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Original Article

Resistive index and chronic allograft nephropathy evaluated in protocol biopsies as predictors of graft outcome

Augusto Vallejos[1], Gabriela Alperovich[1], Francesc Moreso[1], Concepcion Cañas[2], M. Eugenia de Lama[2], Montserrat Gomà[3], Xavier Fulladosa[1], Marta Carrera[3], Miguel Hueso[1], Josep M. Grinyó[1] and Daniel Serón[1]

Departments of [1]Nephrology, [2]Radiology and [3]Pathology, Hospital Universitari de Bellvitge, C/Feixa Llarga s/n, L'Hospitalet, 08907 Barcelona, Spain

Abstract

Introduction. The presence of chronic allograft nephropathy (CAN) in protocol biopsies is negatively associated with graft survival. Although recent studies have indicated that the resistive index (RI) is a predictor of graft failure, it does not correlate with CAN in stable grafts. We therefore studied the relationship between RI and CAN and examined the predictive value of both parameters on graft outcome.

Methods. Included were patients transplanted between 1997 and 2002 and who had protocol biopsies and RI determinations. Renal lesions were blindly evaluated according to Banff 97 criteria. Mean glomerular volume, cortical interstitial volume fraction and intimal arterial volume fraction were estimated using a point counting technique. RI was determined before biopsy in at least two different renal locations. The outcome variable was defined as graft failure or a 30% serum creatinine increase between protocol biopsy and last follow-up.

Results. Eighty-seven patients were included. RI correlated with recipient age ($R = 0.52$, $P < 0.0001$), diastolic blood pressure ($R = -0.36$, $P = 0.0006$), pulse pressure index ($R = 0.27$, $P = 0.009$) and g-score for histological glomerulitis ($\rho = 0.30$, $P = 0.0054$), but there were no correlations between RI and chronic Banff scores or any morphometric parameter. The presence of CAN (relative risk, 3.5; 95% confidence interval 1.2–10.2; $P = 0.02$) but not RI was associated with the outcome variable.

Conclusion. RI was associated with surrogate measures of vascular compliance such as recipient age and pulse pressure index but not with chronic allograft damage, even when it was evaluated by histomorphometry. Our results indicate that histology may be superior to RI in predicting graft function deterioration, at least in patients with stable renal function.

Keywords: chronic allograft nephropathy; protocol biopsies; renal transplantion; resistive index

Introduction

Following transplantation, chronic allograft nephropathy (CAN) is the main cause of graft failure and cardiovascular disease is the primary cause of mortality [1]. The association of various risk factors in both CAN and cardiovascular death, such as older age of the recipient, renal function, hypertension, proteinuria or diabetes, suggest a common pathway between CAN and arteriosclerosis [2].

An increased renal allograft resistive index (RI), which represents the percentage reduction of the end-diastolic flow as compared with the peak systolic flow measured by Doppler ultrasonography [3], is associated with long-term renal allograft survival and patient cardiovascular mortality [4]. These data suggest that RI may represent a surrogate measure of both arteriosclerosis and histological renal allograft damage.

Protocol biopsies allow the early diagnosis of CAN in stable grafts. The presence of CAN in protocol biopsies is associated with a poorer graft survival, and the predictive value of CAN is independent of other factors such as acute rejection, serum creatinine or proteinuria [5,6]. A recent study examining possible correlations between RI and chronic histological damage in protocol renal allograft biopsies found that although both parameters were predictors of graft survival, there was no correlation between RI and histological damage, suggesting that RI is an

Correspondence and offprint requests to: Daniel Serón Micas, MD, Nephrology Department, Hospital Universitari Bellvitge, C/Feixa Llarga s/n, L'Hospitalet, 08907 Barcelona, Spain.
Email: 17664dsm@comb.es

inadequate tool for measuring renal structural damage [4]. Thus, to characterize further the relationship between RI and renal allograft lesions in stable grafts, we studied correlations between RI and renal lesions in protocol biopsies evaluated according to Banff criteria and by morphometric techniques. We also evaluated the predictive values of RI and CAN on graft outcome.

