Transpl Int (1996) 9: 492–498
© Springer-Verlag 1996

ORIGINAL ARTICLE

Stefan H. Jacobson
Georg Jaremko
Frans F. Duraj
Henryk E. Wilczek

Renal fibrosis in cyclosporin A-treated renal allograft recipients: morphological findings in relation to renal hemodynamics

Received: 11 September 1995
Received after revision: 1 March 1996
Accepted: 6 March 1996

S. H. Jacobson
Department of Medicine,
Division of Nephrology,
Karolinska Hospital, Stockholm, Sweden

G. Jaremko
Department of Pathology,
Huddinge University Hospital,
S-141 86 Huddinge, Sweden

F. F. Duraj · H. E. Wilczek (✉)
Dept. of Transplantation Surgery, B56,
Huddinge University Hospital,
S-141 86 Huddinge, Sweden,
Fax: +46 (8) 746 7733

Abstract Nineteen nondiabetic kidney graft patients treated with cyclosporin A for 2 years underwent percutaneous renal allograft biopsy as well as renal hemodynamic examination. Renal allograft fibrosis was quantitatively evaluated as the relative volume of the renal cortical interstitium (V_V %) and as the interstitium/tubuli ratio (I/T ratio). The histological changes were then classified into four groups, depending on the degree of interstitial fibrosis. The glomerular filtration rate (GFR), renal plasma flow (RPF), renal blood flow (RBF), filtration fraction (FF), and fractional clearance of sodium, potassium, phosphate, chloride, osmoles, and free water clearance were determined in all patients and in 13 healthy controls. Kidney graft recipients had significantly lower GFR, lower RPF, and lower RBF than the healthy controls ($P < 0.001$ for all comparisons) while FF was similar in patients and controls. Transplant recipients had a significantly higher fractional excretion of sodium, potassium, chloride, and phosphate than controls. All except one patient had clearly increased V_V values, indicating increased interstitial fibrosis. The mean V_V in renal allograft patients was 35 % ± 10 % (normal < 16 % ± 5 %) and the I/T ratio was 1.07 ± 0.60 (normal < 0.24 ± 0.08). No correlation was found between the quantitative or semiquantitative biopsy analysis and any renal hemodynamic parameter measured. We conclude that renal function is significantly decreased in kidney graft recipients, but that adaptive tubular changes occur in the graft. Interstitial renal fibrosis was common but did not correlate to any renal functional parameter.

Key words Kidney transplantation, fibrosis, cyclosporin A · Fibrosis, kidney transplantation, cyclosporin A

Introduction

The use of cyclosporin A (CyA) as an immunosuppressive agent in transplantation has gained widespread acceptance, but its clinical use is hampered by its nephrotoxicity, which represents the most frequent and serious complication. CyA nephrotoxicity is clinically often difficult to distinguish from rejection or other causes of graft dysfunction in renal transplant recipients. The distinction between nephrotoxicity and renal allograft rejection is important since the management of these two complications is different.

The pathogenesis of CyA nephrotoxicity is not fully understood, although an early, reversible component appears to result from afferent and efferent glomerular arteriolar constriction, accompanied by an increase in renal vascular resistance [1, 6]. Long-term administration of CyA may result in interstitial fibrosis, tubular atrophy, and arteriolar hyalinosis in association with a decrease in glomerular filtration rate (GFR) [9, 13, 17, 25].

Impaired GFR is due to both reduction in the glomerular capillary ultrafiltration coefficient and to reduction in renal blood flow [1, 2]. Direct tubular toxicity appears to be less important in the genesis of renal allograft dysfunction [12]. Development of interstitial fibrosis in renal transplant recipients given CyA has been seen in some studies as soon as 3 months after initiation of CyA therapy, contrary to the findings in renal allograft recipients immunosuppressed with azathioprine [7, 18, 23].

The aim of the present investigation was to study the correlation between morphological findings, mainly interstitial fibrosis, in the renal allograft and renal hemodynamics in nondiabetic renal graft recipients given CyA for 2 years.

