CHAPTER 32

A Report of the Epidemiology of De Novo Donor-Specific Anti-HLA Antibodies (DSA) in "Low-risk" Renal Transplant Recipients

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INTRODUCTION

A continual epidemiological study of donor specific anti-HLA antibody associated allograft destruction commenced in 1999 in the transplant patient population from the Brody School of Medicine at East Carolina University and has continued until present day. This study focuses on the low-immunologic risk (low-risk) patient defined as a primary transplant recipient that is free of DSA on solid phase assay and has a negative crossmatch prior to transplantation. Additionally, this study is limited to those patients transplanted between the dates of March 1999 and February 2006. This study is concerned with the extent and development of DSA in the low-immunologic risk transplant recipient, and with the study of factors associated with DSA onset. Data from each patient was collected at 5 or more time points in the first post-transplant year (1, 3, 6, 9, and 12 months post-transplant) and at least annually thereafter. The present report describes DSA epidemiology observed in the five years following each individual's transplant date.

METHODS

Patients

A total of 224 consecutive patients, who received a renal transplant between the dates of March 1999 and February 2006 at the Brody School of Medicine, East Carolina University (Greenville, North Carolina), were enrolled. At time of transplant, all patients were tested for reactivity to their donor via complement-dependent cytotoxicity crossmatch. Flow cytometric crossmatch was performed on all living-donor transplants. We excluded all patients found to have crossmatch positivity. In addition we excluded patients with alloantibodies present in circulation (and detected via single antigen bead assay) at the time of transplant or within the first month post-transplant that would be reactive to donor typing specificities. All patients received induction therapy with either rabbit antithymocyte globulin or a humanized anti-interleukin-2 receptor monoclonal antibody. Maintenance immunosuppression consisted of a calcineurin inhibitor (cyclosporine or tacrolimus) along with a mycophenolic acid derivative. Patients

received a corticosteroid taper starting at the time of transplant. By two months post-transplant, patients were reduced to and then maintained a level of prednisone 10 mg/day.

Table 1. Completeness of clinical and allograft mortality follow-up five years after transplantation.

Follow-up	Number of Subjects	Percent
Total population	224	100
Subjects with complete 5-year follow-up	183	81.7
Subjects with allograft failure before 5-year exam	21	9.4
Subjects that died before 5-year exam	16	7.1
Subjects lost to follow-up before 5-year exam	2	0.9
Subjects who moved prior to 5-year exam	2	0.9

Study Protocol

Testing and the use of patient data were approved by the East Carolina University Brody School of Medicine Institutional Review Board for human studies. All clinical and research activities are consistent with the Principles of the Declaration of Istanbul.

Follow-up

An analysis of the follow-up status of the 224 subjects who were initially transplanted is shown in Table 1. Nearly all patients were followed for 5 years from the date of transplantation. Less than 1 percent of the total population could not be accounted for at 5 years post-transplantation.

Pre-transplant Screening

All patients underwent a standard pre-transplant evaluation, including cardiac evaluation and screening for cancer. All patients also had an HLA antibody screening by panel reactive antibody (PRA) testing on cyctotoxicity (pre-2002) or ELISA methods (2002 – 2006). Although a small percentage of patients had higher than 20% PRA, retrospective testing of pre-transplant samples using LABScreen beads (described later) indicates that no patients had pre-transplant donor-specific antibodies. Tissue typing was performed using both serology and polymerase chain reaction-single-specific-primer (SSP) methods for HLA-A, -B, -DR, and –DQ antigens.