Materials and methods

Patients

Since 1995, protocol renal allograft biopsies have been performed between the third and sixth month at our centre in patients who gave informed consent and who met the following criteria: (i) serum creatinine <300 μmol/l; (ii) proteinuria <1 g/24 h; and (iii) stable renal function defined as variability of serum creatinine of <15% during 2 weeks before and after biopsy. From 1997, RI were estimated at the time of protocol biopsy.

We thus included all cadaveric transplanted patients between 1997 and 2002 in whom protocol biopsies with sufficient tissue and RI at the time of biopsy were available.

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, presence of hepatitis C virus antibodies, aetiology of end-stage renal disease, time on dialysis, hypertension and total serum cholesterol before transplantation, last panel-reactive antibodies, number of human leukocyte antigen (HLA) mismatches and cold ischaemia time. After surgery, the presence of delayed graft function and acute rejection was evaluated. At the time of protocol biopsies and during follow-up, we recorded serum creatinine, proteinuria, total serum cholesterol, hypertension, cyclosporin (CsA) dose and CsA trough levels or tacrolimus dose and levels.

The number of HLA mismatches was calculated by adding the number of mismatches in the A, B and DR loci. Delayed graft function was defined as haemodialysis requirements during the first week following surgery once accelerated or hyperacute rejection, vascular complications and urinary tract obstruction were ruled out. Acute rejection was defined as an acute rise in serum creatinine >30% that responded to antirejection therapy. A diagnostic biopsy at the time of serum creatinine worsening was done in 89% of patients. Hypertension before and after transplantation was defined as a mean arterial pressure >107 mmHg (blood pressure of ~140/90 mmHg) and/or the requirement for antihypertensive drugs. Pulse pressure index was calculated as the difference between systolic and diastolic blood pressure divided by systolic pressure. Patients were biopsied in a fasting state, and morning doses of antihypertensive drugs were omitted.

An outcome variable was defined to evaluate the association between echographic or histological variables and graft outcome. For this purpose, patients were divided into two groups: (i) renal function deterioration defined as death-censored graft loss or a serum creatinine increase >30% between protocol biopsy and last follow-up; and (ii) stable renal function.

Ultrasonographic determination of the resistive index

The minimal diastolic velocity (V_{min}) and maximal systolic velocity (V_{max}) were determined before protocol biopsy, and the RI was calculated as:

$$RI = [1 - (V_{min}/V_{max})] \times 100$$

RI was determined in each patient in two or three different renal allograft locations at the proximal segmental arteries and was expressed as the mean of these values. All examinations were done by the same observer (C.C.) and the coefficient of variation of RI in the different areas of the graft was 4.9%.

Biopsies

Two cores of tissue were obtained with a 16 gauge needle under ultrasound guidance. Tissue was embedded in paraffin, cut into 4 μm sections, and stained with haematoxylin and eosin, periodic acid–Schiff, Masson's trichrome and silver methenamine. Renal lesions were graded and diagnosed according to the 1997 Banff criteria in the absence of any clinical information by one pathologist (M.C.). CAN was diagnosed in patients in whom the addition of ci-score and ct-score was ≥2. Silver methenamine-stained sections were employed for histomorphometric analysis. Mean glomerular volume (Vg), cortical interstitial volume fraction (Vvinterstitium/cortex) and arterial intimal volume fraction (Vvintima/artery) were estimated by means of a point counting technique as previously described [7].

Statistics

Results are expressed as means ± SD. Comparisons between groups were performed by Student's *t*-tests or the analysis of variance in the case of continuous variables. Spearman correlation analysis was employed to study relationships between ordinal data. Simple regression analysis and stepwise multiple regression analysis were employed to study the relationship between quantitative clinical variables and histomorphometric parameters. Survival analysis was done using Cox's proportional hazard analysis. The weighted kappa statistic was employed to evaluate intraobserver variability [8]. StatView 5.0.1 (SAS Institute Inc., Cary, NC) was employed for the statistical analysis. All *P*-values were two-tailed and a *P*-value <0.05 was considered significant.

