BANTAO Journal 3 (2): p 133; 2005

Early Protocol Renal Allograft Biopsies and Graft Outcome: Effects of Treatment of Borderline and Subclinical Rejections at First Month on Histological Changes and Function at 6 Months

J. Masin-Spasovska[1], G. Spasovski[1], S. Dzikova[1], G. Petrusevska[2], B. Dimova[2], Lj. Lekovski[3], Z. Popov[3], N. Ivanovski[1], M. Polenakovic[1]

[1]Department of Nephrology, [2]Department of Pathology, [3]Department of Urology, Faculty of Medicine, Skopje, Republic of Macedonia

Introduction

Despite improvements in both short- and long-term kidney allograft survival over the last decades, up to 30 % of grafts are still lost in the first 5 years because of a chronic fibrotic process termed chronic allograft nephropathy (CAN), that may be caused by a variety of immunological and clinical factors. CAN progresses rapidly during the first few months and slowly thereafter (1,2). Although the episodes of acute rejection (AR) were most consistently correlated with development of chronic allograft failure, the reported reduction in their incidence over the recent years has not improved long-term graft outcome, neither the incidence of chronic rejection. Since, early AR episodes and CAN are often subclinical causing no measurable decrease in renal function, the possible explanation may lie in the existence of more subtle forms of silent pathogenic inflammation that are undetected but associated with the development of CAN (3). This raises the value of early protocol biopsies of stable allografts and the clinically useful information they provide, determining the incidence of subclinical acute rejection (SAR) or borderline changes (BC) and benefit of treating these histological founding in terms of graft function and longevity. In line with this concept, Shapiro *et al*. reported SAR and BC in about 25% and 21% of the biopsies performed at 1 week after transplantation, respectively (4). Rush *et al*. have even demonstrated the presence of SAR in stable renal function in 29% and BC in 49% of protocol biopsies at 3 months after transplantation, respectively (2). Furthermore, Nickerson *et al*. has reported that corticosteroid treatment of SAR in the first 3 months posttransplant decreases late clinical rejections and improves graft function (5). On the other side, numerous studies have evaluated the prevalence of CAN in well-functioning kidney allograft. Seron *et al*. described a prevalence of CAN in about 42% of protocol biopsies performed 3 months after transplantation (6), while in a recent report of Nankivell *et al*. the percentage was 24% (7). Recently, Rush *et al*. reported improved allograft outcome after treatment of early SAR with high-dose steroids (3,8).

Our study aimed to identify and to evaluate subclinical acute rejections and borderline changes (SAR/BC) and histological findings of chronic allograft nephropathy (CAN) in protocol biopsies at 1 and 6 months after living related kidney transplantation and to determine whether treatment of SAR and BC detected in allograft biopsies at first month postransplant has a beneficial effect on renal allograft histology and/or function at 6 months.

Material and methods

Thirty-five consecutive living related (LR) transplant patients were studied. All patients received their first transplant. Methylprednisolone (500 mg) and Daclizumab (Zenapax; 1 mg/kg BW at implantation and thereafter every 2 weeks x five doses) ware administered as induction therapy. The post-transplant standard triple immunosuppression therapy consisted of: cyclosporine (Neoral; 6 to 8 mg/kg/day) to reach target C2 levels (blood concentration 2 hours after administration of the drug), prednisolone (1 mg/kg/day tapered to 0.1 mg/kg/day after 4 weeks) and mycophenolate mofetil (Cellcept 1 g bid.).

During the first postoperative month patients with delayed graft function (DGF) who suffered post-transplant acute tubular necrosis or experienced a clinical episode of acute rejection (AR) were treated with hemodialysis or pulse corticosteroids, respectively, whenever an increase in serum creatinine >20% or decrease in urine output for 2 consecutive days was observed. These cases were included if their graft function had been stable (no change in serum creatinine > 20%) for at least 2 weeks before the first biopsy. Patients with histology at 1 month biopsy of BC or AR type I or IIA and an increase in serum creatinine between 10 and 20 % from baseline (serum creatinine 2 weeks prior the biopsy) were assessed as SAR and consequently treated with pulse corticoid therapy. The patients with histology of BC or AR followed by rise in serum creatinine < 10% from baseline were not treated.

