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Glomerular Enlargement Assessed by Paired Donor and Early Protocol Renal Allograft Biopsies

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The aim of the study was to evaluate the evolution of glomerular volume 4 months after transplantation. Mean glomerular volume (Vg) was estimated according to the Weibel and Gomez method in a donor and a protocol biopsy done at 139 ± 58 d in 41 stable grafts. Biopsies were also evaluated according to the Banff schema. Vg increased after transplantation from 4.1 ± 1.4 to 5.1 ± 2.4 × 10^6 μ^3 ($p = 0.02$). In patients with chronic allograft nephropathy in the protocol biopsy ($n = 14$), the Vg enlargement was −0.3 ± × 10^6 μ^3 while in patients without chronic allograft nephropathy ($n = 27$), glomerular enlargement was 1.6 ± 2.1 × 10^6 μ^3 ($p = 0.01$). There was a negative association between glomerular volume in the donor biopsy and glomerular enlargement after transplantation ($R = -0.34$, $p = 0.03$). Multivariate regression analysis confirmed that Vg in the donor biopsy and chronic allograft nephropathy in the protocol biopsy were independent predictors of glomerular enlargement after transplantation ($R = 0.48$, $p = 0.01$). Moreover, Vg in the protocol biopsy correlated with creatinine clearance at the time of biopsy ($R = 0.38$, $p = 0.01$). Glomeruli enlarge after transplantation and glomerular volume after 4 months correlates with creatinine clearance, suggesting that glomerular enlargement is a necessary condition for renal adaptation to the recipient. Glomerular enlargement is impaired in patients with chronic allograft nephropathy.

Key words: Chronic allograft nephropathy, glomerular enlargement, glomerular volume, protocol renal allograft biopsies

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Introduction

Total glomerular number in healthy subjects shows an important variability ranging from 0.2 to 1.8 × 10^6 per kidney (1,2). Among dissimilar animals the adaptation of renal function to an increasing metabolic demand mainly depends on an increased glomerular number and only marginally on glomerular enlargement (3). In humans, the number of nephrons is determined by genetic and environmental factors and does not increase after birth. Accordingly, glomerular adaptation to an increased metabolic demand or a reduction in renal mass only depends on glomerular enlargement (1,2). However, it has been demonstrated that the capacity of glomeruli to enlarge is progressively diminished with aging (4,5).

In renal transplantation, it has been proposed that the implantation of one kidney may favor hyperfiltration-mediated damage due to an imbalance between nephron mass and recipient size, triggering glomerular enlargement, glomerulosclerosis and progressive renal failure (6). However, in renal transplants glomerular adaptation may be influenced by pre-existing chronic renal damage and post-transplant injuries such as ischemia-reperfusion lesions or the alloimmune response. Pre-existing tubulointerstitial (7,8) and vascular lesions (9) as well as glomerulosclerosis in donor biopsies (10) are associated with worse allograft outcome. Large glomerular volume in donor biopsies is associated with poor graft function (11) and it has been suggested that extreme glomerular enlargement after transplantation is associated with glomerulosclerosis (12). However, glomerular size adaptation after transplantation has not been systematically studied.

Thus, the aim of the present study was to evaluate mean glomerular volume before and after transplantation using paired pre-implantation and protocol biopsies performed in stable renal allografts.

Patients and Methods

Patients

We have retrospectively reviewed our files to identify patients in whom a donor and an early protocol biopsy with sufficient tissue were available. The timing of early protocol biopsies at our center has been progressively delayed in order to better focus on the study of early chronic lesions. Between 1988 and 1990 the biopsy was done during the first month while later on the biopsy was done between the 3rd and 6th month.

Biopsies

Donor and recipient biopsies were performed and processed for routine light microscopy. Renal lesions in donor and protocol biopsies were graded and diagnosed according to the 1997 Banff criteria in the absence of any

clinical information. For the present study, only biopsies containing at least seven glomeruli and one arterial section were considered (13).

Morphometry

Silver methenamine stained sections were employed for histomorphometric analysis. Mean glomerular volume (Vg) was estimated by a point counting method in all the available glomeruli in one section. For this purpose, a grid of 560 points was displayed on a television screen and the distance between grid points was calibrated with a micrometer ruler. Glomerulus was defined as the area inside the minimal convex polygon described by the outer capillary loops of the tuft. The total number of points hitting the glomerular tuft was counted at 200× magnification. Mean glomerular volume was estimated from the mean glomerular area (A) according to the Weibel and Gomez (W and G) method as (14):

$$Vg = A^{3/2} * \beta / d \quad (1)$$

where β is 1.38, the shape coefficient of the sphere, and d is 1.01, the size distribution of glomeruli assuming a 10% coefficient of variation of the caliper diameter. We estimated mean glomerular volume by means of the W and G method despite the fact that the gold standard to estimate particle volume is the Cavalieri principle (15). This last method is time consuming as it implies counting consecutive serial sections of complete glomeruli and requires knowing the average thickness of biopsy sections. By contrast, the W and G method is an assumption based method requiring only one biopsy section and as we have previously shown, allows obtaining a rather precise estimate of Vg in early protocol renal allograft biopsies (16).

