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DE NOVO ANTI HLA ANTIBODIES AFTER KIDNEY TRANSPLANTATION: CLINICAL SIGNIFICANCE AND ASSOCIATION WITH GRAFT FUNCTION

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Abstract

Background. Kidney transplantation (TR) is the best treatment of chronic kidney disease. Chronic cellular and humoral rejections have still major impact on graft survival. Single antigen bead technology enabled detection of donor specific (DSA) and non-donor specific (Non-DSA) anti HLA antibodies (HLA-Ab). Our study investigates the impact of de novo HLA-Ab on graft function (GF) 12 months after TR.
Material and methods. Fifty pts with living (42) and deceased donor (8) transplantation were included in a 12-month prospective study. HLA-Ab were analyzed using LABScreen mixed kit in the 1st and 12th month after TR. According to the presence of HLA-Ab, pts were divided in group 1 (HLA+) and group 2 (HLA –). Both groups did not differ regarding gender, age, living or deceased donor, immunosuppression, underlying renal disease, rejection episodes, HLA mismatch, cold and warm ischemia time. Serum creatinine (SCr), GFR (Cockroft Gault) and proteinuria (Pr) were analyzed 1st and 12th month after TR.
Results. HLA-Ab were detected in 17 pts (34%), 5 with DSA (10%) and 12 with Non-DSA (24%). Group 1 has a significant worsening of GFR (SCr increased

Résumé

Les anticorps anti HLA de novo après la transplantation rénale: signification clinique et association avec la fonction du greffon

Contexte. La transplantation rénale (TR) est le meilleur traitement de la maladie rénale chronique. Le rejet chronique, cellulaire ou humoral, est toujours présent dans la pathologie du greffon. La technologie Luminex a permis une détection d'anticorps anti HLA (HLA-Ac) spécifiques du donneur (DSA) et du non donneur (non-DSA). Notre travail étudie l'impact des HLA-Ac de novo sur la fonction du greffon.
Matériel et méthodes. 50 pts greffés (42 de donneur vivant et 8 de donneur décédé) ont été inclus dans une étude prospective de 12 mois. Les HLA-Ac ont été analysés à l'aide du kit mixte LABScreen au cours des premier et 12ème mois. Selon la présence de HLA-Ac, les pts ont été répartis dans un groupe 1 (HLA +) et le groupe 2 (HLA –). Les groupes ne différaient pas en ce qui concerne le sexe, l'âge, le donneur, l'immunosuppression, la maladie initiale, les rejets, l'incompatibilité HLA, le temps d'ischémie froide et chaude. La créatinine sérique (Cr), DFG (Cockroft Gault) et la

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from 112.1 to 141.5 (p<0.002) compared with the group 2 where SCr decreased from 116.4 to 111.31 μol/L.(p<0.23). In the same time GFR decreased from 69.7 to 57.09 and increased from 67.8 to 69.3 while Pr increased from 0.42 to 0.58 (p< 0.26) and decreased from 0.81 to 0.32 (p<0.051) in the groups 1 and 2, respectively.

Conclusion. De novo DSA and Non-DSA produce graft injuries in the first 12 months after TR. Regular follow- up of HLA-Ab together with systematic protocol graft biopsy could be essential for further therapeutic interventions.

Key words: kidney transplantation, anti HLA antibodies, DSA and Non-DSA antibodies, graft function.

protéinurie (Pr) ont été analysées en premier et 12ème mois après la TR.

Résultats. Dans 17 pts ont été détectés les anticorps anti-HLA (34%), 5 avec DSA (10%) et 12 avec Non-DSA (24%). Le groupe 1 a une détérioration significative de la FG. La Cr augmente de 112.1 au premier mois à 141.47, (p <0,002) par rapport au groupe 2 où elle diminue de 116 à 111.31 μol/L. (p <0,23). DFG diminue de 69.78 à 57.09 et augmente de 67.78 à 69.88 ml/min dans les groupes 1 et 2, respectivement. La Pr augmente de 0.42 à 0,58 (p <0,26) et diminue de 0.81 à 0.32 gr (p <0,051), dans les groupes 1 et 2.

Conclusion. Les HLA-Ac de novo provoquent des lésions du greffon dans les 12 premiers mois après TR. Le suivi périodique des HLA-Ac ainsi qu'une biopsie systématique du greffon pourraient être essentiels pour d'autres interventions thérapeutiques.

Mots clé: anticorps anti HLA, transplantation rénale, anticorps DSA et Non-DSA, fonction du greffon.

