American Journal of Transplantation 2005; 5: 2877–2882
Blackwell Munksgaard

doi: 10.1111/j.1600-6143.2005.01126.x

Glomerular Size in Early Protocol Biopsies is Associated with Graft Outcome

F. Azevedo[a], G. Alperovich[a], F. Moreso[a], M. Ibernon[a], M. Gomà[b], X. Fulladosa[a], M. Hueso[a], M. Carrera[b], J. M. Grinyó[a] and D. Serón[a,*]

[a] *Nephrology and* [b] *Pathology Departments, Hospital Universitari de Bellvitge, C/Feixa Llarga s/n. 08907 L'Hospitalet, Barcelona, Spain*
* *Corresponding author: Daniel Serón, 17664dsm@comb.es*

Long-term consequences of glomerular enlargement after transplantation are not well understood. The aim is to evaluate the relationship between glomerular volume (Vg) estimated in protocol biopsies, graft function and graft survival. Vg and Banff chronic damage score were evaluated in protocol biopsies at 4 months. Creatinine clearance (CrCl) was estimated by the Cockroft-Gault formula. Vg estimated in 144 patients was 4.8 $\pm$ 2.0 $\times$ $10^6\mu^3$. It was associated with donor age ($r = 0.23$, $p < 0.01$), recipient body mass index ($r = 0.17$, $p = 0.04$), delayed graft function (Vg = 5.9 $\pm$ 2.3 vs. 4.6 $\pm$ 1.9 $\times$ $10^6\mu^3$, $p < 0.01$) and CrCl ($r = 0.17$, $p = 0.04$). The best cutoff of Vg, Banff chronic damage score and CrCl was determined by Cox regression analysis, being 5.0 $\times$ $10^6\mu^3$ for Vg (relative risk (RR): 2.4, 95% confidence interval (CI): 1.03–5.6), >2 for chronic damage score (RR: 3.4, 95% CI: 1.03–8.9) and 60 mL/min for CrCl (RR: 3.5, 95% CI: 1.04–11.9). These variables were independent predictors of death-censored graft survival. According to Vg and CrCl, four groups of patients were defined. Patients with small glomeruli and high CrCl had a 95% graft survival while patients with large glomeruli and low CrCl had a 45% graft survival at 15 years ($p < 0.01$). Large glomerular volume, high Banff chronic score and poor early renal function in stable grafts are independently associated with death-censored graft survival.

Key words: Renal transplantation, protocol biopsies, glomerular volume, graft survival

Received 29 June 2005, revised and accepted for publication 24 August 2005

Introduction

The number of glomeruli is set at the time of birth (1) and shows an important variability between subjects (2,3). There is an inverse relationship between glomerular number and glomerular volume, suggesting that mean glomerular volume (Vg) may represent a rough surrogate measure of renal mass (2). This proposal is further sustained by the observation that aging is associated with fewer nephrons and larger glomeruli, or by the observation that glomeruli enlarge in conditions characterized by reduced nephron number such as oligomeganephronia or hypertension (4,5).

In experimental transplant models (6,7), renal mass is a major determinant of chronic allograft nephropathy (CAN) and surrogate parameters of reduced renal mass in large epidemiological studies are associated with poorer allograft survival (8).

Larger glomerular size evaluated in donor biopsies is associated with late renal allograft dysfunction (9,10). This association is not surprising if we take into consideration that glomerular enlargement is associated with glomerulosclerosis in the native and transplanted kidney (11–14). Taken together, these data suggest that enlarged glomeruli in renal transplants may represent a marker of reduced nephron number and poor graft outcome. However, in a study of paired donor and protocol biopsies done at 4 months, we observed that glomeruli enlarge during the first few months and that larger glomerular size is associated with a better graft function (15). Since graft function is a major determinant of graft survival (16,17), this observation challenges the idea that increased Vg is necessarily a marker of poor prognosis. The aim of the present study is to further characterize the relationship between Vg evaluated in protocol biopsies, graft function and graft survival.

Patients and Methods

Patients

Since 1988, we have been performing an early protocol biopsy in patients who fulfill the following criteria: (i) serum creatinine <300 μmol/L, (ii) proteinuria <1 g/24 h, (iii) stable renal function and (iv) written informed consent. For the present study, we have retrospectively reviewed our files to identify protocol biopsies with at least 10 glomerular sections. 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. For the present study, biopsies performed until December 2001 were considered.

