Incidence and Impact of De Novo Donor-Specific Alloantibody in Primary Renal Allografts

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Background. To date, limited information is available describing the incidence and impact of de novo donor-specific anti–human leukocyte antigen (HLA) antibodies (dnDSA) in the primary renal transplant patient. This report details the dnDSA incidence and actual 3-year post-dnDSA graft outcomes.

Methods. The study includes 189 consecutive nonsensitized, non-HLA-identical patients who received a primary kidney transplant between March 1999 and March 2006. Protocol testing for DSA via LABScreen single antigen beads (One Lambda) was done before transplantation and at 1, 3, 6, 9, and 12 months after transplantation then annually and when clinically indicated.

Results. Of 189 patients, 47 (25%) developed dnDSA within 10 years. The 5-year posttransplantation cumulative incidence was 20%, with the largest proportion of patients developing dnDSA in the first posttransplantation year (11%). Young patients (18–35 years old at transplantation), deceased-donor transplant recipients, pretransplantation HLA (non-DSA)–positive patients, and patients with a DQ mismatch were the most likely to develop dnDSA. From DSA appearance, 9% of patients lost their graft at 1 year. Actual 3-year death-censored post-dnDSA graft loss was 24%.

Conclusion. We conclude that 11% of the patients without detectable DSA at transplantation will have detectable DSA at 1 year, and over the next 4 years, the incidence of dnDSA will increase to 20%. After dnDSA development, 24% of the patients will fail within 3 years. Given these findings, future trials are warranted to determine if treatment of dnDSA-positive patients can prevent allograft failure.

Keywords: Human leukocyte antigen, Donor-specific antibodies, Epidemiology, Allograft survival.

Studies over the last 10 years have established the role of posttransplantation human leukocyte antigen (HLA) antibodies in allograft loss (1–9) and have shown that donor-specific HLA antibodies (DSA) are strongly associated and may be a cause of allograft loss (10, 11). In addition, studies in primates have demonstrated that, if left untreated, an immunologic reaction starting with DSA formation will progress to chronic rejection and allograft loss will occur (12). However, despite knowing that patients with DSA are at risk for allograft loss, the time of de novo DSA (dnDSA) onset (incidence) and the incubation curve from dnDSA to allograft failure (impact) are not clearly defined. To obtain this information, only studies looking at an entire transplanted population from the day of transplantation can provide direct evidence of temporality and understand factors that influence the onset of DSA.

In preformed DSA, the actual survival after the allografts first DSA exposure (day 0 after transplantation) is known (13). However, with dnDSA, it is unknown because previous studies are either cross-sectional tested or used posttransplantation rather than post-DSA survival in the

analysis. This report details the dnDSA incidence and actual 3-year post-dnDSA graft outcomes.

