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Application and interpretation of histocompatibility data in pediatric kidney transplantation

Hilda E. Fernandez, MD, MSCE

Division of Nephrology, Department of Medicine, Columbia University Medical Center, New York, NY

Abstract

Purpose of review—Advances in technology to assess immunologic risk in solid organ transplant offer an opportunity to optimize the approach to pediatric deceased donor kidney transplant (KT) in the setting of a new allocation system in the USA.

Recent findings—Degree of HLA mismatch (MM), Class II HLA MM, unacceptable antigens and donor specific antibody detected by solid phase assays (SPA), and epitope matching pre-transplant affect pediatric KT outcomes. Detection of *dn*DSA (de novo donor specific antibody) antibodies post-transplant has been associated with increased risk of acute rejection and worse allograft function. Development of *dn*DSA occurs in recipients with greater epitope mismatching.

Summary—Improved long-term outcomes may be anticipated in pediatric kidney transplant recipients by incorporating extended HLA MM information and updating the clinical approach to donor kidney matching using available technology to identify clinically relevant immunologic risk.

Keywords

pediatric kidney transplant; histocompatibility

Introduction

The demand for kidney transplantation continues to outstrip the supply for available kidneys worldwide. Since 1999, living related donation in pediatric kidney transplant (KT) programs has decreased from 45% to 30%.¹ Pediatric KT recipient outcomes are linked to age at transplant, degree of alloimmune sensitization, and living versus deceased kidney donors.^{2, 3} Central to these factors are the immunological risk of the donated organ to the recipient. Pre-transplant histocompatibility screening aids in the identification of high-risk KT recipients. Enhancement of histocompatibility tools may improve not only first KT allograft outcomes, but also decrease a lifetime of immunologic risk in future kidney transplants. This review of current histocompatibility techniques aims to guide pediatric transplant clinicians to match the best quality organ to pediatric KT candidates.

Corresponding Author: Hilda Fernandez, Division of Nephrology, Department of Medicine, Columbia University Medical Center, 622 W. 168th Street, PH-4, Suite 124, New York, NY. 10032, hef2112@cumc.columbia.edu; Tel: 212-851-4938; Fax: 212-851-4939.

Conflicts of Interest

None.

Immunobiology in Kidney Transplant

Human leukocyte antigen (HLA) antigen proteins on cell surfaces are key to differentiation of self from non-self proteins.^{4**} Major histocompatibility complex (MHC), a collection of more than 200 genes on chromosome 6p, encodes HLA proteins. The two groups of MHC genes relevant to transplantation are class I and class II. Class I MHC genes (HLA-A, -B, -C) produce class I MHC molecules, which have two polypeptide chains: a polymorphic α chain and an invariable β_2 microglobulin chain. Class I HLA variability is due to the peptide-binding region, the α_1 and α_2 domains. Class I HLA proteins are expressed on the surface of nearly every nucleated cell. There are nine primary MHC class II genes: HLA-DPA1, -DPB1, -DQA1, -DQB1, -DRA, -DRB1, -DRB3, -DRB4, and -DRB5. These class II molecules also consist of two chains, α and β , with the class HLA variability in the peptide-binding regions of the α_1 and β_1 domains. Class II HLA proteins are expressed on antigen presenting cells (APC), such as macrophages, B cells and dendritic cells. Each HLA protein is expressed from two alleles, with a high degree of HLA genetic polymorphism. One exception is HLA-DRA, which is invariant in the peptide-binding regions, and is considered invariant overall.

The adaptive immune response develops immunologic memory and antigen specificity by way of T cells recognition of alloantigens.^{5, 6} Acute cellular and humoral rejections in KT occur as a result of recognition of donor antigens leading to activation of T cells, B cells, creation of donor specific antibodies (DSA), and the antibody-mediated activation of the complement cascade.

The immunologic risk of children as compared to adults has been considered to be lower due to a more naïve immune system (lower T cell activity and lower anti-HLA antibodies prior to transplantation).³ The immunological stakes are higher for a child who will likely need a second and third KT later in life.