Biopsies

Biopsies were obtained under ultrasound guidance with an 18-gauge spring-loaded needle until 1994 and with a 16-gauge needle later on. Biopsies

were formalin fixed and paraffin embedded and processed for routine light microscopy as previously described (15). Renal lesions were graded and diagnosed according to the 1997 Banff criteria in the absence of any clinical information (18). Banff chronic score was calculated as the addition of individual chronicity scores in the four renal compartments (cg, ci, ct and cv).

Silver methenamine-stained sections were employed for histomorphometric analysis. Mean glomerular area (Ag) was estimated by a point counting method at 200× magnification in all the complete and nonsclerosed available glomeruli in one section. Glomeruli lying in the boundary of the biopsy with a sectioned Bowman's capsule were not considered. For this purpose, a grid of 560 points 1 cm apart 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. Vg was estimated from Ag according to the Weibel and Gomez method as (19):

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

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. In a previous study, we already showed that in 4-month protocol biopsies, the size variability of glomeruli fits the Weibel and Gomez assumption (20).

Clinical variables

The following variables were evaluated in each patient at the time of surgery: age and sex of the donor and the recipient, height and weight of the recipient, hypertension, last panel reactive antibodies, number of human leukocyte antigen mismatches and cold ischemia time. After surgery, immunosuppressive therapy, the presence of delayed graft function and acute rejection were evaluated. At the time of protocol biopsy, serum creatinine, proteinuria, hypertension, cyclosporine (CsA) or tacrolimus doses and levels were recorded.

Body surface area (BSA) was calculated from recipient weight and height (21). Body mass index (BMI) was calculated as the weight divided by the squared height.

Creatinine clearance (CrCl) at the time of biopsy was calculated according to the Cockroft-Gault formula (22).

Statistics

Results are expressed as the mean ± standard deviation. Comparison between groups was performed by the Mann-Whitney *U*-test or Kruskal-Wallis test for ordinal variables or not normally distributed continuous variables. Student's *t*-test or analysis of variance and Scheffé test for individual comparisons were employed for continuous normally distributed variables.

Simple regression analysis was employed to study the relationship between normally distributed quantitative parameters and Spearman correlation was employed to study the relationship between ordinal variables. Death-censored graft survival was calculated according to the Kaplan-Meier method and comparison between groups was done by the log-rank test. Univariate and multivariate Cox regression analyses were done to analyze the relationship between clinical or histological data and graft survival.

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

Results

Patients

During the study period, we performed 430 consecutive protocol biopsies and according to the pathology reports, 204 displayed at least 10 glomerular sections. A protocol biopsy containing at least 10 nonsclerosed and complete glomerular sections was available in 144 transplants. Demographic characteristics of patients are summarized in Table 1. Mean follow-up until December 2004 was 95 ± 44 months (range: 12–197). At the end of follow-up, 11 patients died with a functioning graft and 22 patients lost their graft due to CAN (n = 18), chronic glomerulonephritis associated with hepatitis C virus (n = 3) and recurrence of IgA nephropathy (n = 1). In 2 out of 18 patients with CAN, acute rejection grade Ia was also present in the diagnostic biopsy, in 6 out of 18 patients, CAN was associated with transplant glomerulopathy and in 1 case, severe hyaline arteriolar changes suggesting CsA toxicity were observed.

During this period of time, different combinations of immunosuppressive drugs were employed. A CsA and prednisone-based immunosuppression was employed in 123 patients. This treatment was associated with antilymphocytic induction therapy (n = 52), azathioprine (n = 18), mycophenolate mofetil (n = 45) or sirolimus (n = 4). Tacrolimus associated with mycophenolate mofetil and prednisone was employed in 12 patients. In the remaining 9 patients, a calcineurin-free regimen based on

Table 1: Characteristics of patients and clinical evolution after transplantation

