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Nature and extent of glomerular injury induced by cyclosporine in heart transplant patients

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Nature and extent of glomerular injury induced by cyclosporine in heart transplant patients. We sought to clarify whether low-dose cyclosporine (5.0 ± 2.2 mg/kg/day) given for more than two years to prevent cardiac graft rejection induced glomerular injury and to quantify the extent of the lesions. After renal hemodynamic studies, renal biopsy specimens were obtained from 10 patients on cyclosporine and analyzed by a novel morphometric technique consisting of a tridimensional reconstruction of the glomerular tuft. Autopsy kidney specimens from three patients with no clinical history of renal disease, and from four patients who died with dilatative cardiomyopathy served as controls. The glomerular filtration rate and renal plasma flow were significantly depressed below normal values in transplant recipients given cyclosporine, averaging 35 ± 8 and 325 ± 94 ml/min/1.73 m^2, respectively. Conventional light microscopy of specimens from controls and from patients who died with dilatative cardiomyopathy did not reveal renal structural abnormalities. By contrast kidney specimens from cyclosporine-treated patients had obliterative arteriolopathy and ischemic-type changes, with thickening and wrinkling of glomerular capillary wall. Morphometrical analysis of 28 control glomeruli and 40 glomeruli from patients with dilatative cardiomyopathy showed glomerular capillary tuft volumes (V_{CT}) ranging between 1.2 and 2.3 $\mu m^3 \times 10^{-6}$, whereas of 102 glomeruli from cyclosporine-treated patients 42.1% had V_{CT} lower than 1.2 $\mu m^3 \times 10^{-6}$ and 24.4% V_{CT} higher than 2.3 $\mu m^3 \times 10^{-6}$. Tridimensional reconstruction revealed that 40.1% of glomeruli of cyclosporine-treated patients but none of controls were affected by global or segmental sclerosis which was confined to glomeruli with small and normal V_{CT}. Thus, only 2 out of 25 large glomeruli had sclerotic changes involving, however, less then 0.2% of V_{CT}. We conclude that cyclosporine given for more than two years induced moderate to severe renal failure in all patients associated with obliterative arteriolopathy and glomerular ischemia. In these patients two subpopulations of glomeruli of abnormal size emerged. Lower than normal glomeruli had global or segmental sclerosis. Thus, cyclosporine should be better employed for diseases in which the expected benefits are likely to outweigh its potential for inducing major glomerular functional and structural damage.

Cyclosporine has improved allograft survival in renal [1], liver [2], heart [3] and pancreas [4] transplantation. More recently cyclosporine has also been advocated as a promising therapeutic agent for the prevention of graft versus host disease in bone marrow transplantation [5] and for the treatment of some autoimmune diseases including uveitis [6], systemic lupus erythematosus [7], rheumatoid arthritis [8] and various forms of glomerulonephritis [9,10].

However, this drug has major side effects that considerably limit its use in humans, mainly because of liver and renal toxicity [11–13].

One of the main issues related to cyclosporine-induced renal dysfunction is whether this effect is confined to the use of "high" doses, as in the early stages of heart transplantation (that is, 10 to 17 mg/kg/day) [14] or whether it also occurs with the much lower doses that are currently employed to prevent kidney [15] and heart [16] allograft rejection (3 to 7 mg/kg/day). An additional issue that has not been clarified so far is whether cyclosporine nephrotoxicity is reversible or progressive in nature so that terminal renal failure may eventually occur in patients treated for more than two years [17]. The data so far available on this matter are conflicting [17–19].

Recent studies performed in heart transplant patients [17] showed that two years of cyclosporine therapy caused a decline in renal plasma flow (RPF) and glomerular filtration rate (GFR) which was not reversible, and was associated with signs of renal damage which at a given point progressed even if cyclosporine was stopped. At variance, Bantle and coworkers [19] suggested that although cyclosporine treatment produced a decrease in renal function the underlying disease was not necessarily progressive. However, most studies on cyclosporine-induced renal dysfunction did not correlate renal function deterioration with renal structural changes. This is particularly true for glomerular abnormalities which in the majority of the studies so far available have only been investigated by functional measurements.

The present study was designed to correlate renal function deterioration with morphological signs of renal damage in patients with cardiac transplant given cyclosporine for more than two years. Moreover we wanted to better clarify the nature of cyclosporine-induced glomerular damage which so far has not been properly investigated. To this purpose heart transplant patients underwent a renal biopsy and the specimens were analyzed by a novel morphometric technique consisting of a tridimensional reconstruction of the glomerular tuft [20]. This technique allowed to evaluate glomerular volume abnormalities

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and precisely define the extent and the spatial distribution of glomerular changes.