Introduction

There is no doubt that the kidney transplantation is a remarkable achievement in the treatment of chronic kidney disease (CKD). Since more than 50 years, thousands of patients have benefited of a productive life after successful transplant surgery. Either from living or from deceased donor, kidney transplantation remains one of the miracles of the modern medicine. Despite the obvious tremendous success of kidney and other solid organ and tissue transplantation, there are still obstacles and unsolved problems that could have a negative effect on the whole graft and patient survival. Certainly in front of the problem remains cellular or humoral rejection, but other possible significant factors as immunosuppression, medication adherence, recurrence of primary disease are still very important[1-5]. Due to the introduction of new technical facilities, it is possible today to analyze more detailed the process of rejection, especially the so called Antibody Mediated Rejection (AMR) which is responsible for the majority of long term graft loss during a period of follow- up[6-9]. Introduction of solid phase or single antigen bead assay (Luminex) enabled to detect a large amount of HLA antibodies in the recipients before and after the surgery[10,11]. According to the donor HLA typing, the preforming and de novo antibodies could be donor specific (DSA) and non-donor-specific (Non-DSA). The frequency of HLA antibodies among transplant recipients ranges greatly from as low as 4% to more than 50%[11]. 30% of HLA antibodies belong to donor DSA. It is very well known that the appearance of de novo DSA any time after the transplantation has a harmful effect on the graft and negatively contributes to the long term graft and patient survival rate[12-14]. In the same time, there are many reports regarding the possible effect of Non-DSA-HLA antibodies and possible association with a long term graft function and graft structural changes[15-20]. Due to the presence or absence of cross reactive antigens and variability in the immunogenicity of different HLA antigens, it is quite considerable to take into account both, DSA and Non-DSA HLA antibodies when we are talking of AMR after successful kidney transplantation[19,21].

The aim of our study was to investigate the possible association of de novo anti HLA antibodies on kidney function of kidney transplant recipients, 12 months after the surgery.

Material and methods

Fifty patients with predominant haploidentical living (42) and 8 deceased donor transplantation were included in a 12 month prospective study. Renal transplantation was performed at the University Clinical Centre in Skopje, Macedonia, according to the well known principles from the surgical, nephrological and immunological points of view. Hypertension, Glomerulonephritis. Hereditary nephropathies and End Stage Renal Disease (ESRD) were predominant underlying diseases for CKD. The usual pretransplant work-up was done in all potential donors and recipients[22]. According to the centre policy, 50% was a minimum accepted HLA matching in both, living und

deceased donor transplantation. A sequential quadruple Immunosuppression including ATG or Simulect induction and Prednisolon, MMF and CNI as a triple drug maintenance therapy was introduced in all recipients. After the surgery the patients were followed by the same team according to the KDIGO recommendations[23]. Usual Lab analyses, proteinuria, GFR, through immunosuppressant levels, graft ultrasound tomography including Doppler were done practically every month on the outpatient's basis.

Antibodies. The HLA antibodies have been analyzed using LABScreen mixed kit (One Lambda, Canoga Park, CA) and identified with LABScreen single antigen beads (One Lambda) in the first and 12th month after the surgery. Detected anti HLA antibodies were classified as DSA and Non-DSA according to the presence of donor specific antigen while the strength was expressed usually with MIF (Mean Fluorescence Intensity). A MIF value more than 800 was an acceptable cut-off for clinical significance. (Arcdeacon) Luminex analyses were done at minimum two times during the follow- up and/or any time it was necessary in case of potential rejection.

Design of the study. The 12 months prospective study was performed. According to the presence or absence of HLA antibodies, the patients were divided intwo groups: group one with positive HLA antibodies (group 1- HLA+) and group two with negative HLA antibodies (Group 2– HLA–). Both groups did not differ regarding gender, age, living or deceased donor, immunosuppression, underlying renal disease, delayed graft function (DGF), rejection episodes, HLA mismatch, cold ischemia time (CIT), warm ischemia time (WIT). Serum creatinine, kidney function (GFR-Cockroft Gault) and proteinuria were analysed first and 12th month after the surgery.

Statistics. Usual descriptive statistics was used and T-test for significance of differences. A p value less than 0.05 was considered statistically significant.