Subjects and methods

Nineteen nondiabetic kidney graft recipients (13 males and 6 females; mean age 47 ± 17 years, range 20–70 years) who received renal allografts between November 1985 and December 1986 were included in the study. Informed consent was obtained from all patients and the study was approved of by the Ethics Committees of both the Huddinge and the Karolinska Hospitals. Patient demographics are shown in Table 1. Sixteen patients had antihypertensive medication consisting of one or more drugs, e.g., combinations of β-receptor blockers ($n = 12$), diuretics ($n = 11$), calcium channel blockers ($n = 4$), and angiotensin-converting enzyme inhibitor ($n = 1$) at the time of investigation. Mean donor age was 52 ± 15 (range 18–72) years. All transplant recipients underwent renal allograft biopsy and renal function tests as described below. Thirteen healthy controls (mean age 34 ± 5 years, range 26–42 years) underwent renal function tests.

Immunosuppression

Because of an ongoing study at the time, two different immunosuppressive regimens were employed. Thus, nine patients received double drug therapy (CyA and prednisolone) and ten patients were given triple drug therapy [CyA, azathioprine (AZA), and prednisolone]. The CyA dose was adjusted in order to obtain predetermined whole blood levels. The immunosuppressive regimens have previously been described in detail [20]. The CyA trough whole blood levels were measured using the polyclonal radioimmunoassay method (Sandoz, Basel, Switzerland) [10]. Identical prednisolone protocols were used in all patients. Acute rejections episodes were treated with methylprednisolone, 0.5 g, intravenously on the 1st day, followed by 0.25 g/day for another 3 days. If the rejection did not respond to the steroid treatment, additional antithymocyte globulin (ATG-F, Fresenius, Germany) was given in a daily dose of 3 mg/kg for 7 days.

Biopsy technique

All biopsies wee obtained according to a planned protocol for clinical and research follow-up in the Department of Transplantation Surgery at Huddinge Hospital. They were taken as close as practically possible to the second anniversary of the transplantation, irrespective of renal transplant function at the time of biopsy. The percutaneous core needle biopsy method used has been described elsewhere [36]. In short, the location of the kidney graft was determined with the aid of the operation report and manual palpation, and the biopsy was obtained under local anesthesia using a TruCut disposable biopsy needle (outer diameter 2.0 mm; Travenol Labs, Deerfield, Ill., USA). After biopsy, firm manual pressure was applied to the site of puncture for 10 min; thereafter, the patient had to remain in bed for a minimum of 4 h. The patient was allowed to leave the hospital on the day of biopsy after voiding urine.

Biopsy processing and analysis

Biopsies were considered adequate for analysis if they contained cortical tissue with more than seven glomeruli. The smallest area analyzed in a biopsy was 560,000 μm^2. The biopsies were fixed in 3 % buffered formalin and embedded in paraffin according to routine procedures. Sections were cut at 3 μm and stained with hematoxylin-eosin, Ladewig's trichrome stain, and silver methenamine. Microscopy of the biopsies was carried out without knowledge of the immunosuppressive treatment group to which the patient belonged. The relative volume (volume density, V_V) of the renal cortical interstitium was used as a parameter for renal interstitial fibrosis with tubular atrophy, which is the usual finding in chronic CyA nephrotoxicity [18, 31]. For this purpose, the areal density (A_A, relative section area) of the cortical interstitium was determined by point-counting [35], using a 5 × 5 point grid (Integralplatte I, Zeiss, Stockholm, Sweden), which was inserted into the 10 × eyepiece of a Zeiss standard light microscope equipped with a Plan 40 × objective lens. The volume density of the interstitium was calculated according to the formula $P_P = A_A = V_V$ [35]. Another closely related parameter of interstitial fibrosis, the so-called interstitium/tubuli (I/T) ratio, was also used. The I/T ratio was assessed by calculating the ratio of the area occupied by interstitium to that occupied by tubules. Some authors have employed the T/I ratio, which is the same, but inverse, relationship [7]. Both these calculations suffer from the same shortcoming as the V_V of the interstitium in that they can be influenced by pathological processes other than interstitial fibrosis, e.g., interstitial edema, interstitial cell infiltration, etc.

