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Original Article

Impact of the endothelial factors following ischemia/reperfusion injury on the allograft function and histology at 1 and 6 months after renal transplantation

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Abstract

Background. Ischaemia-reperfusion injury (IRI) continues to be one of the leading causes of renal failure following renal transplantation (Tx). Post IRI results in acute endothelial injury. The aim of our study was to evaluate the levels of vasoactive endothelial factors following IRI and to assess the possible impact of the post-IRI effects on the allograft function and histology at 1 and 6 months after Tx.

Methods. Forty consecutive living related kidney transplant recipients were included. Endothelial factors followed before, immediately after Tx and at day 1, and week 1, 2, 3 and 4 after Tx were: endothelin (ET_1), nitric oxide (NO) and free oxygen radicals (FOR). The protocol biopsies performed at 1 and 6 months after Tx were blindly reviewed using Banff' 97 criteria. Patients were divided in two groups according to the occurrence of delayed graft function (DGF) and acute rejection (AR) during the first posttransplant week: Group 1 (G1 - without DGF and AR, n=28) and Group 2 (G2 - with DGF and/or AR, n=12).

Results. The two groups were similar regarding donor and recipient age, gender and body weight, glomerular filtration rate of donated kidney, and HLA matching. However, the groups differed significantly in the mean cold ischemic time (CIT) and previous time on dialysis (3.2±1.1 vs. 4.2±0.6 hours; p<0.006 and 22.2±32.2 vs. 37.2±44.7 months; p<0.05) for G1 vs. G2, respectively. When the groups were compared according to the changes of endothelial factors of IRI, G2 had a significantly higher ET_1 levels after Tx and at day 1 post Tx [102.7±37.1 vs. 44.9±22.4 pg/ml (p<0.001); 76.5±43.7 vs. 40.5±12.8 (p<0.01)], with a significantly lower NO levels at the same time points, [80.8±12.8 vs. 100.6±38.6 μmol (p<0.05); 35.8±19.9 vs. 86.7±20.3 (p<0.001)], respectively. Moreover, a significantly higher levels of FOR were found in Group 2 when compared with Group 1, after Tx, at day 1, and at 1 and 2 weeks post-Tx: [306.3±48.2 vs. 266.6±58.3 CARR units (p<0.001); 420.3±112.8 vs. 319.8±61.6 (p<0.001); 449.3±90.3 vs. 354.6±92.8 (p<0.001), and 345.8±133.3 vs. 256.9±67.5 (p<0.05)], respectively. At 1-month biopsy a higher percentage of acute histological changes was found in G2 compared with G1 (83% vs. 75%). Importantly, the groups differed significantly in the mean HI score (sum of scores for acute and chronic histological changes) at 6 months biopsy [9.1±4.9 (G2) vs. 7.2±2.9 (G1); (p<0.001)]. Thereby, a significantly higher percentage of chronic allograft nephropathy (CAN) progression was found in G2 (75% vs. 57%). However, there was no significant difference in the graft function, i.e. calculated creatinine clearance at 1 and 6 months after Tx, in both groups.

Conclusion. Post IRI is mediated by endothelial release of vasoactive factors such as endothelin, nitric oxide and free oxygen radicals, potentially key molecules in the link of IRI and AR. In fact, the group with DGF and AR early after Tx showed higher percentage of acute histological lesions at 1-month biopsy, and a greater susceptibility for histological deterioration on the 6-month biopsy, accelerating the process of CAN. Endothelial activation may facilitate enhanced graft immunogenecity and induce development of AR, which in turn results in development of chronic allograft nephropathy.

Keywords: kidney transplantation; ischaemia-reperfusion injury; endothelin, nitric oxide; free oxygen radicals; protocol biopsy; delayed graft function; acute rejection

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Introduction

Chronic allograft nephropathy (CAN) has become the leading cause of late kidney transplant failure [1]. Its histological hallmarks are tubular atrophy, interstitial fibrosis, microvascular changes and glomerulosclerosis [2]. CAN is driven by a number of immunological and non-immunological factors such as pre-existing donor pathology, ischaemia-reperfusion injury, delayed graft function and/or acute tubular necrosis, acute rejection, ineffectively and/or un/treated clinical and subclinical rejection, hypertension and calcineurin inhibitor toxicity [3,4].

