# Donor Specific Antibody Surveillance and Graft Outcomes in Pediatric Kidney Transplant Recipients

Rachel M. Engen

A thesis
submitted in partial fulfillment of the requirements for the degree of

Masters of Science

University of Washington
2017

Committee:
Noel S. Weiss
Jodi Smith

Program Authorized to Offer Degree:
Epidemiology

University of Washington

## Abstract

Donor Specific Antibody Surveillance and Graft Outcomes in Pediatric Kidney Transplant Recipients

Rachel M. Engen

Chair of the Supervisory Committee:
Noel S. Weiss, PhD
Department of Epidemiology

**Introduction:** The development of *de novo* donor-specific antibodies (*dn*DSA) has been associated with rejection and graft loss in kidney transplantation, and DSA screening is now recommended in all kidney transplant recipients. However, the clinical significance of *dn*DSA in patients with a stable creatinine remains unclear.

**Methods:** We performed a retrospective cohort study of 103 patients receiving a first, kidney alone transplant between 12/1/2007 and 12/31/2013. Inclusion criteria were age <18 years old at the time of transplant and at least two years of DSA monitoring. All patients underwent DSA screening every 3 months post-transplant with additional testing as clinically indicated. No treatment was given for DSAs in the absence of biopsy-proven rejection.

**Results:** 20 patients (19%) developed *dn*DSA in the setting of a stable creatinine and 13 patients (13%) developed *dn*DSA in the setting of an elevated creatinine. Median follow-up time post-transplant was 4.1 (IQR 2.9-5.7) years. In a Cox proportional hazards regression controlling for age, type of transplant, delayed graft function, cold ischemia time, and baseline eGFR post-transplant, developing *dn*DSA with a stable creatinine was not associated with time to 30% decline in eGFR (HR 0.88, 95%CI 0.30-2.00 p=0.598) or graft loss. *dn*DSA with an elevated creatinine was associated with a 2.8 times increased risk of decline in graft function

(95% CI 1.08-7.27 p=0.034) and a 7.34 times increased risk of graft loss (95%CI 1.37-39.23 p=0.020) compared to transplant recipients who did not develop dnDSA. The presence of *dn*DSA with a stable creatinine was associated with an increased risk of rejection within 3 years (RR 2.53 95%CI 1.49-4.3 p=0.0002) and moderate interstitial fibrosis and tubular atrophy at 2 years post-transplant (aOR 26.8 95%cI 1.91-138 p=0.015).

**Conclusion:** The clinical setting in which *dn*DSA is first detected impacts the association between *dn*DSA and graft function. *dn*DSA with a stable creatinine is associated with future rejection episodes and IFTA, but does not correlate with time to 30% decline in eGFR. *dn*DSA with an elevated creatinine is associated with a 30% decline in graft function, graft loss, and rejection. Further research is needed to clarify the role of *dn*DSA screening in pediatric kidney transplantation.

In 2014 17,814 individuals in the United States received a kidney transplant; 712 of them were children.[1] Over the past 30 years there has been substantial increase in kidney allograft survival, but most of this has been due to improvements in short-term rather than long-term survival.[2] Chronic allograft nephropathy (including interstitial fibrosis with tubular atrophy (IFTA) and transplant glomerulitis) remains the leading cause of graft loss,[3] and HLA antibodies are thought to play a key role in its development.[4]

Donor specific antibodies (DSAs) are antibodies developed by the transplant recipient against HLA antigens present on the donor kidney. Numerous studies have linked the development of *de novo* DSAs (*dn*DSA) after kidney transplantation to poor graft outcomes in both adults and children.[1,5–12] This has resulted in recommendations that patients undergo routine screening for the development of *dn*DSA post-transplant.[13] However, many of these original studies combined screening with testing done in the setting of graft dysfunction[1,6,10,14,15] or screened stored serum without regard to the patient's clinical status.[7–9,12] This raises concern that the association between *dn*DSA and graft outcome seen in prior studies may not be representative of a population with stable kidney function undergoing screening.

Multiple studies have shown that a large subset (34-48%) of patients who develop *dn*DSAs develop neither rejection nor have a decline in graft function.[1,7,9,14,15] In a subgroup analysis of their study of 244 adult patients, Cooper et al reported that the two year graft survival among those with *dn*DSA detected on a protocol test was 93% compared to 97.8% among those with *dn* DSA detected on a non-protocol test, a difference that was not statistically significant.[14] In this study we aim to test whether or not pediatric patients with *de novo* DSAs first detected in the setting of stable pediatric kidney graft function have worse graft outcomes than those with no *dn*DSA.

**Methods**

We performed a retrospective cohort study of all pediatric patients receiving a kidney transplant at Seattle Children's Hospital between 12/1/2007 and 12/31/2013. Inclusion criteria were age less than 18 years, receipt of a primary, kidney-alone transplant, and at least two years of DSA monitoring. Exclusion criteria included a history of prior kidney transplant, previous or concurrent other solid organ transplant, and previous hematopoietic stem cell transplant. All patients had a negative crossmatch and no DSA prior to transplant.

Since January 2008 pediatric kidney transplant patients at Seattle Children's Hospital have undergone DSA screening every 3 months as part of routine clinical care. Patients also undergo surveillance kidney biopsies between three and six months post-transplant, between 6 and 12 months post-transplant, and at approximately 24 months post-transplant. Induction immunosuppression was with methylprednisolone and either thymoglobulin or an IL-2 receptor antagonist. Maintenance immunosuppression was primarily with tacrolimus and mycophenolate mofetil; only patients considered to be at high risk for rejection received maintenance steroids. All patients received antiviral prophylaxis.

BloodWorks Northwest (Seattle, WA), a UNOS-accredited laboratory that specializes in testing for organ transplantation, performed all DSA tests and provided the results; all DSA results within the first 2 years post-transplant were included in analysis. DSA screening was performed by flow cytometry of single-antigen beads using either the Luminex 200 or FLEXMAP 3D platform. All other data were collected from the electronic medical record.

