

## THE EVOLUTION OF UNTREATED BORDERLINE AND SUBCLINICAL REJECTIONS AT FIRST MONTH KIDNEY ALLOGRAFT BIOPSY IN COMPARISON WITH HISTOLOGICAL CHANGES AT 6 MONTHS PROTOCOL BIOPSIES

**J. Masin-Spasovska<sup>1</sup>, G. Spasovski<sup>1</sup>, S. Dzikova<sup>1</sup>, G. Petruševska<sup>2</sup>,  
B. Dimova<sup>2</sup>, Lj. Lekovski<sup>3</sup>, Z. Popov<sup>3</sup>, N. Ivanovski<sup>1</sup>, M. Polenakovic<sup>1</sup>**

*<sup>1</sup>Department of Nephrology, Faculty of Medicine, Skopje, R. Macedonia*

*<sup>2</sup>Department of Pathology, Faculty of Medicine, Skopje, R. Macedonia*

*<sup>3</sup>Department of Urology, Faculty of Medicine, Skopje, R. Macedonia*

**Abstract:** Our study sought to identify the possible implications of histological findings of borderline and subclinical rejections as well as histological markers of chronic allograft nephropathy (CAN) in protocol biopsies at 1 and 6 months after living-related kidney transplantation. Twenty-eight paired allograft biopsies were blindly reviewed using Banff '97 criteria, among which only 10.7% (6/56) showed no histopathological lesions. BR was found in 9/28 (32.1%) and 6/28 (21.4%), and SR in 3/28 (10.7%) and 10/28 (35.7%) of the patients, in the 1 and 6 month biopsies, respectively. The mean CAN score (sum of histological markers for chronicity) increased significantly at 6 months biopsy,  $1.57 \pm 1.36$  vs.  $4.36 \pm 2.32$  ( $p < 0.01$ ).

When compared according to chronicity index ( $CI < 5$ ), the high CI group had a mean CAN score of  $2.36 \pm 1.15$  at 1 month, which increased to  $5.14 \pm 1.99$  at 6 months biopsy (188.9%). The proportion of these changes in low CI group were also increased from  $0.79 \pm 1.12$  to  $3.57 \pm 2.38$  (451.9%).

In conclusion, a protocol 1 month biopsy may uncover a high prevalence of BR or SR in stable allografts. The presence of an untreated BR or SR in biopsies with low chronicity index showed greater susceptibility to histological deterioration on the 6 month biopsy, associated with rapid impairment of graft function and chronic allograft nephropathy.

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

### Introduction

Short- and long-term graft survival after renal transplantation has significantly improved over the last decades. Nevertheless, chronic allograft failure, the dominant cause of late renal allograft loss, may be caused by a variety of immunological and clinical factors. Treatment of chronic graft dysfunction is difficult, particularly in cases with advanced renal lesions. In this regard, the biopsy of a stable allograft is of paramount importance for diagnosis of early rejection, delayed graft function or poor compliance as well as calcineurin-inhibitor cytotoxicity (more likely to develop in kidneys of older donors or in marginal kidneys). Hence, a protocol biopsy may uncover histologic signs of acute rejection without associated graft dysfunction [subclinical rejection (SR) or borderline rejection (BR)]. A number of studies have revealed variable frequencies of these rejections, whereby the highest incidence was reported for the biopsies obtained within the first months after transplantation. Shapiro *et al.* have reported SR and BR in about 25% and 21% of the biopsies performed at 1 week after transplantation, respectively [1]. Rush *et al.* has even reported the presence of clinically quiet rejections in more than 50% of protocol biopsies at 3 months after transplantation [2, 3]. The results of these studies upon the question of treatment of these rejections have documented a beneficial effect of treatment of early subclinical rejection, while the corticosteroid treatment failed in the rejection episodes that occur later than 6 months after transplantation. It remains unclear whether stable graft function could be improved with the same treatment when the biopsy shows borderline rejection [4]. There is also evidence that protocol biopsies of stable renal allografts are valuable for diagnosing chronic allograft nephropathy (CAN), before deterioration of the graft function [5]. Seron *et al.* reported that 42% of cases display CAN at a 3 months biopsy. Moreover, Fujisawa *et al.* [6] observed CAN in 30.4% of stable allografts in pediatric patients at about 100 days after living-related (LR) renal transplantation. With regard to the treatment, Rush *et al.* [7] reported a decrease in chronic histologic changes and an improvement of 24 months allograft function for patients subjected to high-dose steroids in the case of 1–3 month biopsy-proven subclinical rejection when compared with nontreated control patients. It is still uncertain whether corticoid treatment could prevent development of CAN in the episodes of borderline rejections.

