Early renal protocol biopsies: beneficial effects of treatment of borderline changes and subclinical rejections on the histological changes for chronic allograft nephropathy

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Abstract

Protocol biopsies in the early course after renal transplantation revealed that chronic allograft nephropathy (CAN) starts early. Our study aimed to identify borderline changes (BC), subclinical rejections (SR) and histological markers of chronic allograft nephropathy (CAN) in protocol biopsies at 1 and 6 months after living related kidney transplantation, and to determine whether treatment of BC and SR at the 1-month postransplant has a beneficial effect on graft histology and function at 6 months.

Forty paired allograft biopsies were evaluated according to the Banff scoring schema.

BC was found in 13/40 (32.5%) and 12/40 (30%), and SR in 15/40 (37.5%) and 21/40 (52.2%) of patients, on 1 and 6-month biopsies, respectively. The mean HI (histological index/ sum of scores for acute/chronic changes) and CAN score (sum of histological markers for chronicity) increased significantly at 6-month biopsy 5.3±2.9 vs. 7.8±3.6 ($p<0.001$) and 2.1±1.5 vs. 4.6±2.3 ($p<0.000$), respectively. When compared according to the histological index (HI<5>), high HI group had mean CAN score of 2.91±1.24 at 1-month, which increased to 5.09±1.98 at 6-month biopsy (175%). The proportion of these changes in low HI group was increased from 0.94±1.14 to 4.0±2.52 (268 %). At 1-month serum creatinine (sCr) was significantly higher in the high HI group, 134 ± 35.9 vs.113 ± 27.5 ($p<0.04$), while creatinine clearance (Crcl) was respectively lower. No significant difference of these parameters was found at 6 month, between the high and low HI group. When the groups were compared according to the donor age (<55> years), no significant difference was found in sCr and Crcl at 1 month, while at 6 month Crcl was significantly higher in the younger donor group, 52 ± 10.8 vs. 72 ± 22.6 ($p<003$). There was no significant difference in HI score between the two groups. When divided according to the treatment of BC/SR, the group of treated BC/SR at 1 month biopsy had mean HI score of 7.11 ± 1.9, which remained almost the same at 6 months biopsy (7.11 ± 2.32). In contrast, the proportion of these changes in untreated BC/SR group increased from 4.95 ± 1.99 to 8.16 ± 4.30. The mean CAN score in untreated BC/SR group increased from 1.89 ± 1.37 to 4.32 ± 2.24 ($p<0.000$) at 6-month biopsy.

In conclusion, a protocol 1-month biopsy may uncover a high prevalence of BC or SR in stable allografts. The presence of an untreated BC or SR in biopsies showed greater susceptibility for histological deterioration on the 6-month biopsy, accelerating the process of CAN.

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

Introduction

As the leading cause of graft loss, chronic rejection, better defined as chronic allograft nephropathy (CAN), is one of the most important challenges in the field of kidney transplantation (1). CAN may be due to both immunologic and nonimmunologic factors, which lead to the development of nonspecific pathologic features that may be present in well-functioning allografts (2,3). Althought a previous acute rejection episode seems to be the greatest predictor for development of CAN, the presence of "subclinical" rejection also has been suggested to contribute to this process.

Subclinical rejection (SR) is defined as the presence of histological findings meeting the criteria for acute cellular rejection (Banff grade I or greater) in the absence of renal allograft dysfunction (4,5). Several reports suggested the significance of the observation of a high incidence of subclinical tubulitis - "borderline changes" (BC) in the presence of stable renal allograft function (6).

Evaluation of protocol biopsies has revealed a considerably high prevalence of SR, BC and CAN already in early phases, within the first months after transplantation, in stable allografts (7). A number of studies evaluating surveillance biopsies have revealed variable frequencies of SR and BC. Rush *et al*. noted a prevalence of BC in 21% and SR in about 20-50% biopsies performed between 1 and 6 months post-transplantation (8,9). Shapiro *et al*. reported prevalence of BC in 21% and SR in 25% of protocol biopsies performed at 1 week after transplantation (10), while in the recent report of Nankivell *et al*. the percentage was 49% and 29%, in protocol biopsies at 3 months after transplatation, respectively (11). Furthermore, analyzing protocol biopsies performed within the first 6 months after transplantation, Nankivell *et al*. revealed prevalence of CAN from 24% up to 40%, while

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Seron *et al*. reported a prevalence of CAN in about 42% of protocol biopsies at 3 months after transplantation (11,12). Recent reports provide evidence in favor of treating biopsy-proven BC and SR, and that timely therapeutic intervention improves clinical outcomes, thereby preventing development of CAN. This raises the issue of early protocol biopsies of stable allografts and the clinically useful information they provide.