Ischaemia-reperfusion injury (IRI) following kidney transplantation, can result in delayed graft function (DGF), and according to large scale clinical analyses there is consensus that DGF has a significant impact on short and long-term graft survival [5]. Ischaemia and reperfusion induce the development of inflammation and adhesion molecules are essential intermediates between activated endothelial cells and circulating leukocytes. Reperfusion injury represents a cascade of events, initiated by tissue ischaemia and production of free oxygen radicals during the reperfusion process, leading to the development of inflammation, through activation of endothelial cells in the transplant and recruitment of circulating leukocytes [6]. Although the precise mechanisms of IRI have not been clarified, some chemical mediators, such as oxygen radicals and platelet activating factor accompanied by vasculo-endothelial dysfunction, have been suggested to play a role [7]. It is well known that cell damage following ischaemia is a biphasic process: ischaemia initiates injury by depriving cells of the energy needed to maintain ionic gradients and homeostasis, while the reperfusion exacerbates this damage by triggering an inflammatory reaction involving oxygen-free radicals, endothelial factors, and leukocytes [8].

The aim of our study was to evaluate the levels of vasoactive endothelial factors following IRI: endothelin (ET-1), nitric oxide (NO) and free oxygen radicals (FOR), and to estimate the post-IRI effects on allograft function and histology at 1 and 6 months after transplantation (Tx).

Patients and methods

Forty 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) were administered as induction therapy. Maintenance 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 (Cell Cept 1 g/bid).

During the first postoperative month patients with delayed graft function who suffered post-transplant acute tubular necrosis or experienced a clinical episode of acute rejection (AR) were treated with hemodialysis or pulse corticosteroids, respectively.

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 histology was reviewed according to the Banff' 97 scoring schema [2]. 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 a total sum of scores for acute and chronic changes.

Patients with histology at 1-month biopsy of borderline changes (BC) or AR type I or IIA, and an increase in serum creatinine (sCr) between 10 and 20% from the baseline (sCr 2 weeks prior to the biopsy) were assessed as subclinical acute rejection (SAR) and consequently treated with pulse corticoid therapy. The patients with histology of BC or AR followed by a rise in sCr < 10% from baseline were not treated.

Table 1. Clinical data and post-transplant events of all patients

Donor age (yr)	59.3 ± 13.1	
Female/male	16:24	
Recipient age (yr)	34.3 ± 9.8	
Female/male	16:24	
Cause of and-stage renal disease		
	Glomerulonephritis	13
	Diabetes	2
	Hypertensive renal disease	3
	Polycystic renal disease	2
	Reflux nephropathy	7
	Lupus nephropathy	1
	Other	12
Time on dialysis (mo)	26.7 ± 36.5	
Total HLA mismatch score	2.1 ± 1.1	
Mean CIT (h)	3.5 ± 1.0	
DGF (%)	10/40 (25%)	
AR (%)	6/40 (15%)	
DGF and AR (%)	4/40 (10%)	

In order to determine the possible impact of IRI on graft function and histology at 1 and 6 months after Tx, we have divided our patients in two groups according to the occurrence of DGF and AR during the first postransplant week: Group 1 (G1 - without DGF and AR, n=28); Group 2 (G2 - with DGF and AR, n=12).

Endothelial factors (ET_1, NO and FOR) were assessed before, immediately after Tx and at day 1 and week 1, 2, 3 and 4 after Tx. The high sensitivity ^{125}iodine-endothelin 1 assay system with Amerlex-M (Amersham, UK) magnetic separation was used to determinate plasma ET_1 levels. NO was measured by a microplate enzymatic method based on assay kit from OXIS, USA. Colorimetric determination of reactive oxygen metabolites, with

d-ROMs test, (Diacron International S.a.S. Grosseto, Italy) was used for measurement of FOR.

The patient's clinical and biochemical data were recorded at the time of transplantation as well as at 1 and 6 months after Tx. Results were expressed as mean values±SD. For numeric data, an unpaired two-tailed Student`s *t* test was used, and Chi-square analysis for categorical variables. A difference was considered significant if *P* value was <0.05.

Results

The mean age of the entire cohort of donors and recipients were 59.3±13.1 and 34.3±9.8 years, respectively. Demographic characteristics of patients are summarized in Table 1.