Results

A total of 50 patients were tested for presence of potential de novo HLA antibodies were detected, before, 1 month and 12 months after transplantation. In 17 patients, anti HLA antibodies (34%), 5 of them with DSA (10%) and 12 with Non-DSA (24%). Six patients showed HLA antibodies against HLA Class I, 5 against HLA Class II, and 6 against both, Class I and II. The average MFI for both, HLA Class I and II antibodies was 3579.6±2103.3 (range 800- 6600). The most frequent HLA antigens among the HLA antibodies positive patients are: A2, B8, B27, B67, B81, B39 among HLA-Class I and DQ7, DQ8, DQ9, DQ2, DQ4, DR17, DR18 in HLA-Class II antigens. The rest of 33 patients did not show any antibodies after 12 months of follow- up. According to the present antibodies in the serum, the patients were divided

Table 1. Demographic and clinical characteristics of the patients.

Parameters	*Group 1 (HLA+)*	*Group 2 (HLA-)*	*P*
age	32,5 ± 11,76	34,5±11,85	n.s
gender (w/m)	4/13	10/24	n.s
Primary diagnosis			
Hypertension	2	2	n.s.
DM type 1	1	1	n.s.
Glomerulonephritis	4	6	n.s.
FSGS	2	1	n.s.
MPGN	1	3	n.s.
MN	0	1	n.s.
RPGN	0	4	n.s.
Hered. Nephropathies	3	3	n.s.
VUR	2	0	n.s.
ESRD	4	6	n.s.
HLA mismatch	2.9±0.7	3.2±0.4	n.s.
CIT (hours)			
Living donor	≈ 3.8	≈ 3.6	n.s
Deceased donor	≈ 12	≈ 9	n.s.
WIT (min)	≈ 3	≈ 3	n.s.
Rejection	11%	13%	n.s.
Induction therapy SIM/ ATG	3/14	16/14	n.s.
Cyc/Tac	12/5	6/26	n.s.

FSGS – focal and segmental glomerulonephritis, MPGN- membranoproliferative glomerulonephritis, MN – membranous nephropathy, RPGN – rapid progressive glomerulonephritis, ESRD – end stage renal disease, VUR- vesicoureteral reflux, CIT – cold ischemia time, WIT- warm ischemia time, SIM- Simulect, ATG- Anti-thimocyte Globulin, Cyc – Cyclosporin, Tac- Tacrolimus

on group 1 (HLA+) and group 2 (group HLA-). Both groups did not differ regarding baseline clinical and demographic data (Table 1, 2).

Analyzing the results for graft function and proteinuria, it has been shown that the group with confirmed anti HLA antibodies (HLA+) has a significant worsening of graft function (defined by an increase of creatinine value from 112.1±24.4 in the first month to 141.47±40.5 μol/L, (p<0.002) 12 months after transplantation), compared with the group without HLA antibodies (HLA–), where creatinine even decreases from 116±35.9 to 111.31± 26.6 μmol/L(p<0.23) (Table 3, Fig. 1).

In the same time, GFR decreases from 69.78±18.8 to 57.09± 20.7 (p<0.03) and increases from 67.78± 20.1 to 69.88± 19.6 ml/min in the groups HLA+ and HLA-, respectively (Fig. 2).

Regarding proteinuria, the HLA+ group increases not significantly the values of 24 h proteinuria from 0.42±0.37 to 0.58± 0.51gr (p< 0.26) while the HLA- group decreases from 0.81± 0.73 to 0.32±0.38 gr (p<0.051), after 12 months of follow- up (Table 4, Fig. 3).

Discussion

The mild, but significant, worsening of graft function after 12 months in HLA+ patients group confirms the hypothesis that presence of HLA antibodies may produce some degree of graft injury[24-28]. The possible mechanism is a direct effect of donor specific antibodies on the HLA antigens in the endothelial cells of transplanted organ, a reaction that definitely includes the complement cascade which already is very well known[1,29,30]. The ultrastructural changes in the small vessels, peritubular capillaries and glomerula are part of so called BANFF classification[31]. Those histological changes, together with the presence of donor specific HLA antibodies in the serum, are confirmation of AMR, which is now broadly accepted as a clinical entity[31]. There is no doubt that exactly AMR is responsible for the majority of long term graft loss. Despite the fact that deleterious effect of DSA is already widely confirmed among the transplant community, the role of non-DSA antibodies remains controversial. The presence of those antibodies in almost 24% of our patients after 12 months, associated with slight, but significant, decline of GFR, means that some negative effects on renal graft could be associated with the non-donor –DSA antibodies. This phenomenon could be explained with so called cross reactivity among different HLA antigens[21]. Although they are not connected directly with any of the mismatched HLA antigens, the Non-Donor-DSA may react with different epitopes as a part of HLA molecule. Therefore, if the