Variable	Mean ± SD	Range
Donor age (years)	37 ± 16	(12–76)
Donor gender (M/F)	101/43	
Patient age (years)	47 ± 13	(15–72)
Patient gender (M/F)	95/49	
Body mass index (kg/m^2)	23 ± 4	(17–37)
Body surface area (m^2)	1.7 ± 0.2	(1.2–2.5)
Cause of end-stage renal disease		
Glomerular	58	
Interstitial	26	
Polycystic kidney disease	14	
Vascular	3	
Unknown	43	
Panel reactive antibodies (%)	7 ± 18	(0–100)
DR mismatches	0.6 ± 0.6	(0–2)
Cold ischemia time (h)	22 ± 5	(7–38)
Delayed graft function (no/yes)	121/23	
Acute rejection (no/yes)	114/30	
Time of protocol biopsy (days)	125 ± 52	(25–261)
Serum creatinine (μmol/L)	140 ± 44	(72–298)
Creatinine clearance (mL/min)	57 ± 18	(24–144)
Proteinuria (g/day)	0.34 ± 0.28	(0.03–1.00)
Mean arterial blood pressure (mmHg)	101 ± 12	(77–137)

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

anti-lymphocytic therapy, mycophenolate mofetil and prednisone was administered. At the time of biopsy, 122 patients were receiving a CsA-based regimen, 15 patients a tacrolimus-based regimen and 7 patients remained on an anti-calcineurin-free regimen.

Protocol biopsies

Mean number of glomerular sections was 16 ± 5, percentage of glomerulosclerosis was $2 \pm 6\%$ and Vg was $4.8 \pm 2.0 \times 10^6 \mu^3$. Protocol biopsies were classified as normal ($n = 66$), borderline changes ($n = 28$), acute rejection ($n = 5$), CAN ($n = 22$), CAN associated with borderline changes ($n = 16$) and CAN associated with acute rejection ($n = 7$). Vg was not associated with the percentage of glomerulosclerosis or mesangial matrix increase.

Vg and clinical variables

Vg was associated with donor age ($r = 0.23$, $p < 0.01$), recipient BMI ($r = 0.17$, $p = 0.04$), delayed graft function (5.9 ± 2.3 for patients with delayed graft function and $4.6 \pm 1.9 \times 10^6 \mu^3$ for patients with immediate graft function, $p < 0.01$), CsA levels at the time of biopsy ($n = 122$, $r = -0.21$, $p = 0.02$) and CrCl at the time of biopsy ($r = 0.17$, $p = 0.04$).

Vg and graft survival

In order to characterize the predictive value of Vg on death-censored graft survival, the best cut-off for this variable was evaluated considering the following thresholds in an univariate Cox proportional hazard model: 4, 4.5, 5, 5.5 and $6 \times 10^6 \mu^3$. The best prediction of death-censored graft survival was obtained when Vg was binarized as smaller or larger than $5 \times 10^6 \mu^3$ (relative risk (RR): 2.4, 95% confidence interval (CI): 1.03–5.6, $p = 0.04$). Similarly, the best cut-off for chronic score was evaluated considering the following thresholds: 1, 2, 3 and 4. The best prediction of death-censored graft survival was obtained when chronic score was binarized as >2 (RR: 3.4, 95% CI: 1.03–8.9, $p = 0.012$). Finally, the best cutoff for CrCl was evaluated considering the following thresholds: 50, 55, 60 and 65 mL/min. The best prediction of death-censored graft survival was obtained when estimated CrCl was binarized as lower or higher than 60 mL/min (RR: 3.5, 95% CI: 1.04–11.9, $p = 0.04$).

Multivariate analysis showed that Vg, chronic score and CrCl were independent predictors of death-censored graft survival (RR: 3.3, 95% CI: 1.4–7.9, $p < 0.01$; RR: 2.9, 95% CI: 1.1–7.8, $p = 0.04$ and RR: 4.2, 95% CI: 1.2–14.6, $p = 0.02$; respectively). Repeated Cox regression analysis adjusting for donor age, immunosuppressive treatment (CsA vs. non-CsA-based) and acute rejection before the protocol biospy confirmed that Vg (RR: 4.2, 95% CI: 1.6–11.2, $p < 0.01$), chronic score (RR: 3.4, 95% CI: 1.2–9.6, $p = 0.01$) and CrCl (RR: 4.9, 95% CI: 1.3–18.2, $p = 0.01$) were independent predictors of death-censored graft survival.

In order to further explore the relationship between Vg, CrCl and death-censored graft survival, patients were classified in 4 groups according to Vg and CrCl as: (a) small glomeruli with good renal function ($n = 24$), (b) small glomeruli with poor renal function ($n = 60$), (c) large glomeruli with good renal function ($n = 29$) and (d) large glomeruli with poor renal function ($n = 31$). As shown in Figure 1, patients with small glomeruli and good renal function displayed the best graft survival (95%) at 15 years while patients with large glomeruli and poor renal function had the lowest graft survival (45%). Graft survival was intermediate in patients with either large glomeruli and good renal function (73%) or with small glomeruli and poor renal function (78%).

