with high-dose i.v. steroids in all three patients and in two patients additionally with conversion from cyclosporine to tacrolimus. No significant differences in histopathological changes in protocol biopsies or in renal function were

observed in patients with acute rejection compared with patients without acute rejection.

Of the patients analyzed, 32 had pre-existing diabetes (27 patients with type I diabetes, and five with type II diabetes). Other patients had normal fasting plasma glucose before transplantation. In OGTT at 3 months, 30 patients had a normal fasting plasma glucose and normal glucose tolerance, two patients were defined as having IFG, eight patients as having IGT, and three patients as having PTDM. One patient had elevated fasting plasma glucose levels 2 months after transplantation (ranging from 11.6 to 29.6 mmol/l) and was immediately started with subcutaneous insulin treatment, and was defined as having PTDM. Patients with diabetic nephropathy as underlying renal disease received subcutaneous insulin treatment before and after transplantation, except for two of five patients with type II diabetes who were treated with oral hypoglycemic drugs before transplantation and needed insulin treatment only after transplantation, and for one patient with type II diabetes who was treated with oral hypoglycemic drugs also after transplantation. One patient with PTDM was treated with subcutaneous insulin; in the other three patients, glycemic control was achieved by lifestyle changes, weight loss, and steroid dose reduction. Patients with normal glucose tolerance, IFG, IGT, or PTDM (impaired glucose metabolism), and pre-existing diabetes are further characterized in Table 1. Recipients with impaired glucose metabolism were significantly older ($P = 0.005$), received grafts from older donors ($P = 0.04$), and had reduced renal function at 12 months (Table 2, $P = 0.003$) compared to controls. A trend towards increased frequency of delayed graft function and the use of tacrolimus, and longer duration of dialysis was seen in patients with impaired glucose metabolism, but the differences were not statistically significant. One patient had hepatitis C infection already pretransplantation but showed normal postload glucose values at 3 months. Patients with pre-existing diabetes had higher glycosylated haemoglobin at both 3 and 12 months compared with controls or with patients with impaired glucose metabolism ($P < 0.001$) (Table 1). Graft survival both censored for death and uncensored for death was significantly reduced in patients with pre-existing diabetes compared with all the other patients ($P = 0.01$ and $P = 0.005$ respectively).

In addition to biopsies at 0 and 12 months, a protocol biopsy at 3 months was available from altogether 65 patients (25 patients with normal glucose tolerance, 26 with pre-existing diabetes, four patients with PTDM, and 10 patients with IFG or IGT). Reasons for the missing 3 months protocol biopsies were: lymphocele ($n = 1$), increased risk of bleeding because of anticoagulation

Table 1. Detailed characteristics of patients with normal glucose tolerance (controls); impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or post-transplant diabetes mellitus (PTDM); or pre-existing diabetes.

	Controls ($n = 30$)	IFG, IGT or PTDM ($n = 14$)	Pre-existing diabetes ($n = 32$)
Recipient age	44 ± 12	55 ± 11†	47 ± 12
Donor age	49 ± 10	57 ± 6‡	49 ± 12
CIT (h)	20 ± 6	20 ± 3	20 ± 4
DGF (%)	11/30 (37%)	7/14 (50%)	10/32 (31%)
Length of dialysis (months)	21 ± 12	32 ± 25	24 ± 22
HLA mismatch*	2.1 ± 09	2.5 ± 0.7	2.3 ± 0.9
Acute rejection (%)	4/30 (13%)	1/14 (7%)	10/32 (31%)
Methylprednisolone dose at 3 months (mg)	7.9 ± 2.6	8.9 ± 2.4	8.5 ± 2.7
Patients on tacrolimus (%)	5/30(17%)	3/14 (21%)	5/32 (16%)
Patients on ACEI/ARB at 12 months (%)	16/30 (53%)	6/14 (43%)	19/32 (59%)
Total plasma cholesterol at 3 months (mmol/l)	5.4 ± 1.1	5.4 ± 1.3	4.6 ± 0.8
BMI at 3 months	24 ± 3.4	22.3 ± 3.4	24.6 ± 3.5
HbA1c at 3 months (%)	5.8 ± 0.5	6.2 ± 1.2	8.7 ± 1.2§
HbA1c at 12 months (%)	5.8 ± 0.6	5.9 ± 0.5	8.7 ± 1.8§
CMV infections (%)	8/30 (27%)	3/14 (21%)	6/32 (19%)

All data are expressed as mean ± 1 SD.

