The incidence and clinical relevance of anti–HLA and/or MICA antibodies in patients with long–term survival renal allo–grafts

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ABSTRACT

The purpose of this study was to analyze the incidence and clinical relevance of anti–HLA and/or MICA anti–bodies in renal allo–graft transplant recipients with long–term renal survival (>5 years). This retrospective study collected post–transplant serum samples from a total of 110 patients which were used to detect the incidence of an–ti–HLA and/or MICA antibodies as well as anti–HLA donor specific antibodies. Among these 110 patients, 72 pa–tients had antibodies against HLA and/or MICA at the time of test, 61 had anti–HLA antibodies, 31 had anti–MICA antibodies, and 38 were antibody negative. There was no difference in the number of patients developing antibodies against non–donor specific antibodies, donor specific antibodies, Class Ⅰ donor specific antibodies, Class Ⅱ donor specific antibodies or MICA antibodies between normal function group (serum creatinine level < 2.0 mg/dL) and dysfunction group (serum creatinine level > 2.0 mg/dL). For the serum creatinine level, estimated glomerular filtra–tion rate and blood urea nitrogen level, patients with different antibodies were not statistically different to antibody–negative patients. Cox regression analysis showed that the type of transplantation and HLA mismatch number were significant negative risk factors for the development of anti–HLA ($P < 0.05$). Our results suggested that the anti–HLA antibody status has little impact on the renal graft function in the long–term survival allo–graft renal recipi–ents.

Keywords: HLA, DSA, MICA, renal transplantation

INTRODUCTION

In the past two decades, long–term renal graft sur–vival has been greatly improved by the use of more effective immunosuppressive drugs and therapeutic regimens. However, immunological and non–immu–nological factors still exist which can lead to chronic renal allograft dysfunction and loss[1]. Several reports have suggested that the humoral response to allo–antigens is one of the reasons for the development of chronic allograft nephropathy and rejection[2–4].

The role of antibodies against human leukocyte an–tigens (anti–HLA Abs), especially anti–HLA donor–specific antibodies (HLA DSA) in the failure of kidney allografts has been understood more clearly over the past decade[5–7]. The presence of anti–HLA Abs is a major factor causing acute–mediated rejection and also may lead to chronic antibody–mediated allograft rejection[8].

In addition to anti–HLA Abs, antibodies against MHC Class Ⅰ–related Chain A antigen (anti–MICA Abs) also play an important role in the renal allograft rejection, which develops more frequently in patients with graft failure than in those with functioning grafts[9]. Accumulated data shows that specific anti–MICA Abs are related to allograft dysfunction and rejection, even if anti–HLA Abs are absent[10]. MCIA is a member of the non–classical HLA Class–Ⅰ family with a large degree of polymorphism expressed on the surfaces of dendritic cells, monocytes, activated T cells, keratino–

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cytes, fibroblasts, endothelial and epithelial cells[11–13].

The introduction of the Luminex solid−phase tech−nique allows us to detect with more specificity and higher sensitivity HLA Abs and MICA Abs which play detrimental roles in immunologic processes causing renal allograft rejection[14,15]. The purpose of this study was to investigate the prevalence of anti−HLA and anti−MICA Abs in renal transplantation pa−tients with long−term survival of over 5 years by us−ing Luminex solid−phase assay. The correlation of the presence of antibodies against HLA, DSA and MICA with renal function was also investigated.

MATERIALS AND METHODS

Patients

This retrospective study enrolled 110 patients with long−term survival of over 5 years who experienced renal transplantation from 1993 to 2009 at different hospitals and transplant centers. The study was ap−proved by the institutional review boards of Beijing Red Cross Blood Center and all patients signed the written informed consent. The mean age of 110 pa−tients at the time of transplantation as well as their sex, months after transplantation, donor type (deceased or living), HLA mismatch number and therapeutic regi−men [Tacrolimus (FK506) or Cyclosporine (CsA) plus mycophenolatemofetil (MMF) and prednisone] are shown in ***Table 1***. The HLA typing of recipients and donors for HLA−A, −B, −DR and −DQ was available for all cases so the DSA for HLA could be determined but not for MICA.

