*Kidney International, Vol. 51 (1997), pp. 310–316*

# Early protocol renal allograft biopsies and graft outcome

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**Early protocol renal allograft biopsies and graft outcome.** To evaluate whether biopsies performed early after transplantation in stable grafts can predict graft failure due to chronic transplant nephropathy, a protocol biopsy was performed at three months in 98 patients treated with antilymphocytic antibodies, cyclosporine and prednisone. Patients were followed for 58 ± 16 months. Histological diagnosis according to the Banff schema were: normal ($N$ = 41), borderline changes ($N$ = 12), chronic transplant nephropathy (CTN; $N$ = 30), CTN associated to borderline changes ($N$ = 11) and acute rejection ($N$ = 4). Biopsies displaying acute rejection were not considered for statistical analysis. Since clinical characteristics of patients with normal histology and borderline changes, as well as characteristics of patients displaying CTN either with or without tubulitis were not different, biopsies were grouped as presence or absence of CTN. Patients displaying CTN had an increased incidence of acute rejection before performing biopsy (24.3 vs. 3.9%, $P$ = 0.003), a higher mean cyclosporine level until biopsy (242 ± 74 vs. 214 ± 59 ng/ml, $P$ = 0.049) and a lower actuarial graft survival (80.5% vs. 94.4%, $P$ = 0.024). We conclude that early protocol biopsies are useful to detect patients at risk of losing their graft due to chronic transplant nephropathy.

The incidence of acute rejection and consequently, the proportion of grafts lost during the first year after transplantation have decreased since the introduction of cyclosporine [1]. However, the rate of grafts lost after the first year remains unchanged [2]. Chronic transplant nephropathy is the most common cause of late graft failure [3]. The possibility of graft failure is suspected by a sustained and irreversible decline of renal function usually in conjunction with proteinuria and hypertension [4]. Unfortunately, once the clinical diagnosis is histologically confirmed, the degree of renal scarring is usually too advanced to try any potentially effective therapy [5].

The Banff classification of kidney transplant pathology [6, 7] has contributed to international uniformity in the evaluation of renal allograft biopsies. However, limited information is available on the clinical implications of this classification system. This is especially true in the case of protocol biopsies which can display variable degrees of acute and chronic renal lesions [8–12]. Therefore, the aim of the present work was to characterize renal damage in biopsies performed in stable renal allografts early after transplantation according to the Banff schema, and to evaluate whether these lesions may help to predict graft loss due to chronic transplant nephropathy.

Received for publication February 26, 1996
and in revised form July 9, 1996
Accepted for publication August 12, 1996



## Methods

### *Patients*

Between June 1988 and December 1992, a total of 289 patients received a cadaveric renal transplant in our center. A protocol renal allograft biopsy was performed in stable grafts between the second and the fifth months of follow-up if they fulfilled the following inclusion criteria: (a) serum creatinine lower than 200 μmol/liter at the time of biopsy; (b) variability of serum creatinine of less than 15% during two weeks before and after biopsy; (c) proteinuria lower than 1 g/24 hours. Patients who suffered from post-transplant acute tubular necrosis and patients who presented an episode of acute rejection before performing the protocol biopsy were included if they fulfilled the inclusion criteria.

Since the aim of the study was to evaluate whether there is a relationship between biopsy findings in stable grafts and graft failure due to biopsy-proven chronic transplant nephropathy, patients who experienced an episode of acute rejection after biopsy and patients who lost their graft due to any reason other than chronic transplant nephropathy were excluded.

In all patients with a sustained decline of renal function a biopsy was performed to investigate the cause of late graft failure.

### *Definition of clinical variables*

The following variables were evaluated in each patient at the time of surgery: age and sex of the recipient, peak and last panel reactivity antibodies, number of HLA mismatches, donor age, cold ischemia time (CIT) and immunosuppressive treatment. After surgery, the following variables were evaluated: time of biopsy, body wt, post-transplant acute tubular necrosis, acute rejection, proteinuria and serum creatinine at the time of biopsy, evolution of serum creatinine during follow-up, mean cyclosporine dose and mean cyclosporine level between surgery and renal biopsy, and graft loss due to biopsy-proven chronic transplant nephropathy.

