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Reliability of chronic allograft nephropathy diagnosis in sequential protocol biopsies

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Background. Chronic allograft nephropathy (CAN) progresses rapidly during the first few months and slowly thereafter. Although the presence of CAN in protocol renal biopsies is a predictor of outcome, the reliability of this diagnosis according to Banff criteria has not been characterized.

Methods. Renal lesions were evaluated according to the Banff criteria in sequential protocol biopsies performed at 4 and 14 months in 310 biopsies obtained from 155 patients.

Results. CAN progressed from 40 to 53% ($P = 0.001$) while serum creatinine remained stable (146 ± 44 vs. 147 ± 48 μmol/L, P = NS). Graft survival in patients with and without CAN in the first biopsy was 74 versus 91% ($P < 0.05$), and in the second biopsy 75 versus 94% ($P < 0.05$). In 54 patients (35%) no CAN was present in both biopsies, 39 (25%) showed progression to CAN, 19 (12%) showed regression of CAN, and 43 (28%) showed CAN in both biopsies. Graft survival was: 100%, 81.6%, 82.6% and 69.4%, respectively ($P < 0.01$). Assuming that CAN does not regress and sampling error is normally distributed, we estimated that 25% of biopsies cannot be properly classified.

Conclusions. The increase in the incidence of CAN between the 4th and 14th month is lower than the proportion of misclassified biopsies. Thus, monitoring the progression of CAN by means of two sequential biopsies at 4 and 14 months is inaccurate. We suggest that progression of scarring be monitored by means of a donor and a protocol biopsy performed during the first year evaluated with a quantitative approach.

Chronic allograft nephropathy (CAN) is the most common cause of late renal allograft failure [1] clinically characterized by a slow deterioration of renal function [2]. Histologically, CAN is defined according to the Banff schema, a classification system that relies on the semiquantitative evaluation of chronic renal damage in the different renal compartments [3]. Histological confirmation of CAN in patients with slowly deteriorating renal function usually shows that the degree of renal scarring is advanced, probably beyond the threshold of reversibility. Accordingly, it seems unreasonable to expect that at this stage of scarring a therapeutic maneuver may significantly slow renal function deterioration [4].

Some centers have performed protocol biopsies as an attempt to diagnose CAN at an earlier stage. In these studies, the timing of histological evaluation has been targeted during the first two years after transplant. Nevertheless, CAN was a frequent finding in all of the studies, ranging between 25 and 70% [5–9]. In studies of sequential protocol biopsies it has been observed that the incidence and severity of CAN is time dependent [10–14]. Furthermore, in all of the previously mentioned studies there is agreement that the presence of CAN in stable grafts is an independent predictor of renal allograft outcome. Taken together, these results suggest that the presence of scarring in protocol biopsies could be considered as a primary efficacy variable in a hypothetical clinical trial aimed to prevent CAN [15, 16]. However, neither the accuracy of the diagnosis of CAN in sequential protocol biopsies nor the ideal timing of biopsies to detect patients at risk of graft failure have been properly evaluated.

The present study analyzed the evolution of renal lesions evaluated according to the Banff schema in two sequential protocol biopsies performed at 4th and 14th months after transplantation.

METHODS

Patients

Since June 1988 a prospective study of protocol renal allograft biopsies has been conducted in our center [7, 15]. A first protocol renal allograft biopsy was performed at approximately 3 months in patients who gave their informed consent and fulfilled the following criteria: (*a*) serum creatinine < 300 μmol/L; (*b*) proteinuria

Key words: Banff criteria, transplantation, graft outcome, renal lesions, kidney graft, classification of biopsies.

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<1 g/24 h; and (*c*) stable renal function, defined as variability of serum creatinine of less than 15% during two weeks before and after biopsy. A second protocol biopsy was performed at approximately one year of follow-up regardless of serum creatinine or proteinuria. For the present study, only patients biopsied until December 1996 were included.