RESULTS

Patient Demographics

The demographics of the entire patient cohort studied are listed in Table 1. All patients were primary renal transplant recipients that were not lost to follow-up or not DSA positive at 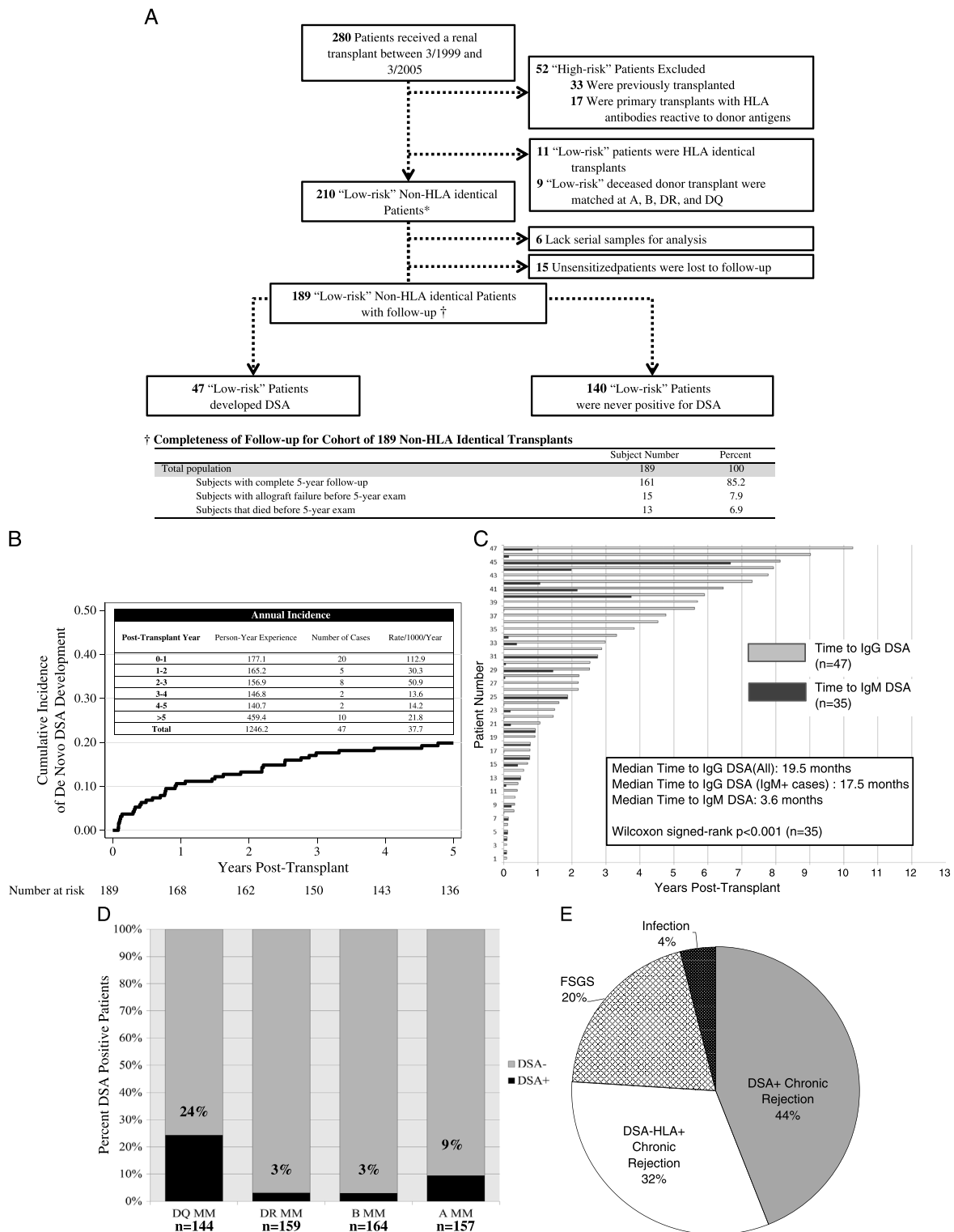the time of transplantation. In this cohort, all patients had 5 years of survival from transplantation or had allograft loss/died before the 5-year visit (Fig. 1A). Of the 189 patients included in this study, 59% were male, 64% were African Americans, and 42% received a deceased-donor transplant. The mean transplant age was 49.7±11.8 years.

In total, 3252 sera were tested, equaling an average of approximately 17 sera tested per patient. Within the 10 years of follow-up, dnDSA developed in 24.8% (47 of 189) of all kidney transplant recipients. The dnDSA-positive cohort was similar to the DSA-negative cohort in many ways.