Patients and methods

The cohort of forty consecutive living related (LR) transplant patients with their first allograft received induction therapy with methylprednisolone (500 mg) and Daclizumab (Zenapax; 1 mg/kg BW at implantation and thereafter every 2 weeks x five doses). The post-transplant standard triple immunosuppression therapy consisted of: cyclosporine (Neoral; 4 to 6 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 SR 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. The same pathologist blindly reviewed the renal lesions for evidence of acute and chronic changes by using descriptive morphologic criteria according to the Banff 97 scoring schema on a scale from 0 to 3 (13). 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 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

Donors and recipients mean age was 59.3±13 and 34.3±9.8 years, respectively. Only 7.5% (6/80) of the biopsies showed no histopathological lesions. BC was found in 13/40 (32.5%) and 12/40 (30%), and SR in 15/40 (37.5%) and 21/40 (52.2%) of patients, on 1 and 6 months biopsies, respectively. The mean HI and CAN score increased significantly at 6-month biopsy, 5.3 ± 2.9 vs. 7.8 ± 3.6 ($p<0.001$) and 2.1 ± 1.5 vs. 4.6 ± 2.3 ($p<0.000$), respectively. The serum creatinine (sCr) and body mass index (BMI) were significantly increased at 6 months after transplantation, while no significant difference was found between calculated creatinine clearance (Clcr) and proteinuria at 1 and 6-month postransplant (Table 1).

Table 1: Biochemical, clinical data and histological findings and scores at 1 and 6 months post-transplantation of all patients

CLASSIFICATIONS	MEAN	SD	MEAN	SD	P value
ALL PATIENTS (n = 40)	**1 month**		**6 months**		
sCr	125.03	33.89	144.15	44.68	***0.005***
Crcl	64.67	16.68	60.04	19.11	n.s.
BMI	22.25	3.95	23.60	4.17	***0.000***
24 h proteinuria	0.72	0.41	0.60	0.60	n.s.
"NO" histological lesions	3/40 (7.5%)		3/40 (7.5%)		n.s.
AR (before 1 month biopsy)	5/40 (12.5%)		/		
AR	3/40 (7.5%)		/		
BC	13/40 (32.5%)		12/40 (30%)		n.s..
SR	15/40 (37.5%)		21/40 (52.2%)		***< 0.05***
CAN score per patient	2.08 ± 1.54		4.63 ± 2.26		***0.000***
HI	5.3 ± 2.9		7.78 ± 3.61		***0.001***

When divided according to the donors age (<55> years), no significant difference was found in sCr and Crcl at 1 month, while at 6 month Crcl was significantly higher in the younger donor group, 52 ± 10.8 vs. 72 ± 22.6 ($p<003$). Furthermore, There was no significant difference in the mean CAN and HI score between the two groups. The percentage of treated BC and SR at 1 month biopsy was significantly higher in the younger donor group, 71% vs. 25%; $p<0.001$ (Table 2).

When compared according to the histological index (HI<5>), high HI group had mean CAN score of 2.91 ± 1.24 at 1 month, which increased to 5.09 ± 1.98 at 6 months biopsy (175 %). The proportion of these changes in low HI group was also increased from 0.94 ± 1.14 to 4.0 ± 2.52 (268 %). At

first month after transplantation sCr was significantly higher in the group with high HI, 134 ± 35.9 vs.113 ± 27.5 (p<0.04), while Crcl was respectively lower. There was no significant difference of these parameters between the high and low HI group at 6 months postransplant. The percentage of treated BC and SR at 1-month biopsy was significantly higher in high HI group, 83% vs. 25%; p<0.001 (Table 3).