The immunosuppressive treatment used in our center has been previously described [13]. Briefly, patients received either antilymphocytic globulin (ALG) or OKT3 as induction therapy and cyclosporine (CsA) and prednisone as maintenance treatment. ALG was given at a dose of 15 mg/kg just before surgery, 12 mg/kg the first day after transplant, followed by four doses of 10 mg/kg on alternate days. OKT3 was given at a dose of 5 mg just before

transplant, followed by four daily doses of 5 mg. Cyclosporine was started at 3 mg/kg i.v. before surgery and continued at 1 mg/kg/12 hours i.v. postoperatively until the oral route could be started at 8 mg/kg/day. Dose modifications were done according to CsA whole blood levels (RIA, monoclonal). Prednisone was started at 0.25 mg/kg/day and was gradually tapered to 0.1 mg/kg/day in two to three months.

Post-transplant acute tubular necrosis was defined as hemodialysis requirements after surgery once hyperacute or accelerated rejection, vascular complications and urinary tract obstruction were ruled out.

The diagnosis of acute rejection was based on classical clinical data and in most instances histological criteria. Rejection episodes were treated with three boluses of 500 mg of methylprednisolone. In this group of patients there were no episodes of steroid-resistant acute rejection.

The mean cyclosporine dose received until biopsy was calculated from the cyclosporine dose at days 1, 7, 14, 30, 60, 90 and the day of biopsy. The mean cyclosporine level was calculated from cyclosporine levels at the same periods of time.

### *Biopsies*

Biopsies were performed with a standard 14 gauge Tru-cut needle (Travenol, Deerfield, IL, USA) until May 1990. At this time, the protocol was stopped since a patient presented an episode of hematuria with ureteral obstruction that was successfully treated. On February 1991 the protocol was restarted using a spring-loaded 18 gauge needle (C.R. Bard Inc., Covington, GA, USA) under ultrasound guidance. Apart from three episodes of minor hematuria, no other complications were seen.

Two cores of tissue were obtained in each case; one was processed for routine histology and the other was snap frozen until use.

*Optical microscopy.* Biopsies were formalin fixed, embedded in paraffin, serially sectioned at approximatelly 4 $\mu$m thick and stained with hematoxylin-eosin, periodic acid-Schiff (PAS), Masson's trichrome and silver methenamine.

Biopsies that did not contain glomeruli or at least one artery were not considered. Renal lesions were evaluated and diagnosed according to the Banff schema [6, 7] by two observers in the absence of any clinical information. Protocol biopsies were not available to clinicians and consequently were not employed to make any clinical decision.

*Morphometry.* Cortical interstitial volume fraction (Vvint/cortex) was measured with a point counting technique at $\times$400 magnification on silver methenamine stained sections as previously described [14].

*Number of infiltrating leukocytes.* Frozen sections were stained using the avidin biotin method with a monoclonal antibody directed against common leukocyte antigen CD45 (PD7/26; Dako, Denmark). The number of positively stained cells was counted at $\times$400 magnification and the result was expressed as the number of infiltrating cells/mm$^2$ of cortical interstitium as previously described [15].

### *Statistics*

Results were expressed as the mean $\pm$ SD.

In the first statistical approach, analysis of variance was employed to compare quantitative data in the different diagnostic categories and the Scheffé test was used for individual comparisons. The Kruskall-Wallis and Chi-square tests were applied for ordinal and categorical data, respectively.

The result of this first analysis suggested to group patients in two new diagnostic categories: presence or absence of CTN. Clinical data in these two new categories were compared either with Student's $t$-test, the Mann-Whitney U-test or $2 \times 2$ contingency tables.

To further analyze independent predictors of CTN, a logistic-regression model was applied [16].

Kaplan-Meier analysis was used to calculate actuarial graft survival and the log-rank test was employed to compare survival between groups. In order to study the independent predictors of graft survival, data were further analyzed by means of Cox's proportional hazard model.

For factors with only two categories, logistic-regression and Cox's proportional hazard model were used to calculate the relative risk estimate. For continuous factors, the relative risk estimate per unit of measurement was calculated [17]. Independent variables were checked for co-linearity.