To further characterize patients according to Vg and CrCl, we analyzed clinical and histological variables in these four groups. In Table 2, clinical variables showing a significant difference between groups are summarized. In Figure 2, acute and chronic Banff scores in these four groups are shown. Despite acute and chronic scores were not different between groups, glomerulitis was significantly lower in patients with small glomeruli and good renal function

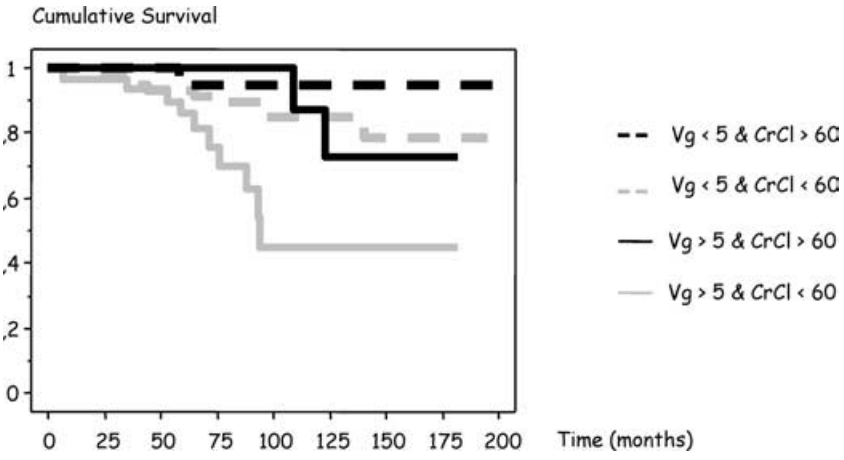

Figure 1: Death-censored graft survival in the four groups of patients defined according to glomerular volume and creatinine clearance ($p < 0.01$).

Table 2: Clinical variables in patients classified according to glomerular volume and creatinina clearance

Vg GFR	Small high	Small low	Large high	Large Low	p-value
N	24	60	29	31	
Vg ($\times 10^6 \mu^3$)	3.2 ± 0.8	3.4 ± 0.9	7.0 ± 1.3	6.6 ± 1.2	
CrCl (mL/min)	73 ± 12	48 ± 9	76 ± 16	45 ± 10	
Donor age (years)	28 ± 12	36 ± 16*	33 ± 12	49 ± 16*,†,‡	0.001
Recipient age (years)	41 ± 14	48 ± 12*	42 ± 12	54 ± 11*,†,‡	0.001
Recipient sex (M/F)	17/7	32/28	24/5	22/9	0.036
DGF (no/yes)	23/1	53/7	20/11	35/4	0.006
BMI (kg/m^2)	24 ± 4	23 ± 3	23 ± 3	25 ± 3†	0.032
BSA (m^2)	1.76 ± 0.19	1.65 ± 0.18*	1.72 ± 0.16	1.74 ± 0.20†	0.046
Proteinuria (mg/day)	330 ± 210	320 ± 240	230 ± 160†	420 ± 270†	0.016
CsA levels (ng/mL)	195 ± 84 (22)	228 ± 107 (52)	166 ± 73† (21)	197 ± 69 (27)	0.049

Vg = mean glomerular volume, ClCr = creatinina clearance, DGF = delayed graft function, BMI = body mass index, BSA = body surface area, CsA levels = cyclosporine levels at the time of biopsy (the number of patients receiving CsA is shown in brackets). *$p < 0.05$ vs. small/high group, †$p < 0.05$ vs. small/low group, ‡$p < 0.05$ vs. large/high group.

