Impact of chronic allograft nephropathy and subsequent modifications of immunosuppressive therapy on late graft outcomes in renal transplantation

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Abstract

Background. Chronic allograft nephropathy (CAN) is the leading cause of organ failure in renal transplant recipients. We retrospectively evaluated the impact of varying immunosuppression in CAN patients on long-term graft survival.

Methods. We retrospectively analysed 158 cyclosporin (CsA)-treated renal transplant recipients with biopsy-proven CAN with follow-up of >1 year. Immunosuppression remained unchanged in 75 (NOVAR) and was modified in 83 patients (VAR). In 36.1% of VAR patients, it was increased; in 63.8%, the addition of other immunosuppressants was associated with a 20% reduction in or withdrawal of CsA. A regression model, for creatinine clearance (CrCl) slope analysis after therapy variation, and Cox’s analysis were applied.

Results. In VAR patients, two-phase regression did not show a correlation between the inflection point in the CrCl slope and treatment variation. Changing immunosuppression gave a borderline advantage in long-term graft survival compared with NOVAR (P = 0.088). In univariate analysis, severe histological lesions, proteinuria >0.5 g/day and CrCl <25 ml/min at biopsy correlated with poor graft outcome (P = 0.0009). In multivariate analysis, only proteinuria and low CrCl remained significant. Stratifying histological lesions in relation to therapy variation showed that severe lesions significantly decreased survival in both VAR and NOVAR groups; however, the highly negative impact of severe lesions in NOVAR patients on graft survival [relative risk (RR) 3.602] was reduced in VAR patients (RR 1.951), with a 10 year graft survival since biopsy of 0.16 vs 0.34 (P = 0.0001).

Conclusions. In transplant patients with CAN, variation of immunosuppression can reduce the negative impact of severe chronic lesions.

Keywords: anti-rejection therapy variation; chronic allograft nephropathy; histology of chronic allograft nephropathy; outcome assessment

Introduction

Although death with a functioning kidney is considered increasingly to contribute to reduced graft survival, chronic allograft nephropathy (CAN), which may be caused by both alloantigen-specific and non-alloantigen-specific mechanisms [1], is still the major long-term cause of graft loss [2]. Histologically, CAN is characterized by interstitial fibrosis, with tubular atrophy, accompanied by a broad spectrum of glomerular and vascular lesions ranging from arteriolar and glomerular sclerosis to chronic transplant arteriopathy and glomerulopathy [3]. With the exception of chronic transplant glomerulopathy, transplant arteriopathy [4] and peritubular capillary basal membrane splitting [5], these lesions are relatively non-specific, making it difficult to distinguish between ongoing smouldering rejection, chronic drug toxicity and adaptation to glomerular hyperfiltration. Moreover, once chronic damage has ensued, the histological counterparts of these different pathological mechanisms often merge with each other. Isoniemi et al. [6] suggested that early fibrosing changes in allografts with stable function, and particularly chronic allograft damage indices, might predict later dysfunction, and this prompted a series of studies on the usefulness of protocol biopsies for predicting and preventing the development of CAN [7]. Only a few reports have evaluated whether
or not any modification of immunosuppressive therapy can reverse the progression of graft dysfunction in the presence of established CAN with renal function already altered [8,9].

The aim of this retrospective study was to determine whether or not a modification of the immunosuppressive scheme might have an impact on graft function in renal transplant patients with biopsy-proven CAN.

Patients and methods

We reviewed the clinical history of 1012 renal transplant recipients who were treated from the beginning with a calcineurin inhibitor and who received their grafts after 1983, when cyclosporin (CsA) became available in our unit. The core of this series consists of a cohort of patients who participated in four prospective trials conducted in our unit since 1983 [10,13–15]. Of the 36 monotherapy patients included, 25 belong to a first single-centre prospective trial comparing CsA monotherapy with double therapy [13]. The remaining 11 monotherapy patients were participants in a second study comparing CsA monotherapy with triple-drug therapy [14]. Of the 75 patients in dual therapy, 50 were participants in a multicentre study that compared CsA monotherapy with dual and triple therapy [10]; the other 25 were part of a single-centre randomized study that compared triple-drug and double-drug therapy [15]. Of the triple-therapy patients, 29 were also part of the multicentre trial [10], while the remaining 13 were enrolled in the trial mentioned last [15].