TABLE 1. Patient demographics

	All	dnDSA−	dnDSA+
Number of subjects	189	142	47
Patient characteristics			
Mean (SD) age at transplantation, yr	49.7 (11.8)	50.0 (10.9)	44.8 (13.5)
18–35	30 (16)	16 (11)	14 (30)
36–50	65 (35)	52 (37)	13 (27)
51–60	57 (30)	45 (32)	12 (25)
>60	35 (18)	27 (19)	8 (17)
Male, n (%)	111 (59)	80 (56)	31 (66)
African American, n (%)	121 (64)	88 (62)	33 (70)
Transplant characteristics			
Deceased donor, n (%)	80 (42)	54 (38)	26 (55)
Delayed graft function, n (%)	6 (3)	3 (2)	3 (6)
Pretransplantation HLA IgG antibody positive (non-DSA)	58 (30)	37 (26)	21 (45)
Mean (SD) total HLA mismatch	4.4 (1.7)	4.2 (1.7)	5.0 (1.5)
A locus mismatches >0	156 (83)	115 (81)	41 (87)
B locus mismatches >0	163 (86)	122 (86)	41 (87)
DR locus mismatches >0	158 (84)	117 (82)	41 (87)
DQ locus mismatches >0	143 (76)	101 (71)	42 (89)
Immunosuppression			
Induction, n (%)			
Daclizumab	162 (86)	122 (87)	40 (85)
Thymoglobulin	25 (13)	18 (13)	7 (15)
Calcineurin inhibitor, n (%)			
Cyclosporine	129 (69)	93 (66)	36 (76)
Tacrolimus	57 (30)	47 (33)	10 (21)
Physician-directed pre-DSA immunosuppression drug change	4 (2)	0 (0)	4 (9)
Documented immunosuppression noncompliance, n (%)	7 (5)	2 (1.4)	5 (11)
Rejection			
T-cell mediated	34 (18)	29 (20)	5 (11)
Antibody mediated (AMR)	0 (0)	0 (0)	0 (0)
Mixed (TCMR with AMR)	14 (7)	3 (2)	11 (23)
Median (range) time to rejection	6.7 (0.2–146.1)	5.0 (0.2–146.1)	13.6 (1.9–52.2)
Rejection before DSA appearance	—	—	4
Rejection after DSA appearance	—	—	11
Rejection at the time of DSA	—	—	1
Median (range) serum creatinine at 1 mo after transplantation	1.5 (0.8–3.7)	1.5 (0.8–3.7)	1.4 (0.8–2.4)
Median (range) serum creatinine at time of DSA appearance	—	1.6 (0.8–4.2)	—
Months of follow-up			
Mean (SD)	92 (33)	95 (31)	84 (38)
Median (range)	92 (10–151)	93 (10–151)	89 (11–142)

AMR, antibody-mediated rejection; SD, standard deviation; TCMR, T-cell–mediated rejection.

A

† **Completeness of Follow-up for Cohort of 189 Non-HLA Identical Transplants**

	Subject Number	Percent
Total population	189	100
Subjects with complete 5-year follow-up	161	85.2
Subjects with allograft failure before 5-year exam	15	7.9
Subjects that died before 5-year exam	13	6.9

B

C

D

E

FIGURE 1. A, flowchart shows the study patients included and excluded from analysis. B, cumulative incidence of de novo anti-HLA DSA. Probability of DSA development based on the year after transplantation. The highest rate of development was in the first year after transplantation. C, IgM appears significantly sooner that IgG to the same DSA specificity indicating that a primary immune response is present and can be observed in most patients. D, number of DSAs relative to the number of mismatches for each HLA loci, indicating that DQ DSA may be more immunogenic. E, number of death-censored allograft failures stratified by cause. The majority of allograft failures were due to chronic rejection of which DSA made up the largest component, indicating that DSA is a major reason for allograft failure. DSA, donor-specific antibody; HLA, human leukocyte antigen.

TABLE 2. Univariate Cox proportional hazards estimates for predictors of DSA appearance (n=189)

Variable	Coefficient (β)	Hazard ratio	SE	P	95% Confidence interval
Univariate analysis					
Patient variables					
Age at transplant 18–35 yr	0.85	2.33	0.75	<0.01	1.24–4.37
Age at transplant 36–50 yr	−0.40	0.67	0.22	0.22	0.35–1.27
Age at transplant 51–60 yr	−0.06	0.94	0.30	0.85	0.50–1.76
Age at transplant >60 yr	−0.35	0.70	0.30	0.42	0.29–1.66
Recipient male gender	0.33	1.40	0.43	0.27	0.76–2.55
African American ethnicity	0.27	1.30	0.41	0.41	0.70–2.43
Transplant variables					
Deceased donor	0.69	1.99	0.58	0.02	1.11–3.53
Delayed graft function	0.78	2.19	1.31	0.19	0.68–7.03
A-locus mismatches >0	0.47	1.61	0.70	0.28	0.68–3.79
B-locus mismatches >0	0.13	1.14	0.50	0.76	0.48–2.69
DR-locus mismatches >0	0.36	1.43	0.62	0.41	0.61–3.38
DQ-locus mismatches >0	1.15	3.14	1.48	0.02	1.24–7.94
Pretransplantation HLA IgG antibodies (non-DSA)	0.77	2.15	0.63	<0.01	1.21–3.82
Immunosuppression variables					
Daclizumab induction	−0.01	0.99	0.39	0.99	0.47–2.13
Tacrolimus maintenance	−0.44	0.64	0.23	0.21	0.32–1.29
Physician-directed pre-DSA immunosuppression drug change	0.48	1.61	0.86	0.37	0.57–4.59
Documented immunosuppression noncompliance	0.71	2.05	1.48	0.32	0.49–8.48
Posttransplantation events					
Acute rejection (before DSA)	−0.57	0.57	0.25	0.19	0.24–1.33
Graft function at 1 mo after transplantation	−0.23	0.79	0.29	0.52	0.39–1.61
Polyomavirus (in situ)	0.35	1.41	1.43	0.73	0.19–10.29
Multivariable analysis					
DQ-locus mismatches >0	1.24	3.48	1.66	<0.01	1.37–8.87
Age at transplant 18–35	0.96	2.62	0.85	<0.01	1.39–4.94
Pretransplantation HLA IgG antibodies (non-DSA)	0.84	2.31	0.69	<0.01	1.29–4.15
Deceased donor	0.70	2.02	0.61	0.02	1.12–3.64