Table 1 **General characteristics of patients with and without anti−HLA and/or MICA antibodies**

Characteristics	HLA and/or MICA Abs (*n*=72)	HLA Abs (*n*=61)	MICA Abs (*n*=31)	Antibody negative (*n*=38)	*P*[*]
Age at time of transplant (year, mean±SD)	39.82±11.13	39.92±11.24	39.06±9.19	37.24±12.34	NS
Time after transplant (months, mean±SD)	114.51±33.73	113.46±32.11	113.68±34.37	130.78±40.36	<0.05
Gender, male (*n*)	33	27	14	21	NS
Scr level(mg/dL, mean± SD)	2.74±2.18	2.84±2.27	2.40±1.43	2.33±1.34	NS
eGFR[a](mean±SD)	39.07±27.98	37.51±27.78	40.43±26.19	45.19±32.62	NS
Baseline immunosuppression (count)					NS
CsA/MMF/prednisone	61	51	29	21	
FK506/MMF/prednisone	11	10	2	17	
BUN level(mmol/L, mean±SD)	12.65±6.70	12.91±6.93	11.97±5.42	13.57±9.32	NS
HLA mismatch number(*n*, mean±SD)	4.58±1.71	4.70±1.76	4.12±1.36	2.97±1.62	<0.01

a, eGFR (mL/min/1.73 m^2) = 186×Scr (mg/dL)$^{-1.154}$×[age (years)]$^{-0.203}$×0.742 (female).

[*]*P* indicates the comparison of inter groups; NS, no significance.

Detection of anti−HLA and MICA Abs

Sera samples were collected when these patients came to hospital for follow−up review from May 2014 to November 2014. Each serum sample was screened firstly for HLA Class Ⅰ, Class Ⅱ and MICA antibod−ies with LABScreen Mixed beads (One Lambda, Inc., Canoga Park, CA). If showing positive results, the sera of patients were then tested by LABScreen Single Antigen beads (One Lambda, Inc., Canoga Park, CA). All tests were performed according to the manufactur−er´s recommendation. Briefly, 20 μL test serum were added to 96−well micro−plate with 5 μL beads in each well, then incubated for 30 min at room temperature in the dark place, and washed 3 times with wash buff−er; 100 μL goat anti−human IgG secondary antibody labeled with R−phycoerythrin (PE) were added and incubated for 30 min in the dark at room tempera−ture, and then washed 3 times again with wash buffer and read on the LABScan 100 flow cytometer (One Lambda, Inc.). A negative control serum (LSNC; One Lambda, Inc.) was also included in each assay, which

was used to normalize the mean fluorescence inten−sity (MFI) of test samples and calculate the normal−ized background ratio (NBG ratio). NBG ratios >2.2 was considered as positive results. The NBG ratio was calculated by the following formula: NBG ratio = (S#N −SNC bead)/(BG#N−BGNC bead). In which, S#N represents sample−specific fluorescent value for bead #N; SNC bead represents sample−specific fluorescent value for Negative Control bead; BG#N represents background NC serum fluorescent value for bead #N and BGNC bead represents background NC serum fluorescent value for Negative Control bead.

Measurement of renal function

The serum creatinine level (Scr, mg/dL) and blood urea nitrogen level (BUN, mmol/L) in the blood samples of patients were tested by Hitachi 7060 Au−tomatic Biochemical Analyzer. The estimated glo−merular filtration rate (eGFR) was calculated with the equation published previously by Schwartz *et al*. [16]. These three measurements were used to assess renal graft function.

Statistical analysis

The continuous variables were expressed as means standard deviations and discrete variables were presented as numbers or percentages. The chi−square test was used to compare the categorical data and the unpaired *t* test or one−way ANOVA analysis was applied for the continuous data. Two−sided *P* values less than 0.05 were considered significant. Risk factors for anti−HLA DSA Abs production were assessed using Cox proportional hazard regression analyses in which the following potential confounders were considered: the year of transplantation; the age of recipients; the type of transplantation (living or deceased); the patient´s sex; number of HLA−A, −B, −DR, −DQ mismatches; type of immunosuppression (FK506/MMF/Prednisone or CsA/MMF/prednisone), and eGFR at the time of testing. Factors found to be significant on univariate analysis (*P* value < 0.1) were introduced into a backward stepwise Cox regression model (multivariate analyses). Final multivariate analysis factors are reported as a hazard ratio with a 95% confidence interval. The software packages SPSS (version 14.0) was used to process data and perform statistical analyses.