Methods

Subjects

The first ten patients (3 female, 7 male; mean age at the time of the study: 46.9 years; range 23 to 57 years) who survived more than two years after cardiac transplantation at the Division of Cardiac Surgery of the Ospedali Riuniti di Bergamo were studied. All patients had been transplanted because of dilatative cardiomyopathy, which was of ischemic etiology in four and idiopathic in six. At the time of the study all patients were in NYHA class I. The mean duration of follow up at time of study was 37.5 months (range: 31 to 48). At time of transplantation the mean oral loading dose of cyclosporine was 7.9 ± 3.1 mg/kg/day. Patients were then immunosuppressed with cyclosporine combined with prednisone or azathioprine, or both; the mean time-averaged dose of cyclosporine along the follow up was 4.8 ± 1.6 mg/kg/day.

Study design

The study protocol, approved by our institutional ethical committee, was illustrated in detail to all patients before admission, and oral informed consent to take part in the study was obtained in each instance. Patients were excluded from the study if a contraindication to percutaneous renal biopsy was present, that is, uncontrolled hypertension, hemorrhagic diathesis (as defined by one or more of the following: template bleeding time > 10 minutes; platelet count $<100{,}000/\mu l$; APTT and PT greater than 1.5 times the control values), solitary kidney, hydronephrosis, multiple renal cysts. Antiplatelet agents were discontinued for seven days before admission in all patients receiving such therapy. Patients were admitted to the Division of Nephrology of the Ospedali Riuniti and on the first hospital day renal function was determined by the clearances of inulin and para-aminohippuric acid (PAH). On the day following the physiologic studies all patients underwent needle renal biopsy and then discharged after a full day of bed rest.

Renal clearance studies

Each cyclosporine recipient underwent renal clearance study. In brief, the clearance of inulin and PAH was performed under a steady state of water diuresis induced by oral water loading. A priming solution containing 10% inulin and 20% PAH was infused, followed by continuous administration of inulin and PAH to maintain constant plasma concentrations of approximately 15 and 1.5 mg/dl, respectively. After an equilibration period of about 60 minutes, three exactly timed urine collections of 30 minutes each were performed. Blood pressure was measured periodically throughout the study. Urine was collected by spontaneous voiding, and blood was collected at the beginning and at the end of each clearance period. Inulin and PAH concentrations in plasma and urine samples were determined using methods previously described [21, 22]. GFR was calculated as the urinary clearance of inulin corrected for body surface area. Effective RPF was calculated by dividing the urinary clearance of PAH by an assumed extraction ratio of 0.8 [23].

Pathological studies

All 10 patients on cyclosporine underwent a percutaneous renal biopsy 31 to 48 months post-transplantation, on the day following the physiological studies. Renal tissue was obtained from renal autopsy specimen of four patients, matched for age, sex, and incidence of hypertension, who died with severe heart failure, due to dilatative cardiomyopathy. Since patients with chronic heart failure were on anticoagulant therapy, it was considered unethical to interrupt the anticoagulation to perform renal biopsy; therefore autopsy specimens were used as control material. Autopsy kidney specimens from three patients with no clinical history of heart and renal diseases and with normal renal function provided normal control values. Further comparison was obtained by performing morphometric analysis on a specimen of normal renal biopsy material.

Renal tissue was processed for light microscopy by fixation in Dubosq-Brazil fluid and embedding in paraffin. Pathological examination and semiquantitative assessment of renal damage were done in at least 18 sections. In cyclosporine-treated patients a total number of 269 glomeruli was evaluated including superficial and juxtamedullary glomeruli. The number and the percentage of globally and segmentally sclerotic glomeruli was determined. Focal and segmental sclerotic lesions were defined as an increase of mesangial matrix substance associated with capillary wall collapse. The sclerotic lesions might or might not be associated with adhesion to Bowman's capsule. The estimation of ischemic glomerular lesions, characterized by thickening and wrinkling of glomerular capillary wall and by thickening of Bowman's capsule, was obtained semiquantitatively using a scoring system from 0 to 3+ (0 = no changes; 1+ = < 30% of glomeruli affected; 2+ = 30 to 60% of glomeruli affected; 3+ = > 60% of glomeruli affected). The hyperplasia of juxtaglomerular apparatus was also graded from 0 to 3+ (0 = no changes, 1+ = mild, 2+ = moderate, 3+ = severe). Interstitial fibrosis and tubular atrophy were graded from 0 to 3+ (0 = no changes, 1+ = changes in < 25% of the biopsy specimens, 2+ = changes affecting 25% to 50% of biopsy specimens, 3+ = changes affecting > 50% of the biopsy specimens). Vascular lesions were evaluated as the presence of PAS positive material permeating the arteriolar wall and narrowing or occluding the vascular lumen or mucoid thickening of intima resulting in a narrowing of vascular lumens and were graded from 0 to 3+ (0 = no changes, 1+ = mild, 2+ = moderate, 3+ = severe). Sclerotic changes in the arteries were graded from 0 to 3+ (0 = no changes, 1+ = mild, 2+ = moderate, 3+ = severe).

