Predictors of Renal Allograft Histologic Damage Progression

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The objective of this study was to analyze factors that are involved in the progression of renal allograft damage in the first 6 mo after transplantation. Donor and 6-mo protocol biopsies of 83 patients who received a renal transplant were classified using the Chronic Allograft Damage Index (CADI). Histologic changes were compared and correlated to clinical parameters at transplantation, at 6 mo, and annually over 2 yr. All CADI components increased significantly in the 6-mo posttransplantation period, except chronic vascular changes and the percentage of glomerulosclerosis. Total cholesterol and LDL- cholesterol at the time of biopsy correlated positively with mesangial matrix increase, and HDL cholesterol correlated negatively with vascular intima increase. High BP at biopsy was associated with tubular atrophy. Diastolic BP at biopsy correlated with 6-mo CADI (CADI-6). Patients with diastolic BP ≥85 mmHg at biopsy had a higher difference between CADI score in protocol biopsies and CADI score in donor biopsies (ΔCADI) and higher creatinine at 1 and 2 yr. CADI in donor biopsies (CADI-0) >1 was more frequently found in older (odds ratio [OR], 1.07; 95% confidence interval [CI], 1.01 to 1.14) and nontraumatic dead donors (OR, 3.89; 95% CI, 1.13 to 13.33). CADI-6 >3 was more frequently found in those with CADI-0 >1 (OR, 3.82; 95% CI, 1.19 to 12.21), older donors (OR, 1.05; 95% CI, 1.01 to 1.10), and number of AB mismatches (OR, 2.36; 95% CI, 1.09 to 5.10). CADI-0, CADI-6, and ΔCADI correlated significantly with serum creatinine at hospital discharge, at 6 mo, and at 2 yr. ΔCADI was affected by initial percentage of glomerulosclerosis (OR, 1.10; 95% CI, 1.02 to 1.19) and creatinine at hospital discharge (OR, 1.01; 95% CI, 1.00 to 1.02). Donor-related as well as nonimmunologic factors, such as hypertension and dyslipidemia, are associated with increased risk for renal allograft damage progression.

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Chronic allograft nephropathy (CAN) can be recognized in early biopsies after transplantation, even before clinical features point to graft dysfunction (1–3). Renal allograft protocol biopsies have become a common practice around the world as a useful tool to detect chronic allograft damage and to predict late graft dysfunction (4–6). Despite the prompt histologic diagnosis of CAN, few options have been identified to prevent or treat them successfully. Although the pathogenesis of this condition remains elusive, various known risk factors for the progression of disease in native kidneys could contribute to allograft damage as well.

Both Banff (7) and Chronic Allograft Damage Index (CADI) (3) classifications have been used to quantify renal allograft histology. Many investigators have demonstrated that use of any of these systems gives a good prediction of graft outcome (1,5,8–10). In both Banff and CADI classifications, the lesions in different renal compartments are evaluated semiquantitatively and then summarized. The origin of these lesions seems to be multifactorial, and, although they may be interrelated, each of them could have different risk factors.

The incidence of CAN is related to the timing of the protocol biopsy, varying from 25 to 50% at 1 yr (3,5,11). The progression from normal histology to CAN or worsening of CAN grade occurs mainly within the first year after transplantation. However, data about the rate of progression of the lesions during this early phase rather than a single time point histologic quantification would help us to detect cases that may probably develop CAN later. Few studies have evaluated the impact of donor histology on the later protocol biopsies, but as marginal donors are accepted more commonly, data about the degree of progression starting from donor histology are mandatory. This single-center study analyzed retrospectively donor and 6-mo protocol biopsies of 83 kidney allograft recipients to identify clinical and histomorphologic risk factors for progression of histologic changes and for renal allograft dysfunction over 2 yr after transplantation.

Materials and Methods

Patients and Samples

Between June 1994 and June 2000, 255 renal transplants were performed in a cohort of 252 patients who lived in the Helsinki University Hospital District. They were followed up at the same single center. Five of them died within the first 6 mo after transplantation; 169 gave their

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consent to a protocol biopsy at 6 mo. Minimum follow-up of the patients was 2 yr or until death. Eighty-three patients in whom donor biopsies also were available were included in this study.