All $P$ values were two tailed and a $P$ value $< 0.05$ was considered significant.

## Results

### *Patients*

One hundred twenty-six patients gave their consent to participate in this study. In 4 cases cortical tissue was not obtained and in 17 cases only one core of frozen tissue was available. These 21 cases were not included in the study. Seven more patients were excluded for the following reasons: death with a functioning graft ($N = 2$), graft loss due to membranoproliferative glomerulonephritis associated to cryoglobulinemia and hepatitis C virus infection ($N = 2$), acute rejection after the protocol biopsy ($N = 2$) and non-treatment compliance ($N = 1$).

Finally, 98 patients were included: 58 males and 40 females of a mean age of 41 $\pm$ 13 years. Eighty-nine patients received a first and 9 a second cadaveric renal transplant. Fifty-one were treated with ALG as the induction therapy and 47 with OKT3. Twelve patients were treated for an acute rejection episode 75 $\pm$ 34 days before the protocol biopsy. Mean follow-up was 58 $\pm$ 16 months (range 25 to 85) and during this period of time 11 patients lost their graft at 38 $\pm$ 9 months (range 25 to 51 months). In these 11 patients urinary tract obstruction, chronic urinary tract infection and renal transplant artery stenosis were ruled out. A diagnostic renal biopsy was performed in all cases. In 7 cases at least one artery showed myointimal proliferation, and consequently they were diagnosed with chronic rejection. In the other 4 patients the renal arteries did not show myointimal proliferation. They were diagnosed with chronic transplant nephropathy grade 2 ($N = 2$) and grade 3 ($N = 2$). In one of these four biopsies, severe hyaline arteriolar lesions were present, however, despite this the patient did not show any episode of acute cyclosporine toxicity.

### *Histological diagnosis*

The mean number of glomeruli per biopsy was 10.2 $\pm$ 5.6. There were 7 or more glomeruli in 70 biopsies. Marginal specimens contained 4.2 $\pm$ 1.3 glomeruli. Frozen sections were available in 77 cases.

**Table 1.** Clinical characteristics of patients at the time of surgery according to histological diagnosis

| | Normal | Borderline changes | CTN | CTN and borderline changes | *P* |
|---|---|---|---|---|---|
| *N* | 41 | 12 | 30 | 11 | |
| Age *years* | 41 ± 13 | 41 ± 14 | 39 ± 14 | 49 ± 10 | NS |
| Sex *male/female* | 21/20 | 9/3 | 15/15 | 9/2 | NS |
| Peak PRA % | 9 ± 19 | 9 ± 17 | 11 ± 21 | 13 ± 20 | NS |
| Last PRA % | 4 ± 15 | 2 ± 4 | 5 ± 17 | 1 ± 4 | NS |
| A+B+DR mismatches | 2.7 ± 1.1 | 2.3 ± 1.1 | 2.9 ± 1.3 | 3.0 ± 0.9 | NS |
| Donor age *years* | 29 ± 13 | 27 ± 14 | 32 ± 14 | 35 ± 17 | NS |
| CIT *hours* | 24 ± 7 | 26 ± 6 | 24 ± 7 | 25 ± 4 | NS |
| Treatment *ALG/OKT3* | 21/20 | 8/4 | 14/16 | 6/5 | NS |

Abbreviations are: CTN, chronic transplant nephropathy; CIT, cold ischemia time; NS, not significant.

**Table 2.** Cortical interstitial volume fracction (Vvint/cortex) and number of interstitial infiltrating CD45 positive cells (leukocytes) in different diagnostic categories

| | Normal | Borderline changes | CTN | CTN+ borderline changes | *P* |
|---|---|---|---|---|---|
| *N* | 41 | 12 | 30 | 11 | |
| Vvint/cortex % | 14.3 ± 4.1 | 14.1 ± 3.7 | 18.1 ± 6.2[a] | 20.1 ± 4.1[ab] | 0.0003 |
| *N* | 34 | 9 | 22 | 10 | |
| CD45 *cells/mm²* | 493 ± 453 | 511 ± 293 | 481 ± 399 | 840 ± 189 | NS |

Abbreviation CTN is chronic transplant nephropathy.
[a] $P < 0.05$ versus normal biopsies
[b] $P < 0.05$ versus borderline biopsies

Histological diagnoses were categorized according to the Banff schema. The diagnostic categories were: normal ($N = 41$), borderline changes ($N = 12$), chronic transplant nephropathy ($N = 41$), and acute rejection ($N = 4$).

