Estimation of Total Glomerular Number in Stable Renal Transplants

XAVIER FULLADOSA,* FRANCESC MORESO,* JOSE A. NARVÁEZ,†
JOSEP M. GRINYÓ,* and DANIEL SERÓN*†
*Nephrology Department, Hospital Universitari de Bellvitge, Barcelona, Spain; and †Institut de Diagnòstic per la Imatge, Hospital Duran i Reynals, Barcelona, Spain

Abstract. Glomerular number (N g ) is considered a major determinant of renal function and outcome. In the dog, it has been shown that Ng can be estimated with reasonable precision in vivo by means of a renal biopsy and magnetic resonance imaging (MRI). Thus, this method was applied to study anatomic clinical correlations in stable human renal transplants. Thirty-nine stable renal transplants were included. A protocol renal allograft biopsy was done at 4 mo. Biopsies were evaluated according to Banff criteria. Glomerular volume fraction (Vglom/cortex) was measured by means of a point-counting method, and mean glomerular volume (V g ) was estimated by means of Weibel and Gomez (V g -W&G) and maximal profile area (V g -MPA) methods. MRI was used to estimate renal cortical volume (V cortex ). N g was calculated as (Vglom/cortex )/V g . GFR was estimated by the inulin clearance. Ten age-matched donor biopsies served as controls for V g . Histologic diagnosis was as follows: normal (n = 20), borderline (n = 7), acute rejection (n = 1), and chronic allograft nephropathy (n = 11). Vglom/cortex was 3.4 ± 1.1%, V cortex was 167 ± 46 cm3, V g -W&G was 3.2 ± 1.2 × 106 μm3, and V g -MPA was 3.3 ± 1.0 × 106 μm3. V g -W&G in donor and recipient biopsies was not different (3.6 ± 1.1 versus 3.2 ± 1.2 × 106 μm3). Total glomerular number estimated by means of V g -W&G (N g -W&G) was 0.73 ± 0.33 × 106 and by V g -MPA (N g -MPA) was 0.74 ± 0.31 × 106. A positive correlation between GFR and N g -W&G (r = 0.47, P = 0.002) was observed. Furthermore, the older the donor, the higher V g -W&G (r = 0.37, P = 0.01) and the lower N g -W&G (r = −0.40, P = 0.01). Total glomerular number can be estimated in stable renal allografts by means of a renal biopsy and MRI. Our data show that N g depends on donor age and positively correlates with GFR.

A wealth of experimental data (1,2) and clinical observations (3–5) support the notion that an imbalance between basal metabolic rate and glomerular number leads to progression of chronic renal failure. Allometric studies have shown that metabolic rate is the primary process that sets GFR, and renal adaptation to body size between species is mainly due to an adaptation to body size between species is mainly due to an

metabolic demand may also contribute to progressive renal function deterioration of the transplanted kidney. However, despite all of these considerations, total glomerular number has never been measured in a clinical setting.

Unbiased stereologic methods have been described to estimate precisely particle number in vitro (13). However, the estimation of total glomerular number in vivo suggests important technical difficulties. Basgen et al. (14) showed that the combination of magnetic resonance imaging (MRI) to measure renal cortical volume (V cortex ) and a biopsy to measure cortical glomerular volume fraction (Vglom/cortex ) and mean glomerular volume (V g ) allows the estimation of total glomerular number with reasonable precision in the dog. In their study, glomerular number estimated in vivo was validated after renal excision by means of the gold standard method for particle counting, the combination of the fractionator and the disector methods. The aim of the present study was to apply the method

between BSA and glomerular number can occur in transplantation patients who receive only one kidney.

Experimentally, it has been shown that the amount of transplanted nephron mass is a determinant of graft outcome (9,10). In transplantation patients, surrogate variables of nephron mass, such as donor age, gender, and race, are associated with renal allograft survival (11). Similarly, surrogate variables of basal metabolic rate, such as body weight and BSA, are also associated with graft outcome (12). These observations have suggested that an imbalance between nephron mass and metabolic demand may also contribute to progressive renal function deterioration of the transplanted kidney. However, despite all of these considerations, total glomerular number has never been measured in a clinical setting.