Glomerular volume enlargement, δVg, was calculated as the difference between Vg in protocol and donor biopsy.

In order to evaluate the presence of shrunken glomeruli, we estimated the mean corpuscle glomerular volume by means of a point counting method and we expressed the result as the proportion of mean tuft volume and mean corpuscle volume (tuft/corpuscle). Glomerular corpuscle was defined as the area inside the Bowman's capsule.

Clinical variables

During this period of time, different combinations of immunosuppressive drugs were employed in our center. Thirty-one patients received a cyclosporine A (CsA) and prednisone-based immunosuppression. This treatment was associated with anti-lymphocytic induction therapy (n = 9), azathioprine (n = 8), mycophenolate mofetil (n = 7) or sirolimus (n = 2). In the remaining 10 cases a tacrolimus and prednisone-based immunosuppression was employed. This treatment was associated with mycophenolate mofetil (n = 9) or sirolimus (n = 1). Exposure to CsA or tacrolimus was evaluated from the mean levels until the protocol biopsy, and calculated from levels at days 7, 14, 30, 60, 90 and the day of the protocol biopsy.

Body surface area (BSA) (17) and body mass index (BMI) were calculated. Creatinine clearance at the time of biopsy was calculated according to the Cockcroft and Gault formula (18).

Statistics

Results are expressed as the mean ± standard deviation. Comparison between paired data was performed using the Wilcoxon *t*-test for ordinal variables or not normally distributed continuous variables and by the paired Student's *t*-test for continuous normally distributed variables. Similarly, comparison between unpaired data was done by means of the Mann–Whitney *U*-test or the Student's *t*-test.

Simple regression analysis and stepwise multiple regression analysis were employed to study the relationship between quantitative parameters. The presence or absence of chronic allograft nephropathy (CAN) in the protocol biopsy was transformed into two dummy variables for the multiple regression analysis.

All p-values were two-tailed and a p-value < 0.05 was considered significant.

Results

A donor and a protocol biopsy containing at least seven glomeruli and one arterial section were obtained in 41 patients. Demographic characteristics of patients are summarized in Table 1.

Evolution of histological lesions

Banff scores in donor and protocol biopsies are summarized in Table 2. A significant increase in the severity of chronic interstitial, tubular and vascular lesions was

Table 1: Demograhic and clinical characteristics after transplantation

Variable	Mean ± SD	Range
Donor age (years)	36 ± 14	(12–60)
Donor gender (male/female)	23/18	
Patient age (years)	42 ± 12	(20–65)
Patient gender (male/female)	27/14	
Body mass index (kg/m^2)	24 ± 4	(17–34)
Body surface area (m^2)	1.7 ± 0.2	(1.4–2.2)
Panel reactive antibodies (%)	3 ± 6	(0–25)
DR mismatches	0.7 ± 0.5	(0–1)
Cold ischemia time (hours)	20 ± 5	(10–31)
Delayed graft function (no/yes)	35/6	
Acute rejection (no/yes)	29/12	
Time of protocol biopsy (days)	139 ± 58	(16–255)
Serum creatinine (μmol/L)	147 ± 56	(78–298)
Creatinine clearance (mL/min)	56 ± 17	(26–90)
Proteinuria (g/day)	0.32 ± 0.25	(0.06–1.00)
Mean arterial blood pressure (mmHg)	103 ± 12	(80–137)

Serum creatinine, creatinine clearance, proteinuria and mean arterial blood pressure were determined at the time of biopsy.

Table 2: Characteristics of donor and protocol biopsies

	Donor biopsy	Protocol biopsy	p
Glomerular sections	19 ± 10	16 ± 7	
Glomerulosclerosis (%)	4.3 ± 7.5	2.9 ± 5.8	NS
Glomerulitis	0	0.19 ± 0.51	0.019
Interstitial infiltrate	0	0.73 ± 0.74	<0.001
Tubulitis	0	0.46 ± 0.74	<0.001
Intimal arteritis	0	0	NS
Arteriolar hyalinosis	0.15 ± 0.42	0.29 ± 0.56	NS
Chronic glomeruli	0.10 ± 0.30	0.15 ± 0.36	NS
Interstitial fibrosis	0.19 ± 0.45	0.49 ± 0.64	0.006
Tubular atrophy	0.15 ± 0.42	0.49 ± 0.67	0.002
Intimal thickening	0.02 ± 0.16	0.19 ± 0.60	0.087
Vg (×10^6 μ^3)	4.1 ± 1.4	5.1 ± 2.4	0.021

Vg: mean glomerular volume.

observed while the increase in chronic glomerular damage and arteriolar hyalinosis did not reach statistical significance.