Patients were divided into 3 categories for analysis: patients who developed *dn*DSA with a stable creatinine, patients with *dn*DSA with elevated creatinine, and those who never developed a *dn*DSA. For all patients with a positive DSA test, the creatinine at the time of the positive test was compared to a baseline in a window 3-6 months prior, defined as the lowest creatinine

measured on two occasions at least one week apart. The stable creatinine group was defined as those who developed *dn*DSA and their creatinine at the time of the first positive DSA test was no more than 0.1 mg/dl higher than the baseline in a window 3-6 months prior. The elevated creatinine group included those patients who developed a *dn*DSA with a creatinine that was at least 0.2mg/dl higher that it had been in the previous three to six months. No threshold was used to define a positive DSA result; the lowest reported MFI in this study was 300. No treatment was given for *dn*DSA in the absence of biopsy-proven acute rejection.

Estimated glomerular filtration rate (eGFR) was calculated using the Modified Schwartz equation[16] for patients less than 18 years old and the CKD-EPI equation[17] once patients were 18 years or older. Baseline eGFR post-transplant was calculated using the lowest serum creatinine obtained on two occasions at least one week apart within the first 60 days after transplant.

The primary outcome of this study was a 30% decline in eGFR, defined as the number of days after transplant when the eGFR fell below 30% of the post-transplant baseline and did not recover within 30 days. A 30% decline in eGFR has been endorsed as a surrogate end point for end stage renal disease that can allow studies to be conducted over shorter time periods and with a smaller number of patients.[18,19] Secondary outcomes included graft loss, eGFR at three years post-transplant, the number of biopsy-proven rejection episodes within 3 years after transplant, and the incidence of IFTA on the 2-year protocol biopsy.

Descriptive statistics used means and standard deviations for normally distributed data and medians and interquartile ranges for skewed data. Using Cox proportional hazards regression, transplant recipients with and without *dn*DSA were compared for the time to a 30% decline in eGFR and time to graft loss. We employed linear regression to examine the relationship with eGFR at 3 years post-transplant and logistic regression to examine the relative incidence of interstitial fibrosis and tubular atrophy. The hazard of rejection was compared using a Cox proportional hazards model with *dn*DSA as a time-varying covariate and allowing for multiple

rejection episodes per transplant recipient. Once recipients developed *dn*DSA they were considered to be DSA positive for the remainder of the follow-up time, even if DSA later resolved. In cases where patients had multiple rejection episodes, time to rejection was calculated from the previous episode. The previous number of episodes was adjusted for in the model by stratification, and robust standard error estimates were used to account for correlation caused by multiple episodes in the same patient. All outcomes were adjusted for confounding by age, type of transplant donor, delayed graft function, cold ischemia time, and baseline eGFR post-transplant, determined on an *a priori* basis. Analysis was conducted using STATA 12 and R version 3.2.2.

This study was approved by the Seattle Children's Hospital Institutional Review Board.

## Results

### *Study population*

138 kidney transplants were performed at Seattle Children's Hospital between 12/1/2007 and 12/31/2013. Among the transplant recipients, 15 were excluded because they were over 18 years old, 12 had a previous solid organ transplant, 1 underwent a multi-organ transplant, and 7 had less than 2 years of DSA screening due to either graft failure prior to 1 year (n=2) or transfer of care to another institution (n=5) (Figure 1). In total 103 patients were included in the study. 81% of patients had six or more DSA tests during the first two years after transplant. Mean follow up time was 4.4 years post- transplant.

33 patients (32%) developed *dn*DSA within two years post-transplant; 20 patients (19%) developed *dn*DSA in the setting of a stable creatinine while 13 patients (13%) did so in the setting of an elevated creatinine. Patients with *dn*DSA and a stable creatinine tended to be younger than those without *dn*DSA, while those with *dn*DSA and an elevated creatinine tended

to be older. Those with *dn*DSA and a stable creatinine were more likely to have had delayed graft function, a longer cold ischemia time, and EBV or CMV mismatch compared to those without *dn*DSA. Patients with *dn*DSA and an elevated creatinine were more likely to have received a deceased donor transplant, have a longer cold ischemia time, and undergo induction with an IL-2 inhibitor. Patients in the three study groups were otherwise similar (Table 1).

### *dn*DSA characteristics

Patients whose *dn*DSA was first detected in the setting of a stable creatinine tended to develop *dn*DSA earlier, and persist longer, than those whose *dn*DSA was detected in the setting of an elevated serum creatinine. Those with *dn*DSA and an elevated creatinine were more likely to have antibodies against Class I HLA (15%) or both Class I and Class II HLA (31%) compared to those with *dn*DSA and a stable creatinine (10% and 10%, respectively). Conversely, those with *dn*DSA with a stable creatinine were more likely to have isolated Class II HLA antibodies (80%). The two groups had similar peak MFIs and rates of C1q positivity. Approximately 30% of detected *dn*DSAs resolved prior to the end of 2 years of monitoring in both groups (Table 2).

### *Outcomes of patients with* dn*DSA*

In a Cox proportional hazards regression controlling for age at transplant, type of transplant donor, incidence of delayed graft function, cold ischemia time, and baseline post-transplant eGFR, there was no significant difference in the hazard for a 30% decline in eGFR between those who developed *dn*DSA with a stable creatinine and those who never developed *dn*DSA (aHR 0.88 95%CI 0.30-2.00 p=0.598) (Table 3; Figure 2). There was no more than a suggestion of an increased risk of graft loss (aHR 1.75 95%CI 0.28-10.73 p=0.546). There was also no difference the eGFR at 3 years post-transplant (difference -1.08ml/min/1.73m$^2$ p=0.616).

Patients with *dn*DSA in the setting of an elevated creatinine did have an increased risk of decline in graft function (aHR 2.80 95%CI 1.08-7.27 p=0.034), increased risk of graft loss (aHR 7.34

95%CI 1.37-39.23 p=0.020), and a lower 3-year eGFR (difference -14.5ml/min/1.73m$^2$ p=0.054) compared to those without *dn*DSA after adjustment for age at transplant, type of transplant donor, incidence of delayed graft function, cold ischemia time, and baseline eGFR (Table 3; Figure 2).