The histological changes were classified into four groups – normal, 1, 2, and 3 – depending on the degree of interstitial fibrosis (Table 1). With regard to our V_V findings in the control group, less than 20 % interstitial fibrosis was considered normal ($n = 1$). Patients with 20 %–30 % fibrosis in the renal allografts were placed in group 1 ($n = 4$), those with 31 %–40 % in group 2 ($n = 9$), and those with more than 40 % interstitial fibrosis in their renal allografts in group 3 ($n = 5$). In addition to this quantitative analysis, the following histological changes were semiquantitatively assessed on a 0–4+ scale: interstitial inflammation, arteriolar hyalinosis, arteriolar smooth muscle degeneration, arteriolar intimal swelling and thrombosis, arterial intimal fibrosis, arterial signs of chronic vascular rejection (intimal cell proliferation, intimal foam cells, myxoid degeneration), and arterial signs of acute vascular rejection (end-arteritis, vascular wall necrosis, thrombosis). For some purposes, the sum of scores for the various arteriolar lesions (range 0–12+) was used as a measure of arteriolopathy. The individual scores for acute and chronic signs of vascular rejection were also sometimes combined (range 0–8+). The occurrence of glomerular changes and of significant interstitial edema was also recorded.

Fifteen kidney graft biopsies, obtained peroperatively and immediately after reperfusion at the time of transplantation, served as histopathological controls. These baseline biopsies and the follow-up biopsies were from different kidney donors. However, there was no difference between the donors with regard to age,

Table 1 Patient demographics after classification into histological groups with regard to kidney graft biopsy findings (*CyA*, cyclosporin A; *AZA*, azathioprine; *Pred*, prednisolone)

	Histological group[a]			
	Normal ($n = 1$)	1 ($n = 4$)	2 ($n = 9$)	3 ($n = 5$)
Living donor kidney	0	2	0	3
Cadaveric donor kidney	1	2	9	2
Recipient age (mean ± SD)	63	44 ± 10	50 ± 17	43 ± 22
(range)		32–55	29–70	20–66
Recipient sex (M/F)	0/1	3/1	6/3	4/1
Donor age (mean ± SE)	44	50 ± 12	53 ± 14	54 ± 21
(range)		35–63	26–72	18–72
Donor sex (M/F)	0/1	3/1	6/3	3/1
Serum creatinine at the time of biopsy (μmol/l)	102	140 ± 24	167 ± 45	207 ± 84
No. of rejection episodes				
0	1	3	5	2
1	0	0	2	0
2	0	1	1	3
> 2	0	0	1	0
Immunsuppression				
Double drug therapy (CyA/Pred)	1	2	2	4
Triple drug therapy (CyA/AZA/Pred)	0	2	7	1
Cumulative CyA dose at the time of biopsy (g)	179	254 ± 117	165 ± 69	299 ± 107
Antihypertensive treatment at the time of biopsy				
No treatment	0	2	1	0
1 antihypertensive drug	0	2	2	2
2 antihypertensive drugs	1	0	4	1
3 antihypertensive drug	0	0	2	2

[a] Normal = interstitial fibrosis < 20 %, 1 = interstitial fibrosis of 20 %–30 %, 2 = interstitial fibrosis of 31 %–40 %, 3 = interstitial fibrosis > 40 %

sex, or number of living related kidneys (Table 2). The volume density (relative volume of the cortical interstitium, V_V) in this control group was 16 % ± 5 %, a finding that is in accordance with observations made by Dunnill and Halley [11] on kidneys obtained at autopsy, in spite of the fact that these authors used a different fixative and processing schedule. The I/T ratio was 0.24 ± 0.08, which is somewhat higher than that reported by Dunnill and Halley [11].

Tests for renal function

GFR was measured as the clearance of inulin and renal plasma flow (RPF) as the clearance of para-aminohippurate, both in terms of their rates per minute and corrected to 1.73 m^2 body surface area. The subjects received 0.5 ml/kg body weight of the solution containing 85 g/l of inulin (Inutest) and 30 g/l of para-aminohippurate (Merck Sharp & Dohme) as a loading dose. The solution was then infused intravenously by a motor-driven syringe at the constant rate of 0.3 or 0.5 ml/min, depending on renal function. The equilibrium time was 60 min. All patients were studied during three 30-min periods. Urine was voided spontaneously after diuresis had been established with oral water loading, and urine was collected at the end of each control period. Concentrations of inulin and paraminohippurate in serum and urine were determined as previously described [16]. Renal blood flow (RBF) was calculated as RPF/1-hematocrit and the filtration fraction (FF) was calculated as the ratio of GFR/RPF and expressed as a percentage. Fractional clearances of sodium, potassium, phosphate, chloride, osmoles, and free water clearances were calculated in relation to clearance of inulin [16].