Table 2: Comparison of biochemical, clinical data and histological findings and scores at 1 and 6 months post-transplantation between the groups according to the donor age (<55 ≥ years)

CLASSIFICATIONS	MEAN	SD	MEAN	SD	P value
	1 month		6 months		
DONOR AGE	< 55 (n =16)		≥55 (n = 24)		
sCr 1 month	121.44	44.72	127.42	25.06	n.s.
sCr 6 months	126.00	45.89	157.13	39.69	***0.032***
Crcl 1 month	70.87	19.45	60.55	13.43	0.076
Crcl 6 months	72.09	22.62	52.01	10.82	***0.003***
DGF	5/16 (31.3%)		5/24 (20.8%)		n.s.
AR (before 1 month biopsy)	2/16 (12.5%)		3/24 (12.5%)		n.s.
AR 1 month	2/16 (12.5%)		1/24 (4.17%)		***< 0.05***
BC 1 month	4/16 (25%)		9/24 (37.5%)		n.s.
SR 1 month	7/16 (42.8%)		8/24 (33.3%)		n.s.
AR 6 month	/		/		
BC 6 months	5/16 (31%)		7/24 (29.2%)		n.s.
SR 6 months	7/16 (43.8%)		14/24 (58.3%)		n.s.
CAN score 1 month	2.06 ± 1.69		2.08 ± 1.47		n.s.
CAN score 6 months	3.94 ± 2.38		5.08 ± 2.1		n.s
HI 1 month	5.75 ± 3.54		5.0 ± 2.43		n.s
HI 6 months	6.56 ± 3.41		8.58 ± 3.59		n.s

Table 3: Comparison of biochemical, clinical data and histological findings and scores at 1 and 6 months post-transplantation between the groups according to the chronicity index (< HI 5≥)

CLASSIFICATIONS	MEAN	SD	MEAN	SD	P value
	1 month		6 months		
CHRONICITY INDEX (HI) at 1 month	HI < 5 (n = 17)		HI ≥ 5 (n = 23)		
sCr 1 month	112.94	27.45	133.96	35.94	***< 0.05***
sCr 6 months	133.12	47.05	153.22	41.43	n.s.
Crcl 1 month	70.84	20.34	60.12	11.87	0.063
Crcl 6 months	66.11	21.44	55.56	16.23	n.s.
DGF	5/17 (29.4%)		5/23 (21.7%)		n.s
AR (before 1 month biopsy)	4/17 (23.5%)		6/14 (42.9%)		***< 0.05***
AR 1 month	/		3/23 (13.04%)		***< 0.05***
BC 1 month	6/17 (35.3%)		7/23 (30.4%)		n.s.
SR 1 month	4/17 (23.5%)		11/23 (47.8%)		***< 0.05***
AR 6 months	/		/		
BC 6 months	5/17 (29.4%)		7/23 (30.4%)		
SR 6 months	8/17 (47.1%)		13/23 (56.5%)		n.s.
CAN score 1 month	0.94 ± 1.14		2.91 ± 1.24		***0.000***
CAN score 6 months	4.0 ± 2.52		5.09 ± 1.98		n.s.
HI 1 month	2.76 ± 1.48		7.17 ± 2.17		***0.000***
HI 6 months	6.88 ± 4.17		8.43 ± 3.07		n.s.

From the cohort of twenty-eight patients with acute histopathological lesions (13 BC + 15 SR) at 1-month biopsy, an increase in sCr between 10 to 20% from baseline (two weeks prior the biopsy) was observed in 2 and 7 patients, respectively, and therefore pulse corticoid therapy was administered. Hence, the entire cohort was stratified to treatment (Tx) group (n=9) and non-treatment (NTx) group (n=19) patients. The groups did not differ in clinical data such as mean age and glomerular filtration rate (GFR) of donors, gender, basic kidney disease and BMI of recipients, except a higher incidence of experienced DGF in the NTx group (5 vs. 1; p<0.05). The Tx group had mean HI score at 1-month biopsy of 7.11 ± 1.9, which remained almost the same at 6 months biopsy (7.11 ± 2.32). In contrast, the proportion of these changes in NTx group increased from 4.95 ± 1.99 to 8.16 ± 4.30 (p<001). Furthermore, NTx group had mean CAN score of 1.89 ± 1.37 at 1 month, which increased significantly to 8.16 ± 4.3 (p<0.000) at 6-month biopsy. However, there was no significant difference in the graft function, i.e. sCr, Crcl and CAN score between the groups neither at 1 nor at 6-month biopsy (Table 4).