Morphometric analysis

Glomerular volume was estimated using serial section reconstruction of individual glomeruli [20]. For each cyclosporine recipient, control, and patient with dilatative cardiomyopathy 81 consecutive sections (4 μ thick) were cut and stained with periodic-acid Schiff's reagent (PAS). A section approximately located in the middle of the tissue, hereafter referred as "starting section", was identified. The profiles of the Bowman's capsule, capillary tuft, and sclerotic changes of all glomeruli in this section were manually outlined using a drawing tube (Zeiss, Germany) mounted on the microscope at the final magnification of ×322. This procedure was repeated for each glomerulus in all the sections preceding and following the

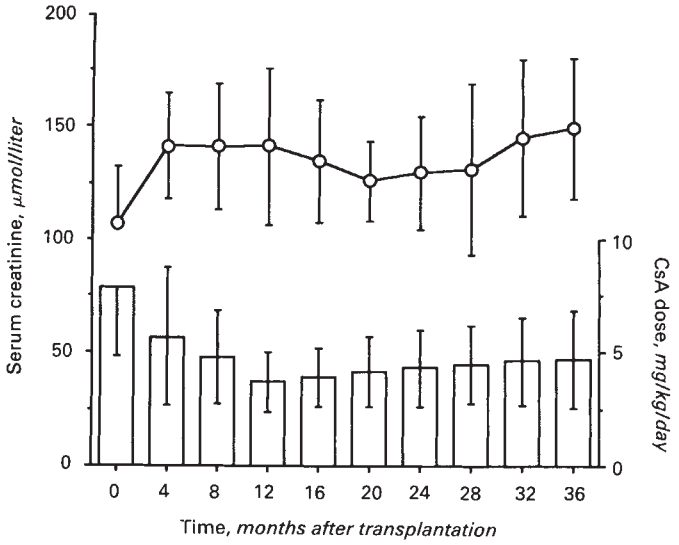

Fig. 1. *Serum creatinine in 10 patients with cardiac transplant at 4 month time intervals after transplantation (solid dots and line).* Columns represent the average cyclosporine dose at the same time points. *$P < 0.05$ vs. month 0.

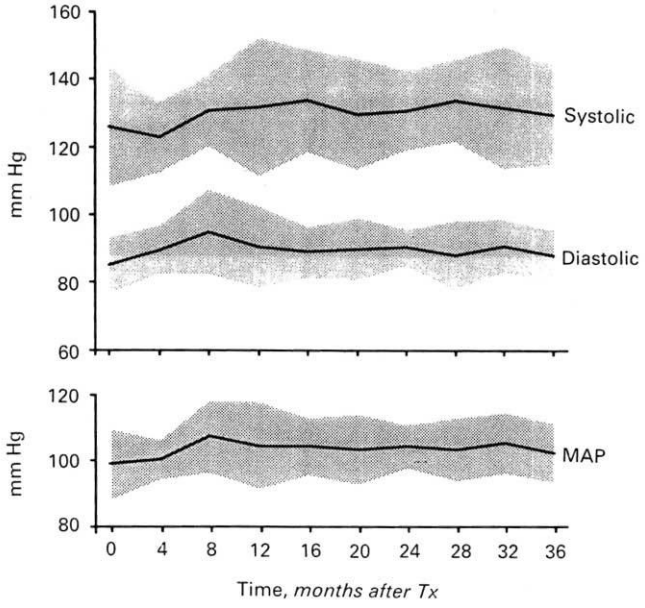

Fig. 2. *Systolic and diastolic blood pressure in cardiac transplant patients during 36 months of follow-up.* Shaded areas represent standard deviations from the mean.

"starting section" until it completely disappeared. Only glomeruli entirely reconstructed were considered for morphometrical analysis (102 glomeruli from cyclosporine recipients, 28 from controls, and 40 from patients with dilatative cardiomyopathy). Surface area of each outline of Bowman's capsule, capillary tuft, and sclerotic regions were determined with stereological methods [24] using a grid 26 × 29 points. Volume of Bowman's capsule, capillary tuft (V_{CT}) and sclerotic regions for each glomerulus was calculated as:

$$V = (\sum_{i=1}^{n} A_i) \cdot d$$

where A_i is the surface area of the outlines of Bowman's capsule, capillary tuft, and glomerular sclerotic regions, d is the section thickness and n is the number of sections for each glomerulus. The percentage of V_{CT} affected by sclerosis was then calculated for each individual glomerulus.

Cyclosporine immunoassay

Cyclosporine trough concentrations were determined on whole blood by a specific radioimmunoassay employing a monoclonal antibody (Incstar Corporation, Minnesota, USA).