Received January 8, 2003. Accepted June 27, 2003.
Correspondence to Dr. Xavier Fulladosa, Nephrology Department, Hospital Universitari de Bellvitge, C/Fixa Llarg a s/n, 08907 L’Hospitalet, Barcelona, Spain. Phone: +34-932607602; Fax: +34-932607607; E-mail: 28383xfo@comb.es
1046-6673/1410-2662
Journal of the American Society of Nephrology
Copyright © 2003 by the American Society of Nephrology
DOI: 10.1097/01.ASN.0000088025.33462.B0
described by Basgen et al. to estimate total glomerular number (N_g) in stable allografts and to explore anatomicclinical correlations.

Materials and Methods

Patients

Since June 1988, a prospective study of protocol renal allograft biopsies has been conducted in our center (15). From July 1996 to June 1997, consecutive renal transplants accomplishing the following criteria were eligible to participate in the present study: (1) serum creatinine <200 μmol/L, (2) stable renal function defined as a variability of serum creatinine <15% during 1 mo before and after the study, and (3) proteinuria <1 g/d. For this purpose, a protocol biopsy was performed at approximately 4 mo. When sufficient tissue was obtained, GFR and effective renal plasma flow (ERPF) were measured 2 wk later. MRI was done to determine total renal volume (V_kidney) and V_cortex. This study was approved by the Ethics Committee of our hospital, and written informed consent was obtained from all patients.

Clinical Variables

The following variables were evaluated at the time of surgery: age and gender of the donor and the recipient, height and weight of the recipient, and cold ischemia time. After surgery, the presence of delayed graft function and acute rejection were evaluated. At the time of protocol biopsy and during follow-up, serum creatinine and proteinuria were recorded. BSA was calculated from recipient weight and height according to the Mosteller formula (16).

Delayed graft function was defined as hemodialysis requirements during the first week after surgery once accelerated or hyperacute rejection, vascular complications, and urinary tract obstruction were ruled out. The diagnosis of acute rejection was biopsy-proven according to the 1997 Banff criteria (17).

Biopsies

Biopsies were obtained under ultrasound guidance with a 16-gauge spring-loaded needle. Two cores were obtained in each patient. Biopsies were processed for routine light microscopy as described previously (15). Tissue was embedded in paraffin; cut into 3- to 4-μm sections; and stained with hematoxylin and eosin, periodic acid-Schiff, Masson’s trichrome, and silver methenamine. Renal lesions were graded and diagnosed according to the 1997 Banff schema by two observers in the absence of any clinical information (17). Protocol biopsies were not available to clinicians and consequently were not used to make any clinical decision. Ten age-matched preimplantation cadaveric donor biopsies served as controls in the estimation of V_g.

None of these 10 age-matched donor controls contributed to the study.

Morphometry

Silver methenamine–stained sections were used to perform the morphometric study. V_g/VEW&G and V_g were estimated.

\[ V_{g/VEW&G} = \frac{V_{g/VEW&G}}{V_{g/VEW&G}} \]

\[ V_{g} = \frac{V_{g}}{P_{2}} \]

reference space (P2) were counted. Accordingly, the area associated with a coarse point corresponds to 16 times the area associated with a fine point. The volume fraction, expressed in percentage, was estimated according to V_g = (P1)/(P2 × 16) × 100.

V_g. Two methods were used to estimate V_g: Weibel and Gomez (W&G) method, which was used for all cases, and maximal profile area (MPA), which was applied to study a subset of 20 cases (18,19).

