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Ischemia-Reperfusion Injury and Acute Rejection - Effects on Nitric Oxide Levels and Allograft Function and Histology at 1 and 6 Months after Kidney Transplantation

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Abstract

Background. Ischemia-reperfusion injury (IRI) is associated with an increased rate of acute rejection (AR), delayed graft function (DGF) or initial poor graft function, development and progression of chronic alograft nephropathy (CAN), leading to a late graft failure and graft loss. Nitric oxide (NO), produced by nitric oxide synthase, has a proven role in both recovery of IRI and promotion of AR. The aim of our study was to (i) analyze the relationship between NO and IRI/DGF and biopsy-proven AR early after transplantation (Tx), and to (ii) estimate the post-IRI effects on allograft function and histology at 1 and 6 months after Tx.

Methods. Forty consecutive living related kidney transplant recipients were included. NO levels were followed immediately after Tx, at day 1, and week 1 and 2 after Tx. Biopsies were performed as clinically indicated. Patients were divided in two groups according to the occurrence of DGF and AR during the first post transplant 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 to the donor and recipient clinical data. The groups differed significantly in the mean cold ischemic time (CIT) and dialysis vintage (3.2±1.1 vs. 4.2±0.6 hours; p<0.01 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 NO assessment, G2 had significantly lower NO levels after Tx, at day 1, and at 1 week post Tx [108.1±8.7 vs. 126.1±14.7 μmol (p<0.001); 36.7±5.9 vs. 126.1±15.2 (p<0.001), and 62.8±14.7 vs. 74.1±10.4 (p<0.05)], respectively. Moreover, the subgroup of G2 patients with DGF and AR (n=6) had significantly higher levels of NO compared with those with DGF but without AR (n=6), at 1 and 2 weeks post-Tx [108.3±16.0 vs. 50.3±5.5 μmol (p<0.001); 112.5±16.1 vs. 64.0±9.0 (p<0.001)], respectively. In addition, G1 had a significantly higher levels of NO early after Tx, at day 1, and at 1 week after Tx, [126.1±14.7 vs. 104.5±7.6 μmol (p<0.001); 126.1±15.2 vs. 35.5±5.5 (p<0.001); and 74.1±10.4 vs. 50.3±5.5 (p<0.001), respectively], when compared with the G2 subgroup with DGF but without AR; significantly higher NO levels at day 1 post Tx [126.1±15.2 vs. 51.0±5.2 μmol (p<0.001)], and a significantly lower NO level at 1 and 2 weeks after Tx, [74.1±10.4 vs. 108.3±16.0 (p<0.001); and 66.6±12.8 vs. 112.5±16.1 (p<0.001), respectively], when compared with the G2 subgroup with DGF and AR. At 1-month biopsy a higher percentage of acute histological changes was found in G2 when compared with G1 (83% vs. 75%). Additionally, the groups differed significantly in the mean HI score (sum of scores for acute and chronic histological changes) at 6-month biopsy [9.1±4.9 (G2) vs. 7.2±2.9 (G1); (p<0.001)]. Thereby, a significantly higher percentage of CAN progression at 6 months was found in G2 (75% vs. 57%). Nevertheless, there was no significant difference in the graft function, i.e. calculated creatinine clearance between and within the groups at 1 and 6 months after Tx.

Conclusion. The patients with DGF and AR had significantly higher levels of NO at 1 and 2 weeks after Tx, when compared with the group without DGF and AR, and patients with DGF but without AR. Furthermore, the group with DGF and AR showed higher percentage of acute histological lesions at 1-month biopsy, greater susceptibility for histological deterioration, and progression of CAN at 6-month biopsy. Endothelial dysfunction following IRI mediated by the NO release may facilitate enhanced graft immunogenecity and induce development of AR, thereby leading to development and progression of CAN.

Keywords: kidney transplantation, ischemia-reperfusion injury, nitric oxide, delayed graft function, acute rejection.

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Introduction

Preservation of graft functions has been one of the most important concerns since the beginning of organ transplantation (Tx). Due to the nature of solid organ transplant procedure, it is not possible to transplant an organ without ischemia and microvasculatory disturbance, which consequently causes reperfusion injury and functional impairment [1]. Ischemia reperfusion injury (IRI) is associated with an increased rate of acute rejection (AR), primary non-function of the graft, delayed graft function (DGF), leading to a late graft failure and graft loss [2]. On the other hand, DGF predisposes the graft to both acute and chonic rejection. Experimental and clinical evidence has identified IRI as an antigen-independent risk factor for chronic allograft nephropathy (CAN) [3,4]. 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 [5]. Nitric oxide (NO), produced by nitric oxide synthase (NOS), has a proven role in both recovery of ischemia and promotion of AR [6,7].