Statistical analysis

All results are expressed as mean ± SD. Data were analyzed using unpaired Student's *t*-test and linear regression analysis. Serum creatinine data were analyzed using one-way analysis of variance and Dunnett test for multiple comparisons [25]. Statistical significance level was defined as $P < 0.05$.

Results

Clinical and laboratory findings

Mean serum creatinine of study population at the time of transplantation was 105 ± 8 μmol/liter. Figure 1 shows the mean serum creatinine at various time intervals after transplantation together with the mean daily dose of cyclosporine. Mean serum creatinine concentration significantly increased ($P < 0.05$) after transplantation and remained elevated above pretransplant value during the entire observation period. Two patients had a transient rise of serum creatinine above 200 μmol/liter during the course of their follow up, but no episodes of acute renal failure were recorded during the entire period of follow up. By the time of renal biopsy, mean serum creatinine concentration of patients in the study group was 140 ± 44 μmol/liter. This value is comparable to that of other patients who received cardiac transplant at our Institution and had been treated with cyclosporine for a similar length of time. Only one patient had measurable proteinuria (0.7 g/24 hr) at time of transplantation. At the time of renal biopsy seven patients were proteinuric, all of them with urinary protein excretion less than 1 g/24 hr.

Both systolic and diastolic blood pressure increased slightly after transplantation in most of the patients; seven of them required antihypertensive therapy, which consisted of low doses of captopril or nifedipine. However as shown in Figure 2 blood pressure was only moderately elevated during three years of follow up. At the time of the study mean arterial blood pressure was 142 ± 12 mm Hg over 89 ± 8 mm Hg.

Renal function studies

Renal functional parameters measured in cyclosporine recipients are summarized in Table 1. Patients were receiving a dose of cyclosporine ranging from 3 to 10 mg/kg/day. Corresponding trough concentrations of immunoassayable cyclosporine were on average 255 ± 151 ng/ml. In all cyclosporine recipients mean arterial pressure was in the normal range. In each patient mean RPF was markedly lower than normal values, averaging 325 ml/min/1.73 m^2. Similarly GFR was reduced as compared to

Table 1. Physiologic findings of cyclosporine recipients

	Mean	Range
Cyclosporine dose *mg/kg/24 hr*	5	3–10
Cyclosporine trough *ng/ml*	255	84–600
MAP *mm Hg*	108	87–119
GFR *ml/min/1.73 m²*	35	18–47
RPF *ml/min/1.73 m²*	325	207–472
Filtration fraction %	10	7–15
Proteinuria *g/24 hr*	0.2	0–0.45

Abbreviations are: MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow.

normal values, averaging 35 ml/min/1.73 m^2 (Table 1). Since the decline in GFR exceeded that in RPF, filtration fraction was lower than normal.

Morphological findings

All the 10 cardiac transplant patients treated with cyclosporine had major changes at renal biopsy. By contrast renal autopsy specimens from controls and from patients who died with dilatative cardiomyopathy did not reveal renal structural abnormalities.

Light microscopic findings in cyclosporine patients are summarized in Table 2. The spectrum of changes was rather broad and involved all anatomical structures of the kidney. Seventy-eight glomeruli of the 269 examined (28.9%) exhibited global (N = 48, 17.8%) or segmental (N = 30, 11.1%) sclerosis. Global glomerular sclerosis was present in all patients but one involving 5.8% to 44.4% of the glomeruli, while segmental sclerotic lesions were observed in all but two patients. Most glomeruli disclosed ischemic changes characterized by thickening and wrinkling of glomerular capillary wall and by thickening of Bowman's capsule. A striking juxtaglomerular apparatus hyperplasia was evident in all glomeruli examined from all patients and ranged from 1+ to 3+ (average 1.8 ± 0.6). All specimens exhibited a pattern of tubular atrophy and interstitial fibrosis, in some cases the changes being nearly diffuse, but most showing narrow striped forms. Interstitial chronic inflammatory reaction was unremarkable. Vascular lesions also included the so-called form of cyclosporine arteriolopathy [16] that was detected in all cyclosporine recipients but one and averaged 1.33 ± 0.50 (mean score). Arteriolar abnormalities consisted of subintimal and medial hyaline deposits with focal replacement of pericytes by PAS-positive hyaline material.

Results of morphometrical analysis are reported in Figure 3. Mean V_{CT} averaged 1.74 ± 0.30 $\mu m^3 \times 10^{-6}$ in controls and 1.58 ± 0.29 $\mu m^3 \times 10^{-6}$ in patients died with dilatative cardiomyopathy, values comparable to those found in cyclosporine recipients (1.66 ± 0.97 $\mu m^3 \times 10^{-6}$). In a specimen of normal renal biopsy material the mean V_{CT} was 1.43 ± 0.34 $\mu m^3 \times 10^{-6}$.