Definition of clinical variables

The following variables were evaluated in each patient at the time of surgery: donor type (heart beating or non-heart beating), age and sex of the donor and the recipient, presence of hepatitis C virus antibodies, etiology of end-stage renal disease, time on dialysis, last panel reactive antibodies, number of HLA mismatches, and cold ischemia time (CIT). After surgery, the presence of delayed graft function and acute rejection were evaluated. At the time of protocol biopsies and during follow-up, serum creatinine, calculated glomerular filtration rate (GFR) by means of the Cockroft-Gault formula [17], proteinuria, blood pressure, cyclosporine (CsA) dose and CsA levels were recorded. Mean CsA level and dose at the time of the first biopsy were calculated as a weighted-time mean from CsA levels and doses at one week, two weeks, one month, two months, and CsA level and dose at the time of the first biopsy. Mean CsA level and dose between biopsies were calculated from CsA levels and doses at four, six, 12 months, and CsA level and dose at the time of the second biopsy.

Total number of HLA mismatches was calculated as the addition of the number of mismatches in the A, B and DR loci. Delayed graft function was defined as hemodialysis requirements during the first week after surgery once accelerated or hyperacute rejection, vascular complications and urinary tract obstruction were ruled out. The diagnosis of acute rejection was defined as an acute rise of serum creatinine >30% that responded to antirejection therapy. A diagnostic biopsy at the time of serum creatinine worsening was done in 75% of patients. Hypertension before and after transplantation was defined as a mean arterial pressure >107 mm Hg (blood pressure of ~140/90 mm Hg) and/or the requirement of antihypertensive drugs. During this period of time, different combinations of immunosuppressive drugs have been employed: (*a*) CsA and prednisone (N = 10), (*b*) concomitant induction therapy with antilymphocytic antibodies, CsA and prednisone (N = 87); (*c*) triple standard regimen with CsA, azathioprine and prednisone (N = 18); (*d*) triple regimen with CsA, mycophenolate mofetil and prednisone (N = 35); and (*e*) induction therapy with antilymphocytic antibodies, mycophenolate mofetil and prednisone (N = 5) [15].

Biopsies

Biopsies were performed and processed for routine light microscopy as previously described [7]. Tissue was embedded in paraffin, cut into 4-μm sections and stained with hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome and silver methenamine. Renal lesions were graded and diagnosed according to the 1997 Banff schema by two observers in the absence of any clinical information [3]. Protocol biopsies were not available to clinicians and consequently were not employed to make any clinical decisions.

Statistics

Results are expressed as the mean ± standard deviation. Comparison between paired data was performed by means of Chi square test, Wilcoxon test and *t* test for repeated measures. Comparison between unpaired data was performed by means of the chi-square test, Student *t* test, Mann-Whitney U test, and the analysis of variance (ANOVA) with the Scheffé test for individual comparisons. Spearman's correlation was employed to analyze the relationship between ordinal parameters. Kaplan-Meier analysis was used to calculate graft survival and the Mantel-Cox log-rank test was used to compare survival between groups. For survival analysis, patients dying with a functioning graft were censored. All P values were two-tailed and a P value <0.05 was considered significant.

RESULTS

Patients

During the study period a first and second protocol biopsy were done in 191 patients. Sufficient tissue for histological evaluation was not obtained in 17 first and 19 second biopsies. Thus, 310 biopsies performed in 155 patients were included. Follow-up time ranged between 5 and 12 years. Demographic characteristics of patients are summarized in Table 1.

Biopsies and sampling adequacy

The first biopsy was performed at approximately 4 months (113 ± 46 days) and the second biopsy was done just after the first year (435 ± 70 days). Mean number of glomeruli was 12 ± 7 and 11 ± 7, respectively. All biopsies contained at least one glomerular and one arterial section and sample size distribution according to 1997 Banff criteria in the 310 biopsies was: 173 (55.8%) biopsies were adequate (≥10 glomeruli, ≥2 arteries), 72 (23.2%) biopsies represented minimum sample adequacy (between 7 and 9 glomeruli and at least one arterial section) and 65 (21.0%) biopsies were inadequate (between 1 and 6 glomeruli and at least one arterial section).