Our study aimed to identify the histological findings of borderline/subclinical rejection or CAN in protocol biopsies at 1 month after living-related kidney transplantation and to compare their evolution with histological changes at the 6 month biopsy.

### *Material and methods*

The cohort of 28 LR transplant patients with their first allograft received induction with methylprednisolone (500 mg) and Daclizumab (Zenapax; 1 mg/kg BW at implantation and thereafter every 2 weeks x five doses). The post-transplant immunosuppression 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.).

The inclusion criteria were serum creatinine < 200  $\mu$ mol/L and proteinuria < 1g/24 hours at the time of the first biopsy, which was defined as a "stable" graft function. 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 haemodialysis or pulse corticosteroids, respectively. During the first postoperative month pulse corticosteroid therapy was administered for AR, whenever there was an increase in serum creatinine > 20% and a decrease in urine output for 2 consecutive days and after haemodynamic or other perisurgical complications had been excluded. These cases were included if their graft function had been stable for at least 2 weeks before the first biopsy.

Protocol biopsies were performed at 1 and 6 months posttransplant using an ultrasound-guided automated biopsy "gun". The formalin fixed biopsies were embedded in paraffin, serially sectioned at 3 to 5  $\mu$ m 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  $\geq$  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 [8]. 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 biopsies were also classified into a chronicity index (CI) according to the total sum of scores for acute and chronic changes as well as arterial hyalinosis: namely, high (CI  $\geq$  5) versus low scores (CI < 5).

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  $\pm$  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 ages of the entire cohort of donors and recipients were  $60.39 \pm 13.63$  and  $35.29 \pm 8.29$  years, respectively. The serum creatinine (sCr) and body mass index (BMI) were significantly increased at 6 months after transplantation, while calculated creatinine clearance (cCrcl) tended to be lower compared to the 1 month values.

Only three biopsies at 1 month and three at 6 months, namely, 6/56 (10.7%) showed no histopathological lesions. BR was found in 9/28 (32.1%) and 6/28 (21.4%), and SR in 3/28 (10.7%) and 10/28 (35.7%) of the patients, at the 1 and 6 month biopsies, respectively. Six patients who experienced an episode of AR ( $n = 7$ ) at the 1 month and two at the 6 month biopsy were classified as SR, respectively. Furthermore, the mean CAN score (sum of histological markers for chronicity) was significantly increased at the 6 month biopsy,  $1.57 \pm 1.36$  vs  $4.36 \pm 2.32$  (Table 1).

Table 1 – Табела 1

*Biochemical, clinical data and histological findings and scores at 1 and 6 months post transplantation in the entire cohort and compared according to the donor age (55 years), and chronicity index (CI 5)*

*Биохемиски, клинички и хистолошки наоди и скорови на 1 и 6 месеци по трансплантација кај целата група, при споредба по возраст на донорот (55 год.) и според индексот на хроничноста (ХИ 5)*

CLASSIFICATIONS	MEAN	SD	MEAN	SD	P value
<b>ALL PATIENTS (n = 28)</b>	<b>1 month</b>		<b>6 months</b>		
sCr	124.14	34.29	148.57	47.20	< 0.05
cCrcl	67.96	18.15	59.72	21.08	n.s.
BMI	22.68	4.42	24.29	4.68	< 0.01
24 h proteinuria	0.79	0.36	0.65	0.63	n.s.
“NO” histological lesions	3/28(10.7%)		3/28(10.7%)		n.s.
AR (n = 7) – histological findings	6/7 (86%) = 6 SR		2/7 (29%) = 2 SR		< 0.05
BR+SR	12/28 (42.8%) = 9BR/3SR		16/28 (57.1%) = 6BR/10SR		n.s.
BR	9/28 (32.1%)		6/28 (21.4%)		n.s.
SR	3/28 (10.7%)		10/28 (35.7%)		n.s.
CAN score per patient	44/28 (1.57+/-1.36)		122/28 (4.36+/-2.32)		< 0.01
<b>DONOR AGE</b>	<b>&lt; 55 (n = 9)</b>		<b>≥ 55 (n = 19)</b>		
sCr 1 month	118.89	50.52	126.63	24.69	n.s.