However, the dnDSA-positive cohort was more likely to be young (18–35 years old at transplantation), to be deceased-donor transplant recipients, to have HLA antibodies to other specificities (not to the donor HLA) before transplantation, and to have a DQ mismatch. At 1 month after transplantation, as well as at the date of DSA detection, the dnDSA patients had stable kidney function that was similar to a DSA-negative patient.

De Novo Donor-Specific Antibody (IgG) Development

Figure 1B shows the cumulative incidence of dnDSA among the entire cohort. The actual 5-year posttransplantation cumulative incidence of dnDSA is 20%. The largest incidence of dnDSA appearance occurs in the first year after transplantation (11.2%). Beyond the first year, the annual rate of new patients who develop dnDSA is as high as 5.1% and as low as 1.3% (Fig. 1B).

As it is classically thought that IgM would appear before IgG in dnDSA development, we examined the IgM on each sample before and including the first IgG-positive sample. We found that, in 35 cases (74%), IgM dnDSA was detectable before or at the time of IgG dnDSA appearance for the same specificity. The median time to IgM dnDSA was much shorter at 3.6 months than the time to IgG dnDSA at 17.5 months (P<0.001; Fig. 1C).

Table 2 shows the univariate and multivariate analyses of the pre-DSA variables that may be precursive to dnDSA development. Univariate analysis showed that most pretransplantation factors have little impact on dnDSA appearance. The factors having significant association to appearance of dnDSA include young age (18–35 years old at transplantation, receiving a deceased-donor transplant, pretransplantation non-DSA, and DQ locus mismatch. In a stepwise multivariate Cox proportional