Histocompatibility Methods

Complement-Dependent Lymphocytotoxicity Crossmatch (CDCXM)

Preformed antibody in the transplant recipient is screened by use of the qualitative CDCXM, where recipient serum is incubated with potential donor B and T cells that express known HLA class I and II antigens, followed by addition of complement and analysis of cell lysis. Panel-reactive antibodies (PRAs) are calculated by the percentage of panel cells that are killed by recipient serum.

The false negative crossmatch rate is improved by the antihuman-immunoglobulin-enhanced CDC (AHG-CDC) assay.⁷⁻⁹ AHG is added prior to complement in order to crosslink a larger number of molecules, enhancing complement-mediated cytotoxic cell death. These CDC methods are qualitative, and their interpretation is subjective.

Flow Cytometry Crossmatch (FCXM)

Fluorescent-labeled reagents directed against anti-HLA antibodies as well as T and B cell-specific cell surface proteins (CD3 for T-cells, CD19 for B-cells) are added to cell samples

to perform the FCXM and detect antibody bound to individual T and B cells. This whole-cell based technique offers definitive antibody and class identification. The addition of pronase (an agent that digests Fc receptors and other cell surface proteins) aids in decreasing non-specific background binding of the Fc receptors and autoantibodies, improving FCXM sensitivity and specificity.¹⁰

Solid-Phase Assays (SPA)

Solid-phase assays detect antibody either through purified HLA antigens affixed to microparticle beads that are analyzed by flow cytometry, or via enzyme-linked immunosorbent assay (ELISA).^{11–15} Candidate serum is added to the SPA, anti-HLA antibodies bind to the antigens, and fluorescent anti-human globulins are added and illuminate when bound to anti-HLA antibodies, measured in mean fluorescence intensity (MFI). The addition of SPA improved the validity of clinically relevant positive crossmatch results by increasing specificity and controlling for non-HLA antibodies and false-positive results. The HLA antigen purification process itself can destabilize the antigen, changing their confirmation and lead to cryptic (previously hidden) antigen expression.¹⁶ A DSA specific to a cryptic antigen may not be clinically relevant, yet a positive result may prevent a recipient from receiving an acceptable donor kidney. Due to the variable antigen density of microparticle beads, there can be inter- and intra-assay variability between SPA, though guidelines now have been suggested for SPA techniques both pre- and post-transplant.¹⁷

Complement-fixing SPA technology determines the ability of DSA to bind complement, potentially offering additional specificity to detecting clinically relevant DSA. The Lumindex® (Life Technologies, Thermo Fisher Scientific Corp, Carlsbad, CA) single-antigen bead (SAB) platform utilizes C1q binding by combining HLA-coated SAB, patient serum and an exogenous source of C1q. Fluorescent anti-C1q antibody is added and illuminates complement-fixing antibodies, independent of the strength of the IgG MFI. The C1q-binding DSA has been correlated to rejection and graft failure in KT recipients, though there is again variability in the availability of C1q assays and performance.^{18–20}

Epitope mapping

Epitopes, the part of the HLA antigen that antibody recognizes, are characterized by short sequences of amino acid residues termed eplets. Duquesnoy, *et al.* developed the HLAMatchmaker, a molecularly based algorithm that determines which eplets are different between donor and recipient.^{21, 22} Epitopes have also been identified through analysis of SAB reactivity of monoclonal antibodies or alloantibodies adsorbed and then eluted on recombinant single antigen cell lines.²³ Electrostatic potentials of HLA epitopes have also been calculated through three-dimensional structural modeling and have been correlated with well-characterized HLA binding assays, suggesting a role for this computational method for predicting HLA antigenicity.²⁴ These advances in epitope mapping may improve probability of transplantation and long-term outcomes.

Pre-Transplant Histocompatibility Listing Factors

Unacceptable Antigens and Calculating cPRA

Candidates undergo HLA typing as well as evaluation for pre-existing anti-HLA antibodies. Patients with well-defined anti-HLA antibodies with confirmation by SPA are listed as unacceptable antigens. As SPA has great sensitivity, some transplant centers may use multiple dilutions of the patient's sera to determine if fluorescence shift is proportional to the titer of the antibody. A transplant center utilizes SPA data to determine individual cut-off levels of MFI to determine unacceptable antigens to be avoided to prevent hyperacute, accelerated antibody-mediated rejections or chronic allograft nephropathy.²⁵ The breadth of sensitization is measured by calculated panel-reactive antibodies (cPRA) in the US and similar statistics in Europe and the UK. The calculation is based on the frequency of donors with unacceptable antigens, and is based on regional differences in HLA antigen distributions.