Statistical analysis

Values are given as mean ± SD. Data were analyzed using linear regression by an analysis of variance (ANOVA). For the comparison of two proportions, Fischer's exact test was used, and for the difference between means, Student's t-test was employed. A P value below 0.05 was considered to indicate a significant difference.

Results

Morphological findings

All but one patient had increased interstitial fibrosis in their biopsy specimen. The mean I/T ratio of the 19 kidney graft patients was 1.07 ± 0.60 (range 0.37–2.68) and the relative volume of the renal cortical interstitium (V_V) was 35 % ± 10 % (range 17 %–56 %), which is higher than the corresponding values obtained from normal kidneys (0.24 ± 0.08 and 16 % ± 5 %, respectively; $P < 0.001$). The mean cumulative dose of CyA at the time of renal biopsy in all 19 patients was 225 ± 56 g. Five of ten patients in the triple therapy group experienced at least one rejection episode compared to three of nine patients in the group given only CyA and prednisolone (P = NS). There was no difference between the histological groups with regard to the number of rejection episodes (Table 1). None of the biopsies showed

Table 2 Demographic comparison of kidney graft donors and of donors providing baseline kidney graft biopsies

	Kidney graft donors	Kidney donors providing baseline biopsies
No. of biopsies	19	15
Living related donors	4	0
Cadaveric donors	15	15
Donor age (mean ± SD) (range)	52 ± 15 18–72	45 ± 15 19–63
Donor sex (M/F)	12/7	9/6

Table 3 Renal hemodynamics in nondiabetic kidney graft recipients and healthy controls (*GFR*, glomerular filtration rate; *RPF*, renal plasma flow; *RBF*, renal blood flow; *FF*, filtration fraction; *FeNa*, fractional excretion of sodium; *FeK*, fractional excretion of potassium; *FeCl*, fractional excretion of chloride; *FeP*, fractional excretion of phosphate; *FeOsm*, fractional excretion of osmoles; *FeH_2O*, free water clearance)

	Kidney graft recipients ($n = 19$)	Controls ($n = 13$)	P value[a]
GFR (ml/min × 1.73 m^2)	42 ± 18	106 ± 13	< 0.001
RPF (ml/min × 1.73 m^2)	205 ± 96	577 ± 105	< 0.001
RBF (ml/min × 1.73 m^2)	339 ± 168	968 ± 177	< 0.001
FF (%)	21 ± 5	19 ± 3	NS
FeNa (%)	9.5 ± 9.0	1.3 ± 0.6	< 0.004
FeK (%)	65 ± 59	27 ± 9	< 0.03
FeCl (%)	9.1 ± 8.1	1.6 ± 0.6	< 0.003
FeP (%)	61 ± 41	16 ± 6	< 0.001
FeOsm (%)	11.1 ± 7.1	3.3 ± 0.9	< 0.001
FeH_2O (%)	8.5 ± 3.6	9.4 ± 2.4	NS

[a] Student's t-test

pronounced interstitial edema or interstitial inflammatory infiltrates. In 17 of the biopsies there were also larger vessels permitting assessment of vascular changes. The occurrence of arteriolar lesions did not differ between the groups as judged by the sum of semiquantitative scores for the various types of arteriolar changes (group 1, 1.0 ± 1.2; group 2, 1.2 ± 1.8; and group 3, 2.0 ± 2.0). Arteriolopathy as described by Mihatsch et al. [21, 26] was not seen in this material. With regard to arterial changes, none of the biopsies showed signs of acute vascular rejection (semiquantitative score 0). In one patient, arterial signs of chronic vascular rejection (semiquantitative score 3) was seen with narrowing of the lumen because of considerable thickening of the intima, while the remaining 16 biopsies demonstrated normal findings (semiquantitative score 0).

Renal hemodynamics

The mean GFR of the patients was 42 ± 18 ml/min × 1.73 m^2 compared to 106 ± 13 ml/min × 1.73 m^2 in the healthy controls ($P < 0.001$, Table 3). RPF and RBF were significantly lower in kidney graft patients than in controls ($P < 0.001$), while no significant difference in FF was observed. The fractional excretion of sodium, potassium, chloride, phosphate, and osmoles was significantly higher in patients than in controls (Table 3). The only exception was the one kidney graft recipient with a normal V_V (< 20 %) who had tubular function parameters comparable to those found in the healthy subjects, although her GFR, RPF, and RBF were also markedly reduced.