Biopsy Technique

Donor biopsies were performed either during the donor operation or after revascularization in the recipient operation, with a Bard Magnum automatic gun and 18-gauge Biopty-cut needle. Six-month biopsies were performed under ultrasound guidance with either Bard Magnum or Bard Biopty devices and 18-gauge Biopty-cut needles. No major complications related to biopsy procedure occurred. Donor biopsies were embedded in paraffin and serial tissue sections stained with hematoxylin/eosin, periodic acid-Schiff, and Masson's trichrome. Protocol biopsies were embedded in Historesin (Leica Instruments GmbH, Heidelberg, Germany) and serial tissue sections stained with hematoxylin/eosin, periodic acid-Schiff, silver methenamine, and May-Grünwald-Giemsa.

Classification of Graft Biopsies

The biopsy sample was considered adequate when there were at least five glomeruli and three arterioles per slice. Fewer than 10% of the biopsies contained no arteries. The average number of glomeruli in the 0-biopsies was 13.6 (range, 2 to 36) and in the protocol biopsies was 9.5 (range, 5 to 30).

Donor and 6-mo protocol biopsies from the 83 renal transplant recipients were scored by the consensus of two observers who were blinded from any clinical information using CADI (3). Interstitial inflammation, interstitial fibrosis, mesangial matrix increase, tubular atrophy, vascular intimal proliferation (cv), and arterial hyalinosis were scored semiquantitatively from 0 to 3 (0, none; 1, mild; 2, moderate; 3, severe) according to the Banff '97 classification (7). In the CADI score system, also the percentage of sclerotic glomeruli (%gs) was scored from 0 to 3 (0, no sclerotic glomeruli; 1, <15% of sclerotic glomeruli; 2, 16 to 50% of sclerotic glomeruli, and 3, >50% of sclerotic glomeruli). CADI has a minimum value of 0 and a maximum of 18, resulting from the sum of interstitial inflammation, tubular atrophy, cv, interstitial fibrosis, mesangial matrix increase, and %gs scores. Arterial hyalinosis was included in the analysis, although it is not a CADI component. The difference between CADI score in protocol biopsies and CADI score in donor biopsies (ΔCADI) was used as a marker of tissue damage progression.

Clinical Variables

The following baseline clinical data were obtained on the day of the transplantation from patient files: Recipient gender and age, cause of ESRD, time on dialysis, dialysis modality, donor type, donor age, donor gender, donor cause of death, number of HLA-AB and HLA-DR mismatches, percentage of panel reacting antibodies (PRA), cold ischemia time (CIT), biochemical laboratory values (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and glycosylated hemoglobin), systolic and diastolic BP, weight, and height. Donor and recipient characteristics are outlined in Table 1. Diagnosis of acute rejection (AR) was based on the clinical status, core biopsy findings classified according to Banff '97 (7), and start of antirejection therapy. Briefly, type I AR is defined by significant interstitial infiltration and foci of moderate to severe tubulitis; type II includes mild to moderate intimal arteritis (subtype A) or severe arteritis (subtype B); type III AR is defined by transmural arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells. Patients with negative PRA constituted 82% of the population.

In the early posttransplantation period, lasting up to the time of hospital discharge (time 0), the following variables were recorded: Early or delayed graft function (DGF; defined as the need for dialysis), infections, AR, graft function (assessed by serum creatinine measurement in μmol/L and 24-h creatinine clearance in ml/min per 1.73 m^2 body surface area), and cyclosporine A (CsA) trough levels.

Thereafter, time intervals were established as follows: From hospital discharge up to the time of the protocol biopsy, and from then up to each annual control. The parameters evaluated at these time points were AR episodes, body mass index (BMI), systolic and diastolic BP, CsA trough levels, biochemical laboratory values as above, and infections. Infections means any virus, bacterial or mycobacterial, that required hospital admission to be treated.

Table 1. Demographic data of the study population[a]

Donor age (yr; mean [range])	41 (14–66)
Cause of donor death (traumatic/nontraumatic)	45%/55%
Donor age in nontraumatically and traumatically dead donors (yr; mean)	48/31
Donor gender (male/female)	64%/36%
Recipient age (yr; mean [range])	48 (26–68)
Recipient gender (male/female)	61%/39%
Time on dialysis (mo; mean [range])	21 (0.4–116)
Type of dialysis (PD/HD)	42%/58%
First transplant	90%
HLA AB mismatches	1.5 (0–3)
HLA DR mismatches	0.8 (0–2)
CIT (h)	21 (15–30)
Patients with PRA >30%	5%

[a]PD, peritoneal dialysis; HD, hemodialysis; CIT, cold ischemia time; PRA, panel reacting antibodies.

