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Predicting renal graft failure by sCD30 levels and *de novo* HLA antibodies at 1 year post-transplantation

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ABSTRACT

HLA antibodies and sCD30 levels were detected in the serum sampled from 620 renal graft recipients at 1 year post-transplantation, which were followed up for 5 years. Six-year graft and patient survivals were 81.6% and 91.0%. HLA antibodies were detected in 45 recipients (7.3%), of whom there were 14 cases with class I antibodies, 26 cases with class II, and 5 cases with both class I and II. Much more graft loss was record in recipients with HLA antibodies than those without antibodies (60% vs. 15.1%, $p<0.001$). Significantly higher sCD30 levels were recorded in recipients suffering graft loss than the others (73.9 ± 48.8 U/mL vs. 37.3 ± 14.6 U/mL, $p<0.001$). Compared with those with high sCD30 levels, recipients with low sCD30 levels (<50 U/mL) had much better 6-year graft survival (92.4% vs. 46.6%, $p<0.001$). Further statistical analysis showed that detrimental effect of *de novo* HLA antibodies and high sCD30 on graft survival was not only independent but also additive. Therefore, post-transplantation monitoring of HLA antibodies and sCD30 levels is necessary and recipients with elevated sCD30 level and/or *de novo* HLA antibody should be paid more attention in order to achieve better graft survival.

1. Introduction

HLA antibodies have been detected in the serum of renal graft recipients for more than four decades [1]. Although many stable recipients with circulating HLA antibodies and excellent graft function have been reported [2,3], the finding that the occurrence of HLA antibodies is strongly associated with subsequent graft loss was confirmed by more and more studies during last decade [4,5]. The major cause of late kidney transplant failure was found to be antibody-mediated microcirculation injury [6]. All of these studies emphasized the importance of monitoring for the presence of *de novo* HLA antibodies post-transplantation.

Soluble CD30 molecule is another immunologic factor closely involved in transplantation, which was researched in detail in recent years [7–11]. Except for the well documented role in predicting acute rejection and graft failure, pre-transplantation sCD30 level is an independent predictor of pneumonia in renal transplant recipients [12]. Therefore, sCD30 levels may reflect an immune state of recipients, which is closely related to patient and graft survival. However, there are relatively few studies with long-term outcomes, which combined the detections of HLA antibodies and sCD30 levels post-transplantation.

In this single center prospective study of 620 renal graft recipients, it was reported that elevated sCD30 levels and the presence of HLA antibodies were associated with an increased risk of subsequent graft failure and that the detrimental effects of elevated sCD30 levels and the presence of HLA antibodies on graft survival are independent and additive.

2. Patients and methods

2.1. Patients

In this prospective study, an overall cohort of 620 recipients was enrolled, who received their deceased donor renal allograft between January 2003 and December 2005 at Fuzhou General Hospital. This was a consecutive series. All the recipients had a functioning graft and were detected for sCD30 levels and HLA antibodies at 1 year post-transplantation. Then they were followed up for at least 5 years. To avoid the effects of preformed HLA antibodies on renal graft survival, recipients with positive PRA (ELISA) pre-transplantation were excluded from this study. Informed consent was obtained from each patient, and the study conformed to the Declaration of Helsinki concerning

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medical research in humans. Donor–recipient blood group matching was identical in all included recipients. HLA crossmatches of patients were negative, which were determined by microdroplet assay of complement-dependent lymphocytotoxicity (CDC). PRA was determined by LAT-M® ELISA (One Lambda, Inc., Canoga Park, CA). Maintenance immunosuppressive regimens almost were standard triple therapy, which consisted of a calcineurin inhibitor (CsA microemulsion–Neoral or Tacrolimus), combined with prednisone and mycophenolate mofetil (MMF). Besides the regimens mentioned above, azathioprine (Aza), mizoribine (MZR) or sirolimus (Rap) were used in a few patients instead of MMF. There were several recipients suffering conversion between CsA and Tacrolimus due to different reasons. The number of those patients was small, so immunosuppressive regimens except standard triple therapy were defined as other regimens.

2.2. Measurement of serum sCD30

Blood samples of recipients were obtained at 1 year post-transplantation. Then sera was separated from cells by centrifugation, and stored at −70 °C until being tested. Human sCD30 instant ELISA kits were obtained from Bender MedSystems (Vienna, Austria). Serum levels of sCD30 were measured in a duplicate manner using ELISA kit according to manufacturers' instructions. As indicated by the manufacturer, the intra-assay and inter-assay coefficients of variation were less than 10% and 20%, respectively.

2.3. HLA-specific antibody detection

All sera were screened by ELISA assays (LAT-M, One Lambda, Canoga Park, CA) to determine the presence or absence of anti-HLA class I or class II antibodies according to the manufacturer's instructions. The positive cutoff score was calculated as 0.2 times the blank adjusted positive control value. Anti-HLA class I antibodies specificities were identified by using a high-definition (HD) single-antigen, ELISA (LAT-1HD, One Lambda), which uses 88 different HLA A and B alleles produced by recombinant technology. For anti-HLA class II antibodies, another ELISA test (LAT 2–40, One Lambda) was performed, which identified DR and DQ subtypes on a panel of purified HLA antigens. Both ELISA tests were performed as recommended by the manufacturer.