<b>sCr 6 months</b>	129.44	53.57	157.63	42.39	0.072
<b>cCrcl 1 month</b>	70.67	20.94	66.67	17.13	n.s.
<b>cCrcl 6 months</b>	66.50	25.34	56.33	18.46	n.s.
<b>DGF</b>	3/9 (33.3%)		5/19 (26.3%)		n.s.
<b>AR ( 7 patients )</b>	3/9 (33.3%)		4/19 (21.1%)		n.s.
<b>BR+SR 1 month</b>	3/9 (33.3%)		9/19 (47.4%)		n.s.
<b>BR+SR 6 months</b>	3/9 (33.3%)		13/19 (68.4%)		<b>&lt; 0.05</b>
<b>CAN score 1 month</b>	17/9 (1.89+/-1.45)		30/19 (1.58+/-1.35)		n.s.
<b>CAN score 6 months</b>	33/9 (3.67+/-2.69)		89/19 (4.68+/-2.08)		<b>&lt; 0.05</b>
<b>CAN score 1-6 m. (increase%)</b>	(194.2%)		(316.5%)		
<b>CHRONICITY INDEX (CI) at 1 month</b>					
	<b>CI &lt; 5 (n = 14)</b>		<b>CI ≥ 5 (n = 14)</b>		
<b>BMI (Donor)</b>	26.10	3.93	24.82	3.79	n.s.
<b>GFR (Donor)</b>	54.38	14.13	48.23	14.22	n.s.
<b>Age (Donor)</b>	57.79	15.56	63.00	11.35	n.s.
<b>sCr 1 month</b>	114.71	29.17	133.57	37.41	n.s.
<b>sCr 6 months</b>	139.14	49.14	158.00	44.19	n.s.
<b>DGF</b>	5/14 (35.7%)		3/14 (21.4%)		n.s.
<b>AR ( 7 patients )</b>	1/14 (7.1%)		6/14 (42.9%)		<b>&lt; 0.05</b>
<b>BR+SR 1 month</b>	6/14 (42.8%)		6/14 (42.8%)		n.s.
<b>BR+SR 6 months</b>	6/14 (42.8%)		10/14 (71.4%)		n.s.
<b>CAN score 1 month</b>	11/14 (0.79+/-1.12)		33/14 (2.36+/-1.15)		<b>&lt; 0.01</b>
<b>CAN score 6 months</b>	50/14 (3.57+/-2.38)		72/14 (5.14+/-1.99)		0.070
<b>CAN score 1-6 m. (increase%)</b>	(451.9%)		(188.9%)		

When divided according to donor age (55 years), the older donor group (n = 19) showed a mean CAN score of  $1.58 \pm 1.35$  which increased to  $4.68 \pm 2.08$  at the 6 month biopsy (316.5%). The proportion of these changes in the younger donor group was lower  $1.89/3.67$  (194.2%). The calculated creatinine clearance (cCrcl) in the older donor group was lower at 1 month and at 6 months, but did not differ significantly between the groups. The proportion of patients with BR and SR among the older donor group was significantly higher at the six month biopsy compared with the younger donor group, namely, 13/19 (68.4%) vs. 3/9 (33.3%).

When compared according to CI, there were no significant differences between the groups in the mean age of donors and glomerular filtration rate (GFR), BMI and serum creatinine (sCr) at the first and at six months, nor in the experience of DGF. There was a significantly greater number of AR episodes in the high CI group 6/14 (42.9%) compared with the low CI group 1/14 (7.1%). The mean CAN score at 1 month was significantly lower in the low CI group

compared with the high CI group, namely  $0.79 \pm 1.12$  vs.  $2.36 \pm 1.15$ , which was not shown at the 6 month biopsy,  $3.57 \pm 2.38$  vs.  $5.14 \pm 1.99$ , respectively.

### *Discussion*

Studies dealing with the clinical value of protocol biopsies performed in stable allografts have uncovered a high prevalence of borderline and subclinical rejection and features of CAN in stable allografts. Importantly, the occurrence of these clinical conditions was found to be associated with the development of chronic damage and deterioration of graft function. Protocol biopsies performed in the early posttransplant period may therefore allow prediction of the long-term allograft outcome.