Medication

Immunosuppressive regimen, antihypertensive medication, and use of statins were recorded during the follow-up. The majority of the patients received a combination of CsA, azathioprine, and steroids to prevent rejection ($n = 63$), but also other immunosuppressives regimens were used. The number of patients on each drug at the protocol biopsy time was as follows: CsA, $n = 81$; mycophenolate mofetil, $n = 12$; tacrolimus, $n = 2$; steroids, $n = 83$; and azathioprine, $n = 63$. The number of patients on antihypertensive drug at protocol biopsy time was as follows: β-blockers, $n = 71$; calcium channel blockers, $n = 41$; and angiotensin-converting enzyme inhibitors, $n = 6$.

Statistical Analyses

The Mann-Whitney *U* test was used to assess differences in rank distributions of continuous variables between two groups. Fisher exact test served to evaluate the relation between two binary variables. Correlations among histologic classifications, histologic scoring, and numerical data and other continuous variables were calculated nonparametrically using Kendall's τ. For logistic regression analyses, the histologic damage and its progression, CADI-0 (in donor biopsies), CADI-6 (at 6 mo), and ΔCADI were converted to binary and used as outcome variables. In each case, the median was used as a cut point to obtain two categories. In the case of CADI-0, the categories were values of ≤1 ($n = 51$) and values >1 ($n = 32$). In the case of CADI-6, the cutoff value was ≤3 ($n = 43$) and values >3 ($n = 40$). In the case of ΔCADI, a group comprised those with minimal or no progression (ΔCADI ≤1

$n = 34$) and those with moderate to marked progression (ΔCADI >1, $n = 49$). A two-sided $P < 0.05$ was considered statistically significant. The calculations were performed using StatsDirect statistical software version 2.2.4.

Results

Clinical Follow-Up

The mean follow-up period was 5.3 yr (range, 1.2 to 8.6). Ten patients died during the follow-up with a functioning graft, two of whom died of infections before the second year. The last serum creatinine value before death was on average 126 μmol/L (range, 68 to 247). One patient returned to dialysis 48 mo after transplantation as a result of CAN. Clinical events during the first 6 mo of follow-up are detailed in Table 2. Events after the protocol biopsy up to 2 yr were late acute rejection ($n = 2$), infections ($n = 18$), and malignancies leading to death ($n = 2$). Evolution of clinical variables over 2 yr is depicted in Table 3. Subclinical AR was found in three protocol biopsies; two of these cases received AR therapy, but one did not because of the high prevalence of chronic changes. Borderline changes were seen in one case. No cases of typical CsA toxicity in 6-mo biopsies were recorded in this cohort.

CADI Score and Graft Function

We studied the correlation between CADI in donor biopsies, CADI at 6 mo from transplantation, and ΔCADI with serum creatinine at the time of hospital discharge, at 6 mo, at 1 yr, and at 2 yr after transplantation (Table 4). CADI scores in donor biopsies and at 6 mo were positively correlated to each other ($\tau = 0.27$ $P = 0.0033$). Although CADI in donor biopsies did not correlate to ΔCADI, there was a positive correlation between chronic damage index in donor kidneys and higher creatinine at two years. ($\tau = 0.29$; see Table 4) Measured creatinine clearance did not correlate to CADI or CADI score components (data not shown).

Logistic regression analyses showed that the risk for having abnormal serum creatinine (>115 μmol) at 2 yr after transplantation was affected by the degree of increase in the CADI score in the first 6 mo (odds ratio [OR] for each point of increase in ΔCADI score, 1.29; 95% confidence interval, 1.02 to 1.63). Other tested variables, such as BMI and BMI increase, lipids, and individual CADI score components, did not reach significance.

Table 2. Events during the first 6 mo of follow-up[a]

Patients with DGF	23%
Mean DGF duration (d [range])	15 (1–56)
Dialysis modality in DGF patients (PD/HD)	42%/58%
Patients with AR	37%
Steroid-sensitive AR	86%
Patients with CMV infection	30%
Patients with infection other than CMV	27%

[a]DGF, delayed graft function; AR, acute rejection; CMV, cytomegalovirus.