Chronic transplant nephropathy (CTN) was mild (grade I) in 34 cases and moderate (grade II) in 7 cases. In 11 out of 41 patients diagnosed of CTN, mild tubulitis was present. These biopsies with CTN and tubulitis were initially analyzed as a separate category (CTN and borderline changes). Mild vascular hyperplasia was observed in 4 cases.

Acute rejection was mild (grade I) in 3 cases and moderate (grade II) in 1 case. The number of biopsies in this diagnostic category was too low for statistical analysis.

Hyaline arteriolar lesions were observed only in four biopsies.

According to the above-mentioned considerations, four diagnostic categories were considered in the first statistical approach: normal ($N = 41$), borderline changes ($N = 12$), CTN ($N = 30$) and CTN + borderline changes ($N = 11$). In these 94 patients, frozen tissue was available in 75 cases. Characteristics of patients at the time of surgery are summarized in Table 1.

### *Interstitial volume fraction and interstitial infiltrating cells*

Cortical interstitial volume fraction (Vvint/cortex) and number of interstitial infiltrating leukocytes were quantified in order to evaluate its relationship to the four histological categories studied (Table 2). Vvint/cortex was increased in patients displaying CTN while the number of CD45-positive interstitial infiltrating cells was higher in the CTN + borderline changes group, but this difference did not reach statistical significance.

### *Clinical variables and diagnostic categories*

Characteristics of patients after surgery according to the four histological categories are summarized in Table 3. The incidence of acute rejection before biopsy was lower in patients with normal histology or borderline changes than in patients with CTN plus or minus borderline changes. Proteinuria was slightly increased in patients with CTN associated with borderline changes than in the other groups. Serum creatinine at the time of biopsy and serum creatinine at the first year of follow-up was higher in patients displaying CTN. For this reason, and in order to increase the number of cases to further analyze data, biopsies were grouped as the presence or abscence of chronic transplant nephropathy.

In this second analysis, the following variables were significantly higher in patients with CTN: the incidence of acute rejection, serum creatinine either at the time of biopsy, at one and two years of follow-up, as well as the mean cyclosporine level (Table 4). To study the independent predictors of CTN, a logistic-regression analysis was performed. The following predictive variables were considered: recipient's age and sex, donor age, body wt, post-transplant acute tubular necrosis, mean CsA level and acute rejection. Only acute rejection ($P = 0.015$, odds ratio: 17.4, 95% confidence interval 1.7 to 179) and mean CsA level ($P = 0.03$, odds ratio: 1.01, 95% confidence interval 1.005 to 1.2) were independent predictors of CTN.

### *Histological damage in biopsies with CTN according to the presence or absence of an episode of acute rejection before biopsy*

Since the incidence of CTN was higher in patients treated for an episode of acute rejection before the biopsy, we further compared biopsies with CTN in patients with ($N = 10$) or without ($N = 31$) a previous episode of acute rejection. Banff scores for acute lesions (glomerulitis, interstitial mononuclear cell infiltration, tubulitis and intimal arteritis) and the number of CD45 positive cells/mm² of cortical interstitium were not different in these two groups of biopsies (data not shown), but patients who were treated for an episode of acute rejection before protocol biopsy had an increased Vvint/cortex (22.3 ± 5.5% vs. 17.4 ± 5.4%, $P = 0.017$).

### *Graft survival according to the presence or absence of CTN*

The number of grafts lost during follow-up was 8 of 41 (19.5%) in patients displaying CTN and 3 of 53 (5.6%) in patients without CTN in the protocol biopsy. Actuarial graft survival (Fig. 1) was significantly lower in patients with CTN ($P = 0.024$).

Since the presence of an episode of acute rejection before protocol biopsy, cyclosporine exposure and serum creatinine at the time of biopsy were associated with the presence of CTN, the Cox's proportional hazard model was employed to analyze the influence of these four factors on graft survival.