Among all biopsies only 7.5% (6/80) showed no histopathological lesions. BC was found in 13/40 (32.5%) and 12/40 (30%), and SAR in 16/40 (40%) and 19/40 (47.5%) of the patients, in the 1- and 6-month biopsy, respectively. The mean CAN score and HI increased significantly from 1 to 6 months. The serum creatinine (sCr) and body mass index (BMI) were significantly increased at 6 months after transplantation while calculated creatinine clearance (cCrcl) was lower compared to the 1-month values, although significant difference was not reached (Table 2).

Table 2. Biochemical, clinical data and histological findings and scores at 1 and 6 months posttransplantation of all transplant recipients (n=40)

parameter	1 month Mean ± St Dev	6 monthc Mean ± St Dev	P value
BMI recipient	22.5 ± 4.0	23.6 ± 4.2	*<0.01*
sCr	125.0 ± 33.9	144.7 ± 44.5	*<0.01*
cCrCl	64.7 ± 16.7	60.0 ± 19.1	n.s.
proteinuria	0.72 ± 0.4	0.60 ± 0.6	n.s.
No lesions	3/40 (7.5%)	3/40 (7.5%)	n.s.
AR	2/40 (5%)	2/40(2%)	n.s.
BC	13/40 (32.5%)	12/40 (30%)	n.s.
SAR	16/40 (40%)	19/40 (47.5%)	n.s.
BC/SAR treated	9/29 (31%)	7/31 (22.6%)	n.s.
CAN score	2.1 ± 1.5	4.6 ± 2.3	*<0.01*
HI	5.3 ± 2.9	7.8 ± 3.6	*<0.01*

n.s. not significant

From the cohort of forty patients with acute histopathological lesions (13 BC + 16 SAR) at 1-month biopsy, an increase in sCr between 10 and 20 % from baseline was observed in 2 and 7 patients, respectively, and therefore pulse corticoid therapy was administered. In 27 patients (33.8%) no CAN lesions were present in both biopsies, 27 (67.5%) showed progression of CAN and 13 (32.5%) presented with stable CAN changes, at 6-month biopsy.

There was no difference between G1 and G2 group in the following parameters: donor age and BMI, recipient age, BMI and time on dialysis, number of HLA matching, GFR of donated kidney, cyclosporine (CyA) levels (C2), sCr, cCrcl, and proteinuria, between the groups neither at 1 nor at 6 months after transplantation. However, the mean cold ischaemic time (CIT) and warm ischaemic time (WIT) were much shorter in the G1 group (Table 3).

Table 3. Comparison of clinical and biochemical data between the groups

parameter	G1-without DGF and AR (n= 28) Mean	St Dev	G2-with DGF and AR (n=12) Mean	St Dev	P value
Donor age	59.8	12.4	57.6	16.8	n.s.
Recipent age	35.1	9.8	32.3	10.0	n.s.
BMI donor	25.7	4.1	26.9	3.7	n.s.
BMI recipient	22.4	4.0	22.8	3.8	n.s.
GFR don. kidney	54.6	16.7	46.7	15.4	n.s.
HLA mismatch	2.1	1.2	2.1	1.1	n.s.
HD duration	22.2	32.2	37.2	44.7	*<0.05*
CIT (h)	3.2	1.1	4.1	0.6	*<0.01*
WIT(min)	3.3	1.3	4.2	0.6	*<0.05*
sCr 1 month	121.3	33.2	133.8	35.4	n.s.
sCr 6 months	144.6	46.2	144.9	42.0	n.s.
cClCr / 1 mo	67.3	17.7	58.6	13.6	n.s.
cClCr / 6 mo	60.7	19.0	58.5	20.1	n.s.
CyA / 1mo (ng/mL)	724.7	175.2	798.1	265.3	n.s.
CyA / 6 mo (ng/mL)	689.8	248.2	632.8	210.2	n.s.

At 1-month biopsy a higher percentage of acute histological changes (AR, BC and SAR) was found in G2 when compared with G1 (83 vs. 75%). As expected, the G2 group had a significantly higher score of acute histologic lesions found at 1- and 6-month biopsy, compared with G1. Importantly, the groups differed significantly in the mean HI score (Table 4).