Results

Patients

A total of 87 stable grafts with sufficient tissue at the time of biopsy were included. Demographic characteristics of patients are summarized in Table1. Immunosuppressive treatment on an intention to treat basis was: CsA, mycophenolate mofetil and prednisone ($n = 46$); CsA, azathioprine and prednisone ($n = 5$);

Table 1. Demographic characteristics of patients

Donor age (years)	41 ± 16
Donor sex (male/female)	60/27
Recipient age (years)	50 ± 14
Recipient sex (male/female)	58/29
Last panel-reactive antibodies (%)	5 ± 16
No. of HLA A + B + DR mismatches	3.1 ± 1.0
End-stage renal disease	
Glomerular	30
Interstitial	12
APKD	7
Ischaemic nephropathy	3
Unknown origin	35
HCV in the recipient (positive/negative)	9/78
Cold ischaemia time (h)	20 ± 5
Delayed graft function (yes/no)	12/75
Acute rejection (yes/no)	19/68

APKD = adult polycystic kidney disease; HCV = hepatitis C virus.

Table 2. Characteristics of patients at the time of biopsy

Variable	Mean ± SD (range)
Time after transplantation (days)	140 ± 35 (93–260)
Serum creatinine (μmol/l)	130 ± 41 (63–300)
Proteinuria (g/day)	0.20 ± 0.22 (0.03–1.0)
Cholesterol (mmol/l)	5.6 ± 1.0 (3.6–7.6)
CsA level (ng/ml)	143 ± 51 (40–285)
Tacrolimus level (ng/ml)	10.1 ± 3.3 (3.7–17.0)
Systolic blood pressure (mmHg)	144 ± 21 (93–220)
Diastolic blood pressure (mmHg)	84 ± 11 (60–105)
Mean arterial blood pressure (mmHg)	104 ± 12 (71–130)
Pulse pressure index	0.41 ± 0.09 (0.18–0.68)

Table 3. Banff scores and morphometric parameters in the protocol biopsies

Banff scores	
g	0.31 ± 0.67
i	0.57 ± 0.69
t	0.53 ± 0.66
v	0.03 ± 0.18
ah	0.29 ± 0.63
cg	0.16 ± 0.37
ci	0.42 ± 0.68
ct	0.37 ± 0.65
cv	0.30 ± 0.63
mm	0.29 ± 0.48
Morphometric parameters	
Vg ($\mu^3 \times 10^6$)	5.8 ± 2.2
Vvinterstitium/cortex (%)	11 ± 5
Mean Vvintima/artery (%)	16.6 ± 3.7

CsA, FTY720 and prednisone ($n = 3$); CsA, rapamycin and prednisone ($n = 7$); tacrolimus, mycophenolate mofetil and prednisone ($n = 17$); and antilymphocytic antibodies, mycophenolate mofetil and prednisone ($n = 9$). At the time of biopsy, 58 patients received a CsA-based immunosuppressive regimen, 22 a tacrolimus-based regimen, and seven patients were treated without anticalcineurin agents. Characteristics of patients at the time of biopsy are summarized in Table 2. Mean time of follow-up was 60 ± 19 months (range 7–94). During this period, six patients died with a functioning graft due to stroke, biliary sepsis, liver cirrhosis, hepatocarcinoma, post-transplant lymphoproliferative disease and peritoneal carcinomatosis. Four patients lost their graft due to CAN ($n = 2$), late acute rejection and chronic glomerulonephritis associated with hepatitis C virus.