Histological classification in relation to renal hemodynamics

In Table 4, the morphological grouping with regard to the degree of interstitial fibrosis is shown in relation to renal hemodynamics. There were no significant differences in GFR, renal perfusion, or tubular function between the three groups with increased interstitial fibrosis. Furthermore, there were no significant correlations between the volume density (V_V) or the I/T ratio and any renal hemodynamic parameters measured.

Discussion

Percutaneous renal allograft biopsies were obtained approximately 2 years after transplantation in nondiabetic adult kidney graft recipients. The histological findings were then correlated to findings in renal hemodynamic studies performed within 1–2 months after the biopsy.

Patients with kidney grafts had significantly lower GFR, RPF, and RBF than healthy controls. The fractional clearances were significantly higher in the kidney graft recipients than in the healthy subjects. This finding is in agreement with animal studies in which intravenous CyA infusion results in increased renal vascular resistance, decreased GFR, RPF, and RBF, and increased proximal fractional tubular reabsorption [8, 24, 30]. The alteration in tubular function observed in these patients is comparable to that associated with mild chronic renal insufficiency of other origin and may, therefore, not be specific manifestations of CyA tubular toxicity. Palestine and coworkers [28] examined renal tubular function in patients treated with CyA for autoimmune uveitis. These investigators were unable to demonstrate significant alterations in tubular function that could be considered specific for CyA nephrotoxicity. Several studies have demonstrated decreased renal function in patients treated with CyA for nonrenal diseases [3, 27,

Table 4 Histological classification according to the degree of interstitial fibrosis found in kidney graft biopsies in relation to renal hemodynamics (*GFR*, glomerular filtration rate, *RPF*, renal plasma flow, *RBF*, renal blood flow, *FF*, filtration fraction, *FeNa*, fractional excretion of sodium, *FeK*, fractional excretion of potassium, *FeCl*, fractional excretion of chloride, *FeP*, fractional excretion of phosphate, *FeOsm*, fractional excretion of osmoles, FeH_2O, free water clearance)

Kidney function	Histological classification				
	Normal ($n = 1$)	1 ($n = 4$)	2 ($n = 9$)	3 ($n = 5$)	*P* value[a]
GFR (ml/min × 1.73 m^2)	43	48 ± 8	38 ± 15	41 ± 30	NS
RPF (ml/min × 1.73 m^2)	190	239 ± 94	209 ± 99	163 ± 116	NS
RBF (ml/min × 1.73 m^2)	322	417 ± 176	362 ± 181	200 ± 123	NS
FF (%)	23	21 ± 5	19 ± 5	24 ± 6	NS
FeNA (%)	1.3	14 ± 16	9 ± 8	7 ± 5	NS
FeK (%)	25	48 ± 40	70 ± 71	84 ± 62	NS
FeCl (%)	0.8	8 ± 11	9 ± 8	8 ± 7	NS
FeP (%)	59	75 ± 80	55 ± 27	62 ± 35	NS
FeOsm (%)	4.8	10 ± 9	12 ± 7	11 ± 10	NS
FeH_2O (%)	5.5	12 ± 3	7 ± 4	8 ± 3	NS

[a] Analysis of variance (ANOVA)

28]. After changing immunosuppressive therapy from CyA to azathioprine, a significant increase in creatinine clearance has been observed [32]. Furthermore, after discontinuing CyA, sodium, potassium, and phosphate balances were maintained by a reduction in the fractional excretion [33]. This would indicate that some sort of adaptive tubular changes occur in the graft and that tubular functional changes during CyA therapy are the result of a decrease in GFR.

In kidney graft recipients, CyA often causes an increase in serum creatinine, but the incidence of acute rejection episodes has, by some authors, been reported to be lower than in patients immunosuppressed with azathioprine and prednisolone [14]. A lower incidence of graft loss due to rejection in CyA-treated patients may indicate that the decrease in GFR most likely is due to CyA nephrotoxicity and not to rejection.