We considered for this analysis patients with at least 1 year of follow-up who developed progressive declines of their graft function or a proteinuria >0.5 g/day, or both, and in whom a graft biopsy demonstrated the presence of CAN. We excluded any non-biopsied patient, and patients who lost their grafts because of acute rejections, recurrence of their original renal diseases in the grafts, urological complications or clear-cut CsA toxicity, as diagnosed by both high CsA trough blood levels and the presence of nodular hyalinosis of the pre-glomerular arterioles. Our aim was to analyse only those patients with histological patterns that did not immediately indicate the underlying pathological processes (either rejection or toxicity). We also excluded patients in whom CAN followed a significant reduction of immunosuppressive therapy due to cancer, severe infection or other severe complications. Of the initial 1012 patients, 158 met the criteria for inclusion in this study.

Baseline immunosuppressive protocols

The immunosuppressive protocols have been reported in detail elsewhere [10]. Briefly, 36 out of 158 patients (22.8%) were on CsA monotherapy: in them, methylprednisolone (MP) was stopped on the 5th post-transplant day and CsA reduced from 12 to 10 mg/kg/day on day 15 and then tapered by 2 mg/kg every 2 weeks to a maintenance dose of 4–5 mg/kg/day, and it was reduced further after the first year. Of the cohort, 75 patients (47.4%) were on dual therapy: their CsA dosage and blood trough levels were the same as for monotherapy patients. Their oral MP was kept at 16 mg/day for 3 months and was then tapered to 8 mg/day by the end of the 6th month. Finally, 42 patients (26.5%) were on triple therapy: in them, the dose of CsA was reduced to 10 mg/kg/day on day 6 and to 8 mg/kg/day from day 15, then tapered to a maintenance dose of 3 mg/kg/day. As in the dual therapy regimen, their MP was reduced to 8 mg/day at the end of month 6. From day 6, azathioprine (Aza) was given to them at a dose of 1.5 mg/kg/day for 3 months, then tapered to a maintenance dose of 1 mg/kg/day. CsA plus mycophenolate (MMF) plus MP was used in five patients, (3.2%), MMF being given at an initial dose of 2 g/day, then adjusted according to the white blood cell count.

Immunosuppressive regimen variation

After graft biopsy, no therapy modification was performed in 75 out of 158 patients with CAN (NOVAR). In the remaining 83 patients, the original immunosuppressive regimen was modified (VAR).

There was not a standardized protocol to modify therapy, but the criteria for changing the therapeutic schedule were based upon the results of biopsies (analysed by the same pathologist throughout the duration of the study), the levels of CsA and the behaviour of serum creatinine over time. As a general rule, whenever a CsA trough level >250 ng/ml or moderate to severe arteriolar hyalinosis, or both, were present, CsA was reduced by 20% and Aza or MMF was added. In the presence of <200 ng/ml CsA, biopsy showing a transplant arteriopathy and chronic transplant glomerulopathy, CsA was left unchanged or increased, and Aza or MMF was added. Intermediate conditions were managed also taking into consideration the rate of the deterioration of graft function: indeed, in some patients with severe histological findings (see definition below), but whose serum creatinine values before biopsy increased slowly, we preferred to adopt a watchful policy before varying therapy.

We used the biopsy which disclosed CAN as the point in time from which survival was measured. Since one of the aims of this study was that of correlating therapy variations with changes in creatinine clearance slope, we considered only those changes of therapy instituted within a reasonable time interval from that biopsy (100 days). If the treatment of such patients was modified after this 100 day period, the changes were not considered as therapy variations for the purpose of this study. Finally, in some patients, Aza or MMF were rapidly withdrawn after addition to the protocol because of intolerance. For these reasons, patients with severe histological lesions were as frequent in the VAR as in the NOVAR group.

CsA whole blood trough levels (WTBLs) were determined at 3 month intervals by polyclonal antibodies until December 1989 and by monoclonal antibodies from January 1, 1990, in 69 of the 83 VAR patients. As it was impossible to compare values obtained with different methods, we report only data obtained with the monoclonal assay.