CIT, cold ischemia time; DGF, delayed graft function; ACEI/ARB, angiotensin converting enzyme inhibitors or angiotensin receptor blockers; BMI, body mass index; HbA1c, glycosylated haemoglobin A1c.

*HLA A-, B-, and DR-mismatch.

†$P = 0.005$ between patients with IFG, IGT, or PTDM and controls, and $P = 0.03$ between with IFG, IGT, or PTDM and patients with pre-existing diabetes (recipient age).

‡$P = 0.04$ between IFG, IGT, or PTDM and controls, and $P = 0.02$ between IFG, IGT, or PTDM and patients with pre-existing diabetes (donor age).

§$P < 0.001$ between patients with pre-existing diabetes and controls or patients with IFG, IGT, or PTDM (HbA1c at 3 and 12 months).

Other differences are not significant ($P > 0.05$).

treatment ($n = 1$), infection at the time of biopsy ($n = 1$), logistic reasons ($n = 4$), patient refusal ($n = 4$). Findings in donor biopsies and in 3 and 12 months protocol biopsies, and the progression of histopathological changes (ΔCADI) in patients with normal glucose tolerance, IFG, IGT, or PTDM, and pre-existing diabetes are presented in Table 2. Patients with PTDM showed a trend towards higher CADI at 12 months (4.8 ± 1.7), and higher ΔCADI between 0 and 12 months (3.8 ± 1.5), but the differences did not reach statistical significance ($P = 0.06$ for CADI at 12 months and $P = 0.16$ for ΔCADI between 0 and 12 months, data nor shown). Patients with impaired glucose metabolism (IFG, IGT or PTDM) showed a nonsignificant trend towards increased CADI at baseline, 3 months, and 12 months, and ΔCADI between 0 and 12 months (Table 2). No significant differences were observed in the individual parameters of CADI or in arteriolar hyaline thickening between the groups (data not shown). No findings of recurrent or *de novo* diabetic nephropathy were recorded in the protocol biopsies.

Correlations between glucose metabolism parameters and CADI scores are presented in Table 3. When 2-h postload glucose values were correlated with histopathological changes, significant correlation was found between CADI at 12 months and 2-h postload glucose in patients with abnormal result in OGTT, i.e. patients with IGT or PTDM ($R = 0.84$, $P = 0.001$, Table 3-a). A statistically significant correlation was similarly seen between ΔCADI between 0 and 12 months and 2-h postload glucose in patients with IGT or PTDM ($R = 0.67$, $P = 0.025$, Table 3-a). In multiple linear regression analysis, these correlations were independent of recipient age, which was significantly higher in patients with PTDM. These correlations were similarly independent of donor age and the occurrence of delayed graft function. Glycosylated hemoglobin at 12 months showed a statistically nonsignificant correlation with ΔCADI between 0 and 12 months in patients with pre-existing diabetes ($R = 0.35$, $P = 0.05$, Table 3-c). No other correlations were observed between the histopathological parameters and parameters of glucose metabolism, and no significant correlations were observed at any time point between renal function and parameters of glucose metabolism (data not shown).

Table 3. (a) Correlations analyzed with linear regression between 2-h postload glucose values and chronic allograft damage index (CADI) at 3 and 12 months, or changes in CADI (ΔCADI) between different time-points of biopsies in patients with abnormal result in oral glucose tolerance test (i.e. patients with IGT or PTDM, $n = 12$). (b) Correlations analyzed with linear regression between glycosylated hemoglobin (HbA1c) values at 3 and (c) at 12 months and chronic allograft damage index (CADI) at 3 and 12 months, or changes in CADI (ΔCADI) between different time-points of biopsies in patients with pre-existing diabetes ($n = 32$)*.