Protocol biopsies were performed using ultrasound-guided automated biopsy "gun". The formalin fixed biopsies were embedded in paraffin, serially sectioned at 3 to 5 µm thickness and stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), Masson's trichrome as well as methenamine silver. Biopsies were considered adequate when they contained ≥7 glomeruli and at least one artery. Renal lesions were blindly reviewed for evidence of acute and chronic changes by the same pathologist using descriptive morphologic criteria according to the Banff 97 scoring schema using a scale from 0 to 3 (9). CAN score was calculated as a sum of scores for the individual histological markers for chronicity: interstitial fibrosis, tubular atrophy, vascular fibrous intimal thickening, arterial hyalinosis and chronic glomerulopathy. The histological

Correspondence to: Goce B Spasovski, MD, PhD, Department of Nephrology, Clinical Center Skopje, University of Skopje, Vodnjanska 17, 1000 Skopje, R.Macedonia, Tel: + 389 70 268 232, Fax: + 389 2 3220 935, e-mail: gspas@sonet.com.mk

index (HI) was calculated as total sum of scores for acute and chronic changes.
The clinical and biochemical data were recorded at the time of transplantation as well as at 1 and 6 months after transplantation. Results were expressed as mean values ± SD. An unpaired two-tailed Student *t* test was used to examine differences in mean values between the groups. Chi square analysis was used to compare the categorical variables.

Results

The mean age of the entire cohort of donors and recipients were 59.60 ± 13.11 and 34.40 ± 9.32 years, respectively. The serum creatinine (sCr) and body weight were significantly increased at 6 months after transplantation, while calculated creatinine clearance (cCrcl) was significantly lower compared to the 1 month values.
Among all biopsies only 18.6% (13/70) showed no acute histopathological lesions. BC were found in 13/35 (37%) and 9/35 (25.7%), and SAR in 15/35 (43%) and 17/35 (48.6%) of patients, on 1 and 6 month biopsies, respectively. The mean histological index (HI), assessed as total sum of scores for acute and chronic changes, increased significantly from 1 to 6 month biopsy (5.14 ± 3.00 vs. 7.94 ± 3.80; p<0.001). Similarly, the mean CAN score (sum of histological markers for chronicity) of 1.83 ± 1.46 at 1 month, increased significantly to 4.66 ± 2.35 (p<0.001) at 6 months biopsy.
From the cohort of twenty eight patients with acute histopathological lesions (13 BC + 15 SAR) at 1 month biopsy, an increase in serum creatinine between 10 and 20 % from baseline (two weeks prior the biopsy) was observed in 2 and 9 patients, respectively, and therefore pulse corticoid therapy was administered. Hence, the entire cohort was stratified to treatment (Tx) group (n=11) and non-treatment (NTx) group (n=17) patients.
The groups did not differ in the clinical data such as mean age and glomerular filtration rate (GFR) of donors, gender, basic kidney disease and BMI of the recipients, experienced DGF, and the incidence of treated AR within the first month postransplantation (Table 1). The Tx group had mean HI at 1 month biopsy of 7.91 ± 2.66, which did not increase at 6 month biopsy (7.91 ± 3.02). In contrast, the proportion of these changes in NTx group was increased from 4.71 ± 1.83 to 8.06 ± 4.85 (p=0.011). However, there was no significant difference in serum creatinine and CAN score between the groups neither at 1 nor at 6 months biopsy. On the other side, at 6 months biopsy, 10/11 patients in Tx group improved and 9/17 in NTx group have worsened their acute histopathological lesions (90.9% and 52.9%, respectively), while 1 patient in Tx group and 6 in NTx group, remained at the same histopatological state.

Table 1: Biochemical, clinical data and histological findings and scores at 1 and 6 months postransplantation according to the treatment of early borderline changes and subclinical acute rejections

	Treatment (Tx) group		**Non-treatment (NTx) group**		***P* - value**
Number of patients	**(n =11)**		**(n = 17)**		
sCr 1 month	126.73	37.57	123.41	28.85	n.s.
sCr 6 months	159.73	43.89	137.88	45.58	n.s.
DGF	3/11 (27.2)		5/17 (29.4%)		n.s.
AR (during 1 mo. postransplant)	2/11 (18.2)		4/17 (23.5%)		n.s.
HI 1 month	7.91 ± 2.66		4.71 ± 1.83		*p<0.01*
HI 6 month	7.91 ± 3.02		8.06 ± 4.85		n.s.
CAN score 1 month	2.18 ± 1.60		1.82 ± 1.42		n.s.
CAN score 6 months	5.27 ± 2.28		4.18 ± 2.53		n.s.