Our previous [9] and present results have shown a higher percentage of patients having BR and SR (43% and 57%) at 1 and 6 months respectively, when compared with previous reports [1, 2, 3]. This might be due to the different sampling time for the biopsies, which is beyond the usual time for AR and DGF, namely, the first two weeks after transplantation. However, the histological findings may be present even after the pulse corticosteroid therapy. Furthermore, our donor group was markedly old (~ 60y) and showed a lot of histological changes for chronicity (CAN score) that almost tripled from the 1 to the 6 month biopsy, a finding that was even more prominent when patients were stratified according to donor age. In the older donor group we observed a substantial rise in the CAN score by 316.5%, while grafts from patients younger than 55 years of age showed only 194.2%. This finding suggests a greater susceptibility to histological deterioration among the older donor population, which was shown by the borderline significant difference in the graft function between the groups at six months ( $p = 0.072$ ).

Trying to solve the question whether we should treat BR or SR even with improving renal function [2, 4], we have documented that, when untreated, the proportion of these subclinical conditions in the group with high CI even progressed from six to ten out of 14 patients (71.4%), while in the low CI group the number of these subclinical findings remained equal at the 1 and 6 month biopsies, respectively. In contrast, the mean CAN score in the group with low CI increased more than fourfold at the 6 month biopsy, compared with a less than twofold increase among patients with a high CI. This observation may to a certain extent be explained by the greater susceptibility to histological deterioration among patients with a lower grade of histological changes and untreated findings of borderline and subclinical rejections. Additionally, the donors in the low CI group tended to be younger ( $57.8 \pm 15.6$  vs  $63.0 \pm 11.4$ ) but experienced a slightly greater number of DGF episodes (5/14 vs 3/14). Hence, the presence of

BR and SR in protocol biopsies performed early after transplantation in patients with well functioning grafts provides relevant information for detecting patients at risk of accelerated graft deterioration towards chronic allograft nephropathy.

### Conclusion

A protocol 1 month biopsy may uncover a high prevalence of BR or SR in stable allografts. The occurrence of these findings was found to be associated with the development of chronic damage and deterioration of graft function. Our findings suggest a greater susceptibility for histological deterioration in organs from the older donor population. The presence of an untreated BR or SR in biopsies with a low chronicity index showed a greater susceptibility to histological deterioration at the 6 month biopsy, associated with rapid impairment of graft function and chronic allograft nephropathy. Future studies testing large patient cohorts will have to clarify the true benefit of protocol biopsy-based therapeutic consequences.

### REFERENCES

1. Shapiro R., Randhawa P., Jordan M.L., *et al.* (2001): An analysis of early renal transplant protocol biopsies-the high incidence of subclinical tubulitis. *Am J Transplant* 1: 47–50.
2. Rush D., Nickerson P., Gough J., *et al.* (1998): Beneficial effects of treatment of early subclinical rejection: a randomized study. *J Am Soc Nephrol* 9: 2129–2134.
3. Rush D. N., Henry S. F., Jeffery J. R., *et al.* (1994): Histological findings in early routine biopsies of stable renal allograft recipients. *Transplantation* 57: 208–211.
4. Jain S., Curwood V., White S. A., *et al.* (2000): Sub-clinical acute rejection detected using protocol biopsies in patients with delayed graft function. *Transpl Int* 13: S52–S55.
5. Seron D., Moreso F., Bover J., Condom E., *et al.* (1997): Early protocol renal allograft biopsies and graft outcome. *Kidney Int* 51: 310–316.
6. Fujisawa M., Ono H., Isotani S., *et al.* (1999): Significance of chronic transplant nephropathy on early protocol biopsies for graft outcome in pediatric renal transplantation. *Transpl Proc* 31: 1687–1690.
7. Rush D., Nickerson P., Jeffery J. (2000): Protocol biopsies in the management of renal allograft recipients. *Curr Opin Nephrol Hypertens* 9 (6): 615–619.
8. Racusen L. C., Solez K., Colvin R. B. *et al.* (1999): The Banff 97 working classification of renal allograft pathology. *Kidney Int* 55: 713–723.

9. Masin-Spasovska J., Spasovski G., Dzikova S., Grcevska L., Petrusevska G., Lekovski Lj., Popov Z., Ivanovski N. (2005): Protocol biopsies in kidney transplant recipients: histologic findings as prognostic markers for graft function and outcome. *Transplant Proc.* 37(2): 705–8.