hazards model, these four variables significantly predicted dnDSA development. The first DQ mismatch increases the risk for dnDSA development by three times (hazard ratio [HR], 3.48; 95% confidence interval [95% CI], 1.37–8.87). Relative to the number of patients mismatched to DQ, DQ dnDSA was much more likely to appear compared with other loci mismatches and their respective dnDSA (Fig. 1D). Being a young
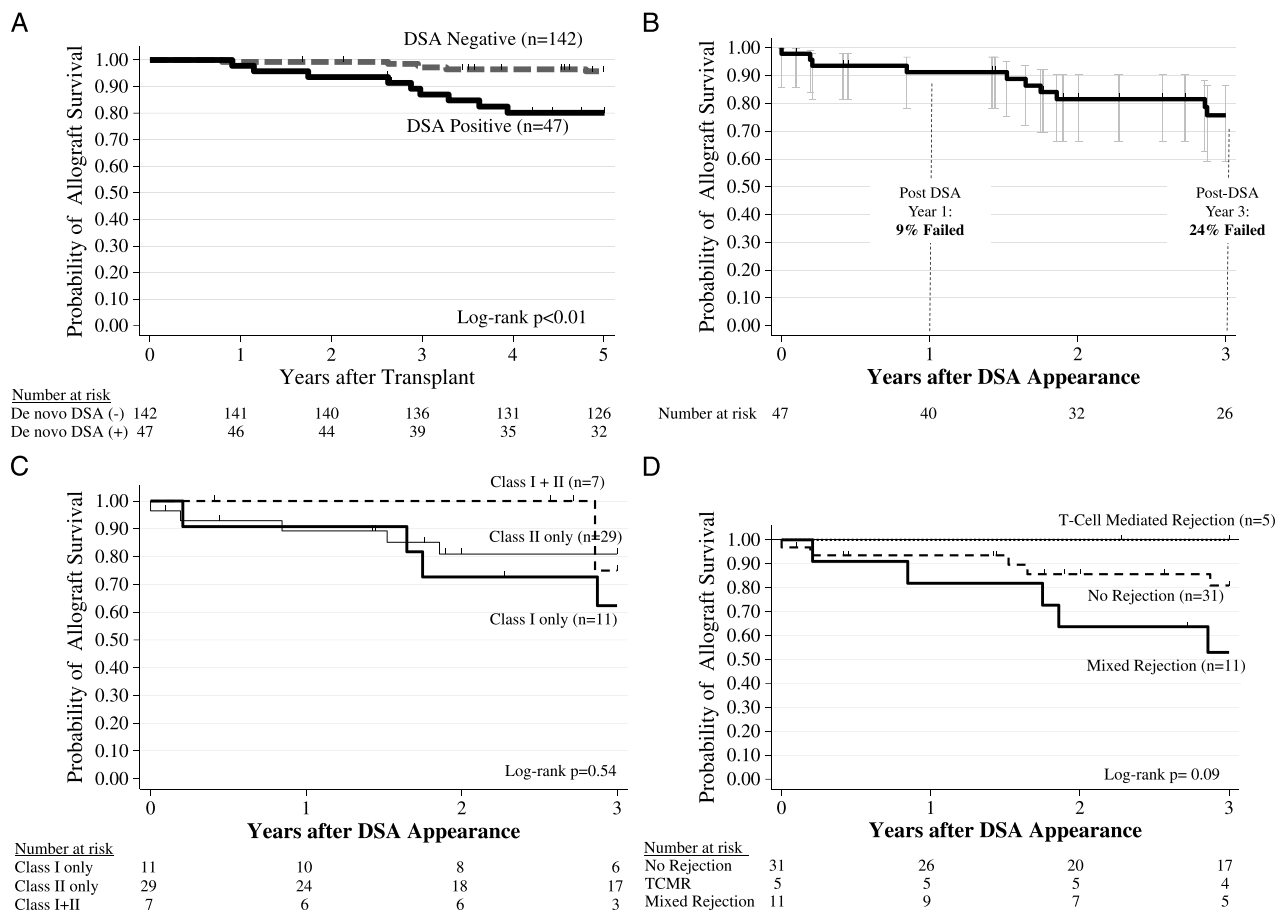
FIGURE 2. A, actual 5-year death-censored graft survival from the time of transplantation showing that dnDSA-positive patients are at a higher risk of failure than DSA-negative patients. B, actual 3-year death-censored graft survival from the time of DSA appearance. C, post-DSA survival stratified by DSA class. D, post-DSA survival based on type of rejection. dnDSA, de novo donor-specific antibody; DSA, donor-specific antibody.

transplant patient (18–35 years old at transplantation) led to a higher risk of dnDSA (HR [95% CI], 2.62 [1.39–4.94]; see **Table S1, SDC,** http://links.lww.com/TP/A756). Having non-DSA pretransplantation increased the risk of dnDSA development (HR [95% CI], 2.31 [1.29–4.15]). Finally, receiving a deceased-donor transplant was another factor predictive of dnDSA (HR [95% CI], 2.02 [1.12–3.64]; see **Table S2, SDC,** http://links.lww.com/TP/A756).

De Novo Donor-Specific Antibody Characteristics

On first appearance of DSA, the most common type of DSA was against class II antigens (68%). Of class II antibodies, DQ predominated (91%). Class I antibodies made up 42% of early DSA (DSA in the first 6 months after transplantation) but were only 29% of late DSA (DSA after 6 months after transplantation). Conversely, class II was 58% of early DSA and 71% of late DSA.