Changes in the CADI Score Components

Interstitial fibrosis, glomerular changes, tubular atrophy, mesangial matrix, and arteriolar hyalinosis increased significantly in the first 6 mo after transplantation. The results are shown in Table 5. Also, CADI increased from mean 1.4 (range, 0 to 6) in donor biopsies to 3.3 (range, 0 to 10) in 6-mo biopsies.

The percentage of glomerulosclerosis did not increase significantly in the first 6 mo. Sclerotic glomeruli were found in 21% of the donors. In this study, the percentage of glomerulosclerosis in the donor biopsy correlated positively with donor age ($\tau = 0.25$; $P = 0.0196$). Sixteen percent of donors who were <45 yr of age (7 of 44) had glomerulosclerosis (range of %gs, 4.4 to 40), whereas 41% the donors who were ≥45 yr of age (16 of 39) had glomerulosclerosis (range of %gs, 3.8 to 16.7; $P = 0.0142$). Nontraumatically dead donors had more frequently glomerulosclerosis ($P = 0.0102$). No association was found between %gs and donor gender, DGF, or serum creatinine at 0, 6, 12, and 24 mo.

Although the cv score did not increase significantly in the first 6 mo after transplantation, 17% of donor biopsies had some vascular changes. In this subset of patients, the cv score was significantly associated with nontraumatic cause of death ($P < 0.001$) and serum creatinine at hospital discharge ($P = 0.026$). No correlation between the cv score and donor age, donor gender, serum creatinine at 2 yr, %gs, or ΔCADI was observed.

Risk Factors for High CADI Score in Donor and Six-Month Protocol Biopsies

Logistic regression analyses showed that the risk for having an elevated (≥2) CADI score in donor biopsies was affected by donor age and nontraumatic cause of death. CADI at 6 mo showed a positive correlation with diastolic BP (DBP) at 6 mo (Kendall's $\tau = 0.20$ $P = 0.0156$). The risk for having elevated (≥4) CADI score at 6 mo was influenced by CADI in donor biopsies, donor age, and AB mismatches. The OR are shown in Table 6.

There was no correlation between DGF, DR mismatches, PRA, or CIT and CADI in donor biopsies. Neither was high CADI at 6 months affected by increase in BMI or the concentrations of glycosylated hemoglobin, serum lipids, or CsA through levels at hospital discharge.

Change in CADI Score Components and Clinical Parameters

We considered the change in each component of CADI between 6-mo and donor biopsies as markers of damage progression in different renal compartments. These changes were correlated to clinical parameters at the time of protocol biopsy (Table 7). Total serum cholesterol and LDL cholesterol correlated to Δ-mm. HDL cholesterol correlated inversely to Δ-cv. Systolic and diastolic BP correlated to Δ-ct. No correlation between ΔCADI components and CIT, BMI, DGF, or glycosylated hemoglobin was recorded.

Risk Factors for High ΔCADI Score

Patients who had moderate to marked histologic progression (ΔCADI >1, $n = 49$) had higher serum creatinine concentration at hospital discharge ($P = 0.0086$), at 6 mo ($P = 0.0154$), at 1 yr

Table 3. Evolution of clinical variables over 2 yr

	At Transplantation	At 6 Mo	At 12 Mo	At 24 Mo
Serum creatinine[a] (μmol/L)	139 (120–158)	121 (114–127)	121 (114–129)	120 (112–127)
Creatinine clearance[a] (ml/min per 1.73 m^2)	57.6 (53.4–62.4)	57.6 (54.6–61.2)	60 (53–63.6)	61.2 (57.6–65.4)
Total cholesterol (mmol/L)	5.5 (5.3–5.8)	5.9 (5.7–6.3)	5.9 (5.7–6.2)	5.6 (5.3–5.8)
LDL cholesterol (mmol/L)	3.1 (2.8–3.4)	3.6 (3.3–3.8)	3.3 (3.1–3.5)	3.2 (2.9–3.4)
HDL cholesterol (mmol/L)	1.3 (1.2–1.4)	1.6 (1.5–1.7)	1.6 (1.5–1.7)	1.6 (1.5–1.7)
Triglycerides (mmol/L)	2.2 (1.8–2.5)	1.9 (1.7–2.2)	1.7 (1.5–2.0)	1.6 (1.4–1.8)
HbA_{1c} (%)	8.3 (7.5–9.1), $n = 24$	7.4 (6.9–7.9), $n = 61$	7.2 (6.7–7.6), $n = 65$	6.8 (6.5–7.2), $n = 66$
Cyclosporin A[a] (μg/L)	212 (201–224), $n = 82$	157 (119–195), $n = 81$	109 (104–113), $n = 81$	105 (100–109), $n = 78$
Body mass index (kg/m^2)	24 (23–24)	25 (24–26)	25 (24–26)	25 (24–26)
Systolic BP (mmHg)	144 (139–150)	142 (138–146)	143 (139–148)	140 (136–144)
Diastolic BP (mmHg)	88 (85–91)	85 (83–88)	86 (84–88)	82 (80–85)