Preliminary and Final Crossmatch

Serum samples from potential KT recipients who are actively waitlisted are routinely screened, and a preliminary crossmatch is made against donor cells using this serum. "Virtual" crossmatch is performed in some centers when the candidate's unacceptable antigens have been entered into the UNOS database (UNET) system. If the preliminary crossmatch is negative, then a final crossmatch is made with either AHG-CDC or FCXM using recent (within the last month) or fresh sera.

Pediatric Kidney Allocation System

With the initiation of the current Organ Procurement and Transplantation Network (OPTN) kidney allocation system in 2014, an emphasis was placed on promoting longevity matching between donor and recipient in order to utilize the maximum amount of graft years through an estimated post-transplant survival score (EPTS).²⁶ Pediatric candidates will continue to have high quality kidneys allocated to children < 18 years of age, with donors with a kidney donor profile index (KDPI) of < 35%. Priority on a sliding scale continues for highly sensitized candidates based on cPRA score starting at a cPRA > 20%, with the largest gains for priority for cPRA > 90%. Therefore, clinical decision making for accepting kidney donors should continue to emphasize for pediatric transplant recipients to receive the best quality kidney donors in order to enhance both patient and allograft survival.

Immune Monitoring and Pediatric Kidney Post-Transplant Outcomes

HLA Mismatch

HLA matching is a well-known predictor of renal allograft survival, though our current allocation system does not account for degree of HLA MM.^{27–29} With a limited organ supply, paucity of living donors, and a desire to decrease waiting time on dialysis, pediatric recipients are transplanted with a high degree of mismatching. Foster, *et al.* demonstrated that in a United States Renal Data System (USRDS) cohort of 8433 pediatric patients from 1988 to 2000, in patients < 21 years of age at first transplant, having 0–1 MM was associated with 15% more time with a functioning graft than in patients having 2–3 MM

with their first allografts, who likewise had 12% more time than those with 4–6 MM allografts.^{30*} In a subset of 2498 patients that were subsequently listed for a second transplant, 0–1 MM first transplant recipients were more 26% more likely to be successfully transplanted with a second transplant.

De novo DSA

The development of *dn*DSA has been associated with higher degree of HLA MM and poorer allograft outcomes.^{31–33} *De novo* DSA was detected in 6% of subjects in a prospective pediatric cohort that monitored HLA antibody and protocol biopsies in a USA pediatric cohort (n=124) on steroid-based (n=60) and steroid-free (n=64) immunosuppression (ISS) protocols.² The development of *dn*DSA was associated with overall worse graft function at 6, 12 and 24 months after transplant (p=0.02), higher risk for acute rejection (p=0.02), and chronic allograft failure (p=0.02) in this cohort. At 2 years post-transplant this cohort had 100% patient survival, but 5 patients had lost their allografts, each associated with both non-adherence to ISS and greater than 3 HLA MM, with no difference in steroid-based or steroid-free ISS. In an Italian cohort of 114 pediatric transplant recipients, 39 patients developed *dn*DSA, 15 of which developed early *dn*DSA, before the first year post-transplant.³⁴ Regardless of timing of appearance, *dn*DSA was associated with late antibody mediated rejection in 47% of early group and 58% of the late group, and allograft loss occurred in early and late *dn*DSA groups (20% and 17%, respectively) in a median follow-up time of 6.7 years.