Temporal evolution of renal lesions

During this period of time no significant modification in the intensity of acute lesions was observed while the

Table 1. Patient characteristics

Variable	Mean ± SD	Range
Donor age *years*	35 ± 17	6–76
Donor sex *male/female*	107/48	
HBD/NHBD	140/15	
Recipient age *years*	43 ± 13	15–71
Recipient sex *male/female*	94/61	
Number of transplant *1st/2nd/3rd*	138/16/1	
HCV *positive/negative*	23/132	
Etiology of end-stage renal disease		
Glomerular	57 (37%)	
Interstitial	37 (24%)	
APKD	23 (15%)	
Nephrosclerosis	8 (5%)	
Unknown	30 (19%)	
Time on dialysis *months*	29 ± 30	0–195
Last PRA %	8 ± 18	0–100
HLA A+B+DR mismatches	2.9 ± 1.1	0–5
Cold ischemia time *hours*	24 ± 6	10–46
Delayed graft function *yes/no*	28/127	
Acute rejection *yes/no*	32/123	

Abbreviations are: HBD, heart-beating donor; NHBD, non-heart beating donor; HCV, hepatitis C virus antibodies; APKD, adult polycystic kidney disease; PRA, panel reactive antibodies.

Table 2. Evolution of histologic lesions

Lesion type	First protocol biopsy	Second protocol biopsy	*P*
g	0.10 ± 0.34	0.13 ± 0.46	NS
i	0.69 ± 0.73	0.58 ± 0.64	NS
t	0.44 ± 1.0	0.30 ± 0.50	NS
v	0	0	NS
ah	0.17 ± 0.4	0.21 ± 0.48	NS
cg	0.17 ± 0.38	0.35 ± 0.56	0.0036
ci	0.46 ± 0.61	0.73 ± 0.77	0.0003
ct	0.46 ± 0.63	0.69 ± 0.75	0.0028
cv	0.06 ± 0.27	0.16 ± 0.50	0.035

Abbreviations are: g, glomerulitis; i, interstitial infiltrate; t, tubulitis; v, vasculitis; ah, arteriolar hyalinosis; cg, chronic glomeruli; ci, chronic interstitium; ct, chronic tubuli; cv, chronic vascular.

severity of chronic lesions increased in all renal compartments (Table 2). Despite progression of renal scarring, serum creatinine and calculated GFR as well as mean arterial pressure at the time of the first and second protocol biopsies remained unchanged. A significant but slight increase of proteinuria was observed. According to usual clinical practice, CsA doses and CsA levels were lower at the time of the second protocol biopsy (Table 3).

To study the degree of correlation between Banff scores in the first and second biopsies, a matrix correlation between Banff scores in both biopsies was built. The following significant positive correlations were observed: g-score in the first and second biopsy (rho = 0.20, $P = 0.01$), i-score in the first and second biopsy (rho = 0.31, $P = 0.001$), ah-score in the first and second biopsy (rho = 0.18, $P = 0.03$), ci-score in the first and second biopsy ($r = 0.30$, $P = 0.0002$) and ct-score in the first and second biopsy (rho = 0.28, $P = 0.004$). No correlation was found between the degree of acute lesions in the first biopsy and chronic lesions in the second biopsy. There was a positive correlation between ci-score and ct-score in the first and cv-score in the second biopsy (rho=0.23, $P = 0.004$, and rho=0.21, $P = 0.007$, respectively).

Table 3. Evolution of clinical parameters

	First protocol biopsy	Second protocol biopsy	*P*
Creatinine *μmol/l*	146 ± 44	147 ± 48	NS
Calculated GFR *mL/min/1.73 m²*	53 ± 14	54 ± 14	NS
Mean arterial pressure *mm Hg*	101 ± 12	104 ± 12	NS
Proteinuria *g/24 hr*	0.31 ± 0.21	0.38 ± 0.52	0.044
CsA dose *mg/kg/day*	5.4 ± 2.3	4.1 ± 1.5	0.0001
CsA level *ng/mL*	216 ± 74	159 ± 48	0.0001

Abbreviations are: GFR, glomerular filtration rate; CsA, cyclosporine.