The development of *dn*DSA was associated with an increased risk of an episode of rejection within 3 years post-transplant regardless of the patient's creatinine at the time of *dn*DSA detection. In a person-time analysis, patients without *dn*DSA developed rejection at an incidence rate of 0.15 episodes per person-year, while those with *dn*DSA and a stable creatinine developed rejection at a rate of 0.36 episodes per person-year and those with *dn*DSA and an elevated creatinine developed 0.71 episodes of rejection per person-year (Table 3). In a Cox proportional hazards analysis with *dn*DSA status as a time varying exposure, allowing for multiple rejection episodes per patient, and adjusted for age at transplant, type of transplant donor, incidence of delayed graft function, cold ischemia time, and baseline-post-transplant eGFR, patients who developed *dn*DSA in the setting of a stable creatinine and those who developed *dn*DSA is the setting of an elevated creatinine had a similar increased hazard for subsequent rejection episodes (*dn*DSA with stable creatinine: aHR 2.41 95%CI 1.27-4.54 p=0.007; *dn*DSA with an elevated creatinine: aHR 2.43 95%CI 1.00-5.90 p=0.497).

Patients who developed *dn*DSA in the setting of a stable creatinine were also more likely to have moderate IFTA on their protocol biopsy 2 years after transplant (aOR 26.81 95%CI 1.91-377 p=0.015) compared to those without *dn*DSA, though the numbers were small. There was no appreciable association between *dn*DSA and mild IFTA. There was no association between *dn*DSA and IFTA among those with an elevated creatinine (Table 3).

In a sensitivity analysis that used different MFI thresholds to define a positive DSA test, there was no change in the primary outcome when using MFI thresholds of 1000, 1500, 2500, or 8000 (Table 4).

## Discussion

In this study we show that clinically stable patients who are diagnosed with *dn*DSAs on a screening test do not have an increased risk of decline in graft function compared to those patients who never develop *dn*DSAs, though they do have an increased risk of acute rejection and moderate IFTA. Consistent with previous studies, we show that patients who develop *dn*DSA in the setting of an elevated creatinine do have an increased risk for decline in graft function and graft loss. To the best of our knowledge, this is the largest study to examine the outcomes of screening *dn*DSA in pediatric kidney transplant patients.

Much of the previous literature on *dn*DSA and graft outcomes has combined data on screening in clinically asymptomatic patients with data from patients with graft dysfunction.[1,5,10,14] While this was logical given the goals of those studies, it may not be appropriate to apply the results to a clinically stable population undergoing DSA screening. Our data and data from Wiebe et al[15] and Cooper et al[14] suggest that patients who are clinically stable and those with graft dysfunction represent distinct groups with different risks of poor graft outcome. This means that the results of those prior studies could overstate the risk of *dn*DSA in clinically stable patients while understating the risk in those with graft dysfunction. This is perhaps best illustrated in the study by Cooper et al, who reported an overall hazard ratio for 2 year graft loss of 7.7 among those with *dn*DSA. However, our review of their reported outcomes shows that, when their patient population was divided into those with *dn*DSA on protocol testing vs nonprotocol testing, the relative risk of 2 year graft loss was 3.2 for the protocol group and 28.6 for the nonprotocol group - a 9-fold difference.[14]

There is already evidence that not all *dn*DSA have equal propensity to damage the kidney. Studies have shown an increased risk of graft loss among patients with anti-HLA class II

antibodies,[20] anti-DQ antibodies,[21] and antibodies with a higher MFI.[8] DSAs are thought to damage the renal microvasculature via complement fixation, and several research groups have found an increased risk of graft loss caused by DSAs that bind complement proteins C1q[22,23] or C3d.[24] However, the strong association between C1q or C3d binding ability and DSA MFI has raised concerns about the validity of these assays.[25,26] It is possible that the types of *dn*DSA that develop in patients who are otherwise clinically stable may differ from the *dn*DSA in patients with an elevated creatinine, which could explain the difference in outcome seen in our study. A higher percentage of patients with *dn*DSA detected in the setting of an elevated creatinine had both class I and class II antibodies; however this study was not designed or powered to detect differences in antibody characteristics. Further research with larger patient populations is needed to better clarify the types of antibodies that are most harmful to the renal allograft.

Both acute rejection[2] and IFTA[27] have been associated with decreased graft survival. However, in our study *dn*DSA detected in a clinically stable patient was not associated with a faster decline in graft function despite being associated with both acute rejection and IFTA. One explanation for this may be that the acute rejection and IFTA observed in this study represent the first steps leading to a later decline in graft function that was not captured in our follow-up time. Alternatively, this finding could be related to our use of clinical pathology reports for the diagnosis of both outcomes. Multiple different pathologists read the biopsies, which raises a concern for information bias due to variability in classification of renal findings. In addition, the Banff criteria for the pathologic classification of kidney transplant biopsies changed just before and again during the course of this study, increasing the potential variation. Yet another explanation is that the association between acute rejection, IFTA and graft function may be more nuanced than previously thought. In their original study of the association between acute rejection and graft loss, Meier Kriesche et al noted that graft survival among patients whose renal function returned to baseline after a rejection episode was no different than patients

without rejection episodes,[2] while Park et al found no association between IFTA and graft function in the absence of ongoing inflammation.[28]

The study utilized a rich clinical data source that included DSA screening results and biopsy data on patients during times of both clinical stability and graft dysfunction. This allows fuller assessment of both *dn*DSA incidence and pathologic changes throughout the post-transplant course. It also took advantage of a large pediatric cohort managed at a single institution under a single standardized set of transplant protocols, decreasing the variation in outcome that could be introduced by variations in immunosuppression management. The use of survival techniques allowed us to take full advantage of the long follow-up times available on many of the study's patients.