The kidney graft recipients in the present study had an increased relative volume of the renal cortical interstitium (V_V) as well as an increased interstitium/tubuli ratio (I/T ratio) in biopsies obtained 2 years after transplantation compared to kidney biopsies taken perioperatively at the time of transplantation. Glomerular, vascular, tubular, and interstitial changes have been observed in CyA-treated patients [4, 5, 13, 18]. One problem with the interpretation and comparison of histological findings in CyA-treated patients from different studies is that most kidney biopsies are done for diagnostic purposes in cases of renal dysfunction. Klintmalm et al. and Wilczek et al. [18, 37] performed annual biopsies for up to 5 years after renal transplantation in patients with stable renal graft function. After only 1 year of CyA treatment, virtually all patients had developed interstitial fibrosis, and atrophic changes in the tubules were always found in areas with interstitial fibrosis. It was concluded that the CyA therapy had contributed to the observed lesions, but that in the majority of patients no progress of the histological lesions could be demonstrated between 1 and 5 years after transplantation. There are also several studies in which no specific CyA-induced morphological changes have been observed despite treatment for up to 1 year [7, 13, 15]. CyA doses were generally higher in those previous studies [7, 13, 18] than in the present investigation.

D'Ardenne et al. [7], using subjective grading and calculation of tubulointerstitial ratios, found an equal prevalence of interstitial renal fibrosis in CyA-treated and non-CyA-treated patients. We used the same, but inverse relationship, namely, the I/T ratio. In addition, we measured the volume density (V_V, relative volume) of the renal cortical interstitium as a parameter for interstitial fibrosis since there is no simple technique to determine the degree of interstitial fibrosis per se. The I/T ratio or T/I ratio suffers from the same shortcoming as the V_V of the interstitium in that it can be influenced by tubulointerstitial pathological processes other than interstitial fibrosis (interstitial edema, interstitial cell infiltration, etc.). By employing the V_V of the interstitium, we tried to avoid the additional influence of different degrees of pathological or artificial tubular epithelial swelling, which may occur in routinely processed kidney biopsies [38]. The cause of interstitial fibrosis in renal allografts exposed to CyA is still not completely known and is most likely multifactorial. Since chronic rejection is one of the conditions that has been suspected to cause the increased fibrosis sometimes seen in renal biopsies from patients not receiving CyA [7, 22, 23, 31, 34], it seems plausible to assume that concomitant chronic rejection and CyA nephrotoxicity may have separate, but additive, effects on the development of interstitial renal fibrosis.

We dit not observe any correlation between the degree of renal interstitial fibrosis and glomerular filtration, glomerular perfusion, or parameters of renal tubular function. Interestingly enough, the one kidney graft recipient with normal interstitial fibrosis demonstrated tubular function parameters comparable to those found in the healthy subjects, although her GFR, RPF, and RBF were markedley reduced. To our knowledge, only few previous reports exist on this issue in which renal

hemodynamics have been examined by a reliable renal clearance technique in humans.

There may be several explanations for the lack of correlation between histological findings and renal hemodynamics. CyA-associated nephrotoxicity is a dose- and time-dependent process [19]. At low trough levels, functional renal changes consisting of reductions in GFR and RPF commonly occur [6, 9, 25]. Superimposed on functional toxicity, morphological changes such as tubular toxicity and vascular interstitial toxicity may develop and progress to chronic renal failure. The threshold for the development of morphological renal lesions and chronic renal failure may depend on each individual patient's sensitivity and on the presence of concurrent risk factors. Even in conditions of functional renal graft stability, some patients demonstrate various degrees of focal interstitial infiltrates of mononuclear cells [29]. Such findings have also been observed in stable kidney graft patients immunosuppressed with azathioprine and prednisolone and, in these patients, probably represent different stages of the rejection process, defined as a silent immunological reaction to allogenic tissue without evident clinical symptoms. The silent immunological reaction may, however, sooner or later interfere with glomerular and tubular renal function and will then manifest itself clinically. Furthermore, the striped focal distribution of the interstitial renal fibrosis often seen in CyA-treated renal allograft recipients may also explain the lack of correlation between morphology and renal function because of the unavoidable sampling problem when core needle biopsy techniques are used to study focal types of changes in the kidney. The presence of hypertension with concomitant vascular lesions in the kidney graft may also play a role. When CyA therapy is continued beyond approximately 1 year, renal dysfunction includes a component that does not improve upon cessation of therapy [15]. Several pathogenic processes, e.g., chronic ischemia due to vasoconstriction and altered extracellular matrix accumulation independent of hemodynamic changes, may contribute to the development of irreversible renal interstitial fibrosis [19]. Cytokines, growth factors, ecosanoids, and thromboxane are also all implicated in the increased matrix synthesis by interstitial cells [19], and it is difficult to estimate to what extent all of these factors may have influenced the renal function studies.