Table 4. Concordance between the diagnosis of chronic allograft nephropathy (CAN) in the first and second biopsies ($P = 0.01$)

	2nd biopsy without CAN	2nd biopsy with CAN
1st biopsy without CAN	54 (34.8%)	39 (25.2%)
1st biopsy with CAN	19 (12.2%)	43 (27.7%)

Temporal evolution of histological diagnostic categories

Histological diagnoses according to the Banff criteria in the first protocol biopsy were: normal ($N = 66$), borderline ($N = 24$), acute rejection ($N = 3$), CAN ($N = 38$), CAN associated with borderline changes ($N = 20$) and CAN associated with acute rejection ($N = 4$). Histological diagnoses in the second protocol biopsy were: normal ($N = 64$), borderline ($N = 8$), acute rejection ($N = 1$), CAN ($N = 53$), CAN associated with borderline changes ($N = 28$), and CAN associated with acute rejection ($N = 1$). Thus, in the first protocol biopsy CAN was diagnosed in 62 of 155 (40.0%) patients and in the second protocol biopsy in 82 of 155 (52.9%) patients ($P = 0.001$). Not only the proportion but also the severity of CAN increased in the second biopsy. Grading of CAN in the first was: absent in 93 (60.0%) patients, mild in 53 (34.2%), moderate in 9 (5.8%), and no cases showed severe CAN. In the second biopsy CAN was: absent in 73 (47.1%), mild in 54 (34.8%), moderate in 27 (17.4%), and severe in 1 (0.6%; $P = 0.01$).

A 2 × 2 contingency table in which the histological diagnosis was summarized as presence or absence of CAN was employed to study the concordance of the diagnosis of CAN in both biopsies (Table 4). In 97 patients (62.5%) there was agreement in the diagnosis obtained in both sequential biopsies. In 39 patients (25.2%)

Table 5. Evolution of CAN in patients with two biopsies containing ≥7 glomeruli (minimum sample adequacy in both biopsies) and in patients with at least one biopsy containing <7 glomeruli (indequate sampling) (P = NS)

	≥7 glomeruli	<7 glomeruli
Both biopsies without CAN	34 (35.1%)	20 (34.4%)
Progression to CAN	22 (22.6%)	17 (30.9%)
Regression of CAN	12 (12.4%)	7 (12.1%)
Both biopsies with CAN	29 (29.9%)	14 (24.1%)
Total	97	58

not showing CAN in the first protocol biopsy, CAN was diagnosed in the second. In 19 patients (12.2%) CAN was diagnosed in the first biopsy but not in the second. In the group of patients not showing CAN in the first biopsy, 27 patients progressed to CAN grade I and 12 patients progressed to CAN grade II or III. On the contrary, all but one of the 19 patients who apparently regressed were diagnosed of CAN grade I in the first biopsy.

Sample size adequacy and diagnosis of CAN

To evaluate whether apparent regression of CAN in the second biopsy is due to inadequate sampling, the concordance of the diagnosis of CAN was studied in patients with at least 7 glomeruli (minimum sample adequacy) in both biopsies and in patients with at least one biopsy containing <7 glomeruli (inadequate sample). As shown in Table 5, evolution of CAN was not different in both groups.