While class I and II HLA MM of HLA-Cw, -DR, -DP, and -DQ are not considered in the organ allocation system, they have been associated with development of *dn*DSA, acute rejection, and worse allograft outcomes.^{35, 36} In a UK pediatric cohort (n=215) with prospective monitoring of HLA antibodies, 35% of patients developed *dn*DSA at a median of 0.25 years post-transplant, with a high prevalence of Class II *dn*DSA (70%), of which 45% were anti-HLA-DQ.³⁷ In 47% of patients who had earlier (< 4 months post-transplant) *dn*DSA detection and lower MFI at detection (< 3500 MFI), *dn*DSA resolved. Patients who were *dn*DSA positive had a more rapid decline in eGFR, with 50% reduction from their post-transplant nadir at 5.3 vs 6.1 years (p=0.02). Analysis of 11,916 pediatric KT recipients from the USA Scientific Registry of Transplant Recipients (SRTR) database from 1990 to 2008, 2704 were re-listed following first kidney allograft failure, of which 15.5% were re-transplanted, and 7.2% remained on the wait list.³⁸ Mean PRA and waiting time increased significantly with degree of HLA-DR MM with first and second KT in this cohort. HLA-DR MM greater than 2 was associated with worse outcomes at five years following second KT, as compared to 0–1 MM.

An Italian pediatric cohort of 48 non-sensitized KT with 65 for-cause biopsy data also demonstrated that high serum *dn*DSA (> 10000 MFI) was associated with intragraft *dn*DSA detected from eluent from graft pellets via SAB.³⁹ Intragraft *dn*DSA, termed graft homing, has been associated with worse allograft outcomes.⁴⁰

C1q+ *dn*DSA has been associated with a higher risk of KT loss than C1q- *dn*DSA.^{18, 20, 41} It has been demonstrated that C1q+ *dn*DSA correlates strongly with high HLA antibody MFI. In a French study of 346 adult non-sensitized KT recipients evaluated at 2 and 5 years post-

transplant, 3.5% and 2.5% had C1q+ *dn*DSA.⁴² Patients with C1q-nonbinding *dn*DSA had worse 10-year allograft survival ($p < 0.001$) than patients with C1q-binding *dn*DSA ($p = 0.002$) compared to patients without *dn*DSA. To better understand the role of C1q and complement fixation in predicting allograft outcomes, C3d-binding and combined C1q and C3d SAB assays were performed in 39 Italian pediatric KT recipients who developed *dn*DSA.^{43**} Patients with persistent non-complement-binding *dn*DSA acquired C1q binding ability, and likewise persistent C1q-binding *dn*DSA acquired C3d binding ability over time. C3d-binding was associated with lower 10-year graft survival probability compared to patients without *dn*DSAs, those with C1q+/C3d- binding *dn*DSA, or with non-complement-binding *dn*DSAs. C3d-binding *dn*DSA was also correlated with AMR development. These data suggest that there is progressive acquisition of *dn*DSA complement-binding ability over time, as detection of C3 fragments is an indicator of ongoing complement-activation. This also supports a paradigm where two separate processes mediate chronic antibody-mediated graft injury initiated by DSA deposition; one that is mediated through antibody-dependent cell cytotoxicity, and the other by the complement classic pathway. Wiebe *et al.* also performed C1q-binding SAB assays in 70 Canadian KT recipients, and found that C1q+ *dn*DSA was associated with allograft loss, as was high *dn*DSA titer, however was not independently associated with outcomes when taking into account clinical phenotype (renal biopsy) and an admission of nonadherence.⁴⁴ These data taken together may suggest that C1q+ *dn*DSA status is a reflection of acute or progressive allograft rejection.

HLA Eplet Matching

Following kidney allograft failure in one adult study of 66 non-sensitized (PRA < 10%) patients at first transplant, immunosuppression withdrawal at allograft failure (discontinuation of anti-metabolite and weaning of CNI), African American race, HLA-DQ eplet mismatching (analyzed using HLAMatchmaker) and graft intolerance syndrome (characterized by allograft pain, fever or hematuria), were associated with becoming highly sensitized, defined as a PRA > 80%.⁴⁵ Use of HLA-DR and HLA-DQ eplet matching may increase acceptable offers to pediatric patients on the waitlist, as demonstrated using HLAMatchmaker on 16 patients.^{46**} It was demonstrated that eplet mismatch is common at HLA-DR/DQ, and that there may be an increased eplet matching that would not have been predicted with traditional DR/DQ matching that would place these potential recipients in a lower risk to develop *dn*DSA. A Canadian study utilizing HLAMatchmaker, with 286 kidney donor-recipient pairs, defined optimal thresholds for epitope mismatching for DR and DQ, and at median of 6.9 years, 0 and 2.7% of patients developed *dn*DSA to DR and DQ, respectively.⁴⁷ While eplet threshold mismatch thresholds are still to be determined in pediatric populations, this is an exciting new tool to aid in kidney allocation.