[a]Measured at hospital discharge at transplantation. HbA_{1c}, glycosylated hemoglobin.

Table 4. Correlation coefficients between serum creatinine at hospital discharge at biopsy time, at 1 yr, and at 2 yr with CADI scores in donor biopsies, in 6-mo biopsies and ΔCADI[a]

	Donor CADI	CADI at 6 Mo	ΔCADI
Creatinine at discharge	0.214 ($P = 0.0125$)[b]	0.324 ($P < 0.0001$)[b]	0.211 ($P = 0.0087$)[b]
Creatinine at 6 mo	0.169 ($P = 0.049$)[b]	0.26 ($P = 0.0012$)[b]	0.192 ($P = 0.0176$)[b]
Creatinine at 12 mo	0.15 ($P = 0.067$)	0.277 ($P = 0.0006$)[b]	0.199 ($P = 0.0137$)[b]
Creatinine at 24 mo	0.286 ($P = 0.0005$)[b]	0.286 ($P = 0.0005$)[b]	0.213 ($P = 0.0097$)[b]

[a]CADI, Chronic Allograft Damage Index; ΔCADI, difference between CADI score in protocol biopsies and CADI score in donor biopsies.
[b]Kendall's τ correlation coefficient with statistical significant P values. $P < 0.05$ is considered significant.

Table 5. Comparison of histologic findings in donor and in 6-mo protocol biopsies

	Donor Biopsy[a]	6-Mo Biopsy[a]	P Values
Interstitial fibrosis	0.50 (0.39–0.61)	0.74 (0.61–0.88)	0.0146[b]
Transplant glomerulopathy	0	0.07 (0.01–0.13)	0.0129[b]
Chronic vasculopathy	0.24 (0.11–0.36)	0.33 (0.20–0.46)	0.1308
Tubular atrophy	0.25 (0.16–0.34)	0.74 (0.63–0.86)	<0.0001[b]
Mesangial matrix increase	0.23 (0.13–0.32)	0.56 (0.45–0.67)	<0.0001[b]
Arteriolar hyalinosis	0.36 (0.20–0.51)	0.57 (0.40–0.75)	0.029[b]
Glomerulosclerosis	3.1 (1.65–4.53)	3.38 (1.66–5.10)	0.4774
No. of glomeruli	13.6 (12.1–15.1)	9.53 (8.61–10.44)	
CADI	1.35 (1.05–1.64)	3.27 (2.82–3.73)	<0.0001[b]

[a]Mean and confidence interval.
[b]Mann-Whitney U test; $P < 0.05$ is considered statistically significant.

($P = 0.0073$), and at 2 yr ($P = 0.0074$). Figure 1 shows graphically these differences. These patients had also higher DBP at 6 mo ($P = 0.019$).

ΔCADI was significantly higher in biopsies from patients with diastolic BP ≥85 mmHg compared with those with diastolic BP <85 mmHg ($P = 0.0177$) at 6 mo, despite similar CADI score in donor biopsies. Patients with elevated DBP at biopsy also had higher serum creatinine at 1 yr ($P = 0.029$) and at 2 yr ($P = 0.026$) compared with normotensive patients. It is noteworthy that a similar percentage of patients received antihypertensive treatment in both groups, and there were no differences in types of antihypertensive drug. The use of statins ($n = 6$) had no impact on histologic damage progression in this population.