De Novo Donor-Specific Antibody Progression and Trends After Initial Onset

A table showing the DSA characteristics of each patient is included as a supplemental table (see **Table S3, SDC,**

http://links.lww.com/TP/A756). At the time of dnDSA appearance, the median (range) mean fluorescence intensity (MFI) of the dnDSAmax (highest intensity MFI dnDSA specificity) antibody was 4924 (1006–23,576). The majority of patients (94%) only had one dnDSA at the initial dnDSA onset. However, 28% of patients developed a second dnDSA specificity after the initial dnDSA onset. Functionally, the patients had stable allograft function at the time of dnDSA (median serum creatinine, 1.6 mg/dL) appearance that was similar to their 1 month posttransplantation function (median serum creatinine, 1.5 mg/dL; Table 1). After the onset of serum dnDSA positivity, 81% of dnDSA persisted and 19% of dnDSA was transient (DSA positivity on only one sample). In patients where dnDSA persisted, the median (range) MFI of the dnDSAmax (6460 [1006–23,576]) was significantly higher than the dnDSAmax MFI of the transient cases (1950 [1029–5648]; *P*<0.01).

De Novo Donor-Specific Antibody and Acute Rejection

In the DSA-negative group, 23% of patients had an acute rejection episode (Table 1). In the dnDSA-positive groups, 33% of patients had an acute rejection episode, the

TABLE 3. Relative risk of graft loss in intervals after first occurrence of dnDSA

Year after event	Beginning total n (loss n)	Probability of graft loss	Relative risk of graft loss
1	47 (3)	0.09	9
2	40 (4)	0.18	6
3	32 (4)	0.24	6

Ratio of the probability (cumulative) of graft loss in the interval after the event (DSA) to the expected probability of graft loss; expected probability of graft loss is estimated with the use of the life table of the East Carolina University patients who were free of DSA throughout transplantation.

majority (12 of 16) of which occurred at or after dnDSA appearance. Mixed rejections accounted for 11 of 16 of the cases in the dnDSA-positive cohort according to histologic findings of both antibody-mediated rejection and T-cell–mediated rejection. Five of these patients with a mixed rejection episode had the finding of C4d positivity.

De Novo Donor-Specific Antibody and Death-Censored Allograft Loss

Overall, chronic rejection (reported as chronic alloantibody-mediated rejection, chronic allograft vasculopathy, or chronic allograft nephropathy) was the primary reason for allograft loss (n=18, 72%; Fig. 1E). Recurrent or de novo glomerular disease (focal segmental glomerulosclerosis) accounted for 20% of allograft loss cases. One patient lost their allograft due to infection. Of the graft loss defined as chronic rejection, 56% of the patients had dnDSA. The remaining patients with chronic rejection were without DSA. However, all of these DSA-negative chronic rejections were positive for non-DSA anti-HLA antibodies after transplantation.

In the 11 dnDSA-positive patients who went on to graft loss with chronic rejection, 8 had a biopsy available approximately 12 months before allograft loss. All biopsies had findings consistent with acute or chronic alloantibody-mediated injury. In addition, nearly all dnDSA patients with chronic rejection had plasma cell/plasmablast infiltrates on the biopsy, which has been previously associated with humoral rejection. The remaining three patients did not have a biopsy within 1 year of allograft loss for an adequate assessment of the chronic antibody-mediated injury.

De Novo Donor-Specific Antibody and Allograft Survival

Figure 2A compares the survival rates from time of transplantation for the patients with dnDSA with those without (a non-DSA cohort is included in **Figure S1, SDC,** http://links.lww.com/TP/A756). DSA positivity leads to poorer survival even in those patients with poor baseline post-transplantation function (serum creatinine >1.6; see **Figure S2, SDC,** http://links.lww.com/TP/A756). However, because patient develop dnDSA at different time points after transplantation, it is not appropriate to calculate survival from transplantation; rather, survival should be assessed from onset of dnDSA. From the onset of dnDSA, 24% of patients lost their allograft within 3 years (Fig. 2B). In comparison, 5-year allograft survival from transplantation for patients without DSA was 96%. The relative risk of allograft loss with DSA was six to nine times higher than the DSA-negative cohort (Table 3).

When looking at other characteristics that may impact post-dnDSA survival, we found that class II antibodies (alone) were not associated with poorer survival when compared with class I only patients and class I and II patients (Fig. 2C). Although the presence of a mixed rejection may be an indicator of poorer prognosis, on survival analysis, there was no statistically significant difference between those DSA patients with a mixed rejection or no rejection (Fig. 2D).