Conclusions

These studies demonstrate that pre- and post-transplant histocompatibility monitoring plays a role in determining immunologic risk before, during and following KT. Degree of HLA MM, including HLA-DR and HLA-DQ, while excluded from the current kidney allocation system, should weigh-in on decisions for accepting donor kidneys. Post-transplant immune monitoring still poses challenges on how to interpret *dn*DSA data in the absence of acute

rejection despite evidence linking it to poorer allograft outcomes. The long-term effect of choosing an appropriately matched donor kidney for a pediatric candidate has long term consequences in kidney allograft and recipient outcomes.

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References

1. Keith DS, Vranic G, Barcia J, Norwood V, Nishio-Lucar A. Longitudinal analysis of living donor kidney transplant rates in pediatric candidates in the United States. *Pediatr Transplant*. 2017; 21(2)
2. Chaudhuri A, Ozawa M, Everly MJ, et al. The clinical impact of humoral immunity in pediatric renal transplantation. *J Am Soc Nephrol*. 2013; 24(4):655–64. [PubMed: 23449533]
3. Dharnidharka VR, Fiorina P, Harmon WE. Kidney transplantation in children. *N Engl J Med*. 2014; 371(6):549–58. [PubMed: 25099579]
- 4**. South AM, Grimm PC. Transplant immuno-diagnostics: crossmatch and antigen detection. *Pediatric nephrology*. 2016; 31(6):897–905. This review of advances in solid phase assays covers the evolution of transplant immuno-diagnostics. [PubMed: 26139577]
5. Truong DQ, Bourdeaux C, Wieers G, Saussoy P, Latinne D, Reding R. The immunological monitoring of kidney and liver transplants in adult and pediatric recipients. *Transpl Immunol*. 2009; 22(1–2):18–27. [PubMed: 19800003]
6. Sarwal M, Chua MS, Kambham N, et al. Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. *N Engl J Med*. 2003; 349(2):125–38. [PubMed: 12853585]
7. Gebel HM, Oldfather JW, Karr RW, Fuller TC, Rodey GE. Antibodies directed against HLA-DR gene products exhibit the CYNAP phenomenon. *Tissue Antigens*. 1984; 23(3):135–40. [PubMed: 6427971]
8. Fuller TC, Fuller AA, Golden M, Rodey GE. HLA alloantibodies and the mechanism of the antiglobulin-augmented lymphocytotoxicity procedure. *Hum Immunol*. 1997; 56(1–2):94–105. [PubMed: 9455498]
9. Fuller TC, Phelan D, Gebel HM, Rodey GE. Antigenic specificity of antibody reactive in the antiglobulin-augmented lymphocytotoxicity test. *Transplantation*. 1982; 34(1):24–9. [PubMed: 6181591]
10. Vaidya S, Cooper TY, Avandsalehi J, et al. Improved flow cytometric detection of HLA alloantibodies using pronase: potential implications in renal transplantation. *Transplantation*. 2001; 71(3):422–8. [PubMed: 11233905]
11. Zachary AA, Delaney NL, Lucas DP, Leffell MS. Characterization of HLA class I specific antibodies by ELISA using solubilized antigen targets: I. Evaluation of the GTI QuikID assay and analysis of antibody patterns. *Hum Immunol*. 2001; 62(3):228–35. [PubMed: 11250040]
12. Zachary AA, Ratner LE, Graziani JA, Lucas DP, Delaney NL, Leffell MS. Characterization of HLA class I specific antibodies by ELISA using solubilized antigen targets: II. Clinical relevance. *Hum Immunol*. 2001; 62(3):236–46. [PubMed: 11250041]
13. Pei R, Wang G, Tarsitani C, et al. Simultaneous HLA Class I and Class II antibodies screening with flow cytometry. *Hum Immunol*. 1998; 59(5):313–22. [PubMed: 9619770]
14. Kao KJ, Scornik JC, Small SJ. Enzyme-linked immunoassay for anti-HLA antibodies—an alternative to panel studies by lymphocytotoxicity. *Transplantation*. 1993; 55(1):192–6. [PubMed: 8420047]
15. Bettinotti MP, Zachary AA, Leffell MS. Clinically relevant interpretation of solid phase assays for HLA antibody. *Curr Opin Organ Transplant*. 2016; 21(4):453–8. [PubMed: 27200498]