Table 2. Frequent HLA antigens, type of de novo anti HLA antibodies and MFI

Anti HLA ab	MFI	Percentage %
Class I	4900 (range 800- 6600)	35,29%
Class II	4866 (range 987- 8470)	29,41%
Class I and II	3422 (range 1323-5000)	35,29%
DSA		10 %
Non DSA		24 %
Most frequent HLA-Ab		
Class 1	A2, B8, B27, B67, B81, B39	
Class 2	DQ7, DQ8, DQ9, DQ2, DQ4, DR17, DR18	

Table 3. Serum creatinine, GFR and 24 h proteinuria 12 months after transplantation

Parameters	*Group 1 (HLA+)*	*Group 2 (HLA-)*	*P*
Serum creatinine	141.47 ± 20.12	111.31±26.6	0.002
GFR	57.09 ±20.72	69.88 ±20.49	0.04
24 h proteinuria	0.58+0.51	0.32+0.38	0.051

Table 4. Difference in serum creatinine, GFR and 24h proteinuria from first to 12th month after transplantation in Group 1 (HLA+)

Parameters	*1 month*	*12 month*	*P*
Serum Creatinine	112.1±24.4	141.47±20.12	0.002
GFR	69.78± 18.81	57,09 ±20,72	0.03
24 h proteinuria	0.42±0.37	0.58±0.51	0.26

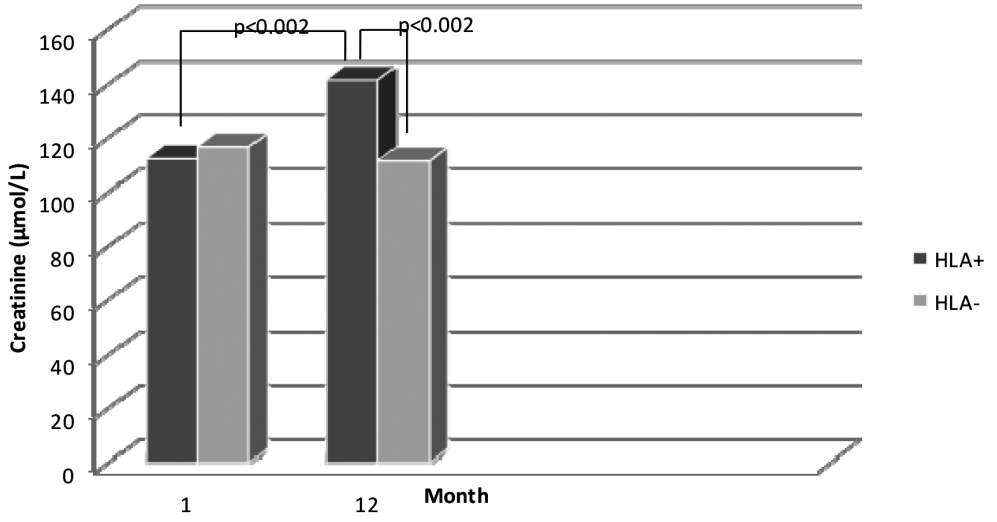

Figure 1. Serum creatinine at 1st and 12th month after transplantation in HLA+ and HLA- group
Increase of creatinine in HLA+ group after 12 months of follow up (p<0.002). Statistically significant difference in creatinine between HLA+ and HLA- group.