W&G Method. Mean glomerular area (A_g) was determined at ×200 magnification by a point-counting method in all available glomeruli in one section. For calibrating the magnification of the digitized image, a micrometer ruler was placed in the center of the stage to calculate the distance between grid points. Mean glomerular volume (V_g/W&G) was obtained according to V_g/W&G = (A_g^3/2 × β)/d, where β is 1.38, the shape coefficient of the sphere, and d is 1.01, the size distribution of glomeruli considering a 10% coefficient of variation of the caliper diameter.

MPA Method. The whole biopsy was sectioned into approximately 3- to 4-μm-thick sections, and every fourth section was numbered. The MPA was estimated at ×200 magnification. Consecutive glomerular profiles of the same glomerulus were digitized and simultaneously displayed on the computer screen. The maximal profile was identified as the glomerular section preceded and followed by two smaller sections, and MPA was measured with a point-counting method. Glomerular volume (V_g-MA) was estimated assuming that glomeruli are spheres.

MRI

Between 2 and 4 wk after biopsy, an MRI of the graft was performed to estimate renal and cortical volumes. Five-millimeter T1-weighted consecutive images were obtained in the axial plane with a repetition time of 550 msec and an echo time of 15 msec on a 1.5 Tesla imaging system (Philips ACS-NT, Eindhoven, The Netherlands). Films were printed at half actual size, and the parenchymal light area was defined as the renal cortex (Figure 1) (20,21). V_kidney and V_cortex were estimated according to the Cavalieri’s principle (22), which states that the volume of a body can be estimated measuring the area of a series of slices through that body and knowing the average thickness of each slice. For this purpose, the area of each image was calculated by means of a point-counting method using a grid of 900 points 0.5 cm apart. Volumes obtained according to the Cavalieri principle may be biased as a result of overprojection, which depends...
on slice thickness (22). For minimizing overprojection, the slice with the largest area was excluded from the estimator according to (23) \( V = d(\bar{a} - a_{\text{max}}) \), where \( V \) is volume, \( d \) is slice thickness, and \( a \) is slice area.

**Glomerular Number**

Because \( V_{\text{cortex}} \) was estimated in vivo and \( V_g \) was estimated in paraffin sections, we applied two correction factors to estimate \( V_g \) in vivo. First, we assumed a 31% reduction of renal volume because of the loss of arterial pressure (14,24), and second, we estimated in our laboratory an area shrinkage factor of 31% in renal slices after paraffin embedding, which corresponds to a volume shrinkage of 43% (22,24). Finally, \( N_g \) was calculated as \( N_g = V_{\text{cortex}} \times V_{\text{glomeruli/cortex}} / V_g \) in vivo.

**GFR and ERPF**

GFR and ERPF were determined approximately 2 wk after renal biopsy by means of the inulin and \( \text{p}-\text{aminohippurate} \) clearances, respectively. A loading dose of 60 mg/kg polyfructosan S (inulin; 1nuest 25%; Lavoosan-Gesellschaft, Linz, Austria) and 15 mg/kg \( \text{p}-\text{aminohippurate} \) (PAH 20%; Merck & Co., Inc.) were administered intravenously followed by a continuous infusion of 50 mg/ml and 20 mg/ml at a constant rate of 30 ml/h to achieve a stable plasma concentration >20 mg/dl and 2 mg/dl, respectively. Blood and urine samples were taken at the midpoint and at the end of each clearance period, respectively. Inulin and \( \text{p}-\text{aminohippurate} \) in plasma and urine were determined by colorimetric methods. GFR and ERPF were calculated as the mean of three consecutive 30-min clearances performed after 1 h of equilibration, and their results were not corrected for BSA. The mean coefficient of variation of GFR and ERPF in the studied set of patients was calculated from these three replicate measurements and was 4% and 6%, respectively.

**Statistical Analyses**

Results are expressed as the mean ± SD. Clinical data from studied patients at the time of biopsy and at 3 yr of follow-up were compared by means of paired t test. Morphometric data of the studied protocol biopsies and the donor biopsies were compared by the t test. Simple linear regression analysis was applied to study the relationship between continuous variables. A Bland-Altman plot was used to compare \( N_g \) obtained by means of W&G and MPA methods (25). \( P < 0.05 \) was considered significant.