HLA typing of transplant recipients was performed by molecular biology (Innolipa HLA typing kit, Innogenetics, Belgium). For all kidney transplant donors, HLA A, B, DR and DQ tissue-typing was performed using the microlymphocytotoxicity technique with One Lambda INC tissue-typing trays and was controlled by molecular biology.

2.4. Patients follow-up

Once detections of sCD30 levels and HLA antibodies were performed, the recipients were routinely followed up on out-patient clinic for at least 5 years or until death. Information on graft function and patient survival was recorded at least every 6 months. Graft loss was defined as a return to chronic dialysis.

2.5. Statistical analysis

EXCEL2003, SAS9.1 and SPSS 13.0 were used for statistical analysis. *T* test or T' test, Analysis of variance of complete random design, Student–Newman–Keuls tests, Chi-Square test, Fisher's Exact Test (2-Tail), Kruskal–Wallis Test, multivariable Cox proportional hazards models, and Kaplan–Meier survival analysis were used for statistical analysis. P values <0.05 were considered significant.

3. Results

3.1. Main outcome

The overall patient number was 715, who received their deceased donor renal allograft between January 2003 and December 2005 at Fuzhou General Hospital. There were 95 recipients excluded from this study, which included 78 recipients with positive PRA pre-transplantation and 17 cases suffering graft loss during the first year post-transplantation. During the 5-year follow-up period, 114 out of 620 recipients (18.4%) suffered graft loss. The median time of graft loss was 45 (15–71) months post-transplantation. Seven recipients died with a functioning graft and 49 recipients died after graft loss during 5-year follow-up period. Six-year graft and patient survivals were 81.6% and 91.0%. The recipients were divided into two groups according to graft status: Group N (with functioning graft, n = 506) and Group F (suffering graft loss, n = 114). Demographic data and other related parameters were shown in Table 1.

3.2. Frequency of HLA antibodies at 1 year post-transplantation

HLA antibodies were detected in 45 recipients (7.3%) at the first year post-transplantation. Antibodies were directed against HLA class I specificities in 14 (31.1%) recipients, class II in 26 (57.7%) and a combination of class I and II in 5 (11.1%). Of the 45 recipients who produced *de novo* HLA antibodies after transplantation, in 38 (84.4%) cases these were donor specific antibodies (DSA). In the 38 recipients who made *de novo* DSA, the majority of antibodies were directed against class II with 24 recipients producing class II antibodies alone, 5 recipients with both class I and II and just 9 recipients class I alone.

3.3. Post-transplant HLA antibodies and graft survival

Much more graft loss (60%, 27 out of 45) was record in recipients with HLA antibodies than those without antibodies (15.1%, 87 out of 575) during 5-year follow-up ($p<0.001$). Six-year survival curve was shown in Fig. 1A. Univariable Cox models were used to further analyze these data. Results showed that *de novo* HLA antibody production after transplant has an obvious detrimental effect on renal graft survival ($p<0.001$, hazard ratio (HR) = 5.483). As shown in Fig. 1B, when *de novo* HLA antibody production was categorized into DSA (n = 38) and non-DSA (n = 7), there was a strong association between DSA and decreased graft survival ($p<0.001$, HR = 6.054) compared with recipients with no detectable DSA (including negative HLA antibodies and non-DSA, n = 575). Because the number of recipients with non-DSA was small, statistical analysis was not performed on the relation between non-DSA and graft loss.

As showed in Fig. 1C, when survival curves were drawn according to donor specific HLA antibody class, the recipients with DSA against both class I and II had the worst 6-year graft survival. Although significant difference of 6-year graft survival was observed between the recipients with DSA against class I and those with class II, both DSA against class I and II had an obvious detrimental effect on renal graft survival. Chi square test and Fisher's Exact Test were also used to access the HR of different HLA antibody classes. Results were showed in Table 2, which were similar to those showed in Fig. 1C. Recipients with HLA antibody against both class I and II had the highest risk of graft loss, and recipients with only class II antibodies had higher risk of graft than those with only class I antibodies.

3.4. Serum sCD30 levels at 1 year post-transplantation

Serum sCD30 levels at 1 year post-transplantation ranged from 12.9 to 323.6 U/mL. The mean sCD30 levels was 44.0 ± 28.4 U/mL, which were similar to that of healthy individuals and much higher than that detected at month 1 post-transplantation reported in our previous study [11,13]. Serum sCD30 levels were not significantly different between recipients with HLA antibodies and those without HLA antibodies

Table 1
Demographics, pre-transplantation status and immunosuppressive protocols of recipients.

Characteristic	Group N	Group F	*P* value
Patient No.(Female)	506 (115)	114 (35)	0.090
Recipient age (year)	39.0 ± 10.8	38.7 ± 10.9	0.428
Donor age (year)	32.9 ± 8.3	33.7 ± 8.4	0.853
Dialysis time (median, month)	11 (0–58)	10 (0–56)	0.915
Dialysis modality			
Hemodialysis	441	99	0.876
Peritoneal dialysis	65	15	
No. of HLA MM	2.8 ± 1.1	2.8 ± 1.3	0.961
HLA MM ≤ 3	412	84	0.070
HLA MM > 3	94	30	
Cold ischemia time (hour)	9.1 ± 2.5	9.6 ± 2.1	0.581
Immunosuppression protocol			
CsA + MMF + Pre	196	55	0.001
FK + MMF + Pre	271	41	
Others	39	18	