The aim of our study was to (i) analyze the relationship between NO and IRI/DGF and biopsy-proven AR early after Tx, and to (ii) estimate the post-IRI effects on allograft function and histology at 1 and 6 months after Tx.

Patients and methods

The cohort of 40 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; 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.). Patients with DGF who suffered post-transplant acute tubular necrosis or experienced a clinical episode of AR (biopsy-proven) were treated with hemodialysis or pulse corticosteroids, respectively. Protocol biopsies were performed at 1 and 6 months postransplant using ultrasound-guided automated biopsy "gun". Renal histology was reviewed according to the Banff 97 scoring schema [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 histological index (HI) was calculated as a total sum of scores for acute and chronic changes. Patients with histology at 1month biopsy of borgerline 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. Nitric Oxide Colorimeric Assay was used for determination of total nitrite as an indicator of NO production. NO levels were followed: before Tx, after Tx, at day 1, at 1,2,3 weeks and at 1 and 6 months after Tx. 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: G1-without DGF and AR (n=28), and G2-with DGF and/or AR (n=12).

The 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 2-tailed Student`s *t* test was used, and Chi-square analysis was used for categorical variables. A difference was considered significant at a *P* value of <0.05.

Results

Table 1. Patients demographic characteristics, clinical data and post- transplant events of all patients

Donor age (yr)	59.6 ± 13.1	
Recipient age (yr)	34.4 ± 9.3	
Female:male	15:20	
Cause of and-stage renal disease		
	Glomerulonephritis	12
	Diabetes	0
	Hypertensive renal disease	5
	Polycystic renal disease	2
	Reflux nephropathy	6
	Other	10
Time on dialysis (mo)	30.1 ± 37.8	
Total HLA mismatch score	2.0 ± 1.2	
Mean CIT (h)	3.4 ± 1.3	
DGF (%)	12/40 (40%)	
AR (%)	6/12 (50%)	

The demographic characteristics of the patients are summarized in Table 1. The mean age of the entire cohort of donors and recipients were 59.3±13.1 and 34.3±9.8 years, respectively. Of the 40 recipients, 12 patients had DGF, and 6 of them were associated with an episode of AR. 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).

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.

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 months 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*

When the patients were divided according to the occurrence of DGF and AR, between the G1 group (without DGF and AR) and G2 group with DGF and/or AR) there was no difference in the following parameters: donor age and BMI, recipient age, BMI and time on dialysis, number of HLA matching, GFR of donated kidney, as weel as in cyclosporine (CyA) levels (C2), sCr, cCrcl, and proteinuria, neither at 1 nor at 6 months after transplantation. However, the mean cold ischaemic time (CIT) and haemodialysis time were much shorter in the G1 group (Table 3).

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 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*
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.

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).

When the groups were compared according to the changes of NO, G2 had significantly lower NO levels after Tx, at day 1, and at 1 week post Tx [108.1±8.7 vs. 126.1±14.7 μmol ($p<0.001$); 36.7±5.9 vs. 126.1±15.2 ($p<0.001$), and 62.8±14.7 vs. 74.1±10.4 ($p<0.05$)], respectively, (Figure 1).

Moreover, the subgroup of G2 patients with DGF and AR (n=6) had significantly higher levels of NO compared with those with DGF but without AR (n=6), at 1 and 2 weeks post-Tx [108.3±16.0 vs. 50.3±5.5 μmol ($p<0.001$); 112.5±16.1 vs. 64.0±9.0 ($p<0.001$)], respectively, (Figure 2).

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*

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

G1-without DGF and AR (n= 28)			
Parameters	**1 mo. (Mean ± SD)**	**6 mo. (Mean ± SD)**	**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 pts (32.1%)	12 pts (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	n.s.
AR gr.: IA, IIA, IIB	9 pts (75%)	9 pts (75%)	n.s.
CAN progression	9/12 (75%)		