**Results**

**Patients**

Forty patients were enrolled, and 39 patients completed the study; one patient was unable to cooperate during the MRI study. Demographic characteristics of patients are summarized in Table 1. Thirty-one patients received a cyclosporin A–based immunosuppression; eight patients who received a transplant from suboptimal donors never received anticalcineuric agents, instead being treated with antithymocytic globulin, mycophenolate mofetil, and prednisone (15,26).

Ten patients experienced acute rejection before protocol biopsy. All rejection episodes were corticosteroidal.

Mean follow-up was 58 ± 6 mo (range, 63 to 71 mo). At the time of biopsy and at 3 yr, serum creatinine and proteinuria remained stable (123 ± 30 versus 133 ± 44 μmol/L [NS]; 0.30 ± 0.19 versus 0.38 ± 0.47 g/24 h [NS], respectively). GFR and ERPF at the time of biopsy were 56 ± 15 and 225 ± 54 ml/min, respectively.

**Histomorphometry and MRI Study**

The mean number of glomeruli in one section was 22 ± 8 (range, 10 to 42). Histologic diagnosis according to 1997 Banff schema was as follows: normal (\( n = 20 \)), borderline (\( n = 7 \)), acute rejection (\( n = 1 \)), and chronic allograft nephropathy (\( n = 11 \)). Morphometric parameters were evaluated in 10 age-matched donor biopsies and in the studied set of protocol biopsies. As shown in Table 2, \( V_{\text{glomeruli/cortex}} \) was lower in protocol biopsies, whereas \( V_g \)-W&G was not different in both sets of biopsies. The coefficient of variation of \( V_{\text{glomeruli/cortex}} \)-MPA was 77%.

In 20 biopsies, the MPA method was used to estimate \( V_g \) that was 3.3 ± 1.0 × 10³ μm³. The number of evaluated glomeruli to estimate \( V_g \)-MPA was 21 ± 13. The average values of \( V_g \)-W&G in this set of 20 patients was 3.4 ± 1.3 × 10³ μm³. Thus, \( V_g \)-MPA was 3% lower than \( V_g \)-W&G, although this difference did not reach statistical significance. The coefficient of variation of \( V_g \)-MPA was 29%.

In the MRI study, the number of films per kidney ranged between 16 and 22, depending on the length of the kidney. \( V_{\text{kidney}} \) and \( V_{\text{cortex}} \) were 254 ± 60 cm³ (range, 158 to 485) and 167 ± 46 cm³ (range, 103 to 372), respectively.

Finally, \( V_{\text{cortex}} \), \( V_{\text{glomeruli/cortex}} \), and \( V_g \) were used to calculate the \( N_g \) of each kidney. Mean glomerular number using \( V_g \) estimated according to the W&G method (\( N_g \)-W&G) was 0.73

**Table 1. Demographic characteristics of patients**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N )</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Donor age</td>
<td>38 ± 18</td>
<td>14–76</td>
</tr>
<tr>
<td>Donor gender</td>
<td>26/13</td>
<td></td>
</tr>
<tr>
<td>Recipient age</td>
<td>46 ± 14</td>
<td>24–69</td>
</tr>
<tr>
<td>Recipient gender</td>
<td>24/15</td>
<td></td>
</tr>
<tr>
<td>Recipient BSA(a) (m²)</td>
<td>1.74 ± 0.19</td>
<td>1.25–2.11</td>
</tr>
<tr>
<td>Cold ischemia time (h)</td>
<td>20 ± 5</td>
<td>11–32</td>
</tr>
<tr>
<td>Delayed graft function (no/yes)</td>
<td>37/2</td>
<td></td>
</tr>
<tr>
<td>Acute rejection (no/yes)</td>
<td>29/10</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) BSA, body surface area.