Only serum creatinine ($P = 0.014$; hazard rate 1.026, 95% confidence interval 1.005 to 1.047) and presence or absence of CTN ($P = 0.03$; hazard rate 5.98, 95% confidence interval 1.15 to 31.25) were independent predictors of graft loss.

**Table 3.** Clinical characteristics of patients during follow-up according to histological diagnosis

| | Normal | Borderline changes | CTN | CTN and borderline changes | *P* |
|---|---|---|---|---|---|
| *N* | 41 | 12 | 30 | 11 | |
| ATN *yes/no* | 5/36 | 4/8 | 8/22 | 1/10 | NS |
| Acute rejection *yes/no* | 0/41 | 2/10 | 7/23 | 3/8 | 0.01 |
| Time of biopsy *days* | 68 ± 39 | 51 ± 27 | 80 ± 43 | 59 ± 32 | NS |
| Body weight *kg* | 61 ± 9 | 61 ± 15 | 63 ± 13 | 63 ± 10 | NS |
| Proteinuria *g/24 hr* | 0.31 ± 0.19 | 0.39 ± 0.17 | 0.27 ± 0.13 | 0.51 ± 0.23[ab] | 0.0024 |
| $S_{Cr}$ at the time of biopsy | 120 ± 31 | 142 ± 40 | 138 ± 32[a] | 161 ± 37[a] | 0.003 |
| $S_{Cr}$ at 1 year of follow-up | 118 ± 24 | 129 ± 34 | 146 ± 41[a] | 150 ± 37[a] | 0.0018 |
| $S_{Cr}$ at 2 years of follow-up | 133 ± 54 | 145 ± 54 | 171 ± 111 | 181 ± 87 | NS |
| Mean CsA dose *mg/kg/day* | 7.8 ± 1.6 | 8.3 ± 1.3 | 7.7 ± 2.0 | 8.0 ± 1.5 | NS |
| Mean CsA levels *ng/ml* | 211 ± 61 | 222 ± 57 | 240 ± 79 | 248 ± 62 | NS |
| Mean follow-up *months* | 58 ± 17 | 61 ± 16 | 51 ± 18 | 57 ± 16 | NS |
| Graft loss *yes/no* | 2/39 | 1/11 | 5/25 | 3/8 | NS |

Abbreviations are: $S_{Cr}$, serum creatinine (μmol/liter); ATN, acute tubular necrosis; CsA, cyclosporine.
[a] $P < 0.05$ versus normal biopsies
[b] $P < 0.05$ versus CTN

**Table 4.** Clinical characteristics of patients according to histological diagnosis

| | No CTN | CTN | *P* |
|---|---|---|---|
| *N* | 53 | 41 | |
| $S_{Cr}$ at the time of biopsy | 125 ± 35 | 145 ± 35 | 0.008 |
| $S_{Cr}$ at 1 year of follow-up | 121 ± 26 | 148 ± 40 | 0.0001 |
| $S_{Cr}$ at 2 years of follow-up | 136 ± 54 | 174 ± 105 | 0.024 |
| AR *yes/no* | 2/51 | 10/31 | 0.003 |
| Mean CsA levels *ng/ml* | 214 ± 59 | 242 ± 74 | 0.049 |

Abbreviations are: CTN, chronic transplant nephropathy; $S_{Cr}$, serum creatinine (μmol/liter); AR, clinical episode of acute rejection before performing the protocol biopsy; CsA, cyclosporine.

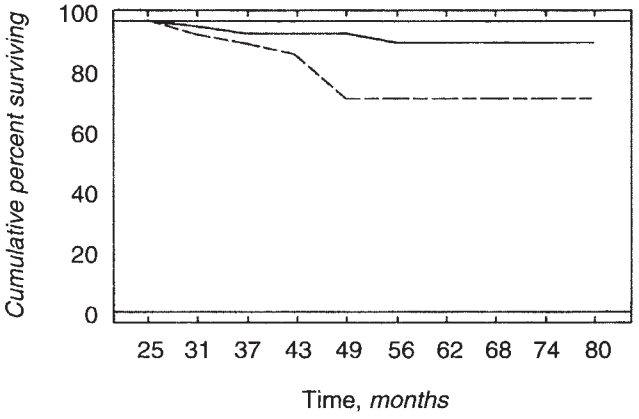


**Fig. 1.** *Actuarial graft survival in patients with chronic transplant nephropathy (dashed line) and in patients without CTN (solid line). P = 0.024.*

### *Histological diagnosis of acute rejection and graft outcome*

None of the four patients who showed histological signs of acute rejection in the protocol biopsy had a previous episode of acute rejection, all of them mantained a functioning graft at 61 ± 11 months (range 58 to 75), and their last serum creatinine was 161 ± 26 μmol/liter (range 143 to 199).