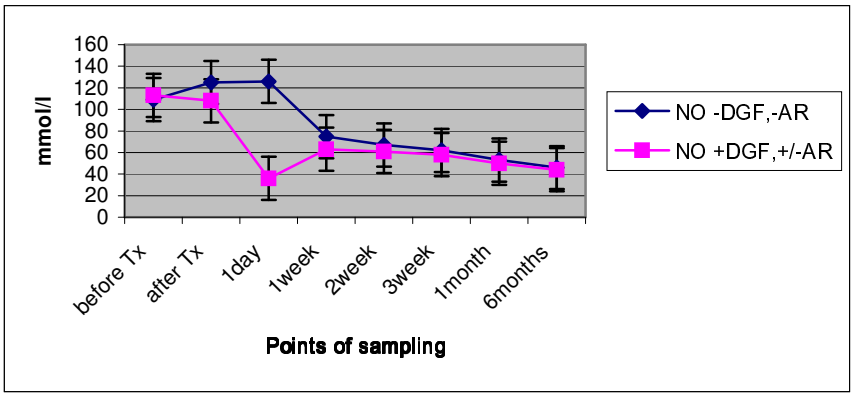

Fig. 1. Comparison of changes of NO between the groups G1 (without DGF and AR) and G2 (with DGF and/or AR)

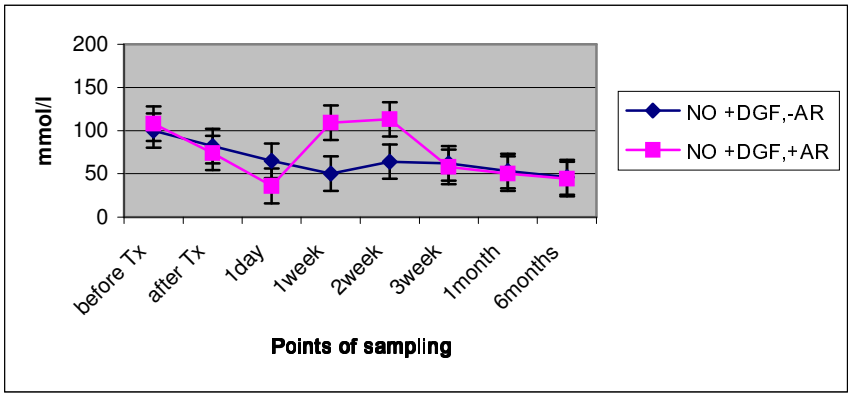

Fig. 2. Comparison of changes of NO between the groups G1 (without DGF and AR) and the subgroup (with DGF and without AR)

In addition, G1 had a significantly higher levels of NO early after Tx, at day 1, and at 1 week after Tx, [126.1±14.7 vs. 104.5±7.6 μmol (p<0.001); 126.1±15.2 vs. 35.5±5.5 (p<0.001); and 74.1±10.4 vs. 50.3±5.5 (p<0.001), respectively], when compared with the G2 subgroup with DGF but without AR (Figure 3).

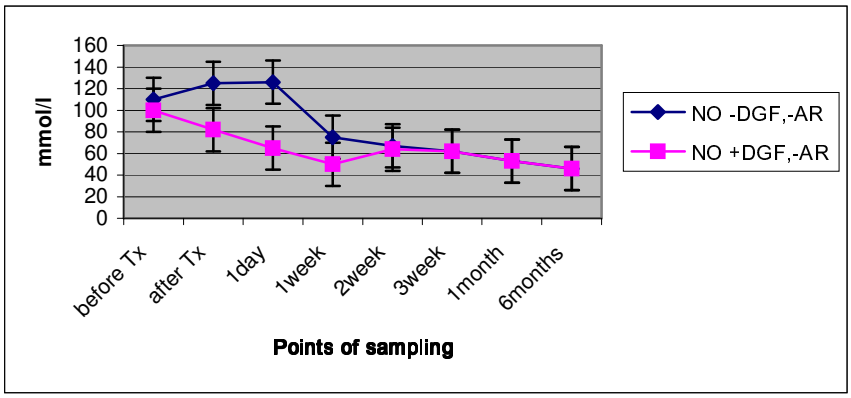

Fig. 3. Comparison of changes of NO between the groups G1 (without DGF and AR) and the subgroup (with DGF and without AR)

Moreover, a significantly higher NO levels at day 1 post Tx [126.1±15.2 vs. 51.0±5.2 μmol (p<0.001)], and a significantly lower NO level at 1 and 2 weeks after Tx, [74.1±10.4 vs. 108.3±16.0 (p<0.001); and 66.6±12.8 vs. 112.5±16.1 (p<0.001), respectively], were found in G1 when compared with the G2 subgroup with DGF and AR (Figure 4).