The chief limitation of this study is that the follow-up period may not be long enough to fully monitor the association between the presence of *dn*DSA and long-term outcome. Antibody-mediated damage is hypothesized to be an indolent process.[15] Terasaki et al published data showing a mean time from detection of *dn*DSA to graft failure of approximately 2.9 years.[29] While the mean post-transplant follow-up time in our study was 4.5 years, 25% of patients in the *dn*DSA-stable creatinine group had less than 2 years of follow-up their first positive DSA test. However, our primary outcome was a 30% decline in renal function, not graft loss. A 30% decline in eGFR is a surrogate endpoint that allows for shorter trial durations while maintaining a chance of type I error of less than 5% in the absence of acute effects on eGFR.[19] In a screening population with a stable serum creatinine there is, by definition, no acute effect of DSA on eGFR. Therefore, the shorter duration of follow-up in our study may be somewhat mitigated.

In this study *dn*DSA was not associated with a decline in graft function in patients with a stable serum creatinine at the time of *dn*DSA detection. Our data showed an increased risk of rejection episodes and IFTA as well as a suggestion of an increased risk of graft loss. Based on this evidence, *dn*DSA would not appear to be an appropriate surrogate endpoint for decline in graft

function in kidney transplant research. Further study is needed to establish the role of *dn*DSA screening in the pediatric kidney transplant population and clarify the types of antibodies that are most associated with harm to the graft.

**References**


1. Kim JJ, Balasubramanian R, Michaelides G, et al. The clinical spectrum of de novo donor-specific antibodies in pediatric renal transplant recipients. *Am J Transplant*. 2014;14(10):2350-2358. doi:10.1111/ajt.12859.

2. Meier-Kriesche H-U, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? *Am J Transplant*. 2004;4(8):1289-1295. doi:10.1111/j.1600-6143.2004.00515.x.

3. El-Zoghby ZM, Stegall MD, Lager DJ, et al. Identifying specific causes of kidney allograft loss. *Am J Transplant*. 2009;9(3):527-535. doi:10.1111/j.1600-6143.2008.02519.x.

4. Terasaki PI. A personal perspective: 100-year history of the humoral theory of transplantation. *Transplantation*. 2012;93(8):751-756. doi:10.1097/TP.0b013e3182483713.

5. Christiaans MH, Overhof-de Roos R, Nieman F, van Hooff JP, van den Berg-Loonen EM. Donor-specific antibodies after transplantation by flow cytometry: relative change in fluorescence ratio most sensitive risk factor for graft survival. *Transplantation*. 1998;65(3):427-433.

6. Mihaylova A, Baltadjieva D, Boneva P, et al. Clinical relevance of anti-HLA antibodies detected by flow-cytometry bead-based assays--single-center experience. *Hum Immunol*. 2006;67(10):787-794. doi:10.1016/j.humimm.2006.07.011.

7. Lachmann N, Terasaki PI, Budde K, et al. Anti-human leukocyte antigen and donor-

specific antibodies detected by luminex posttransplant serve as biomarkers for chronic rejection of renal allografts. *Transplantation*. 2009;87(10):1505-1513. doi:10.1097/TP.0b013e3181a44206.

8. Freitas MCS, Rebellato LM, Ozawa M, et al. The role of immunoglobulin-G subclasses and C1q in de novo HLA-DQ donor-specific antibody kidney transplantation outcomes. *Transplantation*. 2013;95(9):1113-1119. doi:10.1097/TP.0b013e3182888db6.

9. Banasik M, Boratyńska M, Kościelska-Kasprzak K, et al. The impact of de novo donor-specific anti-human leukocyte antigen antibodies on 5-year renal transplant outcome. *Transplant Proc*. 2013;45(4):1449-1452. doi:10.1016/j.transproceed.2012.12.026.

10. Dieplinger G, Ditt V, Arns W, et al. Impact of de novo donor-specific HLA antibodies detected by Luminex solid-phase assay after transplantation in a group of 88 consecutive living-donor renal transplantations. *Transpl Int*. 2014;27(1):60-68. doi:10.1111/tri.12207.

11. Ntokou I-SA, Iniotaki AG, Kontou EN, et al. Long-term follow up for anti-HLA donor specific antibodies postrenal transplantation: high immunogenicity of HLA class II graft molecules. *Transpl Int*. 2011;24(11):1084-1093. doi:10.1111/j.1432-2277.2011.01312.x.

12. Ginevri F, Nocera A, Comoli P, et al. Posttransplant de novo donor-specific hla antibodies identify pediatric kidney recipients at risk for late antibody-mediated rejection. *Am J Transplant*. 2012;12(12):3355-3362. doi:10.1111/j.1600-6143.2012.04251.x.

13. Tait BD, Süsal C, Gebel HM, et al. Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation. *Transplant J*. 2013;95(1):19-47. doi:10.1097/TP.0b013e31827a19cc.

14. Cooper JE, Gralla J, Cagle L, Goldberg R, Chan L, Wiseman AC. Inferior kidney allograft outcomes in patients with de novo donor-specific antibodies are due to acute rejection

episodes. *Transplantation*. 2011;91(10):1103-1109. doi:10.1097/TP.0b013e3182139da1.

15. Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *Am J Transplant*. 2012;12(5):1157-1167. doi:10.1111/j.1600-6143.2012.04013.x.

16. Schwartz GJ, Munoz A, Schneider MF, et al. New Equations to Estimate GFR in Children with CKD. *J Am Soc Nephrol*. 2009;20(3):629-637. doi:10.1681/ASN.2008030287.

17. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612.

18. Thompson A, Lawrence J, Stockbridge N. GFR Decline as an End Point in Trials of CKD: A Viewpoint From the FDA. *Am J Kidney Dis*. 2014;64(6):836-837. doi:10.1053/j.ajkd.2014.09.006.

19. Greene T, Teng C-C, Inker LA, et al. Utility and Validity of Estimated GFR–Based Surrogate Time-to-Event End Points in CKD: A Simulation Study. *Am J Kidney Dis*. 2014;64(6):867-879. doi:10.1053/j.ajkd.2014.08.019.