Histology

Allograft biopsies were classified according to the Banff schema [11]. According to the type of histological features, the patients were grouped into two main categories. Group I—mild: the patients in this group had: (i) absence of significant lesions; and (ii) interstitial fibrosis involving ≤25% of parenchyma (c1), moderate mesangial matrix increase
<25% (mm1) and global sclerosis involving ≥25% of glomeruli (CAN grade 1a). Group II—severe: the patients in this group had: (i) CAN 1a with global sclerosis involving >25% of glomeruli; (ii) interstitial fibrosis involving >25% of parenchyma (ci2-ci3) and moderate to severe arteriolar hyalinosis (ah2, ah3), i.e. CAN 2a; (iii) transplant arteriopathy or transplant glomerulopathy (CAN 1b-2b), or both; or de novo membranous nephropathy (considered a sign of chronic rejection); and (iv) signs of subacute rejection (Banff or transplant glomerulopathy or transplant arteriolopathy and of an ischaemic cardiomyopathy.

In 41 out of 69 patients, at least four serial pre- and four serial post-biopsy determinations of CsA WBTL were made at 3 month intervals. Their mean values were: in the seven patients in whom CsA remained unchanged, 153.2±14.2 ng/ml (median 153.8), with no difference between pre- and post-biopsy levels; in the 31 patients in whom CsA was reduced, 212.7±7.4 ng/ml (median 211.9) before and 112.4±4.8 ng/ml (median 111.5) after biopsy (P=0.000); and in the three patients in whom CsA was increased, 194.6±30.6 ng/ml (median 184.5) before vs 277.2±23.5 ng/ml (median 279.0) after biopsy (P=0.021).

**Analysis of the pattern of creatinine clearance over time**

From the three regression model results, an almost flat pattern of CrCl has been observed in 29 patients (non-significant regression): 13 NOVAR and 16 VAR, i.e. in 16 patients therapy variation had no effect on the evolution of graft function.

A pattern without an inflection point (with a statistical significance in only the linear regression) was observed in 52 patients: 29 NOVAR and 23 VAR. The therapeutic shift therefore did not influence the slope of CrCl in the 23 patients who were subjected to changes of therapy. Of these 52 patients, 44 (84.6%) immunosuppressant was associated with either a reduction in CsA by 20%, in 47 patients, or withdrawal of CsA, in six. In 22 patients (26.5%), MMF was added to or substituted for Aza. In three patients, sirolimus (SRL) was added instead of MMF: in one patient under triple therapy, showing a transplant glomerulopathy and global glomerular sclerosis, CsA was reduced by 20% and SRL added at 2 mg/day; in two other patients receiving MP and CsA, CsA was reduced by 50% and SRL added at 2 mg/day, because of the concomitant presence of a transplant glomerulopathy and of an ischaemic cardiomyopathy.

**Results**

**Characteristics of patients (Table 1)**

Considering only therapy variations performed within 100 days after biopsy, immunosuppressive therapy was unchanged in 75 patients (NOVAR) and was modified in 83 (VAR) (Table 2). There were no significant differences in baseline characteristics between the two groups. However, VAR patients had both significantly higher serum creatinines at biopsy and significantly higher nadir serum creatinines than NOVAR patients.

Table 3 shows the observed histological patterns. In 30 out of the 83 VAR patients (36.1%), immunosuppression was increased (by increasing CsA dosage or by adding another immunosuppressant, or both), while in 53 (63.8%) the addition of a second or third immunosuppressant was associated with either a reduction in CsA by 20%, in 47 patients, or withdrawal of CsA, in six. In 22 patients (26.5%), MMF was added to or substituted for Aza. In three patients, sirolimus (SRL) was added instead of MMF: in one patient under triple therapy, showing a transplant glomerulopathy and global glomerular sclerosis, CsA was reduced by 20% and SRL added at 2 mg/day; in two other patients receiving MP and CsA, CsA was reduced by 50% and SRL added at 2 mg/day, because of the concomitant presence of a transplant glomerulopathy and of an ischaemic cardiomyopathy.