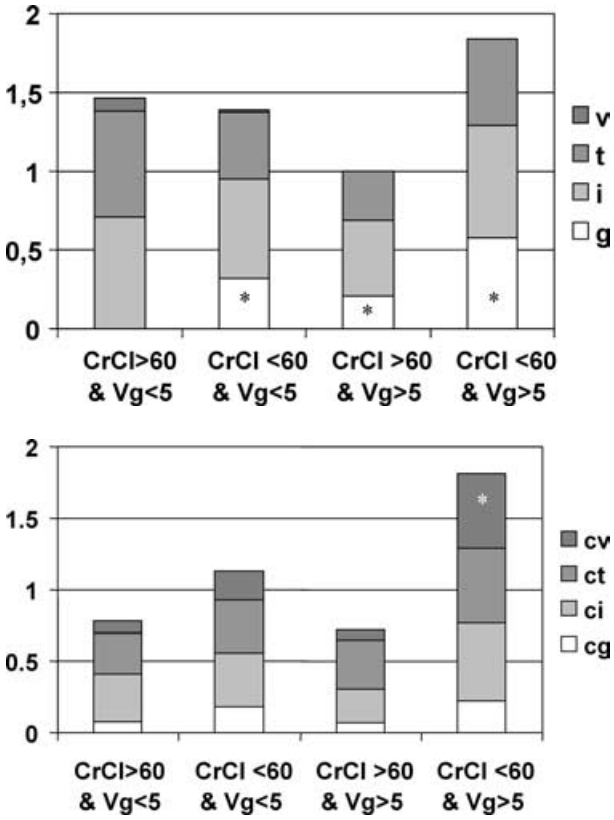

Figure 2: (A) Banff acute scores in the four groups of patients defined according to glomerular volume and creatinine clearance. g-score, glomerulitis; i-score, interstitial infiltrate; t-score, tubulitis; v-score, intimal arteritis. *$p < 0.05$ vs. the group with CrCl >60 and Vg <5. (B) Banff chronic scores in the four groups of patients defined according to glomerular volume and creatinine clearance. cg-score, chronic transplant glomerulopathy; ci-score, interstitial fibrosis; ct-score, tubular atrophy; cv-score, chronic vascular damage. *$p < 0.05$ vs. the other three groups.

($p = 0.013$) and cv-score was significantly higher in patients with large glomeruli and poor renal function ($p = 0.009$).

Discussion

In the present study, we evaluate the relationship between glomerular volume, renal function and graft survival in protocol biopsies obtained at approximately 4 months. Vg was estimated according to the Weibel and Gomez method which is an assumption-based method and consequently may be the source of a systematic error (19). This method assumes that glomeruli are spheres and that their size distribution is known. The Cavalieri or the maximal profile area methods allow a more precise estimation of Vg, but they are more difficult and time consuming to perform since they require the evaluation of serial sections (23,24). The Weibel and Gomez method estimates Vg in one section and accordingly it is easy to perform in a clinical setting. Furthermore, in a previous study, we demonstrated that there is a good agreement between Vg estimated with the maximal profile area and the Weibel and Gomez method in 4-month protocol biopsies (20). Since the association between a morphometric parameter and clinical outcome not only depends on the accuracy of the morphometric method, but also on the sample size, our approach represents a compromise between the accuracy of the estimate of Vg and the number of included cases.

In a study of paired donor and protocol biopsies in which Vg was also evaluated according to the Weibel and Gomez method, we observed that glomeruli enlarge after transplantation and that there is an association between Vg at 4 months and renal allograft function at the time of biopsy (15). In the present study, we confirm that there is a weak but significant association between Vg and CrCl in stable grafts. Taken together, these observations suggest that glomerular size adaptation may be a necessary

condition to achieve an adequate renal function after transplantation. This notion is in agreement with the observation that large glomerular size was associated with a surrogate parameter of increased recipient metabolic demand such as recipient sex and BMI (25,26). Nevertheless, the long-term consequences of this adaptation process after transplantation have not been well characterized since there is a size threshold for glomerular enlargement that leads to glomerulosclerosis and progressive renal failure (27,28). It has been shown that Vg threshold for glomerulosclerosis after transplantation is smaller than in the native kidney (29). In the present study, we observed an association between large Vg and poor allograft survival; despite patients with larger glomeruli have a better renal function at the time of biopsy. Thus, this observation suggests that glomerular enlargement may trigger mechanisms leading to glomerulosclerosis in the long-term follow-up.

In the general population, aging is associated with glomerular enlargement to adapt the filtration surface area to a decreasing glomerular number (2,3). In the present study, we confirm that there is an association between donor age and Vg (9). Taking into consideration that the capacity of glomeruli to enlarge after transplantation is inversely related to donor glomerular volume, the present data do not allow distinguishing whether large glomerular size in protocol biopsies is associated with old donor age or with a proper adaptation to the recipient metabolic demand. Despite this limitation, we observed an association between large Vg and decreased renal allograft survival.