Table 4. Comparison of histological findings and scores at 1 and 6 month posttransplantation between the groups

	G1-without DGF and AR (n= 28)		G2-with DGF and AR (n=12)		
parameter	**Mean**	**St Dev**	**Mean**	**St Dev**	**P value**
AR /1 mo	1/28	3.6%	1/12	8.3%	*p<0.05*
BC+SAR/ 1 mo	20/28	71.4%	9/12	75%	n.s.
Th/BC+SAR/1mo	7/20	35%	2/9	22.2%	*p<0.05*
ac.les.score / 1mo	0.71	0.78	0.98	0.84	*p<0.05*
AR / 6 mo	1/28	3.6%	1/12	8.3%	*p<0.05*
BC+ SAR / 6 mo	23/28	82.1%	8/12	66.7%	n.s.
Th BP +SAR / 6 m	4/23	17.4%	3/8	37.5%	n.s.
ac.les.score / 6mo	0.69	0.79	1.02	1.08	*p<0.05*
CAN score / 1mo	2.2	1.5	1.8	1.7	n.s.
CAN score/ 6 mo	4.5	2.0	5.0	2.8	n.s.
HI / 1mo	5.1	2.9	5.7	2.8	n.s.
HI / 6 mo	7.2	2.9	9.1	4.9	*p<0.05*

Following the evolution of histological lesions and scores at 1- and 6-month biopsy of each group separately, a significant increase of CAN score and HI was found in both groups at 6 months after transplantation (Table 5). A higher percentage and intensity of acute rejection grade and chronic lesions was observed in patients who experienced DGF and AR at first month posttransplantation (G2).

Table 5. Comparison of histological findings and scores at 1 and 6 month posttransplantation within the groups

G1-without DGF and AR (n= 28)			
parameter	**Mean ± St Dev**	**Mean ± St Dev**	**P value**
CAN score	2.2 ± 1.5	4.5 ± 2.0	*<0.05*
HI	5.1 ± 2.9	7.2 ± 2.9	*< 0.05*
ac.les. score	0.71 ± 0.78	0.69 ± 0.79	n.s.
AR gr.: IA, IIA, IIB	9/28 (32.1%)	12/28 (42.9%)	n.s.
CAN progression	16/28 (57%)		
G2-with DGF and AR (n=12)			
parameter	Mean ± St Dev	Mean ± St Dev	P value
CAN score	1.8 ± 1.7	5.0 ± 2.8	*<0.05*
HI	5.7 ± 2.8	9.1 ± 4.9	*<0.05*
ac.les. score	0.98 ± 0.84	1.02± 1.08	*< 0.05*
AR gr.: IA, IIA, IIB	9/12 (75%)	9/12 (75%)	n.s.
CAN progression	9/12 (75%)		

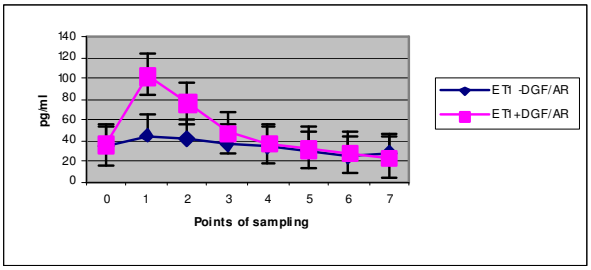


Fig. 1. Comparison of changes of ET1 between the groups

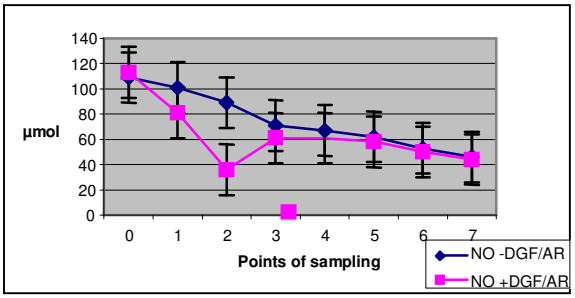
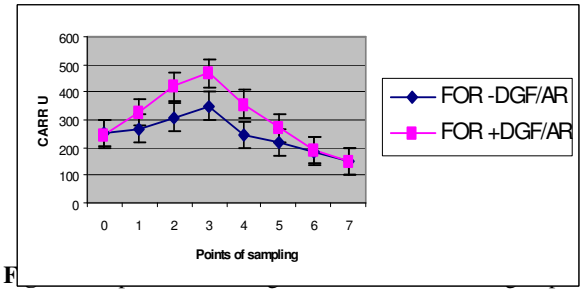


