ACUTE REJECTION FEATURES IN DUAL KIDNEY TRANSPLANT RECIPIENTS FROM ELDERLY DONORS: COMPARISON OF CALCINEURIN INHIBITOR-BASED AND CALCINEURIN INHIBITOR-FREE IMMUNOSUPPRESSIVE PROTOCOLS

F.B. AIELLO, L. FURIAN[1], S. MARINO[2], F. MARCHINI[3], M. CARDILLO[4], N. DE FAZIO[4], P. RIGOTTI[1] and M. VALENTE[5]

Dept. of Oncology and Neurosciences, University of Chieti; [1]Dept. of Surgical Sciences and Gastroenterology, Unit of Kidney and Pancreas Transplantation, University of Padua, Medical School, Padua; [2]Dept. of Mental Health, Azienda ULSS 12, Venice; [3]Unit of Nephrology and Dialysis, Azienda Ospedaliera, Padua; [4]Centre for Organ and Tissue Transplant, IRCCS, "Maggiore" Hospital, Milano; [5]Dept. of Diagnostic Medical Sciences, Section of Special Pathology, University of Padua, Medical School, Padua, Italy

Received May 27, 2009 – Accepted September 24, 2009

Features of acute rejection in dual kidney transplant have not been studied. The aim of this study is to compare acute rejections in dual kidney transplant recipients from elderly donors on different immunosuppressive protocols. Sixty-nine patients were evaluated: 28 received calcineurin inhibitor-based (group 1) and 41 received calcineurin inhibitor-free immunosuppression (group 2). Histology of all donor kidneys was evaluated before implantation. All rejections showed tubulitis in both groups, and were classified as T cell-mediated acute rejections. Incidence and Banff grade of rejections in the two groups were not significantly different. Late rejections however, were observed in group 1 ($P < 0.01$) whereas steroid-resistant rejections occurred in group 2 ($P < 0.03$). C4d deposition was only observed in group 2. Occurrence of acute rejection was significantly associated with graft loss due to interstitial fibrosis/tubular atrophy in both groups. In group 1 mean serum creatinine levels of patients with rejections at six months and one year were higher than those of patients without rejections ($P < 0.03$ and $P < 0.009$, respectively). In group 2 they were higher at six months ($P < 0.01$) but not at one year. In addition, graft loss due to interstitial fibrosis/tubular atrophy occurred in 3/28 patients in group 1 (10.7%, OR= 1.95, 95%CI 1.02-3.71), and in 1/41 patients in group 2 (2.4%, OR= 0.41, 95%CI 0.07-2.24). Taken together these results suggest better renal function in patients on calcineurin inhibitor-free immunosuppression. In conclusion, acute rejections were detrimental irrespective of the type of immunosuppression, but different features were observed with each therapy. A tailored approach should be advantageous for prevention and treatment of acute rejections.

Interstitial fibrosis and tubular atrophy (IF/TA) causes progressive deterioration of the graft function and is the most frequent cause of late graft failure (1-2). It derives from immunologic and non-immunologic injuries including acute and chronic rejection, chronic calcineurin inhibitor (CI)-toxicity,

Key words: dual kidney transplant, acute rejection, immunosuppressive therapy

Mailing address: Marialuisa Valente, MD
Dept of Diagnostic Medical Sciences,
Section of Special Pathology,
Via A. Gabelli 61,
35121, Padua, Italy
Tel: ++39 049 8272274 Fax: ++39 049 8211642
e-mail: marialuisa.valente@unipd.it

0394-6320 (2009)

and others (2-3). IF/TA can begin in the first year following transplant due to ischemia-reperfusion damage and immunologic causes, and increases later on, likely as a consequence of chronic CI-toxicity (1). Acute rejection usually occurs in the first year after transplant, causing variable degrees of tissue injury. How it impacts the outcome of the graft is unclear, and features such as type, number, timing and severity, have been proposed to influence long-term prognosis (3-6). Acute rejection can be antibody-mediated or T-cell mediated (ATCMR). Its histological severity can be evaluated in a semi-quantitative manner using the Banff classification which divides ATCMR into tubulo-interstitial (borderline and type I) and vascular (type II and III) (2, 7). Tubulo-interstitial ATCMR is the most frequent type of acute rejection (1-6).