In epidemiological studies, it has been repeatedly shown that renal function is a major determinant of graft outcome. Furthermore, the presence of chronic allograft damage in protocol biopsies is also an independent predictor of late graft failure (30,31). In the present study, we not only confirm that CrCl and chronic score are associated with death censored renal allograft survival, but we also observed that Vg is an independent predictor of graft survival. This observation raises the question whether Vg may be a useful parameter in the evaluation of renal allograft biopsies to characterize glomerular adaptation after transplantation.

Outcome was the poorest in patients with large glomeruli and poor renal function. This group received kidneys from the oldest donors and the recipient BMI was the highest. These data suggest that this group received kidneys that were unable to properly adapt to the recipient metabolic demand since the potential for glomerular enlargement was already exhausted before transplantation. This notion is reinforced by our previous study of paired donor and early protocol biopsies, in which we observed that the larger the glomeruli in the donor biopsy, the lower glomerular enlargement in the protocol biopsy (15).

On the other hand, patients with large glomeruli and good renal function received kidneys from younger donors and were exposed to lower CsA levels. In this group, graft survival was intermediate. Unfortunately, our data do not allow exploring whether glomeruli were already enlarged in the donor or they represent proper glomerular adaptation to the recipient metabolic demand. The observation that these patients were exposed to lower CsA levels, suggest that CsA may difficult glomerular enlargement after transplantation.

Death-censored graft survival was also intermediate in patients with small glomeruli and poor renal function. In this group, recipients were smaller and probably the stimulus for glomerular enlargement was less intense. Furthermore, CsA levels were higher than in the other groups, once more suggesting that CsA may interfere with glomerular enlargement.

Finally, the best outcome was observed in patients with small glomeruli and good renal function. These patients received kidneys from the youngest donors. Since glomerular size can be considered a surrogate measure of glomerular number (2), we interpret that these patients received the highest nephron number and accordingly, their glomeruli were not forced to growth after transplant since small Vg was sufficient to provide an adequate filtration surface area. It is worth noticing that delayed graft function was very low in these patients while approximately 30% of patients with large glomeruli and good renal function suffered from delayed graft function. Since it has been shown that glomerular number in the general population ranges between 0.2 and 1.8×10^6 (2), we cannot discard that this last group of patients received a low nephron number despite they were transplanted with a kidney from a young donor. Unfortunately, the history of donor hypertension was not properly recorded in order to further explore the hypothesis that this group of donors was endowed with fewer nephrons (4).

In summary, the present data show that large glomerular volume, high chronic score and poor renal function evaluated in stable grafts early after transplantation are independently associated with a decreased death-censored graft survival. These data suggest that the evaluation of Vg in renal biopsies may contribute to characterize glomerular adaptation after renal transplantation.

Acknowledgments

The present work was supported by FIS grants to F. Moreso (PI040164) and D. Serón (PI040086), a Sociedad Española de Nefrología 2004 grant to D. Serón. M. Gomà was supported by a Fujisawa grant. M. Ibernón was supported by an Institut d'Investigació Biomèdica de Bellvitge (IDIBELL) grant.

References

1. Brenner BM, Milford L. Nephron underdosing: a programmed cause of chronic renal allograft failure. Am J Kidney Dis 1993; 21: 66–72.

2. Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K, Bertram JF. A stereological study of glomerular number and volume: preliminary findings in a multiracial study of kidneys at autopsy. Kidney Int 2003; 63: S31–S37.
3. Nyengaard JR, Bendtsen TF. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. Anat Rec 1992; 232: 194–201.
4. Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron number in patients with hypertension. N Engl J Med 2003; 348: 101–108.
5. Ohshima T, Inaba S, Arai M et al. A case of oligomeganephronia, quantitative measurement of the glomerular surface area and the number of glomeuli with a color image analyzer. Nippon Jinzo Gakkai Shi 1994; 3: 271–276.
6. Mackenzie HS, Tullius SG, Heemann UW et al. Nephron supply is a major determinant of long-term renal allograft outcome in rats. J Clin Invest 1994; 94: 2148–2152.
7. Cruzado JM, Torras J, Riera M et al. Influence of nephron mass in development of chronic renal failure after prolonged warm renal ischemia. Am J Physiol Renal Physiol 2000; 279: F259-F269.
8. Terasaki PI, Gjertson DW, Cecka JM, Takemoto S. Fit and match hypothesis for kidney transplantation. Transplantation 1996; 62: 441–445.
9. Abdi R, Slakey D, Kittur D, Birdick J, Racusen LC. Baseline glomerular size as a predictor of function in human renal transplantation. Transplantation 1998; 66: 329–333.
10. Li M, Nicholls KM, Becker GJ. Risk factors for late renal allograft dysfunction: effects of baseline glomerular size. J Nephrol 2002; 15: 620–625.
11. Nishimoto K, Shiiki H, Nishino T, Uyama H, Iwano M, Dohi K. Reversible glomerular hypertrophy in adult patients with primary focal segmental glomerulosclerosis. J Am Soc Nephrol 1997; 8: 1668–1678.
12. Kambham N, Markowitz GS, Valeri AM, Lin J, D´Agati VD. Obesity-related glomerulopathy: An emerging epidemic. Kidney Int 2001; 59: 1498–1509.
13. Li M, Nicholls KM, Becker GJ. Glomerular size and global glomerulosclerosis in normal Caucasians donor kidneys: effects of aging and gender. J Nephrol 2002; 15: 614–619.
14. Bhathena DB. Glomerular size and the association of focal glomerulosclerosis in long-surviving human renal allografts. J Am Soc Nephrol. 1993; 4: 1316–1326.
15. Alperovich G, Maldonado R, Moreso F, Fulladosa X, Grinyó JM, Serón D. Glomerular enlargement assessed by paired donor and early protocolo renal allograft biopsies. Am J Transplant 2004; 4: 650–654.
16. Schwarz A, Mengel M, Gwinner W et al. Risk factors for chronic allograft nephropathy after renal transplantation. Kidney Int 2005; 67: 341–348.
17. Serón D, Arias M, Campistol JM, Morales JM for the Spanish Chronic Allograft Nephropathy Study Group. Late renal allograft failure between 1990 and 1998 in Spain: a changing scenario. Transplantation 2003; 76: 1588–1594.
18. Racusen LC, Solez K, Colvin RB et al. The Banff 97 working classification of renal allograft pathology. Kidney Int 1999; 55: 713–723.
19. Weibel ER. Stereological Methods: Practical Methods for Biological Morphometry. Vol 1. London, UK: Academic Press; 1979: 40–45.
20. Fulladosa X, Moreso F, Narvaez JA, Grinyo JM, Serón D. Total glomerular number and renal function in stable renal allografts. J Am Soc Nephrol 2003; 14: 2662–2668.
21. Mosteller RD. Simplified calculation of body surface area (letter). N Engl J Med 1987; 317: 1098.
22. Cockroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31–41.
23. Lane PH, Steffes MW, Mauer SM. Estimation of glomerular volume with four methods. Kidney Int 1992; 41: 1085–1089.
24. Pagtalunan ME, Drachman JA, Meyer TW. Methods for estimating the volume of individual glomeruli. Kidney Int 2000; 57: 2644–2649.
25. Praga M, Hernandez E, Herrero JC et al. Influence of obesity on the appearance of proteinuria and renal insufficiency after unilateral nephrectomy. Kidney Int 2000; 58: 2111–2118.
26. Kambham N, Markowitz GS, Valeri AM, Lin J, D'Agati VD. Obesity-related glomerulopathy: an emerging epidemic. Kidney Int 2001; 59: 1498–1509.
27. Bhathena DB, Julian BA, McMorrow RG, Baehler RW. Focal sclerosis of hypertrophied glomeruli in solitary functioning kidneys of humans. Am J Kidney Dis 1985; 5: 226–232.
28. Bhathena DB. Focal glomerulosclerosis and maximal glomerular hypertrophy in human nephronopenia. J Am Soc Nephrol 1996; 7: 2600–2603.
29. Bathena DB. Glomerular size and the association of focal glomerulosclerosis in long-surviving human renal allografts. J Am Soc Nephrol 1993; 4: 1316–1326.
30. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. N Engl J Med 2003; 349: 2326–2333.
31. Serón D, Moreso F, Bover J et al. Early protocol renal allograft biopsies and graft outcome. Kidney Int 1997; 51: 310–316.