Fig. 2. Comparison of changes of NO between the groups



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When the groups were compared according to the changes of endothelial factors of IRI, G2 had a significantly higher ET_1 levels after Tx and at day 1 post Tx [102.7±37.1 vs. 44.9±22.4 pg/ml (p<0.001); 76.5±43.7 vs. 40.5±12.8 (p<0.01)], with a significantly lower NO levels at the same time points, [80.8±12.8 vs. 100.6±38.6 µmol (p<0.05); 35.8±19.9 vs. 86.7±20.3 (p<0.001)], respectively, (Figure 1 and 2). Moreover, a significantly higher levels of FOR were found in Group 2 when compared with Group 1, after Tx, at day 1, and at 1 and 2 weeks post-Tx: [306.3±48.2 vs. 266.6±58.3 CARR units (p<0.001); 420.3±112.8 vs. 319.8±61.6 (p<0.001); 449.3±90.3 vs. 354.6±92.8 (p<0.001), and 345.8±133.3 vs. 256.9±67.5 (p<0.05)], respectively, (Figure 3).

Discussion

The full significance of IRI after organ transplantation is still debatable, but is clearly established as major determinant of early graft dysfunction. In renal transplantation clinical practice, it is most commonly recognised as DGF, being largely reversible process with many features in common with the acute tubular necrosis. It is uncertain yet whether IRI manifesting as DGF has long-term sequel following renal transplantation, but there is increasing evidence that it may compromise long-term graft survival [9-11] and contribute to the increased incidence of graft rejection [12,13]. It has been confirmed that ischaemic damage during kidney transplantation is responsible for 20-30% of the worldwide incidence of DGF increasing the incidence of acute rejection, and favoring development of CAN [14,15].

The principal finding in our study was the evidence of DGF in 30% of the patients, whereby 50% of them were associated with an early episode of AR. Furthermore, the group with DGF/AR (i.e. clinical manifestation of IRI) had a significantly longer cold ischemic time in comparison with the group without DGF/AR. These results confirmed the association between the CIT with a higher probability of IRI and the increased risk for DGF [9,12,16]. Moreover, our results have also confirmed the strong correlation between duration of dialysis and the incidence of DGF [17], i.e. the group with DGF/AR had significantly longer dialysis duration.

It has been reported that ischemia not only damages parenchymatous cells but also has a prolonged effect on the function and reactivity of the vasculature of the kidney [18]. Vascular endothelin-1 (ET-1) levels have been reported as elevated during IRI and in patients with acute and chronic renal allograft rejection. Namely, ischemia, hypoxia and vessel wall mechanical stress are the main stimuli to ET-1 production [18]. On the other hand, nitric oxide (NO) produced by the nitric oxide synthase (NOS) enzymes, is a potentially key molecule in the link between IRI and kidney rejection. Decreased NO production following graft reperfusion leads to microvascular constriction and localized reduction in blood flow. In addition, oxidative stress associated with IRI leads to increased production of FOR. Thus, IRI is considered a systemic event resulting in endothelial dysfunction, FOR production, NO depletion, and release of cytokines, leading to the development of an inflammatory response [6,9,19]. In this regard, it is relevant to compare our results of significantly higher ET-1 and FOR levels, and significantly lower NO levels early after transplantation, in the group with DGF/AR, with those of the group without DGF/AR. As expected, the group with DGF/AR showed higher percentage and grade of acute histological lesions at 1- and 6-month biopsy, followed by a greater histological deterioration at 6-month biopsy. The group with DGF/AR was characterized with higher percentage of histological progression of CAN from 1 to 6 months. However, there was no difference in the graft function between and within the groups at 1 and 6 months. A possible hypothesis explaining these findings might be that IRI-mediated tissue injury enhances alloantigen presentation and/or increases graft immunogenesity, predisposing it to a later chronic rejection, especially when a vigorous alloimmune response has been exerted by the occurrence of an acute rejection episode. Other compelling evidence of long term importance of IRI is provided by a randomised study of superoxide dismutase (SOD) administrated intravenously at the time of cadaveric renal transplantation [20]. The hypothesis proposed by Land *et al.* was that early nonspecific reactive oxygen intermediate (ROI) - mediated injury to the graft predisposed to later chronic rejection and that SOD was effective at blocking the early allograft injury [21].

