CAN AN INCREASED NITRIC OXIDE LEVEL BE ACCEPTED AS NON-INVASIVE MARKER FOR SUB/ACUTE REJECTION OF THE KIDNEY ALLOGRAFT?

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INTRODUCTION

Solid organ transplantation is not possible without ischemia and microvasculatory disturbance, which consequently causes reperfusion injury and functional impairment. Ischemic reperfusion injury (IRI) might be associated with primary non-function of the graft, delayed graft function (DGF), and subsequently an increased rate of acute rejection (AR) leading to a late graft failure and graft loss (1,2). Nitric oxide (NO) is well known endogenous immunomodulator involved in immunoregulation and host defense mechanisms (3-5). In addition to the many beneficial effects under physiological conditions, NO may inhibit the oxidative stress, cytokine release, leukocyte endothelial adhesion and apoptosis (6,7). On the other hand, NO overproduction under pathological conditions such as peri/post-transplant events, i.e. surgical stress and infections, may further aggravate the tissue damage although the use of glucocorticoids and calcineurin inhibitors may inhibit its production (8,9). NO is rapidly degraded to stable end-products of nitrite and nitrate, which can be measured in serum and urine, and thereby may also serve as biochemical marker in early detection of allograft dysfunction (10). Data on NO in kidney allografts with acute rejection episodes are scarce and there is no consensus yet on the assay measurements and standardized levels (10,11). Hence, the aim of our study was to analyze the NO levels in patients with protocol biopsies and findings of subclinical acute rejection (SAR), in biopsy-proven AR, and recipients with various causes of allograft dysfunction (serum creatinine >300 μmol/L) occurred within the first posttransplant month: delayed graft function (DGF), urinary tract infection (UTI), and acute cyclosporine toxicity as C2 level above 1200 ng/ml (CyTx). Finally, we analyzed the diagnostic performance of NO levels as non-invasive marker for an early detection of AR and SAR.

MATERIALS & METHODS

We prospectively studied 45 living-related kidney recipients with their first allograft receiving induction therapy with methylprednisolone (500mg) and Daclizumab (Zenapax; 1mg/kg/BW at implantation and thereafter every 2 weeks in five doses). The maintenance immunosuppression consisted of cyclosporine A (Neoral®; 4-6mg/kg/day) initiated at least 36hrs after transplantation to reach target C2 levels, prednisolone (1 mg/kg/day) and mycophenolate mofetil (CellCept® 1 g bid.). Patients with DGF who suffered post-transplant acute tubular necrosis were treated with hemodialysis, and those who experienced an episode of AR (biopsy proven) were treated with pulse corticosteroids or ATG in cases of cortico-resistant AR or vascular lymphocyte infiltration (Banff grade IIB or higher). Protocol biopsies were performed at 1 and 6 months post-transplant. Biopsy specimens were considered as adequate if they contained more than 7 glomeruli and at least one artery that were further histologically processed according to the Banff 97 scoring scheme (11). Protocol biopsies were performed in "stable allografts", meaning stable graft function (no change in sCr > 20%; 2 weeks before the biopsy). In cases of clinically indicated biopsy within one week prior to the 1-month protocol biopsy no other biopsy was performed. For quantitative measurement of serum NO, a Microplate enzymatic method based on assay kit from Oxis (Portland, OR, USA) was used. The kit involves enzymatic conversion of the nitrate into nitrite, followed by determination of the total concentration of NO serum levels. NO levels were measured 20 min after kidney graft reperfusion (NO1), on day 1-(NO2), 5-(NO3) and 14-(NO4) after Tx, and at 1-(NO5) and 6-(NO6) months post-transplant. Clinical and biochemical data were recorded into the database of the study. The diagnostic performance of NO levels by varying the decision thresholds (cut-off values) over the complete range of test results found NO level of 70 μmol/L for AR and 50 μmol/L for SAR to have best discriminatory ability of the test. Sensitivity (the true-positive fraction) and specificity (the ability of a test to correctly exclude the presence of the disease) were defined according to the standard diagnostic performance formulas.