RESULTS

General characteristics of patients with long−term survival

The general characteristics of all patients with survival of over 5 years are shown in **Table 1** according to their different HLA and/or MICA Abs status. The enrolled 110 renal transplants were divided into four groups including anti−HLA and MICA Abs positive group, anti−HLA Abs positive group, anti−MICA Abs positive group and antibody negative group. As shown in **Table 1**, 72 patients had anti−HLA Abs and/or MICA Abs at the time of test, 61 had anti−HLA Abs, 31 had anti−MICA Abs, and 38 were antibody negative. There was no significant difference among these groups in terms of age at time of transplant, gender proportions and baseline immunosuppression. Although the mean value

of Scr level, eGFR or BUN level was higher in the antibody positive group, there was also no statistically significance among the 4 groups as tested by one−way ANOVA method. The patients with anti−HLA or MICA Abs had a significantly longer post−transplant survival time than those without antibodies. Moreover, the HLA mismatch number would significantly affect the development of anti−HLA or MICA Abs because there was a statistically significant difference when comparing this variable among four groups.

The incidence and specificity of anti−HLA and MICA Abs in renal transplant patients

A Luminex Single Bead kit was used to determine the specificity of anti−HLA Abs in the serum showing anti−HLA Abs positive results on mixed beads. All patients were divided into two groups according to their serum creatinine level, i.e. the normal group (with Scr level lower than 2.0 mg/dL) and dysfunction group (with Scr level above 2.0 mg/dL).

The incidence and specificity of anti−HLA and MICA Abs in patients with or without normal renal graft function is listed in **Table 2**. 22 (51.16%) of the patients with normal renal function developed anti−HLA Abs, including 4 (9.30%) non−donor specific anti−HLA antibodies (NDSA) and 18 (41.86%) DSA; 5 (11.63%) developed anti−MICA Abs. In contrast, 39 (58.21%) of patients from the dysfunction group produced anti−HLA Abs including 13(19.40%) NDSA and 26 (38.80%) DSA; 10 (14.93%) produced anti−MICA Abs. Among the HLA DSA in the graft functioning group, 10 (23.26%) were HLA Class Ⅰ and 9 (20.93%) were HLA Class Ⅱ. The numbers of patients with HLA Class Ⅰ and Class Ⅱ DSA in the graft dysfunction group were 8 (11.94%) and 21 (31.34%) respectively. Between two groups, there was no difference in the number of patients developing NDSA, DSA, Class Ⅰ DSA, Class Ⅱ DSA or MICA Abs. The highest mean signal intensity of antibodies as measured by the MFI was higher in the dysfunction group, but there was no statistically significant dif−

Table 2 **The incidence and specificity of anti−HLA and MICA Abs in the patient with or without normal renal graft function**

Antibody status	Normal group (*n*=43)		Dysfunction group (*n*=67)		*P* value[*]	Total (*n*=110)
	Cases (%)	MFI (mean±SD)	Cases (%)	MFI (mean±SD)		
HLA	22 (51.16)		39 (58.21)			
NDSA	4 (9.30)	3,110±5,334	13 (19.40)	3,116±4,394	0.9982	17
DSA	18 (41.86)	1,460±2,441	26 (38.80)	5,331±5,586	0.0086	44
Class Ⅰ	10 (23.26)	997±362	8 (11.94)	2,167±3,207	0.2657	18
Class Ⅱ	9 (20.93)	2,813±4,643	21 (31.34)	5,844±5,886	0.1820	30
MICA	5 (11.63)	752±541	10 (14.93)	5,575±7,191	0.1654	15

[*]*P* value indicates the difference of MFI between two groups.

ference between the two groups in terms of NDSA, Class Ⅰ DSA, Class Ⅱ DSA and MICA. The dif−ference in average MFI was statistically significant only in the case of HLA DSA. The MFI of HLA DSA in patients with dysfunctional renal grafts was much higher than those in patients with normal renal func−tion (1,460$\pm$2,441 *vs.* 5,331$\pm$5,586, *P*=0.0086). Although there was no statistically significant dif−ference between normal and dysfunction groups in terms of anti−MICA Abs (owing to the small sample number and large standard deviation), a large differ−ence was found between their mean MFI (752$\pm$541 *vs.* 5,575$\pm$7,191, *P*=0.1654).