20. Campos EF, Tedesco-Silva H, Machado PG, Franco M, Medina-Pestana JO, Gerbase-DeLima M. Post-Transplant Anti-HLA Class II Antibodies as Risk Factor for Late Kidney Allograft Failure. *Am J Transplant*. 2006;6(10):2316-2320. doi:10.1111/j.1600-6143.2006.01503.x.

21. Willicombe M, Brookes P, Sergeant R, et al. De Novo DQ Donor-Specific Antibodies Are Associated With a Significant Risk of Antibody-Mediated Rejection and Transplant Glomerulopathy. *Transplant J*. 2012;94(2):172-177. doi:10.1097/TP.0b013e3182543950.

22. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-Binding Anti-HLA Antibodies and Kidney-Allograft Survival. *N Engl J Med*. 2013;369(13):1215-1226.

doi:10.1056/NEJMoa1302506.

23. Sutherland SM, Chen G, Sequeira FA, Lou CD, Alexander SR, Tyan DB. Complement-fixing donor-specific antibodies identified by a novel C1q assay are associated with allograft loss. *Pediatr Transplant*. 2012. doi:10.1111/j.1399-3046.2011.01599.x.

24. Comoli P, Cioni M, Tagliamacco A, et al. Acquisition of C3d-Binding Activity by De Novo Donor-Specific HLA Antibodies Correlates With Graft Loss in Nonsensitized Pediatric Kidney Recipients. *Am J Transplant*. 2016. doi:10.1111/ajt.13700.

25. Yell M, Muth BL, Kaufman DB, Djamali A, Ellis TM. C1q Binding Activity of De Novo Donor-specific HLA Antibodies in Renal Transplant Recipients With and Without Antibody-mediated Rejection. *Transplantation*. 2015;99(6):1151-1155. doi:10.1097/TP.0000000000000699.

26. Messina M, Ariaudo C, Praticò Barbato L, et al. Relationship among C1q-fixing de novo donor specific antibodies, C4d deposition and renal outcome in transplant glomerulopathy. *Transpl Immunol*. 2015;33(1):7-12. doi:10.1016/j.trim.2015.06.002.

27. Arias-Cabrales C, Redondo-Pachón D, Pérez-Sáez MJ, et al. Renal graft survival according to Banff 2013 classification in indication biopsies. *Nefrologia*. 2016;36(6):660-666. doi:10.1016/j.nefro.2016.05.018.

28. Park WD, Griffin MD, Cornell LD, Cosio FG, Stegall MD. Fibrosis with Inflammation at One Year Predicts Transplant Functional Decline. *J Am Soc Nephrol*. 2010;21(11):1987-1997. doi:10.1681/ASN.2010010049.