## Discussion

The principal findings of our study are that: (a) about 42% of biopsies performed in stable grafts early after transplantation displayed CTN according to the Banff diagnostic criteria; (b) acute rejection before performing the protocol biopsy and cyclosporine exposure were independent predictors of CTN; and (c) CTN was an independent predictor of graft survival.

The Banff schema [6, 7] has contributed to international uniformity in the evaluation of renal allograft biopsies, but there is still scanty information on the utility of this classification system in clinical practice, especially when it is applied to the evaluation of stable grafts [11, 12]. Renal function is not always included as a defining condition for stable grafts [11, 18], despite the facts that it is well known that serum creatinine and proteinuria are powerful predictors of graft outcome [19, 20], and both parameters correlate with the severity of acute and chronic renal lesions [21, 22]. In the present study, only patients with a serum creatinine level of less than 200 μmol/liter and a proteinuria of less than 1 g/24 hours were included. We used relatively restrictive criteria to define the graft's stability in an attempt to avoid an overestimation of the predictive value of protocol biopsies.

The incidence of chronic renal lesions was to a certain extent higher than expected if the early timing of protocol biopsies is taken into consideration, but the morphometric study confirmed that Vvint/cortex was significantly increased in patients diagnosed of CTN, although in the majority of these biopsies chronic lesions were graded as mild. Isoniemi et al [11] reported that about 2/3 of stable grafts displayed chronic lesions at two years of follow up. In their study, chronic renal lesions were more severe, which is not surprising as protocol biopsies were performed later. By contrast, in sequential biopsies performed in stable grafts during the first year after transplantation, Rush et al [12, 23] reported a higher incidence of the histological diagnosis of acute rejection and a lower incidence of CTN. For example, at six months of follow up, 28% of their biopsies displayed acute rejection and 25% displayed

CTN. Characteristics of patients in their studies differed in some aspects when compared to our study. They performed the protocol biopsy regardless of renal function and the immunosuppression employed was different. They used a triple regime with cyclosporine, azathioprine and steroids, while we employed antilymphocytic antibodies as the induction therapy in addition to cyclosporine and prednisone. In the present work, as well as in the previously mentioned studies [11, 12, 18, 23], marginal specimens (biopsies containing less than 7 glomeruli) were included. Evaluation of marginal specimens implies the risk of underestimating the severity of a given lesion due to its irregular distribution in the whole kidney [6]. For this reason, we cannot rule out the possibility that we might have underscored the severity of acute lesions.

We observed mild tubulitis not only in biopsies with otherwise normal histology, but also in about 25% of cases with CTN. This is in agreement with the results of previous studies that reported that a substantial number of stable grafts display mild tubulitis [6, 10, 23], and confirms that this lesion is also frequently observed in biopsies with CTN [24]. In our study, the clinical evolution of patients with normal histology was not different from patients who displayed borderline changes. Similarly, there were no differences in the clinical evolution of patients who showed CTN either with or without tubulitis. This result confirms previous observations suggesting that the presence of mild tubulitis is not a determinant factor in graft outcome [6].

Acute rejection is an important predictor of late graft failure [25–27], although the effect of mild episodes of acute rejection on graft survival remains a matter of discussion [28]. We observed a strong association between the diagnosis of CTN and the presence of a successfully treated episode of acute rejection before performing the protocol biopsy. It has been proposed that patients who have suffered from an episode of acute rejection may remain immunologically active [29–32]. In the present study, when only patients displaying CTN were considered and grouped according to the presence or absence of acute rejection before biopsy, we did not observe any difference in the degree of acute lesions or in the number of CD45-positive interstitial infiltrating cells. Therefore, our data do not support the hypothesis that the persistence of a slow active inflammation is responsible for the progression of chronic renal allograft damage. Alternatively, acute rejection may be responsible for the induction of irreversible renal lesions that could trigger hyperfiltration mediated damage [33–37]. Anyway, our observation indicates that even episodes of rejection with a good response to antirejection therapy can produce chronic renal damage.