Finally, accruing clinical and experimental evidence suggests that an initial insult to organ allografts may influence both early and late functional survival. This injury may be either immunologic (acute rejection) or antigen independent (ischaemia/reperfusion) [22]. There seems to be a clear association between early (within 6 months of engraftment) acute rejections episodes and late graft loss from chronic rejection [23].

Whether delayed graft function, the principal manifestation of initial IRI, alone affects ultimate graft behavior is under debate, particularly because the authors of many reported series have controlled their studies for the presence of rejection, most studies were retrospective, and some of them required inclusion of grafts surviving >1 year [14,24]. On the other hand, many analyses have reported clear differences. In one such study, the 5-year functional survival rate of renal allografts that had early dysfunction was 69% vs. 79% among those that functioned immediately [23]. In another, the 1-year graft survival was 84% vs. 61% in kidneys with satisfactory and unsatisfactory initial function, respectively [25]. In addition, much of the effect of this early immune-independent event seems to occur during the first year after transplantation.

It is not unreasonable to accept the hypothesis that IRI initiates an inflammatory response that provokes an increased level of acute host immunological reactivity. This would explain the apparent synergy between DGF and episodes of acute rejection, whereby, these two types of events following IRI lead to less favorable graft outcome. Several explanations have been offered for these observations: DGF increases the immunogenesity of the transplanted organ, making it more prone to host alloreactivity, and an acute rejection episode occurring in the functioning graft is difficult to diagnose and may be missed. However, it is possible that increased number of biopsies often performed in grafts with initial poor or absent function may show a higher rate of rejection than appreciated when a biopsy is not undertaken. The early injures may also affect later events: DGF may initiate a programmed inflammatory process within the graft, which leads to chronic changes, while initial acute rejection injury predisposes to chronic graft dysfunction [23-25].

Our data support this view [26]. Immunological inflammation presented with a higher percentage of acute

histological changes: AR, BC and SAR (83% vs. 75%), with a significantly higher percentage of untreated BC and SAR (22.2% vs. 35%; $p<0.05$) at 1-month biopsy, and an evolution towards acute histological deterioration at 6-month biopsy in the Group 2 (with DGF and/or AR), might be an additional explanation for significantly higher percentage of CAN progression in this group (75% vs. 57%). This finding 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 [26-30].

With regard to the possible link between vasoconstriction, ischaemia, and chronic allograft nephropathy development in CyA-treated renal transplant recipients, our study could not confirm any difference in CyA levels at 1 and 6 months after transplantation between the groups.

Conclusions

Post IRI is mediated by endothelial release of vasoactive factors such as endothelin, nitric oxide and free oxygen radicals, potentially key molecules in the link of IRI, DGF and AR. Endothelial activation may facilitate enhanced graft immunogenicity and development of AR, with a greater susceptibility for acute histological deterioration on the 1 and 6-month biopsy, accelerating the process of CAN. This observation may have important implications in the design of clinical trials aimed to promote therapeutic strategies to prevent IRI, and thereby the progression of CAN.

Conflict of interest statement. None declared.