RESULTS

Table 1. Clinical data and peri-operative transplant parameters of the patients

Parameters / data	values
Donor age (years)	58.3 ± 11.5
Gender (F/M)	18/27
Recipient age (years)	35.7±10.4
Gender (F/M)	26/19
Relationship (parent/husband-wife/brother in low)	42/21
Cause of ESRD	(n of patients)
Glomerulonephritis	14
Diabetes	4
Hipertensive	4
Polycystic renal disease	2
Reflux nephropathy	7
Lupus nephropathy	2
Other	12
Hemodialysis duration (months)	27.3 ± 35.7
Total HLA mismatch (score)	2.2 ± 1.1
Cold Ischemia Time - CIT (hours)	3.4 ± 1.1
Delayed Graft Function - DGF (%) 16/45 pts	16/45 (35.5%)
Acute Rejection associated with DGF (%) 9/16 pts	9/16 (56.2%)
Data presented as mean ± SD and percentage	

Table 2. Values of NO serum levels in 6 various time points up to 6 months in patients without graft dysfunction (no GD) and those with different reason for allograft dysfunction (in μmol/L)

Graft dysfunction	NO1	NO2	NO3*	NO4	NO5*	NO6*	P-value ANOVA
No GD, n=29	25±5.3	26±4.5	28±5.7	27±4.1	24±7.5	22±5.7	ns
DGF, n=16	27±7.1	30±5.3	43±6.5	35±5.2	25±4.6	20±6.6	ns
UTI, n=14	26±6.1	28±4.8	35±3.7	37±4.9	21±8.9	21±4.5	ns
CyTx, n=8	25±5.3	29±5.7	49±7.9	38±6.7	22±7.4	20±6.5	p=0.052
SAR1m, n=18	23±4.8	24±5.5	26±6.4	29±6.3	**51±6.3**[†]	24±7.8	p<0.05
SAR6m, n=20	24±5.6	25±4.9	27±6.0	26±5.8	24±6.7	**50±5.5**[†]	p<0.05
AR, n=13	26±6.9	38±5.3	**72±9.8**[#]	58±7.6	36±9.5	21±7.5	p<0.05

Data expressed as means ± SD; P-value (ANOVA); *Kruskall-Wallis $P<0.05$.
Abbreviation: DGF – delayed graft function, UTI – urinary tract infection, CyTx – acute cyclosporine toxicity, SAR1m and SAR6m – subclinical acute rejection at 1-and 6-month protocol biopsy, AR – acute rejection (per clinically indicated biopsy within the first postransplant month).
[†]Student's *t* test P<0.05 versus all other values.
[#]Mann-Whitney P<0.05 versus all other groups.

DISCUSSION

We have evaluated dynamic NO changes as one of the most important biochemical markers of kidney damage and have considered another few peri-operative risk factors that might have an impact on the histology of the kidney. The principal finding in our study was the evidence of DGF in 35.5% of patients, whereas 56.2% of them were associated with an episode of AR (2,3). In addition to the histological diagnosis of AR, protocol biopsies can provide useful information for subclinical, clinically silent changes and an opportunity for individualized immunosuppressive regimen (12). Here, there is a need of a follow-up through any special, non-invasive early marker for the diagnosis of AR (7,16), which is reported as such in our study with various time points of NO sampling prior to the first protocol biopsy at 1 month after transplantation. We observed significantly higher NO levels associated with AR and/or SAR in patients biopsied by indication, and at 1 and 6 protocol compared with groups of confirmed reported by

DISCUSSION (cont.)

Our diagnostic performance of NO cut-off level of 50 μmol/L in discrimination of SAR should be more appropriate parameter obtained from a cohort of 99 biopsies and NO tests (sensitivity and specificity of 78.9% and 75.4%, respectively). In this regard, NO determination should be consider as useful, reliable and cost-effective non-invasive biochemical marker in diagnosis of patients with SAR, which may to a certain degree replace the need of protocol biopsy in the first year after Tx.

CONCLUSIONS

Our study reports significantly higher serum NO levels at day 5 and gradual decrease at day 14 (prior and at the time of clinically manifested AR), and at 1 and 6-month protocol biopsy in SAR patients as compared to all other causes of renal dysfunction. Therefore, in clinical practice NO measurement may have satisfactorily diagnostic performance as useful, reliable, and cost-effective non-invasive biochemical marker not only in AR, but even more importantly, in diagnosis of patients with SAR. Nevertheless, future interventional clinical trials aimed at early diagnosis and treatment of AR and SAR should include measurements of NO before, at the occasion and after the administered therapy for an AR or SAR episode.

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