DISCUSSION

In this study, we analyzed the incidence and impact of dnDSA on primary renal transplant recipients. Our results show that the 5-year cumulative incidence of dnDSA is 20%. We found the dnDSA incidence similar to other reports from longitudinal studies (1, 8, 14). Devos et al., Cooper et al., and Wiebe et al. showed that 18% (62 of 347 with a median follow-up of 26 months), 27% (65 of 244 with a mean follow-up of 19 months), and 15% (47 of 268 with a mean follow-up of 6.2 years) of consecutive renal transplant developed dnDSA, respectively. However, our study expands on previous reports in that, with our analysis of patients who all had at least 5 years of follow-up, we show the annual incidence of dnDSA is highest in the first posttransplantation year (11%). Over the next 4 years, the cumulative incidence of new dnDSA patients increases to 20%, but the annual rate decreases over time and fluctuates between 1% and 5% per year.

The current study supports many previous studies showing that dnDSA is associated with allograft failure (1, 4, 6–9, 11, 14–21). It also supports the previously published data that show class II alloantibodies are more predominant than class I (8, 22). However, our study is the first to assess outcomes after dnDSA development. Once a patient develops dnDSA, the risk of allograft failure in the first post-dnDSA year is 9%, and by 3 years post-dnDSA, 24% of patients will progress to chronic alloantibody-mediated rejection and fail within 3 years. In the year before graft failure, we commonly found evidence of chronic alloantibody-mediated injury with or without acute antibody-mediated rejection in dnDSA-positive patients. One additional finding on the biopsies with chronic alloantibody-mediated injury was the presence of intragraft plasma cells, which has been shown to correlate strongly with the presence of DSA and may be a pool of progenitor cells for long-lived antibody-producing plasma cells, possibly leading to a strengthening of the humoral rejection (23).

One important clinical pearl from this analysis is that, given the allograft function is stable at the time of dnDSA appearance and there is an intermediate period of time it takes for allograft loss to take place, an opportunity for intervention exists. This is intriguing given that it has been

shown in multiple reports that DSA removal may improve allograft survival (*15, 24–26*).

This study also extends our understanding of dnDSA in other ways. Our data indicate that there are four predictive factors for DSA development. First, patients transplanted at 18 to 35 years of age, as has been previously identified (*6*), are at an increased risk of developing dnDSA. Second, deceased-donor recipients are also at a higher risk of developing dnDSA. However, in this cohort, dnDSA patients are more likely to be female, to be African American, to have more HLA mismatches, and to receive thymoglobulin induction and tacrolimus when compared with the living-donor transplants. Combining these differences may be one reason for deceased donors being at higher risk (see **Table S4, SDC,** http://links.lww.com/TP/A756). Third, it is not surprising that DQ mismatch is a major risk factor, given that the majority of DSA in this study is DQ DSA. However, a reason for DQ antigens causing more DSA than other HLA loci needs more investigation. Finally, pretransplantation non-DSA is a dnDSA risk factor because it may be a marker of an immunoreactive patient or non-DSA could be related to future DSA by epitope spreading.

This study also differs from other longitudinal studies in that it has a larger African-American population. Although it is commonly thought that African Americans are at higher risk of rejection (*27*), this risk does not appear to be due to higher rates of DSA because race was not a risk factor for dnDSA development.

Unlike other DSA reports, we were unable to find a clear link between noncompliance and dnDSA (*28*). The dnDSA patients were proportionally more likely to have noncompliance, but because of a lack of prospective surveying, our results may not reflect the true impact on immunosuppression noncompliance. We did, however, find a higher rate of physician-directed immunosuppression reduction or changes in the dnDSA group, which we believe should be looked at more in-depth in future studies to better understand the role of the physician in dnDSA development.

We also show in this study that IgM dnDSA is detectable before IgG dnDSA in the majority of DSA cases. This finding shows that patients have primary and secondary immune responses after transplantation. Because IgM does commonly precede IgG, IgM dnDSA monitoring needs to be evaluated to see if it has usefulness in predicting IgG dnDSA. Also, further analysis of this population to understand if IgM DSA positivity influences the clinical course of the patient is needed and is ongoing.

