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Protocol Biopsies in Kidney Transplant Recipients: Histological Findings and Allograft Function at 1-Year

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Introduction

Protocol renal allograft biopsy has been considered as a potentially valuable diagnostic tool in identifying the histological changes associated with graft prognosis (1). However, the association with the findings of chronic allograft nephropathy (CAN) before the deterioration of the graft function and histological changes of subclinical acute rejection (SAR) or borderline changes (BC) remain less clear, especially concerning the possibility for puls corticosteroid therapy and late allograft failure (2,3). Recent studies have suggested that corticosteroid treatment of subclinical acute rejections (SAR) in early protocol biopsies might decrease late clinical rejections and improve graft function (4,5). Other studies of protocol biopsies taken between 3 months and 2 years after transplantation, have shown the association of histological damage with impaired renal function (4,6). In addition, a variety of clinical factors has been incriminated to influence long-term graft survival: donor age, donor quality, recipient age, basic kidney disease, time on dialysis, HLA mismatching, body mass index (BMI) of recipient, delayed graft function (DGF), urinary tract infection (UTI) etc. (7,8).

The **aim** of our study was to identify SAR and BC as well as histological markers of chronic allograft nephropathy (CAN) among protocol biopsies performed at 1 and 6 months postransplantation and to assess the possible implications of the immunological and clinical factors on the graft function at 1, 6 months and 1 year.

Material and methods

A cohort of thirty living related (LR) first kidney transplant was studied. 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 (sCr) >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 sCr > 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 sCr between 10 and 20 % from baseline (sCr 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 sCr < 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 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, 6 months and 1-year 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

Patients (n=30) were 35.7 ± 8.4 years old at transplantation; 33.3% were female with a mean time on dialysis 31.3 ± 38.6 months. The total HLA mismatch score was 1.9 ± 1.3. Donors were 60.2 ± 13.7 years old with mean glomerular filtration rate (GFR) of the donated kidney of 50.7 ± 13.8 ml/min. DGF occurred in 30% of recipients and was associated with an increased number of acute rejection episodes. Serum creatinine and BMI at 6 and 12 months were significantly increased when compared with 1 month values (124.2 ± 33.3 vs 147.9 ± 46.9; $p<0.01$ and 124.2 ± 33.3 vs 160.1 ± 74.8; $p<0.01$, respectively). Kidney graft function, i.e. mean calculated creatinine clearance (cCrcl) tended to deteriorate from the first to the 6 month value

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(64.1 ± 21.2 vs 59.5 ± 19.4) and remained stable at 12 months after transplantation (59.5 ± 20.4).

Among all protocol biopsies which were performed at 1 and 6 months after transplantation, only 16.6% (10/60) showed no acute histopathological lesions. At the first month BC was shown in 40% and SAR in 30% of the patients. At 6 months the proportion of these findings was even slightly higher, 30% and 46.6%, respectively. Furthermore, the mean CAN score and HI increased significantly on the 6 month biopsy, 1.73 ± 1.46 vs 4.5 ± 2.36 ($p<0.01$) and 4.87 ± 2.8 vs 7.9 ± 4.07 ($p<0.01$), respectively.

In order to assess which variables had the most important influence on the graft function the cohort was divided according to the progressive rise in sCr above 200 μmol/L, or in absolute value more than 20% in the interval from 1 to 12 months, to a group with high Scr (HsCr) and low sCr (LsCr). At baseline, the HsCr group (n=8) presented with significantly higher BMI (25.4 ± 3.8 vs 21.5 ± 3.6, $p<0.052$), much higher percentage of chronic glomerulonephritis as recipient basic kidney disease (62.5 vs 27.2%) and shorter time in dialysis (6.4 ± 5.2 vs 40.7 ± 41.5, $p<0.01$). The characteristic of this group was also the susceptibility for urinary tract infections (2.6 ± 1.3 vs 0.5 ± 0.51, $p<0.01$) per patient for the study period of 1 year. Despite the tendency towards older recipient and donor age, the GFR of donated kidney in HsCr group was slightly higher when compared to LsCr group (53.1 ± 12.8 vs 49.8 ± 14.4). In contrast, this group had higher sCr at 1 month, reaching significant difference in comparison with LsCr group at 6 and 12 months values (200.6 ± 30.9 vs 128.8 ± 35.9; $p<0.01$ and 254.8 ± 80.7 vs 125.6 ± 30.1; $p<0.01$, respectively). There was similar but reversal observation for cCrcl at 6 and 12 months (44.5 ± 5.9 vs 61.8 ± 21.9; $p<0.01$ and 38.2 ± 10.1 vs 63.7 ± 18.6; $p<0.01$, respectively).