(a) Patients with IGT or PTDM	2-h postload glucose at 3 months, *R*-value	*P* –value
CADI at 3 months	0.13	0.71
CADI at 12 months	0.84	0.001
ΔCADI 3–12 months	0.64	<0.05
ΔCADI 0–12 months	0.67	<0.03
(b) Patients with pre-existing diabetes	HbA1c at 3 months *R*-value	*P* -value
CADI at 3 months	0.15	0.5
CADI at 12 months	0.18	0.36
ΔCADI 3–12 months	0.25	0.22
ΔCADI 0–12 months	0.24	0.2
(c) Patients with pre-existing diabetes	HbA1c at 12 months *R*-value	*P* -value
CADI at 3 months	0.02	0.9
CADI at 12 months	0.29	0.12
ΔCADI 3–12 months	0.38	0.06
ΔCADI 0–12 months	0.35	0.05

*Biopsy at 3 months was available from all 12 patients with IFG or IGT and from 26 patients with pre-existing diabetes.
P -values <0.05 are considered significant.

Table 2. Renal function (eGFR) estimated with Cockcroft- and Gault-equation (28), chronic allograft damage index (CADI) at 0, 3, 12 months, and changes in CADI (ΔCADI) between different time-points of biopsies in patients with normal glucose tolerance (controls); impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or post-transplant diabetes mellitus (PTDM); or pre-existing diabetes.

	Controls ($n = 30$)	IFG, IGT or PTDM ($n = 14$)	Pre-existing diabetes ($n = 32$)
CADI at baseline	0.3 ± 0.5	0.9 ± 1.0	0.5 ± 0.8
CADI at 3 months*	0.9 ± 1.1	2.2 ± 2.1	1.2 ± 1.1
CADI at 12 months	1.7 ± 1.6	2.9 ± 2.2	1.6 ± 1.4
ΔCADI 0–12 months	1.5 ± 1.6	2.1 ± 2.1	1.5 ± 1.2
eGFR at 12 months (ml/min)	74 ± 22	54 ± 12†	75 ± 23
eGFR at last follow-up (ml/min)	64 ± 23	52 ± 12	61 ± 26

All data expressed as mean ± 1 SD.
*Biopsy at 3 months was available from 25 patients from the control group, 14 patients with IFG, IGT, or PTDM, and from 26 patients with pre-existing diabetes.
†$P = 0.006$ between IFG, IGT or PTDM and controls and $P = 0.003$ between IFG, IGT or PTDM and pre-existing diabetes (eGFR at 12 months).
All other differences are nonsignificant.

Discussion

To our knowledge, this is one of the first studies analyzing the impact of IGT or diabetes on the progression of histopathological changes attributable to chronic rejection in protocol biopsies. When the impact of disorders of glucose metabolism on sequential protocol biopsy histopathology and graft function was analyzed from renal transplant recipients, pre-existing diabetes was associated with reduced graft survival, but was not associated with decreased renal function or increased histopathological changes. Interestingly, in patients with abnormal result in 2-h OGTT 3 months after transplantation, the 2-h postload glucose values correlated significantly with the progression of histopathological changes. Patients with impaired glucose metabolism were significantly older, had a somewhat longer duration of pre-transplant dialysis period, received grafts from older donors with higher baseline CADI scores, and had a slightly higher frequency of delayed graft function, making these already compromised kidneys probably more susceptible to the damage caused by glucose abnormalities. In these patients with impaired glucose metabolism, renal function at 1 year after transplantation was reduced and a trend towards increased histopathological changes attributable to chronic rejection was recorded.

Previous studies have described increased histopathological changes associated with diabetic nephropathy (especially mesangial matrix increase and arteriolar hyalinosis) in biopsies taken mostly more than 2 years after transplantation in patients with diabetic nephropathy as the underlying cause of transplantation [17,18,21]. We recorded no association of pre-existing diabetes with increased histopathological changes as analyzed with CADI. Our results may be explained by the early timepoint of 12 months for the development of changes associated with diabetic nephropathy. Some evidence shows poorer graft survival and function in patients with diabetic nephropathy as the cause of transplantation [3,29], while some studies show equal graft survival despite diabetes [30]. In our study, no reduced renal function was observed in patients with pre-existing diabetes. Graft survival, however, was significantly reduced in patients with pre-existing diabetes; graft losses were observed during the study period only in patients with pre-existing diabetes.