### Table 1. Characteristics of all patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NOVAR (n=29)</th>
<th>VAR (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>16/13</td>
<td>49/4</td>
</tr>
<tr>
<td>Age at Tx (years: mean±SD)</td>
<td>34.9±12.4</td>
<td>37.6±11.3</td>
</tr>
<tr>
<td>Cadaver/living first Tx</td>
<td>122/25</td>
<td>160/27</td>
</tr>
<tr>
<td>Cadaver/living second Tx</td>
<td>10/1</td>
<td>10/1</td>
</tr>
<tr>
<td>Patients with a PRA titre &gt;50%</td>
<td>15/16</td>
<td>22/1</td>
</tr>
<tr>
<td>Mean dialysis duration (months: mean±SD)</td>
<td>40.0±37.5</td>
<td>41.2±38.6</td>
</tr>
<tr>
<td>Graft function recovery (immediate/delayed)</td>
<td>124/34</td>
<td>166/4</td>
</tr>
<tr>
<td>No. of acute rejection episodes in the first 6 months (0/1/2 or more)</td>
<td>49/80/29</td>
<td>53/81/62</td>
</tr>
<tr>
<td>No. of acute rejection episodes after the 6th month (0/1)</td>
<td>131/27</td>
<td>175/27</td>
</tr>
<tr>
<td>MP pulses (0/1–3 ≥ 3)</td>
<td>41/55/62</td>
<td>44/62/78</td>
</tr>
<tr>
<td>Mean no. of MP pulses (in the first 6 months)</td>
<td>3.1±2.8</td>
<td>3.2±2.9</td>
</tr>
<tr>
<td>OKT3/ATG treatment for steroid-resistant rejection</td>
<td>17</td>
<td>20</td>
</tr>
</tbody>
</table>

PRA = panel reactive antibodies; MP = methylprednisolone; ATG = anti-thymocyte globulin.
showed a decrease in CrCl and eight (15.4%) an increase.

In 72 patients (30 NOVAR and 42 VAR), we observed a hinge in the slope of CrCl (two-phase regression) which was time related to biopsies, but there was no significant difference in the pattern of distribution between the two groups of patients; 14 out of 30 NOVAR vs 24 out of 42 VAR patients had increases and 16 NOVAR vs 18 VAR patients had decreases of CrCl ($P = 0.523$). In 42 out of 72 patients, immunosuppressive therapy was modified and in the other 30, it was maintained unchanged.

Finally, in five patients (two VAR and three NOVAR), we observed a decreasing and then increasing CrCl, or vice versa (quadratic pattern); of them, only one (VAR) showed a decreasing CrCl; all others showed improvements (one in the VAR and three in the NOVAR groups).

Considering only the patients with the two-phase and quadratic regression pattern, an improvement of the CrCl was observed in 42 (54.5%); 25 of the 42 (59.5%) were in the VAR group.

Analysis of the histological pattern

No significant differences in the distribution of histological characteristics were observed between the two groups and no particular histological feature was associated with any regression pattern of CrCl. Also, no significant differences were observed between the 72 patients with mild histological lesions and the 86 patients with severe histology. Respectively, their mean ages at transplantation were $34.3 \pm 12.4$ vs $35.9 \pm 12.4$ years ($P = 0.57$); time spent on dialysis, $40.5 \pm 38.1$ vs $39.7 \pm 37.3$ months ($P = 0.89$); number of MP pulses, $2.8 \pm 2.9$ vs $3.4 \pm 2.7$ ($P = 0.15$);
nadir serum creatinine, $1.4 \pm 0.4$ vs $1.5 \pm 0.5$ mg/dl ($P = 0.17$); serum creatinine at biopsy, $2.3 \pm 0.6$ vs $2.4 \pm 0.7$ mg/dl ($P = 0.74$); mean follow-up from transplantation, $124.2 \pm 53.3$ vs $116.6 \pm 45.7$ months ($P = 0.34$); and patients who increased/reduced immunosuppressive therapy, $27/7$ vs $41/8$ ($\chi^2 = 0.62$). Only the time of biopsy from transplantation was significantly shorter in the mild histology group: $32.5 \pm 28.3$ vs $54.6 \pm 46.1$ ($P = 0.001$).