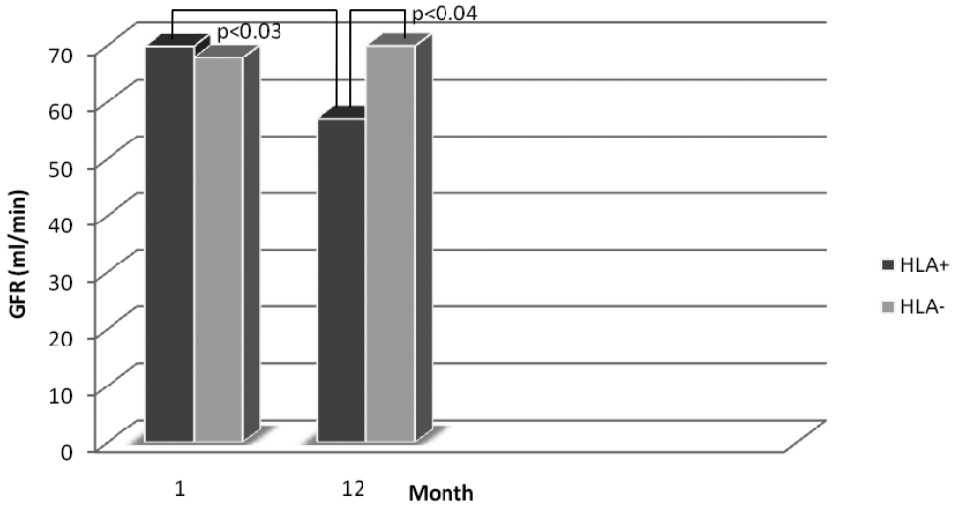

Figure 2. GFR at 1st and 12th after transplantation in HLA+ and HLA- group
Decrease of GFR in HLA+ group after 12 months of follow up (p<0.03). Statistical difference between GFR at 12 months between HLA+ and HLA- groups

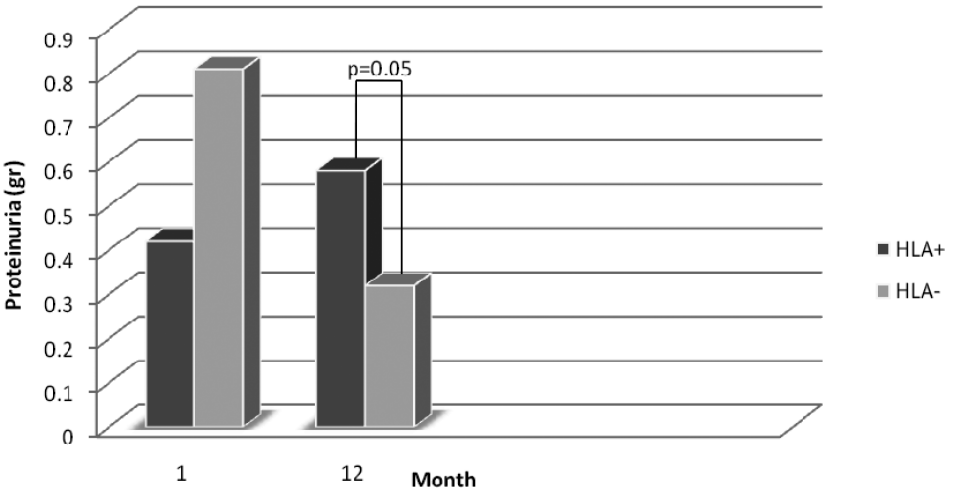

Figure 3. Proteinuria at the 1st and 12th months after transplantation
Increase of proteinuria after 12 months in HLA+ group, and decrease in HLA- group.

Non-Donor-DSA antibodies are produced together with DSA they could share epitopes with mismatch HLA antigens[9,19]. The hypothesis is confirmed if there were no performed DSA and Non-DSA antibodies before transplantation, which is practically our case[9,19]. The fact that no patient was lost during the follow- up, despite the presence of HLA antibodies, could be explained with a relatively moderate MFI, with a maximum peak of 6000 and powerful immunosuppression, including mono and polyclonal induction therapy, as well as triple drug maintenance treatment with steroids, Micophenolat Mofetil (MMF) and Calcineurin Inhibitors (CNI). Therefore, keeping the medications strongly as a long term therapy is necessary for better graft and patient survival[5,32,33]. Regarding the changes in proteinuria, which are also part of the analysis, the same mechanisms could be involved in the process. Despite the difference in proteinuria between both groups did not reach statistical significance after 12 months of follow- up ($p< 0.051$), we could accept that some graft injury is more present in HLA+ group. In the middle there is again AMR, with slow but progressive microvascular destruction, including the changes in glomerula. Proteinuria could be one of the first signs that the AMR is present, even before the decline of GFR.

Introduction of HLA antibodies in a regular follow- up of the transplant recipients enables better control of the rejection, as an immunological phenomenon. They can be used as a diagnostic, predictive and preventive tool. Together with systematic regular protocol biopsies of the graft, the detection of anti HLA antibodies in the serum of transplant recipients may be an alert of, so called, silent rejection, either cellular or humoral[34-36].

In conclusion, our work confirms that de novo HLA antibodies, either DSA or Non-DSA, may produce some injuries of the graft even in the first 12 months after the surgery. Hence, regular follow-up of HLA antibodies in the kidney and other solid organ recipients, together with systematic protocol graft biopsy, could be essential and a key point for further therapeutic interventions.

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