In patients with post-transplant diabetes, changes attributable to diabetic nephropathy have been observed in late biopsies taken several years after transplantation in retrospective analyses [21,22]. However, the progression of histopathological changes during the first year has not been described in detail using protocol biopsies and systematic screening with OGTT. We found a significant correlation between 2-h postload glucose values and progression of histopathological changes associated with chronic rejection during the first 12 months. Our results suggest that abnormalities of glucose metabolism developing after transplantation may be deleterious to the allograft, especially in kidneys from older donors together with other factors, such as higher recipient age and delayed graft function as seen in our study, which are also associated with the progression of histopathological changes

Several risk factors for the development of PTDM have been identified, including increased age, increased BMI, steroid dose, acute rejections, therapy with tacrolimus, CMV and hepatitis C infections [5,7,9,10,29,31,32]. In our study, patients with impaired glucose metabolism were significantly older and received grafts from older donors, but we observed no statistically significant differences in any of these other risk factors between patients with impaired glucose metabolism, pre-existing diabetes or controls. Our study was not designed to investigate the risk factors associated with PTDM or impaired glucose metabolism and the number of patients suffering from PTDM was small, which may limit our analyses. Although the risk of PTDM is the highest during the first months after transplantation with the most intense immunosuppression and the highest steroid dose, PTDM develops in many patients also years after transplantation [33]. We analyzed only the impact of OGTT at 3 months, and no systematic screening with OGTT was applied after that, and some patients may have developed impaired glucose metabolism later after transplantation.

There are several mechanisms by which impaired glucose metabolism may damage the allograft. Changes associated with diabetic nephropathy may occur also in patients with post-transplant diabetes [21,22], although we detected no specific findings of *de novo* diabetic nephropathy in our material. PTDM and IGT are associated with other components of metabolic syndrome i.e. hyperlipidemia, hypertension, and obesity which all may be harmful to the allograft. Severely obese patients are excluded from transplantation in our center, but a previous study from our group showed an association of increased blood pressure and lipid abnormalities with the progression of histopathological changes in protocol biopsies [34]. However, no significant differences were observed in blood pressure control or lipid status between the patient groups in this study.

Optimized glycemic control is shown to delay the progression of diabetic nephropathy in both native and transplanted kidneys [18,35]. We observed a significant correlation between the progression of histopathological changes associated with chronic rejection recorded as CADI score during the first year and 2-h postload glucose values at 3 months, supporting the role of glycemic control in preventing the progression of pathologic changes

in the graft. Surprisingly, we observed no increased histopathological changes or reduced graft function in patients with pre-existing diabetes. However, graft survival was significantly reduced in patients with pre-existing diabetes, as all the grafts lost during the study period were from patients with pre-existing diabetes. A statistically nonsignificant trend of correlation was found between HbA1c levels at 12 months and the progression of histopathological changes. Patients with IFG, IGT or PTDM were older and received grafts from somewhat older donors. Furthermore, these patients with glucose metabolism abnormalities had a slightly higher baseline CADI score, showed a trend towards increased frequency of delayed graft function, and had a slightly longer duration of pre-transplant dialysis period. Although the significant correlations observed in our study were independent of donor or recipient age and delayed graft function in multivariate linear regression, these older and already more damaged kidneys are probably more susceptible to the damage of impaired glucose metabolism, possibly explaining the observed correlations and the findings of reduced kidney function in patients with impaired glucose metabolism. The basal preconditioning of these already compromised kidneys may also be the key factor of the progression of histopathological changes, with the impaired glucose metabolism being associated with further damage, explaining the lack of increased histological changes and lack of correlation of HbA1c and histolopathology in patients with pre-existing diabetes. Almost half of the patients in this study were on ACE inhibitors or angiotensin receptor blocker therapy, which are known to delay the progression of diabetic nephropathy [36] and may be beneficial for the allograft also in nondiabetic subjects [37]. The use of these therapies was somewhat less frequent in patients with impaired glucose metabolism, which may confound our analyses.

In conclusion, our study of the impact of glucose metabolism abnormalities on protocol biopsy histology showed a significant correlation between 2-h postload glucose values in OGTT at 3 months and progression of histopathological changes recorded as CADI score in sequential protocol biopsies in patients with also other significant risk factors for the progression of histopathological changes associated with chronic rejection. These novel findings support the harmful role of PTDM to the allograft, highlighting the importance of adequately screening and treating glucose metabolism abnormalities after transplantation.

Authorship

I.H.: primary researcher in this study (study design, manuscript preparation, clinical data collection); F.O.: clinical data collection, histopathological analyses; A.R-S: histopathological analyses; P.K.: clinical nephrology, study design, head of the project

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