Biopsies

A mean number of 15 ± 8 glomeruli and 3.8 ± 2.1 arterial sections per biopsy were obtained. According to the 1997 Banff criteria, 64 biopsies were adequate (at least 10 glomeruli and two arteries), 15 were marginal (between seven and nine glomeruli and at least one artery) and eight were inadequate (<7 glomeruli and at least one artery). Histological diagnosis according to the 1997 Banff criteria were as follows: normal ($n = 35$), borderline changes ($n = 21$), acute rejection ($n = 7$) and CAN ($n = 24$). Banff scores in each renal compartment and morphometric parameters are summarized in Table 3.

Clinical parameters and resistive index

In the univariate analysis, RI at the time of biopsy positively correlated with recipient age ($R = 0.52$, $P = 0.0001$; Figure 1A), negatively with diastolic blood pressure ($R = 0.36$, $P = 0.0006$; Figure 1B) and positively with pulse pressure index ($R = 0.27$, $P = 0.009$; Figure 1C). Thus, these three variables were considered in the multivariate analysis, and recipient age and diastolic blood pressure were included as independent predictors of RI ($R = 0.57$, $P < 0.0001$).

Even though RI was not different between patients receiving CsA and patients treated with tacrolimus or without anticalcineurin drugs (0.70 ± 0.08, 0.71 ± 0.07 and 0.74 ± 0.11, respectively, P = NS), we observed a negative correlation between CsA whole blood levels at the time of biopsy and RI ($R = -0.27$, $P = 0.04$). This association was not observed in patients receiving tacrolimus. Accordingly, the univariate and multivariate analysis was repeated in the 58 patients receiving CsA. In this subset of patients, recipient age ($R = 0.59$, $P = 0.0001$) and diastolic blood pressure ($R = -0.36$, $P = 0.005$) were also correlated with RI. However, in the multivariate analysis, only recipient age and CsA whole blood level at the time of biopsy were independent predictors of RI ($R = 0.64$, $P < 0.0001$).

Histological parameters and resistive index

RI was not different according to histological diagnosis (Table 4). Glomerulitis was the only histological lesion evaluated according to Banff criteria that was associated with RI (Figure 2). There was no correlation between RI and Vg, Vvinterstitium/cortex or Vvintima/artery. Since glomerulitis was evaluated according to an ordinal scale, the intra-observer variability in the