Antibody-mediated rejection is caused by circulating antibodies to donor endothelial alloantigens. It can be steroid resistant and has a poorer prognosis (8-10). ATCMR can exhibit an antibody-mediated component (8-9). The C4d fragment of the C4 complement component is released during activation of the classical complement pathway that follows an antigen-antibody reaction. Its deposition along endothelial cells of peritubular capillaries (PTC) is associated with circulating donor-specific antibodies, (8, 10-11) and is considered a marker of antibody-mediated reaction. Many centers, however, do not have the facilities to detect circulating anti-donor antibodies. In addition, it can be difficult to find low antibody levels. A diagnosis of suspicious antibody-mediated reaction can be formulated when C4d deposition is associated with one of the following tissue changes: acute tubular necrosis, glomerulitis and polymorphonuclear leukocytes or monocytes in PTC or arteritis (2). Endothelial damage caused by antibodies can increase the severity of ATCMR. In line with this concept, diffuse C4d deposition in type I ATCMR was found to be associated with IF/TA (12- 13).

Marginal kidneys from expanded criteria donors (14) are an important option to counteract the shortage of donor kidneys. Grafts from elderly donors are the most common category, and since they may possess a reduced renal reserve, two kidneys are transplanted in a single recipient, usually age-matched.

It has been recently shown that graft loss in recipients of grafts from donors over 70 years of age was higher than in recipients of grafts from donors 60 to 69 years of age (15) Calcineurin inhibitor-free immunosuppressive therapy based on sirolimus can preserve renal function and decrease the likelihood of IF/TA(16). Thus, in this context it might be particularly advantageous. To the best of our knowledge the impact of acute rejection in dual kidney transplant has not been addressed. A high level of immunogenicity in kidneys from elderly donors has been hypothesized in rats and in humans (17-18), and this could influence acute rejection features. On the other hand, recipients might be at lower immunological risk, due to their older age, which is associated with attenuated inflammatory, cellular and humoral responses.

We therefore evaluated acute rejection features and outcomes in dual kidney transplant recipients from elderly donors (DKT). Moreover, we compared DKT receiving calcineurin inhibitor (CI)-based immunosuppression (group 1) with those receiving CI-free immunosuppression (group 2).

MATERIALS AND METHODS

Study subjects

Sixty-nine dual kidney transplant recipients from elderly donors (DKT) were evaluated: 28 patients undergoing transplant consecutively between October 1999 and June 2003 (mean follow up 59.7 + 28.1 months) who received CI-based immunosuppressive therapy (group 1) consisting of cyclosporine, mycophenolate mofetil (MMF) and corticosteroids; and 41 patients transplanted subsequently from April 2003 to April 2006 (mean follow up 27.0 ± 11.1 months) who received CI-free therapy (group 2) consisting of sirolimus, MMF and corticosteroids. Mean donor age was 71.9 ± 5.3 and 73.6 ± 4.0 years in groups 1 and 2, respectively. Demographics of donors and recipients and clinical data are reported in Table I. All patients had a negative pretransplant cytotoxic crossmatch. One patient in group 1 was PRA test-positive. All acute rejection episodes were associated with an increase in serum creatinine levels, documented by biopsy, and evaluated according to the Banff classification (2, 7). T-cell mediated acute rejection (ATCMR) occurring within six months after transplantation were defined as "early," and those after six months as "late". ATCMRs were treated with methylprednisolone pulses; six were steroid resistant and anti-thymocyte globulin was administered, and in three cases plasmapheresis was also performed.