By contrast the distribution of V_{CT} around the mean was markedly different in controls, patients with dilatative cardiomyopathy, and cyclosporine patients. V_{CT} of glomeruli from controls and patients with dilatative cardiomyopathy ranged between 1.2 and 2.3 $\mu m^3 \times 10^{-6}$, whereas in cyclosporine-treated patients V_{CT} ranged from 0.28 to 4.81 $\mu m^3 \times 10^{-6}$. Forty-two percent of glomeruli had a $V_{CT} < 1.2$ $\mu m^3 \times 10^{-6}$, and 24.5% were > 2.3 $\mu m^3 \times 10^{-6}$, showing that, as compared to control glomerular V_{CT}, two additional subsets of glomeruli with reduced or enlarged V_{CT} were present in the specimens from cyclosporine-treated patients. Similar results were obtained for Bowman's capsule volume (Fig. 4).

Serial section analysis did not show sclerotic lesions in specimens taken from controls and patients with dilatative cardiomyopathy. By contrast, tridimensional reconstruction of 102 glomeruli from cyclosporine-treated patients revealed that 40.1% of glomeruli were affected by sclerosis (8.8% global sclerosis, 31.3% segmental sclerosis). Of note, this percentage was higher than that estimated in the same patients by conventional histological analysis, which averaged 28.9%. Figure 5 illustrates the relationship between volume of glomerular sclerotic lesions and V_{CT} for each individual glomerulus in patients on cyclosporine. No significant correlation ($r = 0.07$, $P = 0.646$) was found between V_{CT} and sclerosis volume. However, glomeruli with V_{CT} larger than normal ($V_{CT} > 2.3$ $\mu m^3 \times 10^{-6}$) were almost never affected by sclerotic changes. Sclerotic lesions were confined to glomeruli with small and normal V_{CT}. Thus for small glomeruli ($V_{CT} < 1.2$ $\mu m^3 \times 10^{-6}$) 28 out of 43 had sclerotic changes, for normal glomeruli (V_{CT} 1.2 to 2.3 $\mu m^3 \times 10^{-6}$) 12 out 34, whereas for large glomeruli ($V_{CT} > 2.3$ $\mu m^3 \times 10^{-6}$) only 2 out 25 had sclerotic changes that however involved less than 0.2% of V_{CT} volume.

To establish whether the reduction in GFR observed in these patients was related to the degree of glomerulosclerosis, in seven patients in which the number of glomeruli used for morphometrical analysis was greater than 10, linear regression analysis was applied to correlate the observed values of GFR with the percent of V_{CT} affected by sclerosis. No significant correlation was found ($r = 0.53$, $P = 0.219$) between these two parameters suggesting that fall in GFR, as measured in this study, is not completely accounted for the degree of glomerulosclerosis in a given patient.

Figure 6 shows the correlation between the time-averaged dose of cyclosporine during the follow up in individual patients, expressed in mg/kg/day, and the mean capillary tuft volume in their renal biopsy. Although a trend exists to smaller glomeruli in patients treated with higher doses of cyclosporine, regression analysis did not reached a significance level.

Discussion

The present study shows that patients on cyclosporine for more than two years to prevent the reject of heart transplant had a severe nephropathy. Renal functional studies showed a marked reduction in RPF and GFR with a proportional greater fall in GFR. This was associated with renal structural changes—at renal biopsy—that included global or segmental glomerulosclerosis, signs of glomerular ischemia, juxtaglomerular apparatus hyperplasia, tubular atrophy, interstitial fibrosis, and hyaline deposits in the arteriolar walls.

Some of these changes had been previously recognized also in the kidneys of patients receiving cyclosporine after liver transplantation [26], or to treat autoimmune diseases such as uveitis [27]. However, one might argue that the lesions observed in our patients may not have been entirely related to the toxic effect of the drug but, at least in part, to a preexisting renal damage possibly related to chronic heart failure. However, renal autopsy specimens taken from patients with severe heart failure and elevated serum creatinine who died before a heart

Table 2. Renal histopathology by conventional analysis

	Months on cyclosporine	GS %	SG %	JGAH	WCW	BCT	IF	TA	A	CsA-AA
B.B.	38	34.8	8.7	2+	0	1+	2+	2+	0	1+
A.G.	39	41.1	11.7	2+	1+	1+	1+	1+	0	2+
G.C.	42	44.4	0	1+	1+	1+	2+	2+	1+	N-A
F.A.	34	43.7	6.8	1+	1+	1+	2+	2+	1+	2+
P.P.	41	0	7.3	2+	1+	2+	2+	2+	2+	1+
S.G.	38	9.6	0	3+	2+	1+	2+	2+	1+	1+
G.D.	34	5.8	17.6	1+	1+	1+	2+	2+	0	1+
F.S.	48	23.5	17.6	2+	1+	2+	2+	2+	1+	1+
T.A.	37	7.4	12.7	2+	1+	1+	1+	1+	1+	2+
C.M.	34	22.7	20.4	2+	1+	1+	2+	2+	0	1+

Abbreviations are: GS, global sclerosis; SG, segmental sclerosis; JGAH, juxtaglomerular apparatus hyperplasia; WCW, wrinkling of capillary wall; BCT, Bowman's capsule thickening; IF, interstitial fibrosis; TA, tubular atrophy; A, arteriosclerosis; CsA-AA, cyclosporine arteriolopathy; N-A, no arterioles available.