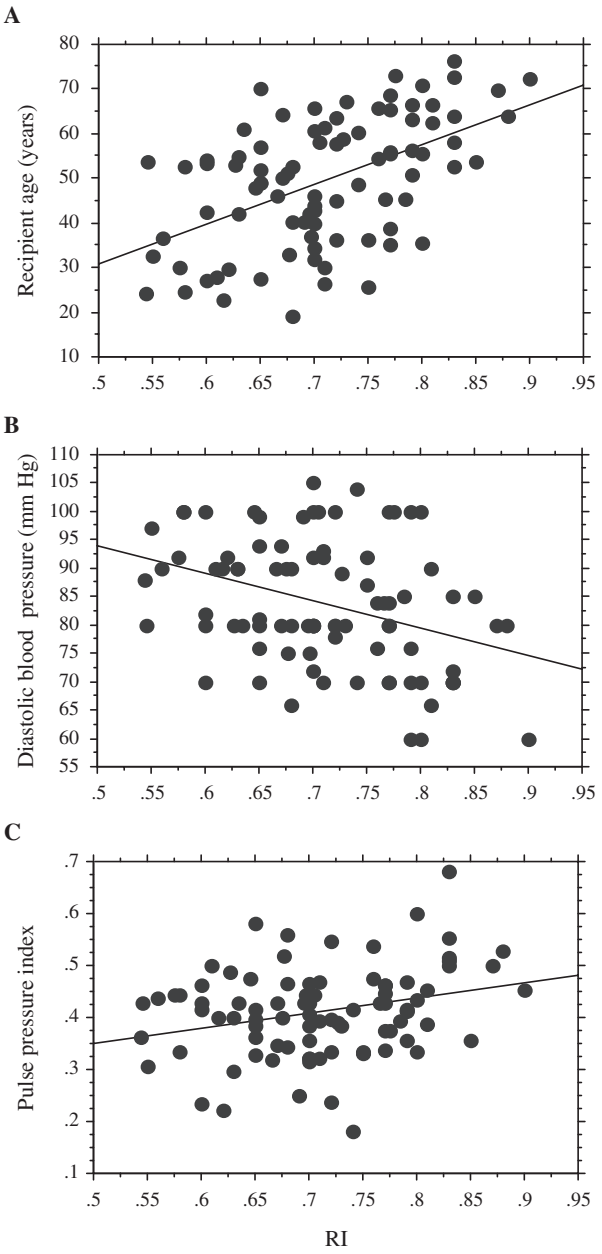

Fig. 1. Simple regression between arterial resistive index and recipient age ($R=0.52$, $P=0.0001$), diastolic blood pressure ($R=-0.36$, $P=0.0006$) and pulse pressure index ($R=0.27$, $P=0.009$).

Table 4. Resistive index according to histological diagnosis

	Normal histology	Borderline changes	Acute rejection	CAN	*P*
n	35	21	7	24	
RI	0.71 ± 0.06	0.72 ± 0.10	0.65 ± 0.10	0.72 ± 0.09	NS

evaluation of this parameter was studied by means of the weighted kappa statistic. Accordingly, glomerulitis was again blindly evaluated by the same observer (M.C.), and the kappa statistic was 0.57, $P<0.05$.

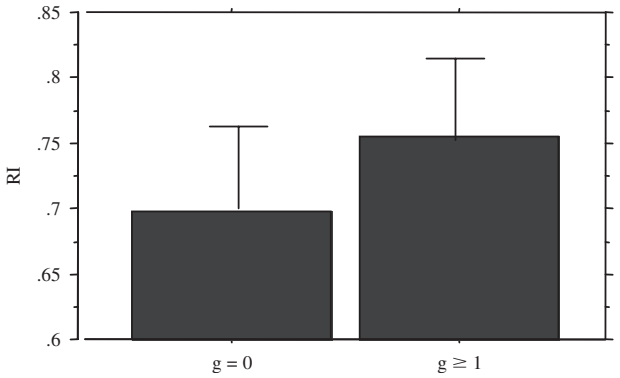

Fig. 2. Resistive index in patients with g $\geq$1 ($n=19$) and without glomerulitis g=0 ($n=68$) determined from the protocol biopsies ($P=0.006$). Glomerulitis was mild (g1) in 13 cases, moderate (g2) in four cases and severe (g3) in two cases.

Clinical and histological parameters associated with resistive index

Since recipient age, diastolic blood pressure at the time of biopsy and glomerulitis were associated with RI, a stepwise multivariate regression analysis was performed to study whether glomerulitis is an independent predictor of RI. This analysis showed that all variables were included in the statitstical model in the following order: recipient age, glomerulitis and diastolic blood pressure ($R=0.62$, $P<0.0001$). Similarly, when this analysis was repeated in CsA-treated patients and included recipient age, CsA whole blood levels at the time of biopsy and glomerulitis, only recipient age and glomerulitis were included in the statisitical model ($R=0.66$, $P<0.0001$).

Graft outcome

There were 17 patients with renal function deterioration and 69 with stable function having mean follow-ups of 54 ± 23 and 62 ± 16 months, respectively. Renal function deterioration at last follow-up was associated with the presence of CAN in the protocol biopsy (relative risk, 3.5, 95% confidence interval: 1.2–10.2; $P=0.02$) but not with RI.