In conclusion, histological evaluation and quantitative measurements of renal allograft biopsies obtained from nondiabetic renal transplant recipients who had been treated with CyA for 2 years showed increased interstitial renal allograft fibrosis in all but one patient, but there was no correlation between the degree of fibrosis and renal functional parameters.

Acknowledgements The authors are very much indebted to the late Professor Sven-Olof Bohman for his invaluable help with the histological evaluation. This study was supported by grants from the Tore Nilsson Foundation, the Magnus Bergwall Foundation, the Swedish Society of Medicine, and the Karolinska Institute.

References

1. Barros EJG, Boim MA, Ajzen H, Ramos OL, Schor N (1987) Glomerular hemodynamics and hormonal participation on cyclosporin nephrotoxicity. Kidney Int 32: 19–25
2. Bennet WM, Houghton DC, Buss WC (1991) Cyclosporine-induced renal dysfunction. Correlations between cellular events and whole kidney function. J Am Soc Nephrol 1: 1212–1219
3. Berg KJ, Forre O, Bjerkhoel F (1986) Side effects of cyclosporine A treatment in patients with rheumatoid arthritis. Kidney Int 29: 1180–1187
4. Bergstrand A, Bohman SO, Farnsworth A, Gokel HM, Krause PH, Lang W, Mihatsch MJ, Oppedal B, Sell S, Sibley RK, Thiru S, Verani R, Wallace AC, Zollinger HU, Ryffel B, Thiel G, Wonigeit K (1985) Renal histopathology in kidney transplant recipients immunosuppressed with cyclosporine A: results of an international workshop. Clin Nephrol 24: 107–119
5. Calne RY, White DG, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Pentlow BD, Rolles K (1978) Cyclosporine A in patients receiving renal allografts from cadaver donors. Lancet II: 1323–1327
6. Curtis JJ, Luke RG, Dubovsky E, Diethelm AG, Whelchel JD, Jones P (1986) Cyclosporine in therapeutic doses increases renal allograft vascular resistance. Lancet II: 477–479
7. D'Ardenne AJ, Dunhill MS, Thompson JF, McWhinnie D, Wood RFM, Morris PJ (1986) Cyclosporine and renal graft histology. J Clin Pathol 39: 145–151
8. Dieperink H, Kemp E, Leyssac PP, Starklint H (1986) Cyclosporine A: effectiveness and toxicity in a rat model. Clin Nephrol 25: S46–50
9. Dieperink H, Leyssac PP, Starklint H, Kemp E (1988) Long-term cyclosporine A nephrotoxicity in the rat. Effects on renal function and morphology. Nephrol Dial Transplant 3: 317–326
10. Donatsch P, Absich E, Homberger M, Traber R, Trapp M, Voges R (1981) A radioimmunoassay to measure cyclosporin A in plasma and serum samples. J Immunoassay 2: 19–32
11. Dunnill MS, Halley W (1973) Some observations on the quantitative anatomy of the kidney. J Pathol 110: 113–121
12. English J, Evans A, Houghton DC, Bennet WM (1987) Cyclosporine-induced acute renal dysfunction in the rat. Transplantation 44: 135–141
13. Farnsworth A, Hall BM, Ng ABP, Duggin GG, Horvath JS, Sheil AG, Tiller DJ (1984) Renal biopsy morphology in renal transplantation. Am J Surg Pathol 8: 243–252
14. Ferguson RM, Rynasiewicz JJ, Sutherland DER, Simmons RL, Najarian JS (1982) Cyclosporine A in renal transplantation: a prospective randomized trial. Surgery 92: 175–182