Histopathology

Biopsy of donor kidneys prior to transplant was performed in all cases. Glomerular sclerosis, interstitial fibrosis, tubular atrophy, and vascular lesions were assessed according to the scoring system by Remuzzi et al. (19). Changes in each component received a score ranging from 0 to 3. The sum of these scores, defined as kidney score could range from 0 to 12. Kidneys with a score from 4 to 6 were considered for use in dual transplants. When one kidney had a score from 0 to 3 and the other of 4 or greater the two kidneys were transplanted in the same patient. Kidneys with a score of 7 or greater were not utilized. When one kidney had a score from 4 to 6 and the other of 7 or greater the kidneys were not used. The number of glomeruli in group 1 donor biopsies was 18.76 ± 7.8 and 18.09 ± 6.14 (right and left kidney, respectively), mean score = 3.78 ± 1.07. The number of glomeruli in group 2 donor biopsies was 16.8 ± 5.9 and 16.1 ± 5.1 (right and left kidney, respectively), mean score = 3.76 ± 1.0. Donor biopsies were evaluated also according to the Banff classification (2, 7). Post-transplant acute rejection biopsies were considered adequate when at least ten glomeruli and two arteries were present.

Serial sections were prepared from formalin-fixed, paraffin-embedded biopsies. Hematoxylin-eosin, PAS, Heidenhain trichrome and Weigert Van Gieson stains were performed. Sections were stained for immunohistochemistry by the Envision technique (Dako, Glostrup, Denmark) using the monoclonal antibody anti-CD68 (Dako, Glostrup, Denmark) after trypsin pre-treatment (Sigma, St Louis, MO, USA). Paraffin sections for detection of C4d were stained by the Envision technique (Dako) after antigen retrieval by pressure-cooking using a polyclonal anti-C4d antibody (Biomedica, Vienna, Austria). Primary antibodies were replaced by irrelevant matched monoclonal antibodies or non-immune serum, as appropriate, as a control for non-specific staining. For C4d staining, renal biopsies from non-transplanted patients (with unrelated pathologies) and sections showing C4d positivity due to acute antibody-mediated rejection were used as negative and positive controls, respectively. Diffuse positive staining, focal positive staining, and PTC score (number of inflammatory cells in PTC) were evaluated according to the Banff classification (2).

IF/TA was diagnosed on explanted kidneys of patients who experienced graft loss (return to dialysis) when the degrees of chronic interstitial and tubular changes, graded according to the Banff classification (2, 7) were higher than those assessed prior to transplant.

Statistical Analysis

Results are expressed as mean ± SD. Pearson's chi-square test, and Student's t-test for non-paired data were performed for statistical analysis.

RESULTS

Evaluation of acute rejections

Sixty-nine DKT were evaluated. Twenty-eight received CI-based immunosuppression (group1), and 41 received CI-free therapy (group 2). There were no significant differences between the groups in terms of age of donors, age of recipients, male/female ratio, cold ischemia time, HLA matching, and delayed graft function (Table I). The histological mean donor kidney score was also not significantly different: 3.78 ± 1.07 in group 1 and 3.76 ± 1.0 in group 2, respectively (mean of right and left kidney scores for each donor), and 4.14 ± 1.10 in group 1 and 4.17 ± 0.94 in group 2, respectively, when the highest score for each donor was considered.

All acute rejection biopsies showed tubulitis and were classified as ATCMRs. The impact of ATCMR on the outcome in each group was evaluated. In group 1 6/28 patients experienced ATCMRs. Seven ATCMR episodes were observed: four borderline,

Table I. *Demographic and clinical data of donors and recipients.*

	GROUP 1[a] (n = 28)	GROUP 2[b] (n = 41)	*P*
Mean donor age (yr)	71.9 ± 5.3	73.6 ± 4.0	NS
HLA match	1.5 ± 1.1	1.2 ± 0.9	NS
Cold ischemia time (hours) (first kidney)	15.2 ± 2.9	15.6 ± 2.8	NS
Cold ischemia time (hours) (second kidney)	17.4 ± 3.2	16.7 ± 2.8	NS
Mean recipient age (yr)	61.7 ± 3.6	61.2 ± 5.2	NS
Male/female ratio	21/7	37/4	NS
Delayed graft function[c]	11	8	NS

[a]Patients on calcineurin inhibitor-based immunosuppression. [b] Patients on calcineurin inhibitor-free immunosuppression. Results are expressed as mean ± SD. [c] Defined as the need for dialysis in the first week after surgery

Table II. *Acute rejections in patients on calcineurin inhibitor-based and -free protocols.*