After testing CADI score components with logistic regression analysis, using HLA-AB and HLA-DR mismatches, CIT, DGF, PRA, AR, and serum creatinine at hospital discharge as independent variables, the risk for ΔCADI >1 was increased signif-

Table 6. Odds for high CADI scores in donor biopsies and at 6 mo and odds for a ΔCADI >1

	Odds Ratio	95% Confidence Interval
Odds for high CADI score at transplantation		
donor age, for 1-yr increase	1.07	1.01–1.14
nontraumatic donor cause of death	3.89	1.13–13.33
Odds for high CADI score at 6 mo		
CADI score in donor biopsies	3.82	1.19–12.21
donor age, for 1-yr increase	1.05	1.01–1.10
HLA-AB mismatches, for 1-unit increase	2.36	1.09–5.10
Odds for a ΔCADI >1		
%gs at transplantation, for 1% increase	1.10	1.02–1.19
serum creatinine at hospital discharge, for 1-μmol/L increase	1.01	1.00–1.02

Table 7. Correlation between clinical parameters and chronic markers increase[a]

	Total Cholesterol	LDL Cholesterol	HDL Cholesterol	Triglycerides	SBP	DBP
Δ-ci	−0.12, $P = 0.17$	−0.04, $P = 0.64$	−0.01, $P = 0.95$	−0.12, $P = 0.17$	0.006, $P = 0.94$	0.06, $P = 0.55$
Δ-ct	0.001, $P = 0.99$	0.06, $P = 0.53$	0.08, $P = 0.41$	−0.15, $P = 0.13$	0.22, $P = 0.034$[b]	0.22, $P = 0.034$[b]
Δ-cg	0.06, $P = 0.75$	0.06, $P = 0.78$	−0.06, $P = 0.77$	0.13, $P = 0.53$	0.21, $P = 0.30$	0.15, $P = 0.48$
Δ-mm	0.22, $P = 0.028$[b]	0.24, $P = 0.014$[b]	−0.03, $P = 0.77$	0.17, $P = 0.08$	0.13, $P = 0.21$	0.11, $P = 0.27$
Δ-cv	−0.08, $P = 0.45$	−0.01, $P = 0.91$	−0.22, $P = 0.034$[b]	−0.03, $P = 0.77$	−0.06, $P = 0.57$	−0.01, $P = 0.91$
Δ-ah	−0.14, $P = 0.14$	−0.18, $P = 0.21$	0.05, $P = 0.62$	−0.17, $P = 0.07$	−0.02, $P = 0.82$	0.09, $P = 0.34$
ΔCADI	−0.05, $P = 0.57$	0.04, $P = 0.58$	−0.05, $P = 0.49$	−0.13, $P = 0.12$	0.06, $P = 0.43$	0.20, $P = 0.014$[b]

[a]ci, interstitial fibrosis; ct, tubular atrophy; cg, transplant glomerulopathy; mm, mesangial matrix increase; cv, chronic vasculopathy; ah, arterial hyalinosis, SBP, systolic BP; DBP, diastolic BP.
[b]Kendall's τ correlation coefficient with statistical significant P values.

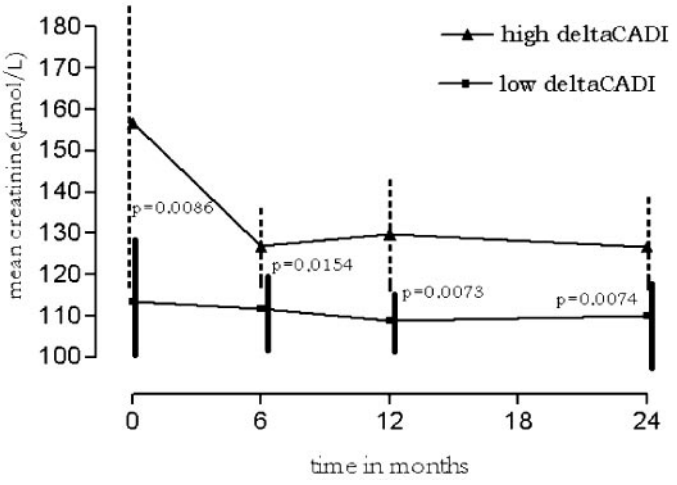

Figure 1. Mean serum creatinine (with 95% confidence interval) in patients with difference between CADI score in protocol biopsies and CADI score in donor biopsies (ΔCADI) >1 and patients with (ΔCADI) ≤1 at hospital discharge, biopsy time, 1 yr, and 2 yr. CADI, Chronic Allograft Damage Index.

icantly by the high degree of glomerulosclerosis in donor biopsy and the high serum creatinine concentration at hospital discharge. The OR for having ΔCADI >1 are shown in Table 6.

