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.

### 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 cytotoxicity (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.

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Number of Subjects</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population</td>
<td>224</td>
<td>100</td>
</tr>
<tr>
<td>Subjects with complete 5-year follow-up</td>
<td>183</td>
<td>81.7</td>
</tr>
<tr>
<td>Subjects with allograft failure before 5-year exam</td>
<td>21</td>
<td>9.4</td>
</tr>
<tr>
<td>Subjects that died before 5-year exam</td>
<td>15</td>
<td>7.1</td>
</tr>
<tr>
<td>Subjects lost to follow-up before 5-year exam</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>Subjects who moved prior to 5-year exam</td>
<td>2</td>
<td>0.9</td>
</tr>
</tbody>
</table>
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.

DSAs 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.

**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.

| 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     | New DSA    | Rate/1000/year |
|                   | Experience      |            | 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 – 5.9 |
| 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 |

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.