It is well established that cyclosporine induces interstitial fibrosis [38, 39]. We observed that cyclosporine exposure was higher in patients displaying CTN, despite the cyclosporine dose being similar in both groups. This result suggests that the fibrogenic effect of cyclosporine appears early after transplantation and that cyclosporine can contribute to the development of CTN. It has been shown that a triple regime with cyclosporine, azathioprine and prednisone decreases the severity of chronic lesions in protocol biopsies performed late after transplantation [11]. In the present study, we used antilymphocytic antibodies as the induction therapy in addition to cyclosporine and prednisone as the maintenance therapy. It is not clear whether the use of a double maintenance regime in our patients may have contributed to the early appearance of chronic lesions either due to a poorer control of the alloimmune response or to the maintenance of higher cyclosporine levels. The relatively low incidence of acute rejection observed in our group of patients [13] argues against the possibility that they received inadequate immunosuppression.

It has been shown that donor age is a determinant factor of graft outcome [40, 41] and that it is also associated with the incidence of chronic renal lesions in protocol biopsies performed at different times of follow-up [11, 18]. The incidence of post-transplant acute tubular necrosis is also a determinant factor of graft survival [42]. Despite the donor age and incidence of post-transplant acute tubular necrosis being higher in patients displaying CTN in the present study, these differences did not reach statistical significance. Other risk factors for late graft failure, such as degree of histocompatibility [43] and cold ischemia time [44], were similar in patients with and without CTN. In the studied set of patients, acute rejection and cyclosporine exposure were independent predictors of CTN.

The presence of CTN was associated with a poorer graft survival. This result is in agreement with Isoniemi et al [11, 45] and Dimény et al [18], who have recently pointed out the importance of chronic renal lesions in protocol biopsies with regard to graft outcome. Since we observed that serum creatinine, cyclosporine exposure and the incidence of acute rejection were higher in patients with CTN, we further studied whether any of these factors contributed to the prediction of graft survival. In the studied set of patients, the presence of CTN and serum creatinine were independent predictors of graft failure. However, graft survival was not influenced by the presence of acute rejection or cyclosporine exposure, suggesting that other factors may have played an important role as a cause of graft loss. Obviously, in the present work the sample size is not large enough to expect that any factor associated with graft survival in a broad series of patients can be identified [42]. Taking into account this limitation, we believe that the most reasonable interpretation of our findings is that CTN observed in early protocol biopsies is the result of many different confluent immunological and non-immunological insults, but the predictive value of CTN on graft survival is probably not dependent on the mechanism of renal injury.

We observed that CTN and serum creatinine were independent predictors of graft survival. In patients with CTN and an increased serum creatinine, the risk for late graft failure was the highest. If we accept that CTN and serum creatinine roughly reflect the balance between renal supply and metabolic demand [34, 46], it seems reasonable to speculate that hyperfiltration mediated damage may play an important role in the progression of renal scarring in this group of patients [33–37].

In summary, we show that the presence of CTN in protocol biopsies performed early after transplantation in patients with well functioning grafts provides relevant information to detect patients at risk of losing their graft due to chronic transplant nephropathy. The predictive value of protocol biopsies is independent of renal functional impairment, at least when patients with a serum creatinine lower than 200 μmol/liter are considered. This observation may have important implications on the design of secondary intervention trials aimed to modify the natural history of chronic transplant nephropathy [47].

## Acknowledgments

This study was supported by FIS grants 95/1088, 96/0785 and a Sandoz SAE grant. F. Moreso was supported by a CIRIT grant (Formació, personal

investigador). We thank Dr. J.M. Ramón from the Epidemiology Department, Ciutat Sanitària i Universitària de Bellvitge, for statistical advice. We thank Ms. Margarita Carmona for her technical assistance.

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