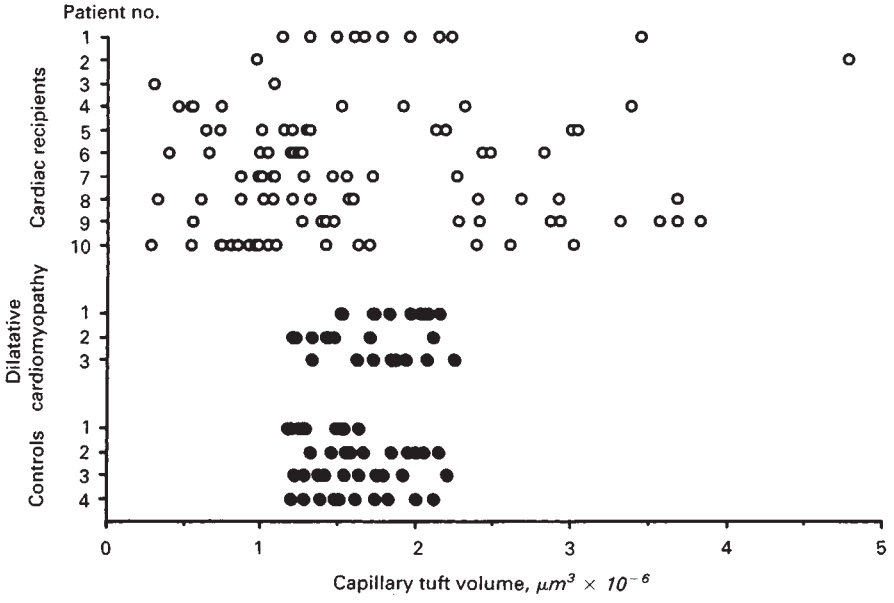

Fig. 3. *Distribution of glomerular capillary tuft volumes in cyclosporine-treated cardiac transplant recipient (○) and control (●).*

transplant became available did not show structural changes. This would suggest that glomerular, interstitial and vascular lesions observed in our cyclosporine patients are likely due to the peculiar renal effect of the drug, rather than a consequence of the underlying hemodynamic changes possibly related to the heart failure.

Similarly, previous studies in autopsied patients who had died with heart failure and reduced GFR [28], as well as more recent reports on heart transplant patients given azathioprine instead of cyclosporine [14, 17], failed to demonstrate glomerular sclerosis, tubulointerstitial fibrosis or hyaline arteriolopathy. All these considerations reinforce the concept that renal structural lesions in cyclosporine-treated patients are likely the consequence of the chronic use of cyclosporine rather than of the pre-existing cardiac failure.

It has been reported that virtually all cardiac transplant recipients treated with cyclosporine develop hypertension, whereas only 20% of the patients treated with azathioprine and prednisone became hypertensive [29–31]. Our patients had normal to mildly elevated blood pressure during the follow up period and seven of them required antihypertensive therapy, which obtained good control of blood pressure (Fig. 2). Studies in early stages of established essential hypertension have shown that GFR is well preserved [32]. Renal deterioration is uncommon in patients with mild hypertension as defined as a diastolic blood pressure of 90 to 104 mm Hg [33]. Most of the patients in whom renal biopsy was performed after 5 to 10 years of untreated essential hypertension show slight to moderate arteriosclerosis [34], and there is correlation between severity of pathological changes and degree of hypertension. Other studies have shown that 15 to 20% with moderate to severe hypertension have little or no intrarenal vascular disease, and almost 50% do not have changes that could be considered "significant" [35–37]. If one considers the short duration and the mild degree of hypertension in our patients, it seems unlikely that the observed severe renal functional impairment and structural damage can be attributed to hypertension.