Anti-HLA-Specific IgG Antibody Detection Protocol

In addition to a pre-transplant sample, patients were routinely monitored at 1, 3, 6, 9, 12 months post-transplant and annually thereafter, for the development of HLA Class I and II antibodies using LABScreen® Mixed beads (One Lambda, Inc., Canoga Park, CA). The LABScreen beads are color-coded and coated with purified HLA Class I and/or Class II antigens. The HLA antigens used to coat the beads consists of a defined pool of HLA antigens, including rare alleles, to increase the chance of detection of all antibodies. Samples that tested positive on LABScreen® Mixed beads were also tested using LABScreen® Single Antigen Class I and II beads (One Lambda, Inc., Canoga Park, CA) to determine antibody specificity. All LABScreen® tests were performed according to the manufacturer's protocol. Briefly, 20µL of test 1:3 serum was incubated with antigen beads for 30 minutes at room temperature in the dark. After three washes, 100µL of anti-human-IgG-PE was added. After an incubation step, samples were washed and read on the LABScan®100 flow analyzer (One Lambda, Inc., Canoga Park, CA). A negative control serum was included in every testing and was used as the background control to normalize the sample data. HLA antibodies were analyzed as mean fluorescence intensity (MFI) values. De novo anti-HLA antibodies were considered positive if it was a new antibody not present at time of transplantation with a normalized intensity via single antigen bead of 1000 MFI or greater. For analysis, the antibody to first appear post-transplant was used. If multiple antibodies appeared at the same time post-transplant, the highest intensity (highest MFI) antibody was chosen for analysis.

RESULTS

Prevalence of De novo DSA In 1999-2006

Of the 224 patients who were studied, de novo donor-specific anti-HLA antibodies were most likely to develop in the first year post transplant.

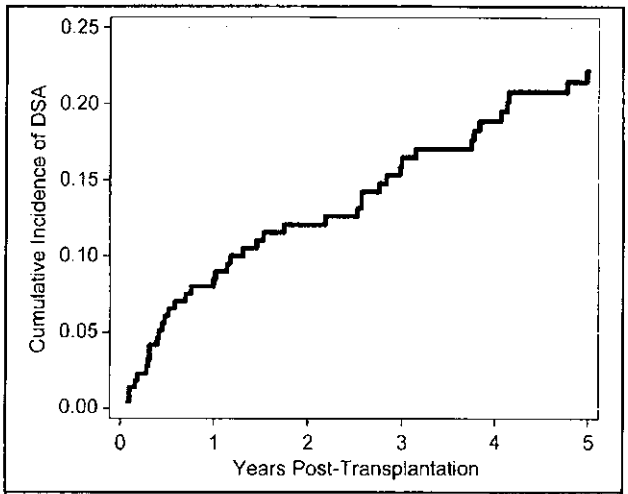

Figure 1. Cumulative incidence rate for de novo DSA.

Table 2. Five-year incidence of DSA among persons free of DSA at the time of transplantation, by donor type, gender, and race.

	Person-Year Experience*	New DSA	Rate/100/ year
Total diagnosed with DSA	902.8	42	4.7
Living-related donor Living-unrelated donor	371.1 178.3	11 8	3.0 4.5
Deceased donor	353.4	23	6.5
Male	519.7	25	4.8
Female	383.2	17	4.4
African American	544.4	28	5.2
Non-African American	358.4	14	3.9

*Person-years: The product of the number of years times the number of members of a population who have been affected by a certain condition (i.e. DSA)

Table 3. Five-year incidence of DSA among persons free of DSA at the time of transplantation, classified according to number of HLA mismatches.

Number of Mismatches	Person-Year Experience	New DSA	Rate/100/ year	95% CI
1 antigen	77.3	0	0	-
2 antigens	72.2	2	2.8	0.69 – 11.1
3 antigens	142.9	4	2.8	1.1 – 7.5
4 antigens	259.2	8	3.1	1.5 – 6.2
5 antigens	117.4	7	6.0	2.8 – 12.5
6 antigens	117.8	10	8.5	4.6 – 15.7
7 antigens	94.5	8	8.5	4.2 – 16.9
8 antigens	358.4	3	14.1	4.5 – 43.7

In total, 27 subjects developed DSA in the first post-transplant year. The prevalence of de novo DSA at 1 year post-transplant was 12.1 cases per 100. When dividing the group by transplant-type it was noted that the 1 year prevalence was slightly higher in those receiving deceased donor transplants compared to living donor transplant recipients (11.6 cases per 100 versus 5.4 cases per 100, respectively). This difference, however, was not statistically significant (p=0.09).

De novo DSA Incidence

Data from 5 years of follow-up in the East Carolina University cohort of patients has allowed ascertainment of the incidence of de novo DSA (Table 2). The average annual incidence rate from this time period of analysis was 4.7 cases per 100. The cumulative incidence plot shows that the incidence is slightly higher in the first year post transplant (approximately 9 cases per 100, Fig. 1). After the first year the incidence rate decreases to 3-4% per year.