Table 4: Comparison of biochemical, clinical data and histological findings and scores at 1 and 6 months post-transplantation between the groups according to the treatment of borderline changes (BC) and subclinical rejections (SR)

CLASSIFICATIONS	MEAN	SD	MEAN	SD	P value
	1 month		6 months		
TREATMENT OF BC/SR	Treatment (Tx) group		Non-treatment (NTx)		
Number of patients	(n = 9)		(n = 19)		
sCr 1 month	130.56	33.09	122.26	29.24	n.s.
sCr 6 months	151.44	38.36	136.11	44.23	n.s.
Crcl 1 month	62.15	15.15	66.38	18.34	n.s.
Crcl 6 months	58.11	21.50	63.35	18.31	n.s.
DGF	1/9 (11.113%)		5/19 (26.32%)		***< 0.05***
AR (before 1 month biopsy)	1/9 (11.113%)		3/19 (15.79 %)		n.s.
AR 1 month	/		/		
BC 1 month	2/9 (22.22%)		11/19 (54.89%)		***< 0.05***
SR 1 month	7/9 (77.78%)		8/19 (42.11%)		***< 0.05***
BC 6 months	4/9 (44.44%)		5/19 (26.32%)		***< 0.05***
SR 6 months	4/9 (44.44%)		12/19 (63.16%)		***< 0.05***
HI 1 month	7.11 ± 1.90		4.95 ± 1.99		0.013
HI 6 month	7.11 ± 2.32		8.16 ± 4.30		n.s.
CAN score 1 month	2.33 ± 1.41		1.89 ± 1.37		n.s.
CAN score 6 months	4.33 ± 1.73		8.16 ± 4.30		n.s.

Discussion

Chronic allograft nephropathy (CAN) causes most kidney allograft losses and despite improvements in immunosuppression, remains the central clinical challenge (14). In protocol biopsy studies, allograft damage is common, time-dependent, progressive and underestimated by measurement of serum creatinine. Rejection leads to allograft damage but the expression depends on its type, timing, severity and persistence. Subclinical rejection (SR) is defined as histologically-proven acute rejection without concurrent renal dysfunction (15), and is influenced by the time after transplantation, prior acute rejections, HLA mismatches, and immunosuppression (9,15). Untreated SR inflicts permanent tubulointerstitial damage and fibrosis (9). There is an evidence that protocol biopsy of stable renal allografts may be valuable tool to uncover early clinically invisible acute histological lesion, such as BC and SR (16). Previous reports show the prevalence of SR approximately in 30-50% of protocol biopsies in the first 3 months after transplantation (9). The principal finding of our study is the histological evidence of BC and SR in relatively high percentage (33 and 38%) at first month, being even higher at 6-month biopsies (30 and 52%), respectively. A comparable experience have been reported by Rush *et al*. (21 and 33-50%), and by Schweitzer *et al*. (23 and 33%), for the prevalence of BC and SR, respectively (4, 9, 17).

Our study demonstrates that corticosteroid treatment of early BC and SR found at 1-month biopsies, is associated with improved histology of acute and chronic lesions at 6 months biopsies. Additionally, 4 patients in Tx group initially diagnosed as SR (classified as type IIA), showed an improvement in histology towards type I A, while 7 patients in NTx group diagnosed as BC at 1-month biopsies, worsened their histology towards SR type IA, at 6-month biopsies. Despite the progression of renal scarring in NTx group (significant increase of mean CAN and HI at 6-month biopsies), both groups presented with similar serum creatinine at the same time point. However, treatment of BC and SR did not result in improved allograft function at 6 months. In contrast, Rush *et al*. reported that the treatment of early SR decreases chronic pathology (CAN), late clinical rejection episodes and improves long-term graft function (9). In addition, Shweitzer *et al*. reported a complete therapeutic response to steroid tretament in 58%, and Gaber *et al*. in 100% of patients with BC (17,18).

Conclusions

A protocol 1-month biopsy may uncover a high prevalence of BC or SR in stable allografts. The presence of untreated BC or SR showed a greater susceptibility for histological acute and chronic deterioration on 6-month biopsy, accelerating the process of CAN. The beneficial effect of the treatment of BC/SR should be confirmed at follow-up of the graft function at 1 and 2 years.

Our preliminary data support the hypothesis that treatment of the subclinical conditions (BC and SR) may counteract the development of CAN, aiming to provide a strong impetus to incorporate early allograft protocol biopsies into a standard clinical practice. Understanding the causes and the mechanisms of chronic allograft nephropathy may suggest targeted therapies to prevent the initiation or progression of CAN.

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