The novelty of the present study consists of the serial section analysis of individual glomeruli to precisely determine the extent of the lesions and to calculate glomerular volumes. Using

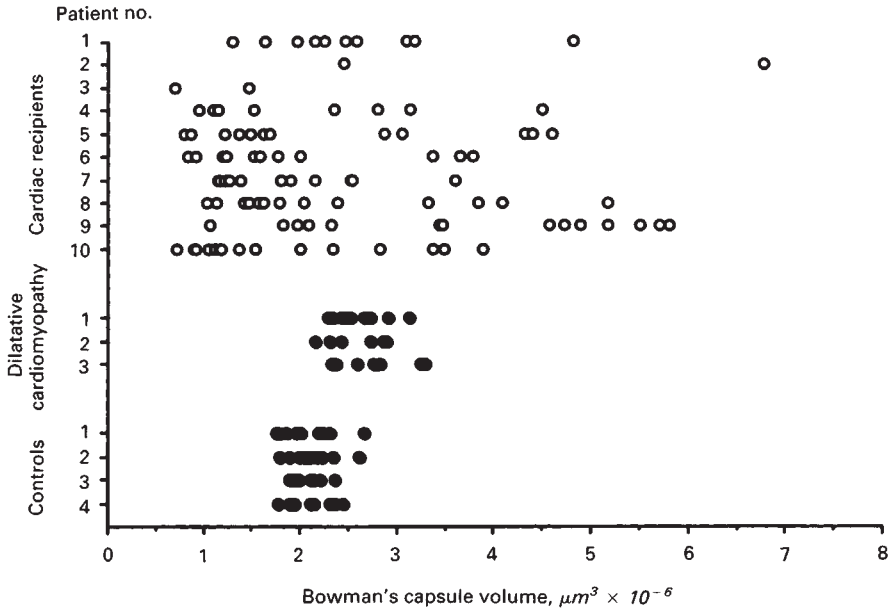

Fig. 4. *Distribution of Bowman's capsule volumes in cyclosporine-treated cardiac transplant recipient (○) and control (●).*

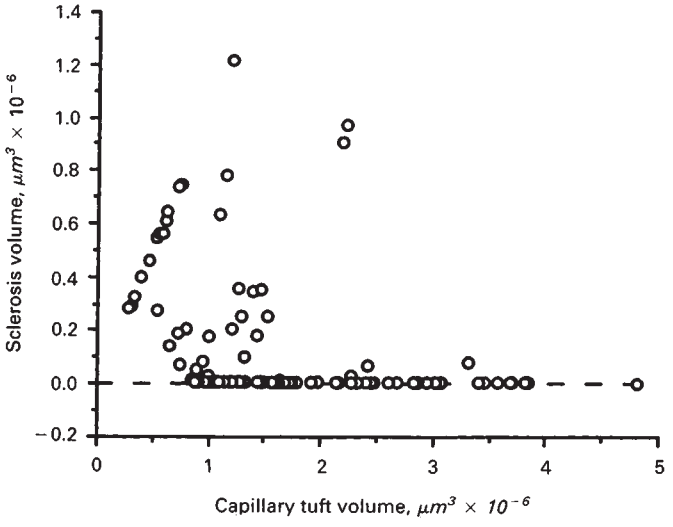

Fig. 5. *Relationship between volume of glomerular sclerotic lesions and glomerular capillary tuft volume for each individual glomerulus in heart transplant recipients on cyclosporine.*

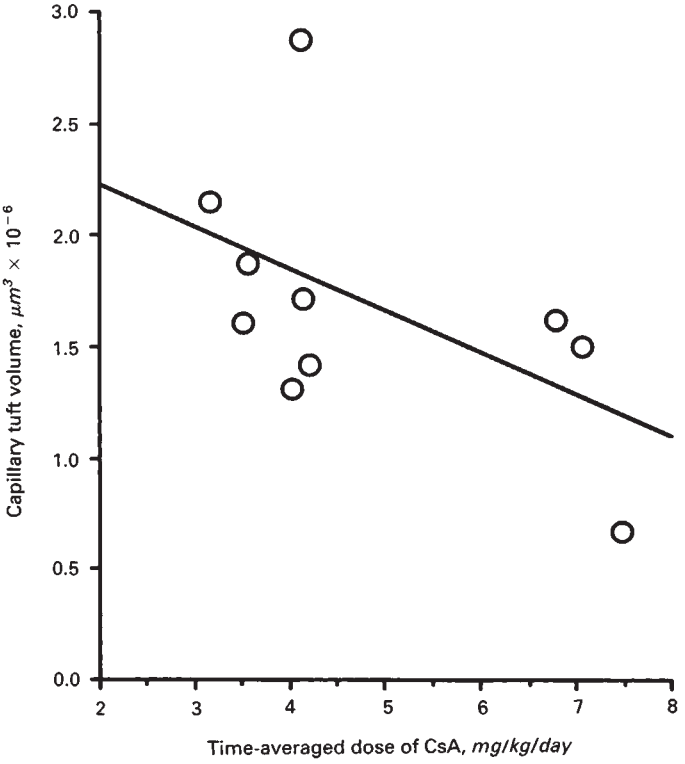

Fig. 6. *Relationship between glomerular capillary tuft volume and time-averaged cyclosporine dose (as mg/kg/day) in heart transplant recipients on cyclosporine.*