16. Gebel HM, Harris SB, Zibari G, Bray RA. Conundrums with FlowPRA beads. *Clin Transplant*. 2002; 16(Suppl 7):24–9.
17. Tait BD, Susal C, Gebel HM, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation*. 2013; 95(1):19–47. [PubMed: 23238534]
18. Yabu JM, Higgins JP, Chen G, Sequeira F, Busque S, Tyan DB. C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. *Transplantation*. 2011; 91(3):342–7. [PubMed: 21116220]
19. 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; 16(1):12–7. [PubMed: 22093755]
20. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. *N Engl J Med*. 2013; 369(13):1215–26. [PubMed: 24066742]
21. Duquesnoy RJ, Askar M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. V. Eplet matching for HLA-DR, HLA-DQ, and HLA-DP. *Hum Immunol*. 2007; 68(1):12–25. [PubMed: 17207708]
22. Tambur AR, Rosati J, Roitberg S, Glotz D, Friedewald JJ, Leventhal JR. Epitope analysis of HLA-DQ antigens: what does the antibody see? *Transplantation*. 2014; 98(2):157–66. [PubMed: 25003284]
23. El-Awar N, Terasaki PI, Cai J, et al. Epitopes of HLA-A, B, C, DR, DQ, DP and MICA antigens. *Clin Transpl*. 2009:295–321. [PubMed: 20524293]
24. Mallon DH, Bradley JA, Winn PJ, Taylor CJ, Kosmoliaptsis V. Three-dimensional structural modelling and calculation of electrostatic potentials of HLA Bw4 and Bw6 epitopes to explain the molecular basis for alloantibody binding: toward predicting HLA antigenicity and immunogenicity. *Transplantation*. 2015; 99(2):385–90. [PubMed: 25606786]
25. Cecka JM., Rajalingam, R., Zhang, J., Reed, EF. Histocompatibility Testing, Crossmatching, and Immune Monitoring. In: Danovitch, GM., editor. *Handbook of Kidney Transplantation*. 5. New York: Wolters Kluwer, Lippincott Williams & Wilkins; 2010. p. 36-60.
26. Organ Procurement and Transplantation Network Center: Concepts for Kidney Allocation. <http://optn.transplant.hrsa.gov/learn/professional-education/kidney-allocation-system>
27. Opelz G, Dohler B. Pediatric kidney transplantation: analysis of donor age, HLA match, and posttransplant non-Hodgkin lymphoma: a collaborative transplant study report. *Transplantation*. 2010; 90(3):292–7. [PubMed: 20548266]
28. Johnson RJ, Armstrong S, Belger MA, et al. The outcome of pediatric cadaveric renal transplantation in the UK and Eire. *Pediatr Transplant*. 2002; 6(5):367–77. [PubMed: 12390422]
29. Takemoto SK, Terasaki PI, Gjertson DW, Cecka JM. Twelve years' experience with national sharing of HLA-matched cadaveric kidneys for transplantation. *N Engl J Med*. 2000; 343(15):1078–84. [PubMed: 11027742]
- 30*. Foster BJ, Dahhou M, Zhang X, Platt RW, Smith JM, Hanley JA. Impact of HLA mismatch at first kidney transplant on lifetime with graft function in young recipients. *Am J Transplant*. 2014; 14(4):876–85. HLA mismatching analysis in United States Renal Data System in pediatric transplant recipients predicts likelihood of successful kidney retransplant. [PubMed: 24612783]
31. Kimball PM, Baker MA, Wagner MB, King A. Surveillance of alloantibodies after transplantation identifies the risk of chronic rejection. *Kidney Int*. 2011; 79(10):1131–7. [PubMed: 21270760]
32. Rebellato LM, Everly MJ, Haisch CE. A report of the epidemiology of de novo donor-specific anti-HLA antibodies (DSA) in "low-risk" renal transplant recipients. *Clin Transpl*. 2011:337–40. [PubMed: 22755428]
33. 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):3355–62. [PubMed: 22959074]
34. Cioni M, Nocera A, Innocente A, et al. De Novo Donor-Specific HLA Antibodies Developing Early or Late after Transplant Are Associated with the Same Risk of Graft Damage and Loss in Nonsensitized Kidney Recipients. *J Immunol Res*. 2017; 2017:1747030. [PubMed: 28367453]