Neither infections in general nor cytomegalovirus infections in particular influenced the histologic progression in this population. The role of polyoma virus cannot fully be ruled out because diagnostics of this infection were not preformed during the study period. AR did not correlate to histologic damage progression. In patients with diabetes before transplantation ($n = 49$), histologic damage seemed more likely to progress, but the difference did not reach statistical significance ($P = 0.061$).

Discussion

The implementation of protocol biopsies to monitor renal allografts has become a valuable tool for a better understanding of the development of CAN. The diagnosis of this condition, however, is complicated by the fact that CAN may develop on a pretransplantation damaged kidney, making the distinction between the two entities difficult in many cases. Thus, the histologic background provided by donor biopsies enables us to discriminate between the effects of various additive insults. To our knowledge, this is the first study to take into consideration the histologic changes developed in the first 6 mo after transplantation and the risk factors involved in that development.

CADI as a classifying system was developed to predict long-term graft function (3). Recently, CADI score was validated in a multicenter study as a surrogate end point for long-term graft function (10).

Hariharan *et al.* (12) reported that creatinine at 6 mo and its

increase up to 1 yr predict allograft outcome in the long term. However, CAN may be diagnosed before allograft dysfunction can be documented clinically with conventional serum creatinine measurement (2,3,9). It was suggested that histologic changes attributable to CAN increase quickly during the first months after transplantation and at a slower rate thereafter (9). In the study of Lehtonen *et al.* (13), the mean CADI score in donor biopsies was 0.74 and it increased to 2.92 at 1 yr. In a multicenter study, Yilmaz *et al.* (10) found that the mean CADI score in donors was 1.3 increasing to 3.3 and 4.1 at 1 and 2 yr, respectively. It is noteworthy that in the latter study, CADI score at 1 yr predicted serum creatinine at 3 yr. Consequently, the increment in histologic damage over the first months after transplantation might anticipate late graft dysfunction better than increase in serum creatinine concentration. These data are in accordance with those in this study.

One of our major findings was that ΔCADI correlated positively to serum creatinine at all time points. This finding supports the idea that the evolution of ΔCADI already over the first 6 mo may predict late graft function. The odds for progression of ΔCADI were not affected by DGF but were increased 10 times for each 10-μmol increase in serum creatinine concentration at hospital discharge. This may be due to insults that occurred during the first month on a suboptimal graft. Among the classically described immunologic and nonimmunologic risk factors, we did not find PRA, HLA mismatches, donor age, donor gender, donor cause of death, DGF, AR, or CsA through levels at hospital discharge to affect ΔCADI. CsA through levels did not correlate to CADI or CADI score components probably because of the lack of specificity of classic CsA-related histologic changes. We should point out, however, that our center strives for optimal HLA matching and does not transplant completely mismatched grafts. Yilmaz *et al.* (10) asserted that acute rejection during the first year and CADI score at 1 yr increased significantly the odds for 3-yr graft loss or patient death. In our study, AR was diagnosed in 35% of the patients and had no impact on ΔCADI. One possible explanation for this is that the majority of the patients included in our study had type I AR: five cases in were class IIa, whereas neither type IIb nor type III rejections were observed.

We also evaluated the biopsies of donor kidneys. Although in this cohort some donor biopsies were taken during harvesting and others were taken after revascularization, we do not believe that it could have affected donor CADI score, as the components of the classification are indicators of chronic changes that demand a relatively long time to develop. We observed that donor CADI score did not correlate to ΔCADI, although donor CADI and CADI at 6 mo correlated positively. However, as the prevalence of CAN increases over time, it is possible that if later biopsies had been available, then the result might have been different. Conversely, as CADI at 6 mo was scored without knowledge of the scores of donor biopsies, it is possible that some lesions that were attributed to CAN could have been present already in the donor.