### Резиме

#### ЕВОЛУЦИЈА НА НЕТРЕТИРАНИТЕ БОРДЕРЛАЈН И СУБКЛИНИЧКИ ОТФРЛАЊА КАЈ БУБРЕЖНАТА АЛОГРАФТ БИОПСИЈА НА ПРВИОТ МЕСЕЦ ВО КОМПАРАЦИЈА СО ХИСТОЛОШКИТЕ ПРОМЕНИ ВО ПРОТОКОЛ БИОПСИИТЕ НА ШЕСТИОТ МЕСЕЦ ПО ТРАНСПЛАНТАЦИЈАТА

Ј. Масин-Спасовска<sup>1</sup>, Г. Спасовски<sup>1</sup>, С. Џикова<sup>1</sup>, Г. Петрушевска<sup>2</sup>,  
Б. Димова<sup>2</sup>, Љ. Лековски<sup>3</sup>, Ж. Попов<sup>3</sup>, Н. Ивановски<sup>1</sup>, М. Поленаковиќ<sup>1</sup>,

<sup>1</sup>Клиника за нефрологија, Медицински факултет, Скопје, Р. Македонија

<sup>2</sup>Институт за патологија, Медицински факултет, Скопје, Р. Македонија

<sup>3</sup>Клиника за урологија, Медицински факултет, Скопје, Р. Македонија

Целта на студијата беше да се идентификуваат можните импликации од хистолошките наоди на бордерлајн (граничните) и субклиничките отфрлања, како и хистолошките маркери за хронична алогографт нефропатија (ХАН) во направените протокол биопсии на првиот и шестиот месец по трансплантација на бубрег од живи сродни дарители. Од направените дваесет и осум двојни алогографт биопсии во првиот и шестиот месец по трансплантацијата, проценети според критериумите на класификацијата Банф 97, само 10.7% (6/56) беа без хистопатолошки лезии. Бордерлајн промени беа најдени кај 9/28 (32.1%) и 6/28 (21.4%), а субклинички отфрлања кај 3/28 (10.7%) и 10/28 (35.7%) од пациентите во биопсиите направени првиот односно шестиот месец по трансплантацијата. Просечниот ХАН скор (збир на хистолошките маркери за хроничитет) бележеше сигнификантен пораст кај биопсиите направени шестиот месец по трансплантацијата,  $1.57 \pm 1.36$  vs.  $4.36 \pm 2.32$  ( $p < 0.01$ ).

При поделбата според индексот на хроничитет (збир на акутните и хронични хистолошки промени) т.е. ( $ХИ < 5 >$ ), групата со висок ХИ беше со просечен ХАН скор од  $2.36 \pm 1.15$  во првиот месец, кој порасна на вредности од  $5.14 \pm 1.99$  во шестиот месец (188.9%). Пропорцијата на овие промени во групата со низок ХИ исто така забележа пораст на вредности од  $0.79 \pm 1.12$  до  $3.57 \pm 2.38$  (451.9%)

Како заклучок, протокол биопсијата во првиот месец по трансплантацијата може да ја открие високата преваленца на бордерлајн и субклинички отфрлања кај пациентите со уредна функција на графотот. Нашите

наоди укажуваат на поизразена суспендибилност за хистолошко влошување кај бубрезите од постари дарители. Присуството на нетретирани борделајн и субклинички отфрлања кај биопсиите со понизок индекс на хроничитет покажаа поголема суспендибилност за хистолошко влошување во биопсијата на шестиот месец, што беше асоцирано со побрзо пропаѓање на функцијата на графтоот и развој на хронична алогографт нефропатија.

**Клучни зборови:** трансплантација, алогографт биопсија, бордерлајн, субклинички отфрлања, (ХАН) хронична алогографт нефропатија, (ХИ) индекс на хроничитет.

**Contact address:**

**Goce B Spasovski, MD, PhD, Sc. Res.**

**Department of Nephrology**

**Clinical Centre Skopje**

**University of Skopje**

**Vodnjanska 17**

**1000 Skopje**

**Macedonia**

**Mob. phone: +389 70 268 232**

**Fax: +389 2 3231 501**

**E-mail: [gspas@sonet.com.mk](mailto:gspas@sonet.com.mk)**