35. Bachelet T, Martinez C, Del Bello A, et al. Deleterious Impact of Donor-Specific Anti-HLA Antibodies Toward HLA-Cw and HLA-DP in Kidney Transplantation. *Transplantation*. 2016; 100(1):159–66. [PubMed: 26262501]
36. Wiebe C, Nevins TE, Robiner WN, Thomas W, Matas AJ, Nickerson PW. The Synergistic Effect of Class II HLA Epitope-Mismatch and Nonadherence on Acute Rejection and Graft Survival. *Am J Transplant*. 2015; 15(8):2197–202. [PubMed: 26095765]
37. 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):2350–8. [PubMed: 25167892]
38. Gralla J, Tong S, Wiseman AC. The impact of human leukocyte antigen mismatching on sensitization rates and subsequent retransplantation after first graft failure in pediatric renal transplant recipients. *Transplantation*. 2013; 95(10):1218–24. [PubMed: 23478360]
39. Nocera A, Tagliamacco A, Cioni M, et al. Kidney Intra-graft Homing of De Novo Donor-Specific HLA Antibodies Is an Essential Step of Antibody-Mediated Damage but Not Per Se Predictive of Graft Loss. *Am J Transplant*. 2017; 17(3):692–702. [PubMed: 27501275]
40. Bachelet T, Couzi L, Lepreux S, et al. Kidney intra-graft donor-specific antibodies as determinant of antibody-mediated lesions and poor graft outcome. *Am J Transplant*. 2013; 13(11):2855–64. [PubMed: 24102857]
41. Freitas MC, 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):1113–9. [PubMed: 23514959]
42. Guidicelli G, Guerville F, Lepreux S, et al. Non-Complement-Binding De Novo Donor-Specific Anti-HLA Antibodies and Kidney Allograft Survival. *J Am Soc Nephrol*. 2016; 27(2):615–25. [PubMed: 26047793]
- 43**. 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; 16(7):2106–16. Outcomes for C1q and C3d-binding *de novo* DSA in pediatric renal transplant suggest progressive acquisition of complement activity associated with acute and ongoing kidney allograft rejection. [PubMed: 26725780]
44. Wiebe C, Gareau AJ, Pochinco D, et al. Evaluation of C1q Status and Titer of De Novo Donor-Specific Antibodies as Predictors of Allograft Survival. *Am J Transplant*. 2017; 17(3):703–11. [PubMed: 27539748]
45. Singh P, Filippone EJ, Colombe BW, et al. Sensitization trends after renal allograft failure: the role of DQ eplet mismatches in becoming highly sensitized. *Clin Transplant*. 2016; 30(1):71–80. [PubMed: 26529289]
- 46**. Bryan CF, Chadha V, Warady BA. Donor selection in pediatric kidney transplantation using DR and DQ eplet mismatching: A new histocompatibility paradigm. *Pediatr Transplant*. 2016; 20(7): 926–30. HLA class II eplet mismatching is common in pediatric kidney transplant candidates, and through use of eplet matching strategies, there may be better potential matching of donor/recipient pairs. [PubMed: 27448994]
47. Wiebe C, Pochinco D, Blydt-Hansen TD, et al. Class II HLA epitope matching-A strategy to minimize de novo donor-specific antibody development and improve outcomes. *Am J Transplant*. 2013; 13(12):3114–22. [PubMed: 24164958]
48. Weinstock C, Schnaidt M. The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera. *Int J Immunogenet*. 2013; 40(3):171–7. [PubMed: 22913810]
49. Barocci S, Valente U, Gusmano R, et al. Autoreactive lymphocytotoxic IgM antibodies in highly sensitized dialysis patients waiting for a kidney transplant: identification and clinical relevance. *Clin Nephrol*. 1991; 36(1):12–20. [PubMed: 1889147]
50. Rodey GE, Neylan JF, Whelchel JD, Revels KW, Bray RA. Epitope specificity of HLA class I alloantibodies. I. Frequency analysis of antibodies to private versus public specificities in potential transplant recipients. *Hum Immunol*. 1994; 39(4):272–80. [PubMed: 7520897]