Protocol biopsies were classified as normal (n = 19), borderline changes (n = 6), acute rejection (n = 2) and CAN (n = 14). Patients were divided in two groups according to the presence (n = 14) or absence of CAN (n = 27). No association between acute or chronic Banff scores in the protocol biopsy and creatinine clearance was observed.

There was a significant increase in glomerular size after transplantation (Table 2). However, the correlation between Vg in the donor and in the recipient was in the limit of statistical significance (R = 0.27, p = 0.08).

Factors associated with Vg in the donor and protocol biopsies

Neither donor age nor sex was associated with Vg in donor biopsies.

The only clinical variables associated with Vg in the protocol biopsy were serum creatinine (R = 0.31, p = 0.04) and creatinine clearance at the time of biopsy (R = 0.38, p = 0.01). Vg in the protocol biopsies was not associated with donor or recipient age and gender, BSA, immunosuppression, delayed graft function, acute rejection and the timing of protocol biopsy.

In order to further characterize the relationship between creatinine clearance at the time of biopsy and Vg, univariate and multivariate stepwise regression analyses were performed considering all clinical and histological variables. Vg in the protocol biopsy and donor age were the only independent predictors of creatinine clearance (R = 0.53, p = 0.002).

Factors associated with glomerular enlargement

Patients with CAN in the protocol biopsy had smaller glomeruli than patients without CAN. In agreement with this observation, glomeruli did not enlarge after transplantation in patients with CAN while there was a significant enlargement of Vg in patients without CAN. The coefficient tuft/corpuscle was not different in both groups. As shown in Table 3, no other clinical variables were associated with the presence or absence of CAN.

Moreover, an inverse association between Vg in the donor biopsy and glomerular enlargement was observed (R = −0.30, p = 0.05). The stepwise multivariate regression analysis showed that both variables, CAN in the protocol biopsy and Vg donor, were independent predictors of glomerular enlargement (R = 0.48, p = 0.007). The first variable entered into the model was the presence of CAN (R = 0.37, p = 0.017).

Table 3: Clinical data at transplantation and at the time of biopsy in patients with and without chronic allograft nephropathy in the protocol biopsy

Variable n	No CAN 27	CAN 14	p
Donor age (years)	37 ± 13	35 ± 16	NS
Donor gender (male/female)	16/11	7/7	NS
Patient age (years)	43 ± 13	40 ± 12	NS
Patient gender (male/female)	18/9	9/5	NS
Body surface area (m^2)	1.74 ± 0.19	1.76 ± 0.18	NS
Body mass index (kg/m^2)	24 ± 4	24 ± 4	NS
Panel reactive antibodies (%)	2 ± 5	3 ± 7	NS
A+B+DR mismatches	3.0 ± 1.0	3.1 ± 0.8	NS
Cold ischemia time (hours)	20 ± 5	19 ± 4	NS
Delayed graft function (no/yes)	23/4	12/2	NS
Acute rejection (no/yes)	21/6	8/6	NS
Time of protocol biopsy (days)	139 ± 60	139 ± 58	NS
Serum creatinine (μmol/L)	142 ± 54	158 ± 60	NS
Serum cholesterol (mmol/L)	5.5 ± 1.1	5.8 ± 1.2	NS
Proteinuria (g/day)	0.3 ± 0.2	0.4 ± 0.2	NS
Cyclosporine (yes/no)	23/4	9/5	NS
Mean cyclosporine level (μg/L)	200 ± 106	270 ± 77	NS
Vg D ($x10^6$ μ^3)	4.1 ± 1.3	4.1 ± 1.7	NS
Vg R ($x10^6$ μ^3)	5.8 ± 2.5	3.9 ± 1.9	0.015
Tuft/corpuscle	0.73 ± 0.11	0.74 ± 0.15	NS
Vg R – Vg D ($x10^6$ μ^3)	1.6 ± 2.1	−0.3 ± 2.6	0.017

Vg D: donor mean glomerular volume; Vg R: recipient mean glomerular volume.

Discussion

In the present study, we have observed that glomeruli enlarge during the first 4 months after transplantation in stable renal allografts. The adaptation of glomerular volume after transplantation has not been systematically investigated. Some studies have suggested that glomerular volume in renal transplants is not different from nonpaired donor biopsies (16–20). However, glomerular volume adaptation after transplantation has not been explored using paired biopsies done before and after transplant. Because the range of Vg in normal adults is rather wide, only studies using paired data will allow controlling the variability of Vg between donors.