**Table 2. Morphometric evaluation in donor and protocol biopsies\(a\)**

<table>
<thead>
<tr>
<th></th>
<th>Donor (Mean ± SD)</th>
<th>Recipient (Mean ± SD)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N )</td>
<td>10</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>( V_{\text{glomeruli/cortex}} ) (%)</td>
<td>5.3 ± 1.7</td>
<td>3.4 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( V_g )-W&amp;G ((\times 10^6) μm³)</td>
<td>3.6 ± 1.1</td>
<td>3.2 ± 1.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(a\) \( V_{\text{glomeruli/cortex}} \), cortical glomerular volume fraction; \( V_g \)-W&G, mean glomerular volume estimated according to Weibel and Gomez method.
The correlation between the \( N_g\text{-W&G} \) and \( N_g\text{-MPA} \) methods was \( r = 0.9 \) \((P < 0.0001)\); Figure 2). A Bland-Altman plot was used to assess the agreement between both methods, the mean difference being \( 0.05 \pm 0.14 \times 10^6 \) glomeruli (Figure 3).

### Structural and Functional Correlations

GFR correlated with \( N_g\text{-W&G} \) \((r = 0.47, P = 0.002)\); Figure 4). Because \( N_g\text{-W&G} \) was calculated from  \( V_{glomerulus/cortex} \), \( V_{cortex} \), and \( V_{g-W&G} \), we studied whether any of these parameters also correlated with GFR. The only variable that showed a positive correlation with GFR was  \( V_{glomerulus/cortex} \) \((r = 0.43, P = 0.005)\). Donor age correlated positively with \( V_{g-W&G} \) \((r = 0.37, P = 0.01)\) and negatively with \( N_g\text{-W&G} \) \((r = -0.40, P = 0.01)\); Figure 5), whereas recipient BSA did not correlate with \( V_g\text{-W&G} \). \( N_g\) did not correlate with serum creatinine or proteinuria neither at the time of biopsy nor at 3 yr of follow-up.

### Discussion

In the present study, we applied the validated method described by Basgen et al. (14) in dogs to estimate the number of glomeruli in stable renal allografts. This method combines an MRI study to estimate  \( V_{cortex} \) and a renal biopsy to estimate  \( V_{glomerulus/cortex} \) and \( V_g \). Despite that this method suggests a maximal intraindividual error of 36% in the estimation of \( N_g \) in the dog and its application in stable grafts does not allow evaluation of its precision by means of an unbiased method, we decided to apply it assuming that determination of \( N_g \), even with moderate precision, may be useful to explore and understand the role of nephron mass in renal transplants.

As expected, we observed a great variability in \( N_g \) of transplantation patients and a positive correlation between \( N_g \) and GFR. Despite the potential sources of systematic error in the estimation of \( N_g \), the observation of this anatomoclinical correlation reinforces the previously stated idea that the estimation of \( N_g \) with the present method can be useful to study the relationship between glomerular mass and graft outcome.

\( V_{cortex} \) was estimated \textit{in vivo} with a graft MRI that allows an accurate estimation applying the Cavalieri method, because average thickness of renal slices is determined precisely. However, it has to be taken into consideration that overprojection effect may constitute a source of systematic error that can be
almost completely removed applying a simple modification to the Cavalieri method (23).

The agreement between V_cortex measured directly on ex vivo sliced kidneys and V_cortex estimated by MRI has been characterized precisely in the rat but not in the human kidney (27). Thus, cortical area was defined as the parenchymal light area from T1-weighted MRI films as it has been previously described (20,21). The volume reduction that occurs after renal excision is unknown in stable renal allografts. For this reason, we assumed a 31% volume reduction according to the shrinkage factor obtained by Basgen et al. (14) in healthy dogs. Because paraffin embedding suggests a further renal shrinkage (22,24), a second volume correction factor of 43% was applied according to measures obtained at our laboratory. Obviously, the assumptions and corrections applied suggest a potential source of systematic error (27).