15. Humes HD, Jackson NM, O'Connor RP, Hunt DA, White MD (1985) Pathogenetic mechanisms of nephrotoxicity: insights into cyclosporine nephrotoxicity. Transplant Proc 17: 51–62
16. Jacobson SH, Eklöf O, Eriksson CG, Lins LE, Tidgren B, Winberg J (1989) Development of hypertension and uraemia after pyelonephritis in childhood: 27 year follow-up. BMJ 299: 703–706
17. Klintmalm GBG, Iwatsuki S, Starzl TE (1981) Nephrotoxicity of cyclosporine A in liver and kidney transplantation. Lancet I: 470–471
18. Klintmalm G, Bohman SO, Sundelin B, Wilczek H (1984) Interstitial fibrosis in renal allografts after 12 to 46 months of cyclosporine treatment: beneficial effect of low doses in early post-transplant period. Lancet II: 950–954
19. Kopp JB, Klotman PE (1990) Cellular and molecular mechanisms of cyclosporine nephrotoxicity. J Am Soc Nephrol 1: 162–179
20. Lundgren G, Albrechtsen D, Brynger H, Flatmark A, Frödin L, Gäbel H, Persson H, Groth CG (1987) Improved early course after cadaveric renal transplantation by reducing the cyclosporin dose and adding azathioprine. Transplant Proc 19: 2074–2079
21. Mihatsch MJ, Thiel G, Spichtin HP, Oberholzer M, Brunner FP, Harder F, Olivieri V, Bremer R, Ryffel B, Stöcklin E, Torhorst J, Gudat F, Zollinger HU, Loertscher R (1983) Morphological findings in kidney transplants after treatment with cyclosporine. Transplant Proc 15: 2821–2835
22. Mihatsch MJ, Thiel G, Ryffel B (1988) Histopathology of cyclosporine nephrotoxicity. Transplant Proc 20: 759–771
23. Morris PJ, Chapman JR, Allen RD, Ting A, Thompson JF, Dunnill MS, Wood RF (1987) Cyclosporine conversion versus conventional immunosuppression: long-term follow-up and histological evaluation. Lancet II: 586–591
24. Murray BM, Paller MS, Ferris TE (1985) Effect of cyclosporine administration on renal haemodynamics in conscious rats. Kidney Int 28: 767–774
25. Myers BD (1986) Cyclosporine nephrotoxicity. Kidney Int 30: 964–974
26. Nizze H, Mihatsch MJ, Zollinger HU, Brocheriou C, Gokel JM, Henry K, Sloane JP, Stovin PG (1988) Cyclosporine-associated nephropathy in patients with heart and bone marrow transplants. Clin Nephrol 30: 248–260
27. Palestine AG, Nussenblatt RB, Chan CC (1984) Side effects of systemic cyclosporine in patients not undergoing transplantation. Am J Med 77: 652–656
28. Palestine AG, Austin HA, Nussenblatt RB (1986) Renal tubular function in cyclosporine-treated patients. Am J Med 81: 419–424
29. Savoldi S, Scolari F, Sandrini S, Scaini P, Sacchi G, Tardanico R, Camerini C, Salerni B, Tonini G, de Nobili U (1988) Cyclosporine chronic nephrotoxicity: histologic follow-up at 6 and 18 months after renal transplantation. Transplant Proc 20: 777–784
30. Sullivan BA, Hak LJ, Finn WF (1985) Cyclosporine nephrotoxicity: studies in laboratory animals. Transplant Proc 17: 145–149
31. Thiru S, Maher ER, Hamilton DV, Evans DB, Calne RY (1983) Tubular changes in renal transplant recipients on cyclosporine. Transplant Proc 15: 2846–2851
32. Versluis DJ, Wenting GJ, Derkx FH, Schalekamp MA, Jeekel J, Weimar W (1987) Who should be converted from cyclosporine to conventional immunosuppression in kidney transplantation and why. Transplantation 44: 387–389
33. Versluis DJ, Wenting GJ, Jeekel J, Weimar W (1988) The influence of cyclosporine A on proximal tubular function in renal allografts. Transplant Proc 20: 686–690
34. Wallace AC (1985) Histopathology of cyclosporine. Transplant Proc 16: 117–122
35. Weibel ER (1979) Stereological methods I. Practical methods for biological morphometry. Academic Press, London, pp 101–161
36. Wilczek HE (1990) Percutaneous needle biopsy of the renal allograft. A clinical safety evaluation of 1129 biopsies. Transplantation 50: 790–797
37. Wilczek H, Bohman SO, Klintmalm G, Groth CG (1988) Five year serial renal graft biopsy study in cyclosporine-treated patients. Transplant Proc 20: 812–815
38. Zollinger HU, Mihatsch MJ (1978) Renal pathology in biopsy. Light, electron and immunofluorescent microscopy and clinical aspects. Springer Verlag, Berlin