Discussion

In the present study, we evaluated possible associations between RI and acute or chronic histological lesions in early protocol renal allograft biopsies. In agreement with previous studies [4,9,10], clinical parameters associated with increased RI were older recipient age, elevated diastolic blood pressure and pulse pressure index. We failed to observe an association between elevated renal allograft RI and the presence of any chronic lesion evaluated according to Banff criteria, and this is in agreement with data reported by Radermacher *et al.* [4] who evaluated possible associations between RI and chronic damage in protocol

biopsies at 6 months. In our study, biopsies were also evaluated by means of a morphometric technique in order more accurately to test a possible association between RI and structural damage. We evaluated Vg, Vvint/cortex and Vvintima/artery because they are surrogate measures of CAN [7,11]. However, none of these parameters was associated with the RI, which indicated that RI cannot be considered as a surrogate measure of chronic structural damage, at least during the first few months of follow-up. In the present study, we confirmed that patients displaying CAN in the protocol biopsy were at increased risk for graft function deterioration [5,6], whereas we were unable to find any association between RI and graft outcome. This result supports the concept that histology is superior to RI for predicting graft function deterioration, at least in patients with stable renal function. In contrast, Pape *et al*. [12] reported an association between RI and chronic damage evaluated in sirius red-stained biopsies in deteriorating paediatric renal allografts. In the current study, both RI and chronic damage were independent predictors of graft function deterioration. However, it should be recalled that RI in adult populations is strongly influenced by aortic compliance, but this is probably not the case in children. Thus, we speculate that in children, the association between RI and renal structural damage may be more intense than in adults since there is less interference by aortic stiffness.

Although RI has been employed to evaluate renal transplant dysfunction, it has been shown to have a low specificity and sensitivity for the diagnosis of acute or chronic dysfunction [13]. Despite the low utility of RI as a diagnostic tool, it recently has been shown to be a powerful predictor of late graft failure [4]. This discrepancy between the diagnostic and prognostic utility of RI raises questions about its physiological significance. It has been proposed that RI provides a surrogate measure of renal vascular resistance; however, experimental and clinical data indicate that this parameter is better related to vascular compliance [10,14]. In an isolated perfused kidney model, the physiological parameter that most closely correlates with increased RI is an increased pulse pressure index. This correlation explains the observed associations between age and RI in the general population, and the association between recipient age and RI but not that of donor age in transplanted patients [9].

In agreement with Radermacher *et al*. [4], we observed a negative association between CsA levels and RI. Since CsA treatment has been associated with various cardiovascular changes, such as vasoconstriction and tachycardia, further studies will be needed to characterize the relationship between these two variables.

We observed an association between RI and glomerulitis, but not between RI and interstitial infiltration, tubulitis or vasculitis. The evaluation of biopsies according to Banff criteria is associated with a significant intra- and inter-observer variability [15]. Even though the degree of glomerulitis was mild in the majority of our patients, we evaluated intra-observer variability for glomerulitis and found a reasonable reproducibility. The association between RI and glomerulitis was confirmed in the multivariate analysis in which clinical parameters associated with RI were also considered. To our knowledge, this association has not been described previously because other studies did not perform correlations between RI and acute lesions in protocol biopsies [4]. Previous studies have demonstrated associations between increased RI and carotid intimal thickening [16] or left ventricular hypertrophy [17], suggesting that increased RI may contribute to end-organ damage. Thus, it is tempting to speculate that glomerulitis may be the consequence of increased RI, and as such may represent a structural link between increased RI and decreased long-term renal graft survival. However, glomerulitis has also been associated with antibody-mediated rejection. Unfortunately, we were not able in the present study to evaluate C4d deposition, which is a specific marker of humoral rejection. Nevertheless, in a multicentre study which included 551 protocol biopsies, we observed that the incidence of diffuse peritubular capillary C4d deposition was as low as 2% [18].

Large epidemiological studies have repeatedly demonstrated that even though elderly recipients have a lower incidence of acute rejection, older recipient age is a major risk factor for late graft failure [19]. This discrepancy between acute rejection and chronic allograft failure in elderly recipients has not been adequately explained [20]. Since vascular compliance decreases whereas RI increases with ageing, we suggest that increased RI may contribute to renal allograft damage and shortened graft survival.