29. Terasaki PI. Humoral theory of transplantation. *Am J Transplant*. 2003;3(6):665-673.

**Figure 1:** Study Flow Diagram

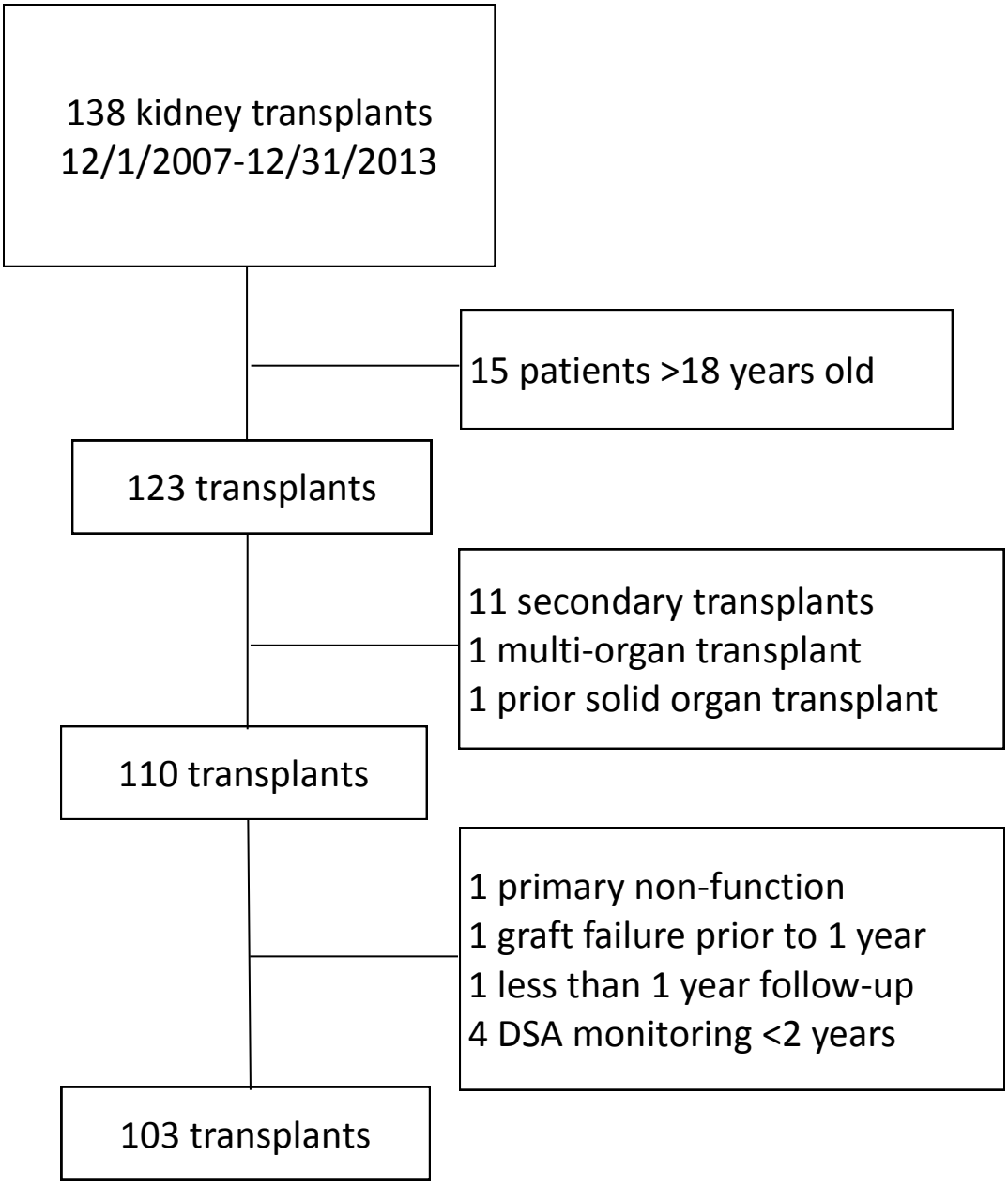


**Table 1:** Baseline characteristics, immunosuppression management, and transplant characteristics of 103 pediatric renal transplants performed 12/1/2008-12/31/2013, subdivided by subsequent diagnosis of *dn*DSA and creatinine at time of *dn*DSA detection.

| | **No *dn*DSA** n=70 | | ***dn*DSA with stable creatinine** n=20 | | ***dn*DSA with elevated creatinine** n=13 | |
|---|---|---|---|---|---|---|
| **Age (years)** (median (IQR)) | 12.2 | (7.4-14.8) | 6.8 | (3.8-16.3) | 16 | (6.5-16.3) |
| **Male** (n(%)) | 44 | (63%) | 15 | (75%) | 8 | (62%) |
| **Caucasian** (n(%)) | 46 | (66%) | 11 | (55%) | 6 | (46%) |
| **ESRD cause** (n(%)) | | | | | | |
| **CAKUT** | 36 | (54%) | 9 | (45%) | 8 | (65%) |
| **Other** | 34 | (46%) | 11 | (55%) | 5 | (35%) |
| **Deceased donor transplant** (n(%)) | 31 | (44%) | 10 | (50%) | 11 | (85%) |
| **Induction**(n(%)) | | | | | | |
| **Thymoglobulin** | 56 | (80%) | 17 | (85%) | 8 | (62%) |
| **IL-2 blockade** | 14 | (20%) | 3 | (15%) | 5 | (38%) |
| **# of thymoglobulin doses** (mean(sd)) | 4.4 | (1.2) | 4.6 | (1.1) | 4.8 | (1.6) |
| **Steroids at induction** (n(%)) | 61 | (87%) | 19 | (95%) | 12 | (92%) |
| **Maintenance MMF** (n(%)) | 70 | (100%) | 20 | (100%) | 13 | (100%) |
| **Maintenance Immunosuppression** (n(%) | | | | | | |
| **Tacrolimus** | 68 | (97%) | 19 | (95%) | 13 | (100%) |
| **Sirolimus** | 2 | (3%) | 1 | (5%) | 0 | (0%) |
| **Maintenance steroids** (n(%)) | 4 | (6%) | 1 | (10%) | 0 | (0%) |
| **Warm ischemia time (min)** (median (IQR)) | 556 | (44-67) | 52 | (45-59) | 51 | (47-60) |
| **Cold ischemia time (min)** (median (IQR)) | 178 | (126-714) | 449 | (139-691) | 698 | (494-996) |
| **Delayed graft function** (n(%)) | 4 | (6%) | 3 | (15%) | 0 | (0%) |
| **cPRA** (median(IQR)) | 0 | (0-0) | 0 | (0-0) | 0 | (0-0) |
| **# Class I HLA mismatches** (mean(sd)) | 4.2 | (1.6) | 4.8 | (1.2) | 4.9 | (1.1) |
| **# DQ mismatches** (mean(sd)) | 1.2 | (0.73) | 1.2 | (0.75) | 1.2 | (0.69) |
| **# DR mismatches** (mean(sd)) | 0.8 | (0.73) | 1 | (0.73) | 0.9 | (0.86) |
| **EBV D+/R-** (n(%)) | 21 | (30%) | 9 | (45%) | 2 | (20%) |
| **CMV D+/R-** (n(%)) | 20 | (29%) | 9 | (45%) | 4 | (33%) |
| **Baseline eGFR (ml/min/1.73m$^2$)** (mean(sd)) | 85.7 | (21.2) | 93.9 | (36.6) | 89.0 | (20.1) |
| **Time in Study (years)** (mean(sd)) | 4.5 | (1.6) | 4.5 | (1.7) | 3.2 | (1.8) |