	GROUP 1[a]	GROUP 2[b]	P
Patients with acute rejection	6/28 (21.4%)	7/41 (17%)	NS
Rejection episodes	7	11	NS
Histological severity	1.81 ± 0.75	1.57 ± 0.78	NS
Late rejections	3/7 (42.9%)	0/11 (0%)	<0.03
Steroid resistant rejections	0/7 (0%)	6/11 (54.5%)	<0.01

[a] *Patients on calcineurin inhibitor-based immunosuppression.* [b]*Patients on calcineurin inhibitor-free immunosuppression.* [c] *Score of tubulitis expressed as mean ± SD.*

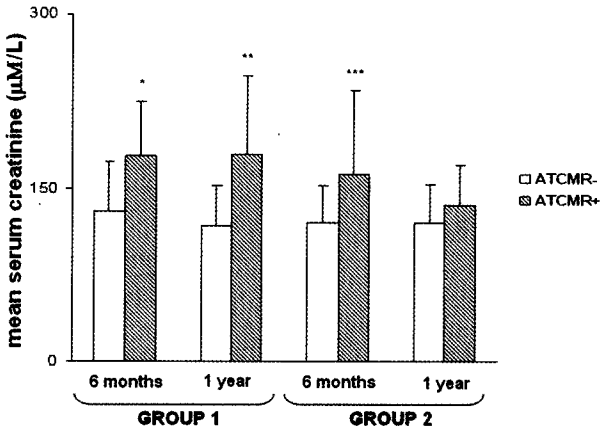

Fig. 1. *Serum creatinine levels in patients of group 1 and 2 with and without ATCMRs. Serum creatinine values, expressed at means ± SD, were examined six months and 1 year after transplant. * $P < 0.03$; ** $P < 0.009$; *** $P < 0.01$.*

two type IA and one type IB. Mean serum creatinine levels of these patients at six months and one year were significantly higher than those of patients without ATCMRs ($P < 0.03$ and $P < 0.009$, respectively (Fig.

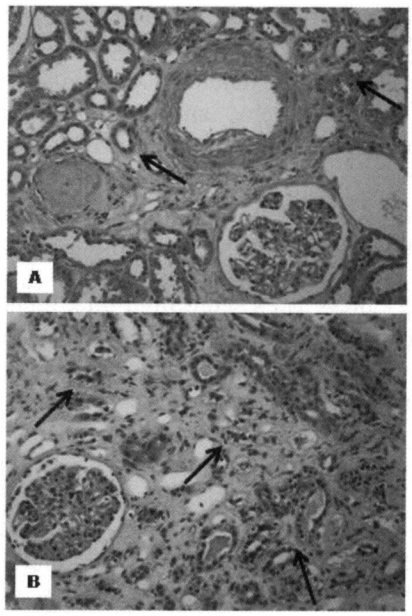

Fig. 2. *Patient of group 1. A) Donor pre-transplant biopsy. B) Interstitial fibrosis and tubular atrophy in the explanted kidney at the time of graft loss. Atrophic tubules are indicated by arrows (H and E original magnification x20).*

1). Graft loss due to IF/TA occurred in two of the six patients with ATCMRs (33%), and in one of the 22 patients without ATCMRs (2.9%) ($P < 0.04$).

In group 2, 7/41 patients experienced ATCMR. Eleven ATCMR episodes were observed: four borderline, five type IA, one type IB and one type II. The mean serum creatinine level was significantly higher than that of patients without ATCMRs at six months ($P < 0.01$) but not at one year (Fig. 1).

Graft loss due to IF/TA occurred in one of the seven patients with ATCMR (14.3%), while among patients without ATCMR it did not occur (0%) ($P < 0.02$). Thus, in both groups graft loss due to IF/TA was significantly higher in patients who experienced ATCMRs.