We thereafter focused on each CADI component and found that patients with higher ΔCADI received allografts with higher percentage of glomerulosclerosis. In a recent study, the percentage of glomerulosclerosis showed an adverse impact on graft outcome (14). It was estimated that a 1% increase in glomerulosclerosis in donor biopsy is responsible for a 0.8-ml/min drop in GFR at 4 yr (14). In our study, a 1% of increase of glomerulosclerosis in donor biopsies increased the risk for an increase in CADI score of more than one unit over 6 mo by 10%. This could be explained by the hyperfiltration of the remaining nephrons and consequent progressive damage.

As previously observed (15), the percentage of glomerulosclerosis increases with donor age. Nevertheless, we also observed that 16% of young donors had some degree of glomerulosclerosis. The percentage of glomerulosclerosis in donor biopsies had no correlation to creatinine at any time point. This can be partially explained by the relatively low number of patients with any glomerulosclerosis (21%) and low percentage of sclerosis (mean 3%) in those who had this finding. These low values compared with other studies (14,15) can be explained by the relatively low mean donor age in our population. We observed that kidneys from nontraumatically dead donors, which are more frequently older donors, had a higher percentage of glomerulosclerosis. Older donors are more likely to die from cerebrovascular disease, which is frequently associated with hypertension. The impact of cause of death on graft function (16,17) and on histology (18) has been studied before, but the results have been ambiguous. We did not find any association between female donor gender and worse graft survival, as other authors have suggested (19).

We found ΔCADI to be positively correlated to DBP measured at 6 mo. Legendre *et al.* (2) reported that histologic deterioration was associated with elevated BP and proteinuria, although serum creatinine remained normal. However, Serón *et al.* (9) reported that the increase of renal scarring within the first 14 mo posttransplantation was not associated with high serum creatinine concentration or with increment in BP. In a recent publication, Nankivell *et al.* (11) did not find any independent effect of hypertension degree on the risk for CAN in patients who had type 1 diabetes and received pancreas-kidney grafts. In our study, patients who had DBP >85 mmHg had higher ΔCADI and higher serum creatinine at 2 yr. Among the CADI score components, increase of tubular atrophy showed a positive correlation to both SBP and DBP. However, it is not possible to evaluate whether elevated BP is caused by renal parenchyma damage or is the promoter of these histologic changes.

Kuypers *et al.* (20) reported on a significant increase in tubular atrophy and interstitial fibrosis in 3-mo biopsies when DGF was present. Similar results were achieved by Di Paolo *et al.* (13). We could not see any correlation between the DGF and tubular atrophy increment or any other individual chronic marker increment in concordance with a previous study (21). One possible explanation is that in our cohort, the DGF rate was lower than in the studies mentioned above.

Serón *et al.* (22) found high total serum cholesterol before transplantation to be a significant risk factor for intimal thickness in the 3-mo protocol biopsies, and patients with transplant vasculopathy at 3 mo had a significantly worse prognosis at 10 yr. Moreso *et al.* (23) reported also that chronic transplant vasculopathy at 3 mo was associated with donor vasculopathy,

histoincompatibility, and high serum cholesterol concentration. In this study, we found that hypercholesterolemia and high LDL cholesterol levels were associated with mesangial matrix increase, whereas high HDL cholesterol was associated with less vascular intima thickening. When all of these data are taken together, dyslipidemia seems to possess a deleterious effect on early posttransplantation histology, although donor vasculopathy could erroneously be attributed to CAN if baseline biopsy data were not available. There was an association between donor vasculopathy and serum creatinine at hospital discharge but not with subsequently measured creatinines, as others also have noted (23). Holdaas *et al.* (24) studied the effect of fluvastatin on graft loss or doubling of serum creatinine, without finding statistically significant differences between treatment and placebo groups during 5 yr of follow-up. In this study, in which 8% of the patients were taking statins at protocol biopsy time, we did not find any effect of lipid-lowering drugs on ΔCADI.

In conclusion, the progression of chronic kidney allograft damage is strongly influenced by the donor graft quality. Despite the relatively low number of patients and biopsy sampling bias that limit the conclusions of this study, the change in CADI over 6 mo seems to be a useful tool to distinguish patients with progressive histologic allograft damage early. Classical risk factors of atherosclerosis increased ΔCADI in this cohort. Thus, a more aggressive treatment of dyslipidemia and hypertension could possibly diminish the rate of CAN progression and subsequent graft loss.

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