**Graft survival**

Graft survival 10 years after biopsy was better in VAR patients (30 events in 83 patients) than in NOVAR (43 events in 75 patients), although this did not reach statistical significance (50.3 vs 30.2%; log-rank: $P = 0.088$) (Figure 1 and Table 4). Also better, although still non-significant, was 10 year death-censored after-biopsy graft survival among VAR patients at 60.1% (24 events) vs 36.4% in NOVAR patients (35 events) (log-rank: $P = 0.11$) (Table 4).

From among the patients with more severe histologies, considering separately only those 39 patients displaying chronic rejection (CAN grade 1b or 2b), there was no significant difference among the 20 NOVAR and 19 VAR patients in any of: mean age at transplantation ($33.87 \pm 12.51$ vs $36.19 \pm 12.98$ years, respectively, $P = 0.57$), months on haemodialysis ($31.95 \pm 31.81$ vs $52.16 \pm 45.57$, $P = 0.12$), mean donor age ($30.5 \pm 13.95$ vs $37.11 \pm 12.04$ years, $P = 0.12$), mean number of MP pulses for rejection ($3.45 \pm 3.22$ vs $3.75 \pm 2.35$, $P = 0.75$), nadir creatinine ($1.50 \pm 0.41$ vs $1.62 \pm 0.45$ mg/dl, $P = 0.39$) and creatinine at biopsy ($2.32 \pm 0.76$ vs $2.44 \pm 0.76$ mg/dl, $P = 0.95$). However, in this particular subgroup of patients, graft survival was significantly worse at 10 years in NOVAR patients when compared with VAR patients ($0.08$ vs $0.44$ $P = 0.034$). The same was true for death-censored graft survival (NOVAR 0.11 vs VAR 0.54, $P = 0.033$).

Among VAR patients, there were no long-term differences in graft survival between those in whom CsA dosage remained unchanged or was increased vs those in whom CsA dosage was reduced or withdrawn (log-rank: $P = 0.34$).

Table 4 also shows the results from a log-rank test of the effect of other parameters on graft- and death-censored graft survivals and the results of the Cox multivariate analysis of the final model of graft survival. Histological lesions were significantly correlated with graft outcome in univariate analysis, but turned out to be non-significant in the multivariate one. Thus, in multivariate analysis, only proteinuria and CrCl were associated with long-term graft survival.

The expected survivals of grafts, based on a Cox model, which took into account both histological grading and any modification of therapy, are shown in Figure 2. The 10 year expected graft survivals

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Table 4. Univariate and multivariate analysis of the prognostic values of some relevant parameters at biopsy for graft- and death-censored graft survival at 10 years after biopsy

<table>
<thead>
<tr>
<th>Univariate analysis</th>
<th>Graft survival</th>
<th>Death-censored graft survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapy change (yes vs no)</td>
<td>0.0888</td>
<td>0.1102</td>
</tr>
<tr>
<td>Serum creatinine at biopsy ($\leq 2.2$ vs $&gt; 2.2$ mg/dl)</td>
<td>0.4085</td>
<td>0.0833</td>
</tr>
<tr>
<td>Histology (mild vs severe lesions)</td>
<td>0.0009</td>
<td>0.0025</td>
</tr>
<tr>
<td>Proteinuria at biopsy ($\leq 0.5$ vs $&gt; 0.5$ g/day)</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>CrCl at biopsy ($&gt; 25$ vs $\leq 25$ ml/min)</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean arterial blood pressure ($\leq 110$ vs $&gt; 110$)</td>
<td>0.7476</td>
<td>0.8581</td>
</tr>
<tr>
<td>Use of converting enzyme inhibitors (yes vs no)</td>
<td>0.0812</td>
<td>0.1265</td>
</tr>
<tr>
<td>Addition of MMF or not</td>
<td>0.6656</td>
<td>0.7339</td>
</tr>
<tr>
<td>Serum cholesterol levels ($\leq 200$ vs $&gt; 200$ mg/dl)</td>
<td>0.1341</td>
<td>0.2437</td>
</tr>
<tr>
<td>Serum triglycrides levels ($\leq 160$ vs $&gt; 160$ mg/dl)</td>
<td>0.3528</td>
<td>0.7721</td>
</tr>
<tr>
<td>Serum HDL levels ($\leq 60$ vs $&gt; 60$)</td>
<td>0.1179</td>
<td>0.2678</td>
</tr>
</tbody>
</table>