this morphometrical technique 102 glomeruli from 10 heart transplant patients given cyclosporine, 28 glomeruli from control subjects and 40 glomeruli from patients who died for a dilatative cardiomyopathy were reconstructed. Forty percent of glomeruli in renal biopsies taken from cardiac transplant patients had global or segmental sclerotic changes, while no glomerulosclerosis was found in any of the two "control" group glomeruli. This estimation of glomerulosclerosis is at variance with the data of conventional histological analysis we performed in the same patients. Actually conventional histology showed sclerotic lesions in only 28.9% of the glomeruli examined. This difference indicates that the conventional analysis of renal histological changes based on the examination of selected sections underestimates the actual number of glomeruli affected by the sclerotic lesions.

In the attempt to quantify the nature of cyclosporine-induced glomerulopathy Myers and coworkers [16] have measured the distribution of glomerular cross-sectional areas in heart transplant patients treated with "high" and "low" dose of cyclo-

sporine. They found that the bell-shaped distribution of glomerular area observed in normal subjects was lost in patients on cyclosporine for at least 12 months who had an increased prevalence of smaller and higher than normal glomerular areas. The authors' interpretation of their findings was that the subset of glomeruli that had escaped sclerosis underwent hypertrophy as a possible compensatory mechanism to the loss of filtration surface by the damaged glomeruli. The results we obtained by serial section reconstruction of individual glomeruli are remarkably similar to those reported by Myers and coworkers [16]. After more than two years of cyclosporine therapy smaller and larger than normal glomeruli appeared. Thus the relative distribution of the 102 glomeruli analyzed showed that 42.1% were smaller, and 24.5% were larger than normal range.

The interpretation that the subset of larger than normal glomeruli had undergone a process of compensatory hypertrophy [16] is not in contrast with our findings. In fact, after 31 to 48 months of cyclosporine, none of the hypertrophic glomeruli had sclerotic lesions, the majority of glomeruli with global or segmental sclerosis (64.2%) belonging to the subset of small glomeruli. It is possible that large glomeruli, exposed to major adaptive hemodynamic changes, will eventually be damaged as shown in experimental animals with surgical reduction of renal mass [38, 39]. With time this might lead to an inexorable progression of the renal damage. However, at the time of the present analysis we have been unable to find a correlation between the decline in GFR and the degree of sclerotic lesions in individual patients, suggesting that the development of glomerulosclerosis is not the only factor responsible for the reduction in GFR. Besides, we have observed a rather important reduction of RPF below normal values which could account, at least partially, for the observed reduction in GFR. That cyclosporine treatment is associated with a reduction in RPF has been also reported by Myers et al [16], and it is likely the consequence of the sustained increase in preglomerular vascular resistance observed in heart transplant patients given "low" dose cyclosporine [16]. Moreover, there is increasing experimental evidence that cyclosporine-induced renal impairment is caused by vasoconstriction of afferent arteriole [40]. Signs of glomerular ischemia were the pronounced wrinkling and thickening of glomerular capillary wall and the marked hyperplasia of juxtaglomerular apparatus observed in all the specimens we examined from cyclosporine-treated patients. In turn glomerular ischemia leads to a reduction of efferent arteriolar compliance so that peritubular arteriole perfusion is reduced. This is the most likely explanation for the lesions reported by Mihatsch and coworkers some years ago [41] who described tubulointerstitial lesions characterized by severe tubular atrophy, thickening of tubular basement membrane and the peculiar striped form of interstitial fibrosis in renal transplant patients on cyclosporine.

In conclusion we have documented that patients with heart transplantation given cyclosporine at a dose that is considered "low" by the current standards [14, 16] have major renal functional and structural changes. Whether more prolonged treatment would result in progressive and possibly irreversible renal damage is, at the present, open to speculation. On the other hand we cannot exclude that patients treated with lower doses of cyclosporine and/or for shorter periods of time than the present population of patients may have only mild signs of renal involvement, or none at all.

The morphometric approach we employed allowed to define for the first time that beside tubulointerstitial and vascular changes the chronic treatment with cyclosporine is associated with a global or segmental glomerulosclerosis which is the likely consequence of afferent arteriole vasoconstriction. These findings raise the issue of the theoretical benefit of cyclosporine weighed against its adverse effect on the kidney. We believe that the risk of cyclosporine nephropathy might be acceptable for patients with cardiac transplant as long as no alternative immunosuppressive therapy is practicable. However, treatments for more than two years have to be considered with more caution to prevent renal allograft rejection, and this kind of toxicity is probably unacceptable for the prevention of rejection of isolated pancreas graft in non-uremic diabetics [42].

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