Table 3 displays the characteristics of all DSA(+) patients including those with HLA Class Ⅰ and Ⅱ Abs status, HLA ABDRDQ mismatch with donor and specific type of HLA DSA against each type of Class Ⅰ and Class Ⅱ molecules with the correspond−

Table 3 **Characterization of donor specific antibody (DSA) positive patients**

DSA (+) patient ID	Class Ⅰ and Ⅱ Abs status	HLA MM with donor (A/B/DR/DQ)	DSA Class Ⅰ (MFI)	DSA Class Ⅱ (MFI)
1	−/+	2/1/1/−	−	DR4 (3,144)
2	−/+	0/2/2/1	−	DR9 (556), DQ9 (521)
3	+/−	1/2/1/1	B60 (395)	−
4	−/+	2/2/2/2	−	DR7 (1,636), DQ4 (1,219)
5	−/+	1/2/2/2	−	DR4 (10,795), DR15 (9,396), DQ6 (6,413), DQ8 (10,436)
6	+/−	1/1/0/0	A2 (1,625), A75 (964)	−
7	−/+	2/2/1/1	−	DR17 (1394), DQ2 (489)
8	−/+	2/2/2/1	−	DR9 (534), DR10 (4,565), DQ9 (2,306)
9	+/−	1/1/0/1	B75 (2,884)	−
10	−/+	2/2/1/1	−	DR17 (1,592)
11	+/+	2/2/2/−	−	DR4 (250), DR12 (331)
12	+/−	1/1/1/−	A11 (1,016), B48 (298)	−
13	−/+	1/1/2/1	−	DR12 (9,086), DR15 (3,470)
14	+/−	2/1/0/−	A2 (1,593)	−
15	−/+	0/2/1/1	−	DR8 (6,998), DQ4 (8,726)
16	+/−	1/2/1/1	B7 (613)	−
17	+/−	1/1/1/−	A11 (1,455), B48 (473)	−
18	+/+	1/2/2/1	A30 (577)	DR7 (418),DR12 (768)
19	−/+	1/1/1/−	−	DR15 (409)
20	−/+	2/2/2/1	−	DR9 (1,482), DR10 (8,839), DQ9 (5,410)
21	+/−	1/1/1/2	A33 (764), B52 (355)	−
22	+/1	2/2/1/1	A2 (708), B54 (421)	−
23	−/+	2/0/1/2	−	DR12 (7,519), DQ7 (8,321), DQ8 (3,399)
24	−/+	1/1/1/−	−	DR15 (336)
25	−/+	2/2/2/1	−	DR9 (1,314), DR10 (8,014), DQ9 (4,782)
26	+/−	1/1/1/2	A33 (403)	−
27	−/+	0/2/1/1	−	DR8 (8,038), DQ4 (9,229)
28	+/+	1/2/1/1	B7 (595)	DR4 (1,138)
29	−/+	1/2/2/2	−	DR4 (9,879), DR15 (9,464), DQ6 (6,805), DQ8 (7,382)
30	+/−	2/1/0/−	A2 (1,037), B48 (452)	−
31	−/+	1/1/2/1	−	DR12 (8,408), DR15 (2,801)
32	+/−	1/1/1/−	A11 (748)	−
33	−/+	0/1/2/1	−	DR7 (521), DR17 (834), DQ4 (358)
34	+/+	2/2/1/1	A2 (804)	DR13 (205)
35	−/+	0/1/1/−	−	DR15 (883)
36	+/+	1/1/1/−	A11 (9,836), B48 (1,953)	DR12 (265)
37	−/+	1/1/1/−	−	DR15 (366)
38	+/−	2/2/1/1	A2 (505), B54 (792)	−
39	−/+	1/2/2/2	−	DR4 (9,779), DR15 (8,375), DQ6 (10,686), DQ8 (11,032)
40	+/−	2/1/0/−	A2 (1,203), B48 (501)	−
41	−/+	1/1/1/−	−	DR15 (286)
42	−/+	0/2/1/1	−	DR8 (6,620), DQ4 (8,796)
43	+/+	1/2/1/1	A24 (16,527),B46 (20,077),B35 (24,296)	DR4 (24,514),DQ9 (23,185)
44	−/+	1/1/1/−	−	DR15 (874)

ent MFI value. There were 44 patients who developed HLA DSA after transplantation. In these 44 DSA−positive patients, 18 had HLA Class Ⅰ DSA and 30 patients had HLA Class Ⅱ DSA, whereas 6 displayed both HLA class Ⅰ and Ⅱ DSA.

The correlation of antibody status with renal allo−graft function

The Scr level, eGFR calculated according to Scr, and BUN level are three important indicators for re−nal graft function. ***Table 4*** displays their values in patients with different antibody statuses. For the Scr, eGFR and BUN level, patients with anti−HLA and/or MCIA Abs, anti−HLA Abs, anti−HLA DSA, anti−Class Ⅰ DSA, anti−Class Ⅱ DSA or MICA Abs were not statistically different to antibody−negative pa−tients. These results implied that the levels of anti−HLA and/or MICA antibodies had no direct associa−tion with renal graft function in long−term survival renal recipients.