We then examined whether ATCMRs had different features in the two groups. The percentage of patients with ATCMRs, the number of rejections and their histological severity were not significantly different. However, steroid resistant ATCMRs were observed in group 2 ($P < 0.01$) whereas late ATCMR occurred in group 1 ($P < 0.03$) (Table II). All three

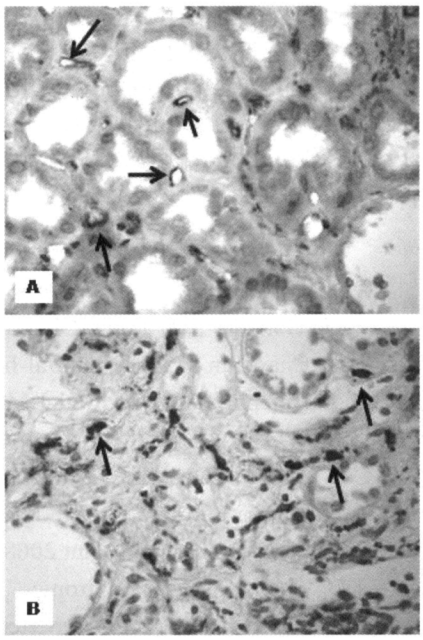

Fig. 3. *Patient of group 2. **A)** Diffuse C4d positivity. C4d-positive PTC are indicated by arrows. **B)** CD68-positive monocytes were present in PTC (arrows) and in the interstitium (original magnification x40).*

late ATCMRs (100%) occurred in patients who suffered graft loss due to IF/TA, while only two of the 15 early ATCMRs (13.3%) were associated with graft loss ($P < 0.002$). An example of interstitial and tubular changes in the pre-transplant biopsy and at the time of graft loss in a patient who experienced a late ATCMR (type IB) is shown in Fig. 2 (A and B).

In all group 1 biopsies and in all but one biopsy of group 2, C4d deposition was evaluated. C4d positive staining was not observed in group 1. In contrast, in group 2 C4d positivity was found in 4 cases. The biopsy of a steroid-resistant early ATCMR showed diffuse positivity with glomerulitis, and PTC score was 1 with monocytes predominating (Fig. 3 A and B). Focal positivity was observed in three early ATCMRs; glomerulitis was observed in two cases, PTC score was 0 in one case and 1 in two cases, with monocytes predominating, and two cases were steroid resistant.

Evaluation of clinical data and outcome

Mean serum creatinine levels at six months and one year in the two groups were not significantly different (group 1 = 141.4 ± 48.3 μM/L, 129.7 ± 49.1 μM/L *vs* group 2 = 126.6 ± 42.4 μM/L, 122.4 ± 49.1 μM/L). As above-mentioned, however, in group 1 both at six month and one year after transplant, patients with ATCMRs had mean serum creatinine levels significantly higher than those of patients without ATCMRs (Fig. 1). In contrast, one year after transplant, patients of group 2 with and without rejections had similar mean serum creatinine levels (Fig. 1).

In group 1 graft loss occurred in 6/28 patients (21.4%), which was due to IF/TA in three cases, thrombosis of renal veins in one case, and cardiovascular diseases in two cases (death with functioning graft), OR = 1.81 (95%CI 1.02-3.21). In group 2 graft loss occurred in 3/41 patients (7.3%), due to IF/TA in one case, vascular rejection in one case, and thrombosis of renal veins in one case, OR = 0.52 (95%CI 0.2-1.35). Thus, the proportion of patients with graft loss due to IF/TA in group 1 was 3/28 (10.7%), OR 1.95 (95%CI 1.02-3.71), while it was 1/41 (2.4%), OR = 0.41 (95%CI 0.07-2.24) in group 2. In both groups graft loss due to IF/TA occurred within the third year after transplant.

DISCUSSION

Marginal kidneys are an important option to expand the donor pool. Kidneys from elderly donors may have a reduced renal reserve, and chronic CI-toxicity can further impair their function. Sirolimus, administered alone to animals, was devoid of nephrotoxicity, (20-21) and, when included in CI-sparing and CI-free protocols, preserved renal function in humans (16). In previous reports on single kidney transplant recipients from standard donors, when sirolimus was added to CIs the number of ATCMRs was lower than that observed using CI-based protocols (22). In the absence of CIs, however, it was similar or higher (23-24) depending on number, type and doses of the other immunosupressive drugs used in combination with sirolimus (25-26, 16).