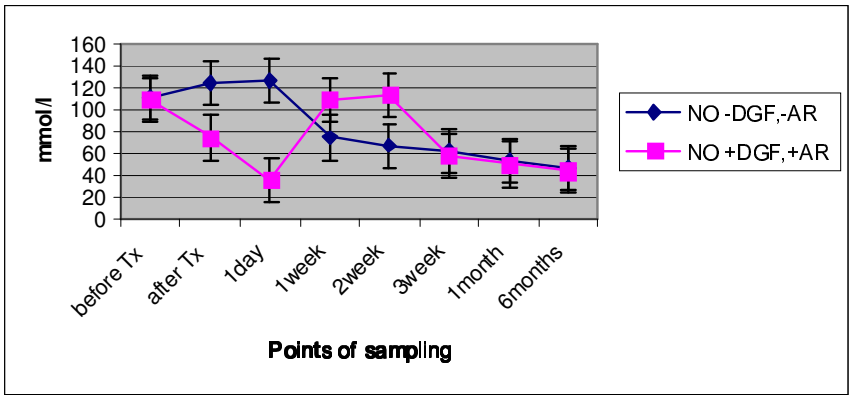

Fig. 4. Comparison of changes of NO between the groups G1 (without DGF and AR) and the subgroup (with DGF and AR)

Discussion

IRI occurring secondarily to renal retrieval, storage, and transplantation, is responsible for 20-30% worldwide incidence of DGF and may increase the incidence of AR, as well as favor CAN [1-3,9]. IRI was correlated with the incidence of AR in several clinical series [10-13]. The principal finding in our study was the evidence of DGF in 30% of the patients, and 50% of them were associated with an episode of AR. Furthermore, the group with DGF and/or AR had a significantly longer CIT when compared with the group without DGF and AR. These results confirmed the association between the CIT with the higher probability of IRI [1,2]. Moreover, our results confirmed the strong correlation between duration of dialysis and incidence of DGF [14]. Namely, the group with DGF and/or AR had significantly longer dialysis duration. Thus, IRI is a systemic event resulting in endothelial dysfunction, production of oxygen radicals, NO depletion, and release of cytokines, leading to the development of an inflammatory response [4-7]. 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 a microvascular constriction and localized reduction in blood flow. In addition, oxidative stress associated with IRI leads to increased production of free oxygen radicals and decreased NO production [5,7]. In this regard, it is relevant to compare our results of significantly lower NO levels early after Tx, in the group with DGF and/or AR, with those in the group without DGF and AR. On the other hand, the subgroup of patients with DGF and AR had a significantly higher NO levels at 1 and 2 weeks post-Tx, when compared with the group with DGF but without AR, and those in the group without DGF and AR. These findings could be explained, as a response to various cytokines that participates in the process of rejection, and by activated macrophages express of inducible nitric oxide synthase (iNOS), enzymes that convert L-arginine to increased NO production during AR [4,7,15-16]. Moreover, our results are comparable with those reported by Khanafer at al. who found a significant increase of NO during episodes of acute rejection when compared with other causes of allograft dysfunction, such as postrenal uroinfections, Tacrolimus toxicity, DGF, and temporary increase in serum creatinine of >10% [7].

Our study the group with DGF and/or 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. Additionally, the group with DGF and/or 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.

These findings might be explained by the definition of IRI, as a complex sequence of events that influence the early phase of recovery following kidney transplantation and has also been identified as an antigen-independent risk factor for CAN [13]. Moreover, recent studies suggest that allografts exposed to IRI have an increased immunogenesity, leading to increased rate of acute rejection episodes, which is well known risk factor for chronic allograft damage [3,7,15]. In addition, IRI may cause a release of cytokines and grow factors associated with CAN. Namely, many of the inflammatory cytokines and other chemical mediators released during recovery from ischemic renal damage are identical to those released during alloimmune response, including interferon-γ, transforming growth factor-β, interleukin-6, prostaglandins and nitric oxide [5,17].

Conclusions

A significantly higher levels of NO associated with AR, in comparison with other causes of renal dysfunction were found in our study. Thus, this significant increase of NO levels in patients with AR may be an additional useful biochemical marker which contributes to the diagnosis of AR. In addition, the group with DGF and AR was characterized with higher percentage of acute histological lesions at 1-month biopsy, greater susceptibility for histological deterioration, and progression of CAN at 6-month biopsy. It is likely that IRI increase the immunogenecity of the graft and probability of AR, which in turn contribute with the development and progression of CAN.

Conflict of interest statement. None declared.

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