Adaptation of glomerular volume to either renal mass ablation or increasing metabolic demand has been explored in different experimental and clinical settings. In experimental studies of renal mass ablation, glomeruli enlarge in proportion to parenchymal mass reduction (21,22). Whereas uninephrectomy in the rat does not lead to end-stage renal disease, 5/6 ablation is associated with marked glomerular enlargement, glomerular hypertension and glomerulosclerosis leading to progressive renal failure (23). In humans, glomerular volume enlarges many folds during childhood to adapt glomerular filtration rate to body growth (2,24). In adults, after uninephrectomy glomerular volume increases by a twofold factor, but glomeruli usually do not develop sclerosis. On the contrary, in patients with

unilateral renal agenesia, a condition associated with reduced nephron number, or in patients with oligomeganephronia, glomerular volume enlarges by a five- to eightfold factor and is associated with glomerulosclerosis (25,26). Thus, it is accepted that the capacity of glomeruli to enlarge is age dependent and nephron loss via glomerulosclerosis may be initiated when compensatory glomerular hypertrophy has reached a critical volume threshold.

In the present study, mean glomerular volume enlargement during the initial months after transplantation was approximately 20%. Bhathena observed a trend for glomerular enlargement during the first 2 years although it was not statistically significant in comparison to nonpaired donor biopsies. In biopsies performed after the second year, he showed that the presence of glomerulosclerosis was associated with a glomerular diameter enlargement of 37%, which corresponds to a volume enlargement of approximately 250%. However, glomerular enlargement in renal transplants with glomerulosclerosis was smaller than glomerular enlargement in congenitally nephronopenic native kidneys (12), suggesting that the critical volume threshold for glomerulosclerosis is lower in transplanted than in native kidneys. In our study, protocol biopsies were performed earlier and we were not able to detect any association between glomerular volume and glomerulosclerosis. Unfortunately, we were not able to explore the timing to maximal glomerular enlargement as well as the critical volume threshold for glomerulosclerosis.

In the present study the presence of CAN in the protocol biopsy and larger glomerular volume in the donor biopsy were associated with an impaired capacity of the glomeruli to enlarge. The presence of CAN was the variable that explained the higher proportion of the glomerular enlargement variability. These data suggest that preservation of tubulointerstitial structures is a necessary condition for glomerular adaptation to an increased metabolic demand after transplantation. A similar observation has been done in microalbuminuric diabetic patients in whom an inverse relationship between interstitial volume fraction and glomerular volume has been shown (27).

In stereological studies performed in autopsies, it has been consistently described as an inverse relationship between glomerular number and glomerular volume (1). Thus, a large glomerular volume may be considered a surrogate measure of glomerular number and may reflect predisposition to renal disease (6,28). In studies performed in donor biopsies, an inverse association between donor age and glomerular volume has been described (11,16). Abdi et al. have shown that an increased glomerular volume in donor biopsies correlates with renal allograft dysfunction (11). We observed that the larger Vg in the donor biopsy the smaller glomerular enlargement after transplantation. This result suggests that kidney with larger glomeruli have already been adapted to the donor metabolic demand and, accordingly, their potential for a further adaptation is limited.

In the present study, we were not able to find any relationship between BMI or BSA and glomerular enlargement even though an association between glomerulomegaly and obesity has been described in the native kidney (29,30). Noticeably there was no association between timing of protocol biopsy and glomerular enlargement suggesting that major glomerular volume increase occurs early after transplantation.

Chronic tubulointerstitial damage did not correlate with creatinine clearance. This is not surprising if we take into consideration that at this early stage of chronic damage, structural and functional correlations in well-functioning grafts are weak (31). On the contrary, glomerular volume at the time of protocol biopsy correlated with estimated creatinine clearance, suggesting that glomerular enlargement during the early post-transplant period may be a necessary condition for the transplanted kidney to adapt to the recipient metabolic demand and to achieve an adequate renal function. Unfortunately, the present data do not allow us to study whether this adaptation process implies enlargement over the threshold leading to glomerulosclerosis in a proportion of cases. Nevertheless, this result suggests that impaired glomerular enlargement may be associated with a poor outcome.

In summary, we interpret that glomerular enlargement is a necessary condition for the transplanted kidney to achieve an adequate renal function. Glomerular enlargement depends on donor glomerular volume, suggesting that donor kidneys with larger glomeruli and presumably with lower glomerular number have already exhausted their ability to adapt after transplantation. Moreover, this process is impaired in patients with tubulointerstitial chronic lesions, suggesting that early stages of CAN are not only characterized by tubulointerstiutial damage but also by an insufficient glomerular adaptation.

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