Concordance of the diagnosis of CAN and outcome

To evaluate whether apparent regression of CAN in a proportion of patients represents the inaccuracy of the diagnostic criteria or a real regression of chronic lesions, serum creatinine, proteinuria at the time of first and second protocol biopsies and renal allograft survival were compared in the four previously mentioned groups. As shown in Table 6, serum creatinine and proteinuria values were low in patients not displaying CAN in both biopsies, intermediate in patients showing either progression or regression of CAN and high in patients diagnosed of CAN in both biopsies. Similarly, renal allograft survival was excellent in patients without CAN, intermediate in patients in whom CAN apparently progressed or regressed, and poor in patients displaying CAN in both biopsies (Table 6). Despite patients who progressed and patients who regressed showed a similar clinical evolution, the probability to progress was higher than the probability to regress (25% vs. 12%, $P < 0.05$).

Clinical and histological variables associated with the progression of CAN

Patients were classified as progression or no progression of CAN. Progression of CAN was defined as the presence of CAN in the second biopsy in patients with a first biopsy not showing CAN or the increase in the CAN grade between the first and second biopsy. The degree of chronic lesions in the first biopsy was lower in patients who progressed. Mean CsA level until the first biopsy was similar in both groups while the mean CsA level between the first and second biopsies was lower in patients who progressed. However, the CsA dose until the first biopsy and between biopsies was similar in both groups (Table 7). None of the following variables was different between the no progression and progression groups: donor age (35 ± 18 vs. 37 ± 15 years) and sex (29 vs. 35% females), recipient age (44 ± 14 vs. 43 ± 12 years) and sex (37 vs. 43% females), last panel reactivity antibodies (6 ± 20 vs. 10 ± 20%), number of HLA mismatches (2.8 ± 1.1 vs. 2.9 ± 1.0), cold ischemia time (24 ± 7 vs. 23 ± 6 hours), the proportion of patients treated with mycophenolate mofetil (25 vs. 27%), delayed graft function (19 vs 16%), acute rejection (21 vs. 20%), serum creatinine (144 ± 41 vs. 148 ± 49 μmol/L), calculated GFR (54 ± 14 vs. 52 ± 14 mL/min/1.73 m^2), mean arterial blood pressure (101 ± 11 vs. 102 ± 14 mm Hg) or proteinuria (0.28 ± 0.19 vs. 0.32 ± 0.21 g/day) at the time of the first biopsy.

Prediction of graft survival

In order to study which timing of protocol biopsy allowed a better prediction of outcome, graft survival according to the presence or absence of CAN in the first and second protocol biopsies was studied separately by means of Kaplan-Meier analysis. Graft survival in patients with and without CAN was 74 and 91% in the first biopsy ($P < 0.05$) and 75 and 94% in the second biopsy ($P < 0.05$).

DISCUSSION

The present study shows that chronic renal allograft lesions progress between the 4th and 14th month, while serum creatinine, calculated GFR and arterial pressure remain stable. The only clinical modification between the two protocol biopsies was a slight increase of proteinuria that, despite reaching statistical significance, was too small to be considered of any clinical relevance. During this period of time, the CsA dose was tapered and accordingly CsA levels decreased. This modification may partly explain the lack of deterioration in renal function despite the progression of renal scarring [18]. Together these results point out that the correlation between histological damage and clinical parameters in transplanted patients is not good enough to consider either renal function or proteinuria as a reliable marker of the progression of CAN [6, 7, 15]. This observation reinforces the notion that protocol biopsies allow information to be obtained that is not contained in clinical data.

Table 6. Serum creatinine (μmol/L) and proteinuria (g/24 h) in the first and second protocol biopsy in patients displaying: both biopsies without CAN, progression of CAN, regression of CAN and both biopsies with CAN

	No CAN	Progression	Regression	CAN in both biopsies	*P*
N	54	39	19	43	
1st biopsy					
S_{Cr}	131 ± 31	147 ± 51	158 ± 48[a]	158 ± 44[a]	0.001
Proteinuria	0.22 ± 0.12	0.31 ± 0.17[a]	0.34 ± 0.17[a]	0.39 ± 0.28[a]	0.0003
2nd biopsy					
S_{Cr}	128 ± 32	151 ± 48[a]	151 ± 37	169 ± 60[a]	0.0003
Proteinuria	0.18 ± 0.12	0.29 ± 0.22	0.38 ± 0.42	0.71 ± 0.85[a,b,c]	0.0001
Survival %	100	81.6	82.6	69.4	0.0020