Surprisingly, large but comparable number of acute inflammatory activity (BC/SAR) was present in both groups. However, there was much higher percentage of moderate grade (type IIB) AR episodes in the HsCr group at 1 and 6 months biopsy (25 vs 4.5 and 25 vs 0%), respectively. The groups didn't differ in HI and mean CAN score on 1 month biopsy, while there was significant increase in both parameters in the HsCr group on the 6 month biopsy (10.3 ± 3.0 vs 7.1 ± 4.1; $p<0.05$ and 5.8 ± 1.8 vs 4.1 ± 2.5; $p<0.05$, respectively). Interestingly, the predominant chronic histological changes were present on the vascular structures (cv: 1.63 ± 0.52 vs 0.73±0.63; $p<0.01$).

Discussion

The impressive reduction in acute rejection and early graft loss with modern immunosuppression has focused attention on the long-term outcome of kidney transplants and the factors that lead to chronic graft failure. Both donor characteristics and early postoperative events are critical to the long-term outcome, with higher rates of failure associated with donor quality, acute rejection, and clinical risk factors such as DGF, BMI, UTI (7,8,10-12). However, the relationship between these factors and histological appearance are not clear. A controversy also exists, concerning the puls corticoid treatment in cases of clinically silent acute and chronic histological lesions (2-4). In the present study, we examined the incidence of SAR, BC and histological markers of CAN in protocol biopsies at 1 and 6 months postransplantation, and determined the clinical risk factors which might influence graft function at 1, 6 months and 1 year.

Our study confirmed previous reports on the high incidence of subclinical acute and chronic histological changes which occur early after transplantation (1,3-6). Consequently, sCr was steadily increased at 6 months, accompanied with slight decrease in cCrcl. Both parameters of graft function remained stable thereafter at 12 months.

The question remained over the factors which have possibly influenced the higher sCr progression in the HsCr group. Among clinical parameters we included the quality of the donated kidney. At baseline, the HsCr group presented with slightly advanced donor age but somewhat higher donor GFR. However, at 1-month the cCrcl in the HsCr group remained stable, but deteriorated at 6 and further at 12 months, what might be in line with the reports for the association of advanced donor age and poor graft function and outcome (10). Cold ischemia time, female to male donation, DGF and episodes of AR during first week postransplantation were comparable between the groups. However, the fact is that we did not biopsy every graft experiencing DGF within one week, thus it is conceivable that we could have omitted some very early rejection episodes. In present study, this is in line with the observation of two fold greater percentage of treated patients with DGF in LsCr group. Interestingly, there were 4 vascular Type IIB, AR in HsCr group (2 at 1 and 6 month biopsy) associated with DGF episode in three patients. In contrast, only one patient in LsCr group presented with the same type of rejection at 1 month biopsy.

Further potential contributors for worsening of the graft function at 6 and 12 months in HsCr group were increased BMI, greater susceptibility to urinary tract infection and greater number of patients with glomerulonephritis as basic kidney disease. While pre-emptive transplant has been shown to be advantageous to the graft survival in several studies (13), the patients of HsCr group in our study has been characterised by much shorter dialysis duration, observation, which needs further clarification.

The histological finding of chronic intimal vascular thickening (cv) at 6 month biopsy in HsCr group might be considered a consequence of previous and present vascular type of rejections. In addition, the greater CAN score in this group might be also associated with chronic infections, partially explained by the greater number of urinary tract infections.

Conclusions

In conclusion, 1 and 6 months biopsy may be valuable to determine borderline and subclinical rejection and to prognosticate the outcome of renal allograft function. The presence of vascular type of acute rejection associated with

delayed graft function, underdiagnosed and untreated early acute rejections, progressive rise in BMI and greater susceptibility to urinary tract infection might lead to a rapid impairment of the graft function accelerating the process of chronic allograft nephropathy.

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