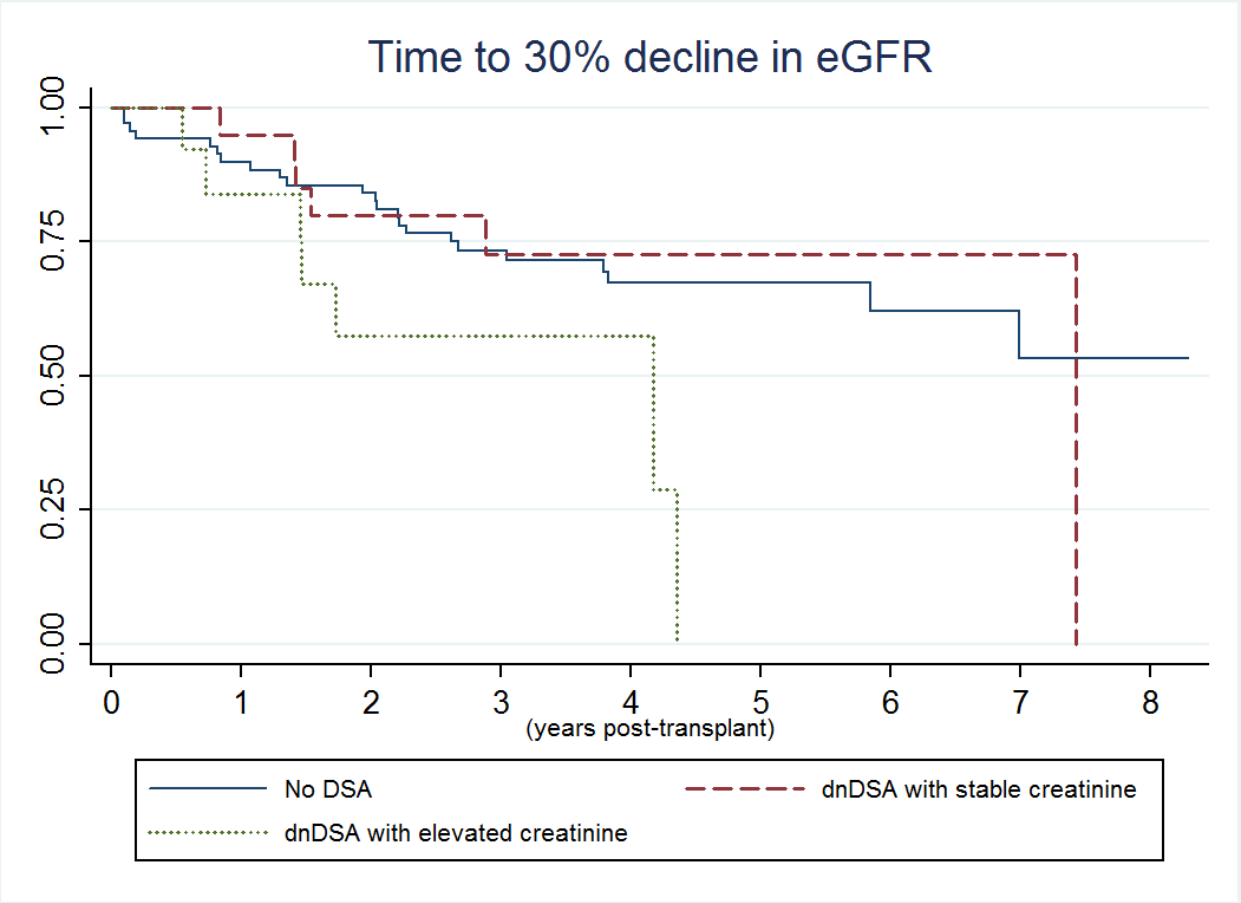
**Table 2:** Characteristics of *de novo* donor specific antibodies detected in pediatric patients after kidney transplant, by creatinine stability at time of *dn*DSA detection.

| | ***dn*DSA with stable creatinine** n=20 | | ***dn*DSA with elevated creatinine** n=13 | |
|---|---|---|---|---|
| **≥6 DSA tests in 2 years of monitoring** | 17 | (85%) | 10 | (77%) |
| **Time to DSA (days)** (median(IQR)) | 332 | (87-738) | 543 | (394-630) |
| **Class of Initial DSA** (n(%)) | | | | |
| **Class I** | 2 | (10%) | 2 | (15%) |
| **Class II** | 16 | (80%) | 7 | (54%) |
| **Class I & Class II** | 2 | (10%) | 4 | (31%) |
| **Initial DSA MFI** (median(IQR)) | 2500 | (1050-4350) | 2400 | (1300-3400) |
| **Class of DSA overall** (n(%)) | | | | |
| **Class I** | 2 | (10%) | 2 | (15.4%) |
| **Class II** | 13 | (65%) | 7 | (15%) |
| **Class I & Class II** | 5 | (25%) | 4 | (31%) |
| **Peak MFI overall** (median(IQR)) | 6150 | (2750-11600) | 4100 | (2200-8900) |
| **C1q positive** (n(%)) | 6 | (43%) | 2 | (40%) |
| **Biopsy within 3months of positive DSA** | 10 | (50%) | 10 | (77%) |
| **Antibody-mediated rejection** | 1 | (10%) | 1 | (10%) |
| **Acute cellular rejection** | 3 | (30%) | 6 | (60%) |
| **Duration of DSA (days)** (median(IQR)) | 378 | (85-596) | 172 | (85-241) |
| **DSA resolve before 2 years** (n(%)) | 6 | (30%) | 4 | (31%) |

**Table 3:** Outcomes among pediatric kidney transplant patients subdivided by presence of *dn*DSA and creatinine stability at the time of *dn*DSA detection. Results are adjusted for age at transplant, type of transplant donor, incidence of delayed graft function, cold ischemia time, and baseline estimated glomerular filtration rate.

| **Outcome** | **No *dn*DSA n=70** | | | ***dn*DSA with stable creatinine n=20** | | | | | ***dn*DSA with elevated creatinine n=13** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **aHR** | | | **aHR** | **(95%CI)** | | **p** | | **aHR** | **(95%CI)** | | **p** | |
| **eGFR 30% decline** | Ref | | | 0.88 | (0.30-2.00) | | 0.598 | | 2.80 | (1.08-7.27) | | 0.034 | |
| **Graft loss** | Ref | | | 1.75 | (0.28-10.73) | | 0.546 | | 7.34 | (1.37-39.23) | | 0.020 | |
| | **Mean** | **SD** | | **Mean** | | **SD** | **p** | | **Mean** | | **SD** | **p** | |
| **eGFR at 3 years** (ml/min/1.73m$^2$) | 75.34 | 23.65 | | 74.26 | | 36.20 | 0.632 | | 60.84 | | 30.31 | 0.054 | |
| | **n=244.7 person-yrs** | | | **n=41.8 person-yrs** | | | | | **n=22.5 person-yrs** | | | | |
| | **IR** | **RR** | | **IR** | **aHR** | **(95% CI)** | **p** | | **IR** | **aHR** | **(95% CI)** | **p** | |
| **Rejection** (at 3 years) | 0.15 | Ref | | 0.36 | 2.41 | (1.27-4.54) | 0.007 | | 0.71 | 2.43 | (1.00-5.90) | 0.050 | |
| | **n=57** | | | **n=17** | | | | | **n=11** | | | | |
| **IFTA** (at 2 years) | **n** | **(%)** | **aOR** | **n** | **(%)** | **aOR** | **(95%CI)** | **p** | **n** | **(%)** | **aOR** | **(95%CI)** | **p** |
| None | 26 | (46%) | - | 5 | (29%) | - | - | - | 4 | (36%) | - | - | - |
| Mild | 28 | (49%) | Ref | 8 | (47%) | 2.03 | (0.52-7.93) | 0.308 | 6 | (55%) | 1.15 | (0.22-5.91) | 0.865 |
| Moderate | 3 | (5%) | Ref | 4 | (24%) | 26.8 | (1.91-377) | 0.015 | 1 | (9%) | 5.58 | (0.22-138) | 0.294 |