Tubulo-interstitial ATCMR is the most frequent type of acute rejection (1-6). We have studied its features in groups of dual kidney transplant recipients on different immunosuppressive protocols. Mean serum creatinine levels and graft loss due to IF/TA were

significantly higher for patients with ATCMRs in both groups, thus, irrespective of the immunosuppressive therapy ATCMRs were detrimental.

The percentage of patients with ATCMRs, the number of rejections and their histological severity were not significantly different. However, late rejections were present only in the group of patients treated with CI-based therapy, and, as reported for single kidney transplant patients (6, 27-28), were significantly associated with IF/TA development.

In our study, steroid resistant ATCMRs were observed only in patients treated with CI-free therapy, however, with the exception of one patient who experienced graft loss due to vascular rejection, all patients underwent rejection reversal and their graft is currently functional.

Withdrawal of calcineurin inhibitors has been found to be associated with an increased incidence of antibody-mediated acute rejections (29-30). Interestingly, C4d deposition was observed only in ATCMR biopsies of CI-free patients (group 2).

In group 1, mean serum creatinine levels of patients with ATCMRs were significantly higher than those of patients without ATCMRs both at six months and one year after transplant, whereas, CI-free patients with or without ATCMR (group 2), at one year, had similar mean serum creatinine levels. Moreover, in group 1, despite the absence of steroid-resistant rejections, more graft losses were observed. Notably, in both groups graft losses due to IF/TA occurred within the third year after transplant, thus the higher number of losses in group 1 was not attributable to differences in follow-up time. These results suggest that the CI-free protocol exhibited lower nephrotoxicity, although a greater number of patients is needed to determine the advantages of CI-free therapy.

In conclusion, dual kidney transplant recipients from elderly donors on CI-based and CI-free immunosuppressive protocols experienced ATCMRs showing different features. Irrespective of the type of immunosuppression, ATCMRs were associated with a poor prognosis. Thus, a tailored approach for patients on different immunosuppression should be useful for prevention and treatment of acute rejections.

ACKNOWLEDGEMENTS

We are grateful for the technical assistance of Elisabetta Baliello, Luca Braghetto, and Alessandra Dubrovich and for the secretarial assistance of Alessandra Cervellin. We thank Drs Mila Della Barbera and Cesare Guarena for helpful discussion, and Prof. Franco Ranelletti for critical review of the manuscript.

REFERENCES

1. 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; 349:2326-33.
2. Solez K, Colvin RB, Racusen LC, et al. 2007 Banff 07 classification of renal allograft pathology. Updates and future directions. Am J Transplant 2008; 8:1-8.
3. Cornell LD, Colvin RB. Chronic allograft nephropathy. Curr Opin Nephrol Hypertens 2005; 14:229-34.
4. Matas A. Chronic rejection in renal transplant recipients – risk factors and correlates. Clin Transplant 1994; 8:332-5.
5. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 2000; 342:1837-8.
6. Joosten SA, Sijpkens YW, van Kooten C, Paul LC. Chronic renal allograft rejection: pathophysiologic considerations. Kidney Int 2005; 68:1-13.
7. Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int 1999; 55:713-23.
8. Manuiyyedi S, Crespo M, Collins AB, et al. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification.. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. J Am Soc Nephrol 2002; 13:779-87.
9. Regele H, Exner M, Watschinger B, et al. Endothelial C4d deposition is associated with inferior kidney allograft outcome independently of cellular rejection. Nephrol Dial Transplant 2001; 16:2058-66.
10. Bohmig GA, Exner M, Habicht A, et al. Capillary C4d deposition in kidney allograft: a specific marker of alloantibody-dependent graft injury. J Am Soc