Discussion

Chronic allograft nephropathy (CAN) leads to chronic allograft dysfunction and loss. There is increasing evidence that protocol biopsies performed in stable allografts may be a valuable tool to uncover early clinically unapparent acute histopathological lesions, named borderline changes and subclinical acute rejections. Both conditions have been suggested as causes of CAN and deterioration of graft function.
When compared with previous reports the prevalence of borderline changes and subclinical acute rejections in our previous (10) and present report is somewhat higher (37 and 43%) at 1-month and (25.7 and 57.5%) at 6 month biopsy (1,2,3). This might be due to the different sampling time for the biopsies (1-month), which is beyond the usual time for AR and DGF, namely, the first two weeks after transplantation. Secondly, the existence of a subtle form of clinically silent immunological inflammation presented with a high number of untreated borderline changes (n=11) at 1-month biopsy and an evolution towards SAR in a substantial proportion of these patients at 6-month biopsy (7/11), might be an additional explanation for this observation. Morover, 5 patients in NTx group initially diagnosed as SAR and 5 patients in Tx group classified as SAR (type IIA), were still classified as SAR at 6-month biopsy. The improvement in histology towards type I AR after pulse corticoid therapy in the Tx group of patients, goes in line with the reports from recent studies that corticosteroid treatment of early subclinical rejection is associated with better outcomes in renal transplant patients (3,9).
Trying to solve the question whether treatment of BC or SAR detected in routine allograft biopsies 1 month after transplantation has a beneficial effect on allograft function

at 6 months, we have documented that the substantial number of patients with subclinical acute histological lesions in NTx group has evolved towards worsened pathohistological grade of these findings. In line with this observation is the significant increase in HI from 1 to 6 month biopsy in NTx group of patients, while both groups presented with similar CAN scores and serum creatinine at the same time points. This further confirms the value of protocol biopsies in determining the incidence of subclinical acute rejection and/or borderline changes and the important contribution they provide on the decision how to individualize the treatment of these histological changes for a better graft function and survival. However, the definitive result from this study concerning the latter histological deterioration and impairment of allograft function could be expected from the 1 and 2 years follow–up results.

Conclusion

In conclusion, a protocol 1-month biopsy may be valuable to determine the prevalence of BC or SAR and to individualize the treatment of these subclinical conditions in stable allografts. Untreated BC and SAR at 1-month biopsy showed greater susceptibility for acute histological deterioration on the 6 months biopsy. A beneficial effect of the treatment of SAR and BC should be confirmed at the follow up of the graft function at 1 or 2 years.

Key words: kidney transplantation, protocol biopsy, borderline changes, subclinical acute rejection, chronic allograft nephropathy.

References

1. Gloor MJ, Cohen AJ, Lager DJ, *et al.*: Subclinical rejection in tacrolimus-treated renal transplant recipients. *Transplantation* 2002;73: 1965-1968
2. Seron D, Moreso F, Fulladosa X, Hueso M, *et al*.: Reliability of chronic allograft nephropathy diagnosis in sequential protocol biopsies. *Kidney Int* 2002;61:310-316
3. Rush D, Nickerson P, Gough J, *et al*.: Beneficial effects of treatment of early subclinical rejection: a randomized study. *J Am Soc Nephrol* 1998;9:2129-2134
4. Shapiro R, Randhawa P, Jordan ML, *et al*.: An analysis of early renal transplant protocol biopsies-the high incidence of subclinical tubilitis. *Am J Transplant* 2001;1:47-50
5. Nickerson P, Jeffery J, Gough J, *et al.*: Effect of increasing baseline immunosupression on the prevalence of clinical and subclinical rejection: A pilot study. *J Am Soc Nephrol* 1999;10:1801-1805
6. Seron D, Moreso F, Bover J, Condom E, *et al*.: Early protocol renal allograft biopsies and graft outcome. *Kidney Int* 1997;51:310-316
7. Nankivell BJ, Fenton-Lee CA, Kuypers DJ, *et al.*: Effect of histological damage on long-term kidney transplant outcome. *Transplantation* 2001;71:515-523
8. Rush DN, Jeffery JR, K.Trpkov, *et al.*: Effect of subclinical rejection on renal allograft histology and function at 6 months. *Transpl Proc* 1996;28:494-495
9. Racusen LC, Solez K, Colvin RB *et al*.: The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999;55:713-723
10. Masin-Spasovska J, Spasovski G, Dzikova S *et al*.: Protocol biopsies in kidney transplant recipients: histologic findings as prognostic markers for graft function and outcome. *Transplant Proc* 2005;37:705-708