References

1. 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: 605-612.
2. Racusen LC, Solez K, Colvin RB *et al.*: The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; 55: 713-723.
3. Nankivell BJ, Borrows RJ, Fung CL, O`Connell PJ, Allen RD, Chapman JR. Chronic allograft nephropathy: surrent concepts and future directions. *Transplantation* 2006; 81: 643-654.
4. Chapman JR, O`Connell PJ, Nankivell BJ *et al.* Chronic renal allograft dysfunction. *J Am Soc Nephrol* 2005; 16: 3015-3026.
5. Cecka JM, Cho YW, Terasaki PI *et al.* Analysis of the UNOS scientific renal transplant registry at 3 years. Early events affecting transplant success. *Transplantation* 1992; 53: 59.
6. Hourtman M, Vasse N, Le Mauff B, Soulillou JP. The role of adhesion molecules in ischemia-reperfusion injury of renal transplants. *Nephol Dial Transplant* 1997; 12: 2485.
7. Lefer AM, Lefer DJ *et al.* Pharmacology of the endothelium in ischemia-reperfusion and circulatory shock. *Ann Rev Pharmacol Toxicol* 1993; 33: 71-90.
8. Torras J, Cruzado JM, Grinyo JM *et al.* Ischemia nad Reperfusion Injury in Transplantation. *Transplant Proc* 1999; 31: 2217-2218.
9. Weight SC, Bell PR, Nicholson ML *et al.* Renal ischemia-reperfusion injury. *Br J Surg* 1996; 83:162.
10. Lim EC, Terasaki PI *et al.*. Early graft function. *Clin Transpl* 1991, p 401.
11. Cacciarelli T, Sumrani N, Delaney V, *et al.* The influence of delayed graft renal allograft function on long-term outcome in the cyclosporine era. *Clin Nephrol* 1993; 39: 335.
12. Ojo AO, Wolfe RA, Held PJ, *et al.* Delayed graft function: risk factors and implications for renal allograft survival. *Transplantation* 1997; 6: 968.
13. Howard RJ, Pfaff WW, Brunson ME, *et al.* Increased incidence of rejection in patients with delayed graft function. *Clin Transplant* 1994; 8:527
14. Troppmann R, Gillingham KJ, Beneditti E, *et al.* Delayed graft function, acute rejection and outcome after cadaveric renal transplatation. *Transplantation* 1995; 59: 962.
15. Moreso F, Gallen M, Garcia-Osuna R, *et al.* Multivariate analysis of prognostic factors in renal transplantation. *Transplat Proc* 1995; 27: 2226.
16. Menger MD, Vollmar B *et al.* Role of microcirculation in transplantation. *Microcirculation* 2000; 7: 291-306.
17. Keith DS, Cantarovich M, paraskevas S and Tchervenkov J. Duration of dialysis pretransplatation is an important factor for delayed recovery of renal function following deceased donor kidney transplantation. *Transpl Int* 2007; 21(2): 126.
18. Levin FH. Endothelins. *N Engl J Med* 1995; 333: 356.
19. Espinosa G, Lopez-Farre A, Cernadas R, *et al.* Role of endothelin in the pathophysiology of the renal ischemia-reperfusion in normal rabbits. *Kidney Int* 1996; 50: 776-782.
20. Land W, Schneeberger H, Schleibner S, *et al.* The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal tranplants. *Transplantation* 1994; 57:211.
21. Land W, Messmer K *et al.* The impact of ischemia/reperfusion injury on specific and nonspecific, early and late chronic events after organ transplantation. *Transplant Rev* 1996; 10: 108.
22. Tullius SG, Tilney NL *et al.* Both antigen dependent and independent factors influence chronic allograft rejection. *Transplantation* 1995; 59: 3113.
23. Neimark DMJ, Cole E *et al.* Determinants of long-term renal allograft survival. *Transplant Rev* 1994; 3: 93.
24. Troppman C, Gillingham KJ, Gruessner RWG, *et al.* Delayed graft function in the absence of rejection has no long-term impact. *Transplantation* 1996; 61: 1331.
25. Heil J, Sutherland DER, Najarien JS, *et al.* Acute tubular necrosis of cadaveric renal allografts does not correlate with organ sharing or preservation time but portends a poorer prognosis for long-term graft survival. *Transplantation* 1983; 16: 270.
26. Masin-Spasovska J, Spasovski G, Dzikova S, *et al.*: Do we have to treat subclinical rejections in early protocol renal allograft biopsies? *Transplant Proc* 2007; 39(8): 2550-3.
27. Rush D, Karprinski M, Nickerson P, Dancea S, *et al.*: Does subclinical rejection contribute to chronic rejection in renal transplant patients? *Clin Transplant* 1999; 13: 441.
28. 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.
29. Chapman JR, O`Connell PJ, Nankivell BJ *et al.* Chronic Renal Allograft Dysfunction. *J Am Soc Nephrol* 2005; 16: 3015-3026.
30. Masin-Spasovska J, Spasovski G, Polenaković M, *et al.* Chronic allograft nephropathy (CAN) in early renal protocol biopsies: does treatment of borderline and subclinical acute rejections prevent development and progression of CAN? *Prilozi* 2005; 26(2): 91-103.