To determine if any pre-transplant factors influenced incidence, we evaluated multiple variables. The first, donor type, was shown to have some impact on incidence rates. Deceased donor transplants had an annual incidence of 6.5%. This was nearly 2 times the rate in living related (3.0%) and living unrelated (4.5%) transplants. Second, African-Americans were shown to have an incidence of 5.2 per 100, per year. Non-African-Americans had a lower incidence of 3.9 cases per 100, per year. Finally since it is generally believed that females might be at a higher risk of DSA because of previous pregnancies, we investigated to determine if gender impacted incidence. From this population, no difference was seen between male and female transplant recipients.

DSA can only occur if a HLA mismatch is present, therefore HLA mismatch may be an important factor to determine those at highest risk of DSA. Table 3 shows that incidence rate of patients according to their number of HLA mismatches. The incidence rate indicate a trend from the low of 2.8 cases per 100 in those with 1 mismatch to a high of 14.1 cases per 100 in

patients with 8 antigen (A, B, DR, and DQ) mismatches. Because a high degree of mismatch was shown to have a higher incidence of DSA, we looked specifically at each type of mismatch (Table 4). DQ mismatch was significantly associated with DSA (p=0.036). All other mismatches were not found to be associated with DSA.

Table 4. Five-year incidence of DSA among persons free of DSA at the time of transplantation, classified according to type of HLA mismatch.

	Person-Year Experience	New DSA	Rate/100/ year	95% CI
A-locus mismatch>0	712.8	40	5.6	4.1 – 4.2
B-locus mismatch>0	757.4	38	5.0	3.7 – 6.9
DR-locus mismatch>0	732.4	38	5.2	3.8 – 7.1
DQ-locus mismatches>0	640.0	38	5.9	4.3 – 8.2

DISCUSSION

This prospective study of consecutive "low-risk" renal transplant patients examines the epidemiology of de novo DSA. Nearly all patients had at least five years of follow-up or failed prior to the fifth year. Over the five year period, 18.8 percent of patient developed DSA. The incidence of DSA was approximately 5 cases per 100/year. The incidence was highest in the first year post transplant and reduces but remains consistent in the years following. The groups with the highest incidence of DSA are recipients of a deceased donor transplant and African-American transplant patients. Although these factors showed a higher incidence rate, they were not statistically associated with DSA. This study identified only one characteristic of transplant recipients which is frequently present in advance of DSA. This factor is a DQ mismatch.

Moving forward with this study, there is a hope that new findings will shed light on why some patients develop DSA and some do not. In the initial years of this study, medication compliance rates were not well documented. However, in the years since 2006, the ability to capture this finding has improved. It is possible that non-compliance has a major role in DSA development. Future analysis of patients transplanted after 2006 will be able to determine this.

In all, these findings are the basis for both understanding the severity of the current problem and investigating DSA in the future. As we shift from an era of T-cell centric immunosuppression to an era where both the B- and T-cell are recognized and immunosuppressed, an understanding of the historical epidemiology of DSA as presented here is necessary. The hope is that future immunosuppression can successfully remove DSA in transplant patients leading to prolonged allograft survival.

SUMMARY

The donor specific anti-HLA antibody (DSA) has been increasingly recognized as the major cause of allograft loss. Despite this, no published reports exist describing the true epidemiology of de novo DSA. Here we describe the epidemiology of DSA based on the results of one of the longest running antibody study in consecutive renal transplant recipients. The study includes 224 non-sensitized, non-HLA-identical patients who received a primary kidney transplant between 3/1999-3/2006. Protocol testing for DSA was done pre-transplant, at 1, 3, 6, 9, and 12 months, and then annually. DSA was tested using single antigen beads. Data from the East Carolina University transplant cohort indicate that the prevalence of DSA in the first year post-transplant is 12.1 cases per 100. The average annual incidence of DSA is 4.7 per 100 cases, per year. The highest incidence of DSA was in the first year post transplant. Although deceased donors and African-Americans have a higher incidence rate of DSA than the comparator living donors and non-African American groups, respectively, these factors were not associated with DSA onset. The one factor found to be predictive of DSA was DQ mismatch (p=0.036). Based on these epidemiologic findings in combination with previous reports showing DSA is a cause of allograft failure, it seems reasonable that at least annual testing should be done even in "low-risk" transplant patients, because every year a new 5% of patients will develop DSA.