To fully interpret this study, the limitations of the study should be considered. First, our definition of DSA positive using a MFI of 1000 or greater, although commonly used, has not been established as a clinically relevant cutoff. Further studies investigating the appropriate clinically relevant cutoff as well as confirming the data using other manufacturer's single antigen assays may also be useful. Second, the small number of DSA-positive patients makes it difficult to assess further variables (rejection, DSA class, etc.) that stratify failure in the DSA-positive cohort. Third, this study is unique in that it has a large population of African-American transplant recipients; however, in some transplant centers where the recipients are primarily non–African American, the findings here may not be applicable. Finally, as with all studies,

defining causation is difficult. In this study, DSA is strongly associated with allograft failure, but other unknown variables may also have an impact on survival.

In summary, these data suggest that approximately 11% of patients without detectable DSA at the time of transplantation will have detectable DSA at 1 year and that, over the next 4 years, the incidence of detectable DSA will increase to 20%. Once DSA appears, the probability of graft loss within 3 years after DSA appearance is 24%. Relative to those without DSA, the relative risk of graft loss is nine times higher at 1 year after DSA appearance.

MATERIALS AND METHODS

Patients

We enrolled all renal transplant patients receiving a living-donor or a deceased-donor transplant between March 1999 and February 2006. All patients underwent a standard pretransplantation evaluation. At time of transplantation, all patients were tested for reactivity to their donor via complement-dependent cytotoxicity crossmatch (XM). Flow cytometric XM was performed on all living-donor transplants. Testing using LABScreen beads (described later) indicates that no patients had pretransplantation DSA. Tissue typing was performed using both serology and polymerase chain reaction single-specific primer methods for HLA-A, HLA-B, HLA-DR, and HLA-DQ antigens. We excluded all patients found to have donor-reactive alloantibodies present in circulation (and detected via XM or single antigen bead assay).

Study Protocol

Testing and 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.

Immunosuppression

Per protocol, patients with a panel reactive antibody of less than 20% and without delayed graft function received daclizumab induction, whereas patients with a panel reactive antibody of more than 20% or delayed graft function received rabbit antithymocyte globulin induction. Maintenance immunosuppression included a calcineurin inhibitor, a mycophenolic acid derivative, and a prednisone taper starting at the time of transplantation that was reduced to and then maintained a level of 5 mg/day by 1 month after transplantation.

Rejection Pathology

Acute rejection was defined as an increase in serum creatinine at least 20% above baseline serum creatinine with histologic evidence on renal allograft biopsy by Banff 1997 criteria (update 2005) (*29, 30*).

Anti-HLA-Specific IgG Antibody Monitoring and Testing

In addition to a pretransplantation sample, patients were routinely monitored at 1, 3, 6, 9, and 12 months after transplantation, then annually and when clinically indicated, for HLA class I and II antibodies development using LABScreen Mixed beads (One Lambda, Canoga Park, CA). Samples tested positive on LABScreen Mixed beads were also tested using LABScreen Single Antigen Class I and II beads (One Lambda, Canoga Park, CA) to determine antibody specificity. If a patient was found to be positive on LABScreen Single Antigen, all previous samples tested with the LABScreen Mixed antigen product were tested via the single antigen platform. All LABScreen tests were performed according to the manufacturer's protocol. For the IgM assay, the IgG detecting antibody was replaced with an IgM detecting antibody (R-phycoerythrin–conjugated AffiniPure F(ab) fragment donkey anti-human IgM obtained from Jackson ImmunoResearch, West Grove, PA). HLA antibodies were analyzed as MFI values. dnDSA were considered positive if it was a new IgG antibody not present at time of transplantation and the normalized intensity via single antigen bead of 1000 MFI or greater.

Statistical Methods

All statistical analyses were performed using Stata/MP version 10.1 (College Station, TX). Two-sided $P<0.05$ was considered statistically significant. In the incidence rate calculation, once patients were positive for a single IgG DSA, they were censored. On Cox proportional hazards analysis, variables with a significance level of $P<0.15$ in the univariate analyses were selected for inclusion in the multivariate analyses. A stepwise model selection method (threshold of $P=0.05$) was used to arrive at a final regression model. The final model was assessed for goodness-of-fit. Kaplan-Meier analysis with log-rank test was used to assess survival (allograft loss). Allograft loss was a return to dialysis.

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