Nephrol 2002; 13:1091-99.
11. Racusen LC, Colvin RB, Solez K, et al. Antibody-mediated rejection criteria – an addition to the Banff 97 classification of renal allograft rejection. Am J Transplant 2003; 3:708-14.
12. Herzenberg AM, Gill JS, Djurdjev O, Magil AB. C4d deposition in acute rejection: an independent long-term prognostic factor. J Am Soc Nephrol 2002; 13: 234-41.
13. Jianghua C, Wenqing X, Huiping W, Juan J, Jiandoyong W, Qiang H. C4d as a significant predictor for humoral rejection in renal allografts. Clin Transplant 2005; 19:785-91.
14. Metzger RA, Delmonico FL, Feng S, Port FK, Wynn JJ, Merion RM. Expanded criteria donor for kidney transplantation. Am J Transplant 2003; 3:114-25.
15. Chavalithdhramrong D, Gill J, Takemoto S Madhira B R, Cho Yw, Shah T, Bunnapradist S. Patient and graft outcomes from deceased kidney donors age 70 years and older: An analysis of the Organ Procurement Transplant Network/United Network of Organ Sharing Database. Transplantation 2008; 85: 1573-79.
16. Mehrabi A, Fonouni H, Kashfi A et al. The role and value of sirolimus administration in kidney and liver transplantation. Clin Transplant 2006; 20:30-43.
17. Reutzel-Selke A, Jurish A, Denecke C, et al. Donor age intensifies the early immune response after transplantation. Kidney Int 2007; 71:629-36.
18. Sanchez-Fructuoso A, Naranjo Garcia P, Calvo Romero N, Ridao N, Naranjo Gomez P, Conesa J, Barrientos A. Effect of the brain-death process on acute rejection in renal transplantation. Transplant Proc 2007; 39:2214-16.
19. Remuzzi G, Grinyo J, Ruggenenti P, Beatini M, Cole EH, Milford EL, Brenner BM. Early experience with dual kidney transplantation in adults using expanded criteria donors. J Am Soc Nephrol 1999; 10:2591-98.
20. DiJoseph JF, Sharma RN, Chang JY. The effect of rapamycin on kidney function in the Sprague-Dwaley rat. Transplantation 1992; 53:507-13.
21. Golbaekdal K, Nielsen CB, Djurhuus JC, Pedersen EB. Effects of rapamycin on renal hemodynamics, water and sodium excretation, and plasma levels of angiotensin II, aldosterone, atrial natriuretic peptide, and vasopressin in pigs. Transplantation 1994; 58: 1153-57.
22. Kahan BD, Julian BA, Pescovitz MD, Vanrenterghem Y, Neylan J. Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in caucasian recipients of mismatched primary renal allografts: a phase II trial. Rapamune Study Group. Transplantation 1999; 68:1526-32.
23. Groth CG, Backman L, Morales JM, et al. for the Sirolimus European Renal Transplant Study Group. Sirolimus (Rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Transplantation 1999; 67:1036-42.
24. Kreis H, Cisterne JM, Land W, et al. for the Sirolimus European Renal Transplant Study Group. Transplantation 2000; 69:1252-60.
25. Johnson RWG, Kreis H, Oberbauer R, Brattstrom C, Claesson K, Eris J. Sirolimus allows early cyclosporine withrawal in renal transplantation resulting in improved renal function and lower blood pressure. Transplantation 2001; 72:777-86.
26. van den Akker JM, Hené RJ, Hoitsma AJ. Inferior results with basic immunosuppression with sirolimus in kidney tranplantation. Neth J Med 2007; 65:23-28.
27. Humar A, Kerr S, Gilligham KJ, Matas AJ. Features of acute rejection that increase risk for chronic rejection. Transplantation 1999; 68:1200-03.
28. Pallardo Mateu LM, Sancho Calabuig A, Capdevila Plaza L, Franco Esteve A. Acute rejection and late renal transplant failure: risk factor and prognosis. Nephrol Dial Transplant 2004; 19:iii38-iii42.
29. Knechtle SJ, Pirsch JD, Fechner J, et al. Campath-1H induction plus rapamycin monotherapy for renal transplantation: results of a pilot study. Am J Transplant 2003; 3:722-30.
30. Hazzan M, Buob D, Labalette MF, Glowacki F, Hoffman M, Copin MC, Noel C. Assessment of the risk of chronic allografts dysfunction after renal transplantation in a randomized cyclosporine withdrawal trial. Transplantation 2006; 82:657-62.