Key Points

- Degree of HLA matching affects long-term outcomes for pediatric transplant recipients in both first and future kidney transplants.
- Advances in histocompatibility tools increase degree of matching between donor and recipients.
- Decreasing immunologic risk through eplet matching may provide better utilization of donor kidneys to the best HLA-matched transplant recipients.
- Post-transplant monitoring of *dn*DSA plays an important role in detecting acute rejection and may be used to identify those at risk for worse long-term allograft outcomes.

Table 1

Histocompatibility techniques in kidney transplantation.

Antibody Detection Technique	Use in Transplantation	Limitations
Complement-Dependent Lymphocytotoxicity Crossmatch (CDCXM)	<ul style="list-style-type: none"> • Screens preformed antibody in transplant recipient • Calculates PRA • Qualitative 	<ul style="list-style-type: none"> • Low sensitivity: Fewer donor B cells to recognize class II antibody following purification process • Autoantibodies (usually IgM) cause false positive results[*] • Low specificity: Confounded by non-specific cell injury and CREGs[‡]
Antihuman-immunoglobulin-enhanced CDC (AHG-CDC)	<ul style="list-style-type: none"> • Enhanced complement-mediated cytotoxic cell death • Qualitative 	<ul style="list-style-type: none"> • Does not control for non-HLA antibody or autoreactive IgM binding • Methodological variation between transplant centers (i.e. extended incubation, Amos-modification and crossmatching at 4°C and 37°C)
Flow Cytometry Crossmatch (FCXM)	<ul style="list-style-type: none"> • Detect antibody bound to T and B cells • Whole-cell based • Pronase increases sensitivity and specificity • Qualitative 	<ul style="list-style-type: none"> • High false positive result rate (e.g. setting of immunoglobulin therapy) • Hemodilution creates false negative result • Prolonged pronase use leads to HLA antigen loss.
Solid-Phase Assays (SPA)	<ul style="list-style-type: none"> • Detects bound antibody via flow cytometry or ELISA • Quantitative, reports fluorescent intensity 	<ul style="list-style-type: none"> • Anti-HLA antibodies detected on lymphocytes may be undetectable on beads, and vice-versa • False negative results can occur when CREGs and beads with multiple alleles for the same antigen lead to dilution of anti-HLA antibodies • Prozone can lead to false negative results through competitive displacement of HLA-antibody by complement factor C1⁴⁸ • False negative reactions result from distortions of HLA molecule masking appropriate epitope
Clq Luminex® assay	<ul style="list-style-type: none"> • Identifies complement-fixing antibody, independent of strength of IgG MFI 	<ul style="list-style-type: none"> • Presence of denatured antigen leading to decreased bead intensity • Need high antigen density, high complement and high

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		IgG levels to avoid false negative results
Epitope Mapping	<ul style="list-style-type: none">HLAMatchmaker is a computational method to predict HLA antigenicity	<ul style="list-style-type: none">Need to develop eplet threshold exclusions, based on known allele level typing of HLA class I and II antigens

PRA: Panel reactive antibody

* Addition of dithiothreitol (DTT) reduces false positive IgM autoantibody reactions.⁴⁹

[‡]CREGs: Cross reactive epitope groups - Shared HLA epitopes that can cause a single antibody to react to several HLA molecules.⁵⁰