Cox model, final

<table>
<thead>
<tr>
<th>Univariate analysis</th>
<th>Graft survival</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria at biopsy ($\geq 0.5$ vs $\leq 0.5$ g/day)</td>
<td>0.0001</td>
<td>3.517</td>
<td>2.005–6.168</td>
</tr>
<tr>
<td>CrCl clearance at biopsy ($&gt; 25$ vs $\leq 25$ ml/min)</td>
<td>0.0001</td>
<td>0.276</td>
<td>0.159–0.479</td>
</tr>
</tbody>
</table>
after biopsy were: 0.34 [95% confidence interval (CI) 0.22–0.52] in VAR patients with severe lesions (curve D) vs 0.16 (95% CI 0.08–0.33) in NOVAR patients with severe lesions (curve B), and 0.63 (95% CI 0.50–0.79) in VAR patients with mild lesions (curve C) vs 0.47 (95% CI 0.34–0.64) in NOVAR patients with mild lesions (curve A). There was a significant linear increment of the relative risk (RR = 1.60; 95% CI 1.24–1.96; \( P = 0.001 \)) from the best to the worst of the four groups. Comparing the curves, A vs C showed an RR of 1.34 (95% CI 0.61–2.95); D vs C an RR of 1.95 (95% CI 0.91–4.17); and B vs C an RR of 3.60 (95% CI 1.73–7.49), with an overall significance among the four curves of \( P = 0.0012 \). Similar results have been obtained for death-censored graft survival.

Discussion

In this retrospective study of patients with histological features of CAN, we tried to determine if modifying the therapy of established CAN could have an impact on the subsequent evolution of the graft. We used two methods: an analysis of the slope of CrCl and a multivariate analysis of parameters measured at biopsy. Most of the patients in the present series were part of a randomized population participating in four prospective trials conducted in our unit since 1983 [10,13–15], and have now been followed-up for as long as 20 years. Most of these patients were on the older formulation of CsA, administered either alone or in combination with steroids or steroids and Aza. Biopsy-proven CAN with histological features suggestive of chronic rejection was detected in 36% of patients. In these cases, we attributed the development of CAN to inadequate immunosuppression, and reinforced therapy either by adding steroids in patients receiving CsA monotherapy or by adding Aza to patients on dual therapy. In the remaining 64% of cases, in whom interstitial fibrosis, focal glomerular sclerosis and arteriolar sclerosis of variable degrees were the main histological findings, we could not exclude concomitant chronic calcineurin inhibitor-related toxicity. In these cases, the addition of another immunosuppressant was coupled either with a reduction of CsA by ~20% (88.6%) or with the withdrawal of CsA (11.3%). In the presence of such an ill-defined histological picture, we tried to maintain adequate immune surveillance while contemporaneously reducing iatrogenic nephrotoxicity. At any rate, among patients whose therapy was adjusted, a significantly better clinical graft outcome was not observed in those individuals in whom CsA was reduced or withdrawn, compared with those in whom CsA was maintained (or even increased). Only in more recent years was MMF added. Although in univariate analysis changing to MMF from Aza did not show an effect, the number of patients in this series who were converted to MMF was too small to allow a reliable analysis of its role on allograft survival.

In 1997, Weir et al. [16] showed that, in a small cohort of patients with progressive deterioration of their graft function, reducing CsA dosage by 50% and adding MMF was significantly associated with improvement of allograft function. Although prospective, this first study was short, with a mean observation period after change of therapy of 7.2 ± 0.2 months. In a second paper, the same authors [8] confirmed their conclusions, reporting on 118 patients followed for a mean of 651 days after changes in their therapies, and suggest that long-term attrition of graft function was due mostly to CsA-related toxicity. They used a two-phase regression model to demonstrate that changes in therapy were time related to the improvement in serum creatinine. Variability in the course of declining renal function makes the prediction of graft outcome from a simple linear relationship problematic; for this reason, Fink et al. [17] proposed the two-phase regression model based on the reciprocal of serum creatinine as the standard method to model renal function decline in renal transplant recipients.