V_{V glom/cortex} was estimated with low precision. Because a biopsy constitutes only a small fraction of the whole kidney, the evaluation of any parameter raises the question of its representativeness (28,29). The precision of the estimate of V_{V glom/cortex} was low because of the small sample size provided by a needle biopsy and also by the relative volume of the structure of interest (14). The smaller the structure of interest in the same reference space, the higher the coefficient of variation.

V_g was estimated by two different model-based methods: the W&G and the MPA (18,19). Both methods assume that glomeruli are spheres, whereas a second consideration of the W&G method is that the coefficient of variation of size distribution is known. The W&G method allows the estimation of V_g in one section, and the MPA method requires the evaluation of multiple sections. The first method is quick to perform, whereas the second one is time consuming but allows a similar estimation of V_g when compared with the Cavalieri method, the gold standard for the estimation of V_g (30). We observed that despite that the W&G method slightly overestimates V_g, the correlation between both methods was within the range described by others (19), suggesting that the W&G method allows a reasonable estimate of V_g. Furthermore, the coefficient of variation of V_g calculated by the MPA method was low when compared with the coefficient of variation of V_{V glom/cortex}. Accordingly, our data suggest that the error in the estimation of N_g is more dependent on the estimation of V_{V glom/cortex} than on the estimation of V_g.

Thus, we estimated in our set of patients a mean number of glomeruli of 0.73 ± 0.33 × 10^6 by means of W&G, and there was no difference between N_g obtained with W&G or with MPA methods. An important variability in glomerular number has been reported in studies that used different methods to count glomeruli (31,32). Nyengaard and Bendtsen (7) reported a mean N_g of 0.62 × 10^6, and Bertram and colleagues (8) reported a mean N_g of 0.81 × 10^6, a figure within the range reported in our study. They used an unbiased stereologic method, the combination of the fractionator and the disector, which represents the gold standard for particle counting.

A correlation between decreased N_g and increased V_g has been described in different experimental and clinical settings (33–35). Thus, glomerular enlargement is accepted to represent an adaptation mechanism to a low nephron mass or to an increased metabolic demand (6,36). We observed in renal transplants that donor age correlates negatively with N_g and positively with V_g. The association between increasing age and reduced glomerular number has already been described by others (7,8). In donor biopsies, Abdi et al. (37) described a positive relationship between age and glomerular size and showed that glomerular size in donor biopsies but not donor age is an independent predictor of late allograft dysfunction. It can be interpreted that older donors with fewer glomeruli have adapted their filtration surface area by means of glomerular enlargement. Despite all of these considerations, it has not been established whether glomeruli enlarge after transplantation. The glomerular capacity to enlarge after renal ablation depends...
on the age of the subject. In congenital unilateral renal agenesis, $V_g$ increases by a fivefold factor, whereas in adult patients, glomerular enlargement after nephrectomy only increases by a twofold factor (38). It has been suggested that there is a critical glomerular enlargement that is associated with an increased risk of glomerulosclerosis in either the native or the transplanted kidney (39,40). In our study, biopsies were done 4 mo after transplantation, a sufficiently prolonged follow-up to expect an adaptation of glomeruli. However, we failed to observe any association between recipient BSA and $V_g$, suggesting that after transplantation, the capacity of the glomeruli to adapt to the recipient metabolic demand is impaired. This notion is reinforced by the observation that mean $V_g$ in age-matched donor biopsies was not different from glomeruli to adapt to the recipient metabolic demand is im-

In summary, we have estimated $N_g$ in renal allograft patients by means of a renal MRI and an allograft biopsy. Despite that it is not possible to assess in vitro the precision of the estimate of $N_g$ obtained in grafts with stable function, our data show that the total number of transplanted glomeruli is a major determinant of renal allograft function, and, consequently, these data are in agreement with the hypothesis that glomerular number is a determinant of graft outcome.

Acknowledgments

The present work was supported by a Fondo de Investigación Sanitaria (FIS) grant (96/0856), a PENSA-Esteve grant from the Catalan Society of Nephrology, and a Fundación La Marató TV3 grant.

References