Abbreviations are: CAN, chronic allograft nephropathy; S_{Cr}, serum creatinine.
[a, b and c] $P < 0.05$ between that value and No CAN group, progression and regression groups respectively (Scheffé test)

Table 7. Clinical and histological characteristics of patients according to the progression of CAN between the first and second biopsies

	No progression of CAN	Progression of CAN	*P*
N	104	51	
First biopsy Banff scores			
g-score	0.11 ± 0.37	0.10 ± 0.30	NS
i-score	0.71 ± 0.76	0.67 ± 0.68	NS
t-score	0.36 ± 0.57	0.16 ± 0.21	NS
v-score	—	—	
ah-score	0.17 ± 0.18	0.16 ± 0.21	NS
cg-score	0.19 ± 0.39	0.14 ± 0.35	NS
ci-score	0.57 ± 0.65	0.23 ± 0.43	<0.001
ct-score	0.57 ± 0.68	0.25 ± 0.44	<0.001
cv-score	0.07 ± 0.30	0.06 ± 0.26	NS
N	102	48	
Mean CsA dose at 1st biopsy	5.2 ± 1.7	5.3 ± 2.1	NS
Mean CsA dose between biopsies	4.0 ± 1.3	4.2 ± 1.8	NS
Mean CsA levels at 1st biopsy	218 ± 72	217 ± 74	NS
Mean CsA levels between biopsies	165 ± 50	149 ± 41	0.04

Cyclosporine (CsA) levels (ng/mL) were determined in 102 and 48 patients in the no progression of CAN and progression of CAN groups, respectively, since five patients did not receive cyclosporine.

The severity of acute and chronic lesions observed in a renal compartment in the first biopsy correlated with the same type of lesion in the same compartment in the second biopsy. In contrast with the progression of chronic lesions, acute lesions remained stable. In sequential protocol biopsies, Rush, Jeffery and Gough [10] and Legendre et al [12] observed that acute lesions peak during the first few months and decrease thereafter, while chronic lesions progress during the first year. In contrast, Nankivell et al showed that acute lesions peak at three months while chronic lesions remain stable during the first year [19]. We previously observed that the number of interstitial infiltrating cells assessed with monoclonal antibodies decreased during the first year, while acute lesions assessed with an ordinal scale apparently remained stable [20]. We did not find any correlation between acute lesions in the early biopsy and chronic lesions in the second one, in contrast to other reports [10, 14, 19, 21]. It is difficult to find a reasonable explanation for these differences, but the timing of biopsies, patient characteristics and immunosuppression were not the same in the different centers. Also, inter-observer variability could account for some of these differences [22].

In a subset of patients diagnosed of CAN at four months no chronic lesions were observed in the one-year biopsy. This result raises the question whether apparent regression of CAN reflects sampling error, intraobserver variability or a real regression of chronic damage. The present study's biopsies were evaluated according to Banff criteria, which have been devised to evaluate diagnostic but not protocol biopsies. Despite that CAN represents a continuum**,** according to the Banff schema it is diagnosed when tubular atrophy is present and interstitial fibrosis is observed in more than 5% of the biopsy [3]. The extension of interstitial fibrosis and tubular atrophy is used to grade CAN as mild (up to 25%), moderate (26–50%) and severe (>50%). In such a grading system, intra- and interobserver variability increases as the extension of damage approaches the cut-off separating two categories. In the present study, the majority of patients displayed either no CAN or grade I CAN. Accordingly, in a proportion of biopsies, the degree of chronic damage should have been in the cut-off separating both catego-

ries. This notion is reinforced by the observation that graft survival was low in patients displaying CAN in both biopsies, excellent in patients not displaying CAN in any biopsy, and intermediate in patients with progression or regression of CAN. We interpret that in patients displaying CAN in both biopsies, the degree of interstitial fibrosis and tubular atrophy was clearly over the threshold of 5% and in patients not displaying CAN in both biopsies, no chronic lesions were present at all. In patients with either apparent progression or regression, the degree of chronic lesions was around the cut-off separating CAN from no CAN, and accordingly the probability to misclassify such a pair of biopsies was the highest.