**Figure 2:** Kaplan-Meier survival curves for (A) time to a 30% decline in estimated glomerular filtration rate and (B) time to graft loss among pediatric kidney transplant patients, subdivided by presence of *dn*DSA and creatinine stability at the time of *dn*DSA detection.



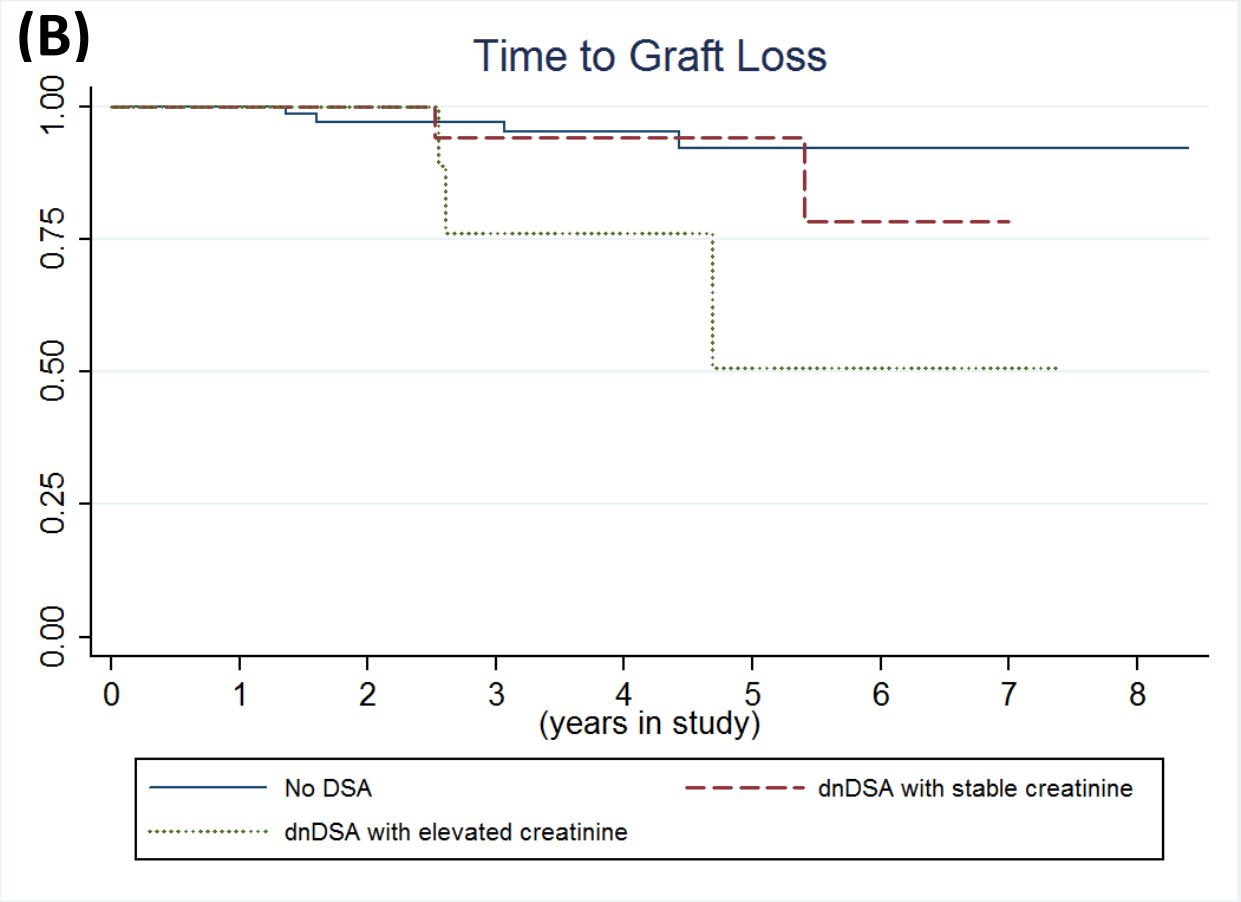

(B)
Time to Graft Loss
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
5
6
7
8
(years in study)
No DSA
dnDSA with stable creatinine
dnDSA with elevated creatinine

**Table 4:** Risk of 30% decline in estimated glomerular filtration rate among pediatric kidney transplant patients, comparing patients with *dn*DSA and a stable serum creatinine to patients who did not develop *dn*DSA within 2 years of kidney transplant, using different MFI thresholds to adjudicate DSA positivity. Results are adjusted for age at transplant, type of transplant donor, delayed graft function, cold ischemia time, and baseline estimated glomerular filtration rate.

| | No *dn*DSA | *dn*DSA with stable creatinine | 30% decline in renal function: No *dn*DSA vs *dn*DSA with a stable creatinine | | |
|---|---|---|---|---|---|
| | n | n | aHR | (95%CI) | p |
| Any MFI | 70 | 20 | 0.88 | (0.30-2.00) | 0.598 |
| MFI ≥1000 | 72 | 17 | 1.01 | (0.38-2.69) | 0.976 |
| MFI ≥1500 | 75 | 17 | 0.97 | (0.33-2.85) | 0.955 |
| MFI ≥2500 | 79 | 17 | 1.04 | (0.39-2.76) | 0.938 |
| MFI ≥8000 | 90 | 9 | 0.99 | (0.23-4.29) | 0.994 |