Table 4 Comparison of eGFR between anti−HLA and/or MICA antibody− positive and negative patients

HLA and/or MICA antibody status	Scr (mg/dL)	P^*	eGFR (mL/min/1.73 m^2)	P^*	BUN (mmol/L)	P
Antibody negative (*n*=38)	2.33±1.34	–	45.18±32.62	–	13.57±9.32	–
HLA and/or MICA Abs positive (*n*=72)	2.74±2.18	0.2927	39.07±27.98	0.3064	12.83±6.70	0.6326
HLA Abs positive (*n*=61)	2.84±2.27	0.2128	37.51±27.78	0.2147	13.13±6.93	0.7888
HLA DSA positive (*n*=44)	2.80±2.44	0.2936	39.81±28.33	0.4273	12.61±7.07	0.5980
Class Ⅰ DSA positive (*n*=19)	2.56±2.96	0.6867	47.49±32.30	0.8013	12.66±8.43	0.7215
Class Ⅱ DSA positive (*n*=32)	2.85±1.95	0.1925	36.04±26.74	0.2097	12.62±6.04	0.6220
MICA Abs positive (*n*=31)	2.40±1.43	0.8347	40.43±26.19	0.5140	11.97±5.42	0.4008

$^*P$ indicates the difference with antibody −negative patients

Risk factors for anti−HLA DSA production assessed by Cox proportional hazard regres−sion analyses

The Cox proportional hazard method was used to examine the relationship between the presences of anti−HLA DSA among different subpopulations of patients (***Table 5***). Univariate analysis of risk factors for development of anti−HLA DSA showed patients´ age at transplant and eGFR at the time of testing to be insignificant ($P > 0.1$). Two variables of recipient gender and immunosuppression were also not signifi−cantly associated with the development of anti−HLA DSA on univariate analysis if $P < 0.05$ was considered as significant, but the type of transplantation and HLA mismatch number were significantly associated with the production of anti−HLA DSA($P < 0.05$). Four co−variables with $P < 0.1$ on univariate analysis were introduced into a backward stepwise Cox regression model (multivariate analyses) and the results suggest−ed that the type of transplantation and HLA mismatch number were significant negative risk factors for the development of anti−HLA DSA ($P < 0.05$).

DISCUSSION

The Luminex platform was used in this study to detect HLA and MICA Abs of post−transplant serum samples in a group of long−term survival patients with renal allo−graft. The prevalence of anti−HLA and MICA Abs as well as the relation−ship between humoral responses and allo−graft function were also analyzed in order to understand the meaning of anti−HLA/MICA antibody moni−

toring and to predict the risk of graft failure.

The occurrence of HLA Abs, especially HLA DSA, is known to play an important role in renal graft dysfunction and loss by initiating the pro−cess of damage and repair in the endothelium of blood vessels. Many earlier studies have also pro−vided numerous evidence showing the importance of anti−HLA antibodies to allo−graft longevity. However, we should interpret the antibody moni−toring results with caution, especially for patients

Table 5 Cox proportional hazard analysis of risk fac−tors for HLA DSA development

Variable	Hazard ratio	P	95%CI
Univariate analysis			
Female gender	0.616	0.058	0.373–1.017
Age at transplant	0.990	0.435	0.965–1.105
Living donor	2.189	0.025	0.044–0.809
HLA−ABDRDQ mismatch number			
0–2	1		
3–4	1.848	0.048	1.005–3.398
5–6	1.966	0.102	0.875–4.420
FK506/MMF/predisone regimens	2.163	0.056	0.313–4.014
eGFR at the time of testing			
<30 mL/min/1.73 m^2	1		
30–60 mL/min/1.73 m^2	1.303	0.509	0.595–2.853
>60 mL/min/1.73 m^2	1.248	0.429	0.721–2.158
Multivariate analysis			
Female gender	1.559	0.199	0.791–3.070
Living donor	2.072	0.034	0.721–3.814
HLA−ABDRDQ mismatch number			
0–2	1		
3–4	2.572	0.045	1.023–6.466
5–6	4.409	0.003	1.631–11.924
FK506/MMF/predisone regimens	2.060	0.247	0.607–6.992