In summary, the presence of CAN but not RI was associated with graft function deterioration in early protocol biopsies. We failed to detect an association between chronic renal damage and RI, even when damage was evaluated with a morphometric technique. The only structural lesion associated with RI was glomerulitis, suggesting that this lesion may provide a structural link between increased RI and decreased long-term renal graft survival.

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Conflict of interest statement. None declared.

References

1. Pascual M, Theruvath T, Kawayt T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long term outcomes after renal transplantation. *N Engl J Med* 2002; 346: 580–590
2. Seron 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

3. Tublin ME, Bude RO, Platt JF. The resistive index in renal doppler sonography: where do we stand. *Am J Roentgenol* 2003; 180: 885–892
4. Radermacher J, Mengel M, Ellis S *et al*. The renal resistance index and renal allograft survival. *N Engl J Med* 2003; 349: 115–124
5. Seron D, Moreso F, Bover J *et al*. Early protocol biopsies and graft outcome. *Kidney Int* 1997; 51: 310–316
6. 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
7. Moreso F, Lopez M, Vallejos A *et al*. Serial protocol biopsies to quantify the progression of chronic allograft nephropathy in stable renal allografts. *Am J Transplant* 2001; 1: 82–88
8. Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. *Psychol Bull* 1968; 70: 213–216
9. Krumme B, Grotz W, Kirste G, Schollmeyer P, Rump LC. Determinants of intrarenal doppler indices in stable renal allografts. *J Am Soc Nephrol* 1997; 8: 813–816
10. Tublin ME, Tessler FN, Murphy ME. Correlation between renal vascular resistance, pulse pressure and the resistive index in isolated perfused rabbit kidneys. *Radiology* 1999; 213: 258–264
11. Alperovich G, Maldonado R, Moreso F, Fulladosa X, Grinyo JM, Serón D. Glomerular enlargement assessed by paired donor and early protocol renal allograft biopsies. *Am J Transplant* 2004; 4: 650–654
12. Pape L, Mengel M, Offner G, Melter M, Ehrich JHH, Strehlau J. Renal arterial resistance index and computerized quantification of fibrosis as a combined predictive tool in chronic allograft nephropathy. *Pediatr Transplantat* 2004; 8: 565–570
13. Genkins S, Sanfilippo F, Carroll B. Duplex doppler sonography of renal transplants: lack of sensitivity and specificity in establishing pathological diagnosis. *Am J Roentgenol* 1989; 152: 535–539
14. Bude RO, Rubin JM. Relationship between the resistive index and vascular compliance and resistance. *Radiology* 1999; 211: 411–417
15. Furness PN, Taub N. Convergence of European Renal Transplant Pathology Assessment Procedures (CERTPAP) Project. International variation in the interpretation of renal transplant biopsies: report of the CERTPAP Project. *Kidney Int* 2001; 60: 1998–2012
16. Shimizu Y, Itoh T, Hougaku H *et al*. Clinical usefulness of duplex ultrasonography for the assessment of renal arteriosclerosis in essential hypertensive patients. *Hypertens Res* 2001; 24: 13–17
17. Alterini B, Mori F, Terzani E *et al*. Renal resistive index and left ventricular hypertrophy in essential hypertension: a close link. *Ann Ital Med Int* 1996; 11: 107–113
18. Mengel M, Bogers J, Bosmans JL *et al*. Incidence of C4d stain in protocol biopsies from renal allografts: results from a multicenter trial. *Am J Transplant* 2005; 5: 1050–1056
19. Moreso F, Ortega F, Mendiluce A. Recipient age as a determinant factor of patient and graft survival. *Nephrol Dial Transplant* 2004; 19 [Suppl 3]: 16–20
20. Meier-Kriesche HU, Ojo AO, Sibrik DM *et al*. Relationship of recipient age and development of chronic renal allograft failure. *Transplantation* 2000; 70: 306–310

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