In the present series, by analysing the pattern of the behaviour of CrCl rather than of the reciprocal of serum creatinine, we tried to find an inflection in the slope of CrCl that could be related to therapy modification. In 51.3% of the patients, we found either a relatively flat slope or only a linear regression. These profiles precluded any analysis of the effect of varying therapy on the CrCl pattern in those patients who underwent a change of therapy. A relationship between a change in the steepness of the slope of CrCl and change of therapy was detected, instead, in about half of the cases with the two-phase or the quadratic regression patterns. A further decline of the CrCl slope was observed in about half of the NOVAR and VAR group patients, while a further increase of CrCl or a reversal of its decline was observed in 57% of VAR and in 46.6% of NOVAR patients, the difference between the two groups being insignificant. Cumulatively, CrCl improved concomitantly with

![Figure 2](image_url)

**Fig. 2.** Expected graft survival according to the severity of lesions and the introduction or not of a therapy modification.
therapy modification in ~60% of VAR patients when analysed with the two-phase and quadratic regression patterns. Thus, the use of this method for evaluating the impact of therapy modification on allograft function was of limited value in this series, and we think that its usefulness as a generalized clinical tool requires further confirmation.

On the other hand, the standard survival analysis reveals a marginally significant long-term benefit from therapy shift in the presence of CAN. Indeed, the 10 year survival rates calculated from the time of biopsy showed a graft survival rate of 0.50 among VAR patients vs 0.30 among NOVAR patients. The 10 year death-censored graft survival also showed a non-significant trend towards better results in the group of patients undergoing therapy changes (0.60 vs 0.36). These data, in this particular set of patients, might have clinical relevance beyond their borderline statistical significance, especially if one considers that patients who changed therapy had significantly higher serum creatinines at biopsy. Moreover, graft- and death-censored graft survival was significantly better in the VAR patients of the subgroup displaying chronic rejection.

Therapy modification had only a borderline correlation with allograft outcome by univariate analysis, and an even less significant correlation in the final Cox model. In agreement with other studies [18], in our series the presence of mild lesions was also predictive of better long-term graft- and death-censored graft survival by univariate analysis. In multivariate analysis, however, the strongest predictors of bad long-term allograft function were proteinuria and low CrCl at biopsy; severe histological lesions lost the negative predictive value observed in univariate analysis. The data of this series suggest that both treatment and histological grading had little impact on graft outcome when considered separately. However, when the impact of treatment was evaluated according to the histological grading, the graft survival of patients with severe lesions who were subjected to therapy modification was significantly better than that of patients whose treatment was not modified, with a significant linear increment of the RR among the various groups. In patients with severe lesions, the graft survival rate 10 years after biopsy was indeed 0.34 in the VAR group vs 0.16 in the NOVAR group. On the other hand, in the patients with mild lesions, 10 year graft survival was 0.63 in the VAR group and 0.47 in the NOVAR group.

Therefore, a compromised histological picture is predictive of a worse graft outcome in patients not undergoing any therapy adjustment; the negative impact of these lesions, however, may be significantly reduced, provided that an adjunct immunosuppressant is given and, in cases with co-existent signs suggestive of nephrotoxicity, CsA is simultaneously reduced. In this respect, our data confirm the experiences of others with MMF [8,19], although most of the changes we instituted consisted of the addition of Aza to CsA.

In recent years, the need to identify early histological markers of prognostic value in steady-state clinical conditions has prompted the widespread use of protocol biopsies and has further stimulated studies to correlate histology to long-term graft survival [20]. Our present data seem to confirm the usefulness of early treatment after biopsy identification of CAN. We think that any attempt to relate the results of early biopsy findings to the outcome of the graft should also take into account any intervening changes of therapy, which may in the long-term modify the prognostic value of histological findings.

The retrospective nature of this analysis, the absence of a standardized therapy shift protocol, as well as the fact that the addition of either Aza or MMF was associated with a reduction of CsA dosage, might dampen to some degree the relevance of our results. We would like to point out, however, that to our knowledge no other study has analysed the effects of the treatment of CAN at 10 years after biopsy. The difficulties inherent in designing and completing a prospective study of such duration are considerable.

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Conflict of interest statement. None declared.

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