with normal−function grafts as suggested by Bartel *et al.* [17]. In this study, all 110 enrolled patients received renal transplants for at least 5 years. Anti−HLA antibody−positive patients, even DSA−positive patients, had comparable Scr, eGFR and BUN levels with antibody−negative patients (**Table 4**). Between patients with and without normal graft function, there was no statistically significant difference *no matter what their antibody statuses were*. Thus, it´s clear that allo−grafts can survive for many years in the presence of antibodies. Although many groups had demonstrated the presence of DSA is associated with a significantly decreased graft survival, there were some other papers which showed a similar incidence of rejection, similar serum creatinine levels and a similar graft survival in patients with and without DSA[18]. Possible reasons for this include: the border between positive and negative reactions differs between centers and amongst studies; some DSA is non−complement fixed, which doesn´t mediate rejection; HLA antibodies are frequently observed in the sera from healthy males with no history of allo−sensitization.

This study performed a retrospective analysis of post−transplant anti−HLA and/or MICA Abs development in a cohort of 110 long−term survival renal transplantation patients. Among them, 44 (40%) patients developed anti−HLA DSA Abs after transplantation. According to an investigation of former studies, the prevalence of alloantibodies against donor HLA molecules was highly variable, from 1.6% to 60% of the patients [19]. This great variation is most likely due in part to a *different* MFI cut−off value, which is used to distinguish the positive from negative result and which is different, being set by various studies and authors [20]. Many centers use a threshold of over 1,000 for HLA DSA positivity which results in a close apparent association between the development of DSA and graft damage. However, a few studies with a lower MFI threshold of $\geqslant 300$[21] or even $\geqslant 100$ [22], for DSA positivity also demonstrated the clinical importance of anti−HLA DSA Abs to graft function and failure. Unfortunately, there is no a consensus at the moment on MFI cut−off value, even though it is highly desirable for solid organ transplantation to have standardized guidelines for testing and management of DSA [23]. The higher frequency of anti−HLA DSA Abs (40%) in this study may have the following two reasons. Firstly, the positive reaction was set as NBG ratios >2.2 rather than a certain MFI value. Secondly, kidney transplantations of all patients involved in this analysis were carried out

at least 5 years ago and the chance of developing anti−HLA DSA Abs would be increased over time as suggested by Lee *et al.* [24].

Although the number of patients with differing antibody statuses in the graft functioning group had no statistically significant difference with those in the graft dysfunction group, the average MFI was higher in the latter group for those patients with anti−HLA DSA, Class Ⅰ, DSA, Class Ⅱ DSA and MICA antibodies. Importantly, the difference between the two groups had a statistical significance in terms of anti−HLA DSA Abs ($P=0.0086$). The lack of statistical significance for Class Ⅰ, DSA, Class Ⅱ DSA and MICA may have resulted from small sample volume and large standard deviation. These results repeatedly indicate the big challenge in determining the cut−off value of MFI for HLA DSA positivity.

Alternatively, previous studies have suggested that antibodies against MICA also have an adverse effect on graft survival, as MICA Abs could be detected among 37% patients without anti−HLA antibodies and whose grafts had failed [9]. However, in this study, we didn´t find a correlation between MICA anti−Abs and allo−graft function.

The occurrence of post−transplant NDSA also had adverse effects on renal allo−grafts as shown in an earlier study [25], but there was no evidence showing a significant relationship of NDSA with renal allo−graft function in this analysis, because the average MFI of NDSA in the graft functioning group was comparable with that of NDSA in the graft dysfunction group.

In summary, our study retrospectively analyzed a group of long−term survival patients with renal allo−transplantation and the results showed that a relatively high proportion of patients produced post−transplant anti−HLA and/or MICA antibodies, some of them with anti−HLA DSA Abs. We did not find a strong association between the occurrence rates of either *antibody with renal function*, which is in contrast with most previous studies. We believe it´s very important to monitor the development of anti−HLA and/or MICA antibodies in patients with renal allo−grafts, so as to offer the timely adjustment of immunosuppression regimens of post−transplantation. Over the last decade, advances in antibody detection, especially after introduction of Luminex single−antigen microsphere technology, have revolutionized our approach to kidney transplantation. However, its high sensitivity, which although is able to detect antibodies earlier, is a double−edge sword for doctors and

patients. We have to *carefully interpret* the results and estimate its effect on *graft function and survival*. Furthermore, the cost of regularly detecting anti−HLA/MCIA antibodies by LABScreen method is relative high for most Chinese patients. So currently, we need *much more evidence* to assess the importance of anti−HLA/MICA antibody monitoring in the future.

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