Progression of CAN was observed in 25% of patients while regression was observed only in 12%, confirming that the probability to progress was higher than the probability to regress. Approximately half of the patients who progressed displayed CAN grade II or III in the second biopsy while the other half displayed CAN grade I. Nearly all patients with apparent regression displayed CAN grade I in the first biopsy. Accordingly, this result suggests that apparent regression reflects the difficulty to properly classify biopsies with mild interstitial fibrosis and tubular atrophy.

The present data allow an evaluation of the accuracy of Banff criteria to diagnose CAN in protocol biopsies. If we assume that during the study period real regression of scarring has not occurred, then 12% of the patients with apparent regression of CAN were misclassified. A similar proportion of patients with progression to CAN must also have been misclassified if we assume that the distribution of sampling error follows a normal distribution. Thus, in our hands the evaluation of protocol biopsies with Banff criteria implies a misclassification of approximately 25% of the cases. This number represents both sampling error associated with the performance of two sequential biopsies and intraobserver variability.

Despite the estimated error of 25% in the diagnosis of CAN, we studied which clinical variables and histological lesions in the first biopsy were associated with the progression of CAN. Patients displaying less severe chronic lesions at four months had a higher probability to progress. This result is probably a consequence of the grading system employed. Mild progression of fibrosis in patients not displaying CAN in the first biopsy (interstitial fibrosis in less than 5% of biopsy surface) will result in progression to CAN. On the contrary, in patients already displaying CAN grade I (interstitial fibrosis affecting 6 to 25%) mild progression of interstitial fibrosis, for example from 10 to 20%, will not result in the progression of CAN. On the other hand, an association between progression of CAN and lower mean CsA levels between the first and second biopsy was observed. The interpretation of this result is difficult since the immunosuppression was heterogeneous in the studied set of patients, but raises concern about a possible detrimental effect of low cyclosporine levels during the first year [23].

According to Banff schema an adequate sample must contain at least ten glomeruli and two arteries. This condition has been defined to properly diagnose acute rejection but not CAN, since the distribution of acute lesions is rather variable [3]. No relationship could be established between biopsy adequacy and the frequency distribution of the diagnosis of CAN, suggesting that the sample size requirement for a diagnosis of CAN may be less than for acute rejection. This observation suggests that even small samples can be considered viable to evaluate the extension of chronic tubulointerstitial damage, especially if they are evaluated by means of quantitative measures [11, 16, 24–26].

The incidence of CAN progressed from 40 to 53% during the study period in the overall group. This increase is relatively moderate if we take into consideration that the incidence of lesions that mimic CAN in blindly evaluated donor biopsies at our center is 15% [15]. Thus, renal allograft scarring progresses rapidly during the first few months and slowly thereafter. This observation may have important implications in the design of trials aimed to prevent renal scarring. In a trial in which progression of scarring is evaluated by means of two sequential biopsies, the ideal timing of biopsies to reduce sample size requirements will be defined according to the shortest follow-up period in which the highest progression occurs. The present data do not favor the utilization of two protocol biopsies done during the first year, but suggest that the best strategy to monitor progression of chronic lesions is to perform a donor and a protocol biopsy during the first year [15, 16].

In summary, only a moderate increase in the incidence and severity of CAN occurs between the 4 and 14 month. Moreover, in approximately 25% of biopsies the diagnosis of CAN is misclassified. Taken together, these data suggest that evaluation of two sequential biopsies by means of the Banff schema is not the ideal strategy to monitor progression of renal scarring. Thus, we suggest monitoring the progression of scarring by means of a donor and a protocol biopsy performed during the first year and evaluated with a quantitative approach.

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