1. Analysis of Luminex-based Algorithms to Define Unacceptable HLA Antibodies in CDC-crossmatch Negative Kidney Transplant Recipients.

Zecher D, Bach C, Preiss A, *et al*.

Transplantation. 2018 Jun;102(6):969-977. doi: 10.1097/TP.0000000000002129.

ABSTRACT

Background: HLA-specific antibodies detected by solid phase assays are increasingly used to define unacceptable HLA antigen mismatches (UAM) before renal transplantation. The accuracy of this approach is unclear.

Methods: Day of transplant sera from 211 complement-dependent cytotoxicity crossmatch-negative patients were retrospectively analyzed for donor-specific anti-HLA antibodies (DSA) using Luminex technology. HLA were defined as UAM if DSA had mean fluorescence intensity above (I) 3000 (patients retransplanted and those with DSA against HLA class I and II) or 5000 (all other patients), (II) 5000 for HLA-A, -B, and -DR and 10 000 for HLA DQ or (III) 10 000 (all HLA). We then studied the accuracy of these algorithms to identify patients with antibody-mediated rejection (AMR) and graft loss. UAM were also determined in 256 transplant candidates and vPRA levels calculated.

Results: At transplantation, 67 of 211 patients had DSA. Of these, 31 (algorithm I), 24 (II) and 17 (III) had UAM. Nine (I and II) and 8 (III) of 11 early AMR episodes and 7 (I), 6 (II) and 5 (III) of 9 graft losses occurred in UAM-positive patients during 4.9 years of follow-up. Algorithms I and II identified patients with persistently lower glomerular filtration rate even in the absence of overt AMR. Of the waiting list patients, 22-33% had UAM with median virtual panel reactive antibody of 69.2% to 79.1%.

Conclusions: Algorithms I and II had comparable efficacy but were superior to Algorithm III in identifying at-risk patients at an acceptable false-positive rate. However, Luminex-defined UAM significantly restrict the donor pool of affected patients, which might prolong waiting time.

2. Antibody-mediated rejection: analyzing the risk, proposing solutions.
Arias M, Rush DN, Wiebe C, *et al.*
Transplantation. 2014 Aug 15;98 Suppl 3:S3-21. doi: 10.1097/TP.0000000000000218.

NO ABSTRACT AVAILABLE

3. Antibody-Mediated Rejection Due to Preexisting versus De Novo Donor-Specific Antibodies in Kidney Allograft Recipients.

Aubert O, Loupy A, Hidalgo L, *et al.*

J Am Soc Nephrol. 2017 Jun;28(6):1912-1923. doi: 10.1681/ASN.2016070797. Epub 2017 Mar 2.

ABSTRACT

Antibody-mediated rejection (ABMR) can occur in patients with preexisting anti-HLA donor-specific antibodies (DSA) or in patients who develop de novo DSA. However, how these processes compare in terms of allograft injury and outcome has not been addressed. From a cohort of 771 kidney biopsy specimens from two North American and five European centers, we performed a systematic assessment of clinical and biologic parameters, histopathology, circulating DSA, and allograft gene expression for all patients with ABMR (n=205). Overall, 103 (50%) patients had preexisting DSA and 102 (50%) had de novo DSA. Compared with patients with preexisting DSA ABMR, patients with de novo DSA ABMR displayed increased proteinuria, more transplant glomerulopathy lesions, and lower glomerulitis, but similar levels of peritubular capillaritis and C4d deposition. De novo DSA ABMR was characterized by increased expression of IFNγ-inducible, natural killer cell, and T cell transcripts, but less expression of AKI transcripts compared with preexisting DSA ABMR. The preexisting DSA ABMR had superior graft survival compared with the de novo DSA ABMR (63% versus 34% at 8 years after rejection, respectively; P<0.001). After adjusting for clinical, histologic, and immunologic characteristics and treatment, we identified de novo DSA ABMR (hazard ratio [HR], 1.82 compared with preexisting DSA ABMR; 95% confidence interval [95% CI], 1.07 to 3.08; P=0.03); low eGFR (<30 ml/min per 1.73 m2) at diagnosis (HR, 3.27; 95% CI, 1.48 to 7.23; P<0.001); ≥0.30 g/g urine protein-to-creatinine ratio (HR, 2.44; 95% CI, 1.47 to 4.09; P<0.001); and presence of cg lesions (HR, 2.25; 95% CI, 1.34 to 3.79; P=0.002) as the main independent determinants of allograft loss. Our findings support the transplant of kidneys into highly sensitized patients and should encourage efforts to monitor patients for de novo DSA.

4. Analysis of predictive and preventive factors for de novo DSA in kidney transplant recipients.

Hirai T, Furusawa M, Omoto K, *et al.*

Transplantation. 2014 Aug 27;98(4):443-50. doi: 10.1097/TP.0000000000000071.

ABSTRACT

Background: Development of de novo donor-specific anti-HLA antibodies (dnDSA) has been associated with poor graft outcome, although the preventive factor for its production is still elusive. We analyzed the incidence of dnDSA within 5 years posttransplant in 562 living-kidney transplant recipients to evaluate predicting and preventive factors for dnDSA development.

Materials And Methods: All patients were considered to be non-HLA sensitized, as determined by the preoperative single-antigen bead assay (SABA), although they included various ABO blood type compatibilities. Preoperative administration of rituximab was indicated for 48% of patients, mainly for ABO incompatible transplantation. We retrospectively compared the patients with dnDSA and those without.

Results: Development of dnDSA was observed in 27 of the total 562 patients (5%). Chronic rejection was more frequently observed in patients with dnDSA than in those without (41% vs. 6%, P<0.001). The dnDSA-positive patients showed decreased graft function and poorer graft survival rates than those who tested negative. In multivariate analysis, higher likelihood of dnDSA production was observed in male recipients (odds ratio 6.57, P=0.012) and patients with a higher number of HLA-DR mismatches (odds ratio 2.41, P=0.008), whereas lower likelihood was observed in patients treated with rituximab induction (odds ratio 0.33, P=0.040).

Conclusion: Results suggest that rituximab induction as a standard immunosuppression protocol may have a preventive effect for dnDSA production in the non-HLA sensitized low immunologic risk patients.

5. Analysis of preformed donor-specific anti-HLA antibodies characteristics for prediction of antibody-mediated rejection in kidney transplantation.

Malheiro J, Tafulo S, Dias L, *et al.*

Transpl Immunol. 2015 Mar;32(2):66-71. doi: 10.1016/j.trim.2015.01.002. Epub 2015 Feb 7.

ABSTRACT

Background: The relevance of preformed donor specific antibodies (DSA) detected by Luminex assays, with a negative complement-dependent cytotoxicity (CDC) crossmatch, remains unsettled in kidney transplantation (KT). We aimed to analyze the impact of preformed DSA characteristics on kidney graft outcomes.

Methods: In 462 patients that received a kidney graft in our unit, between 2007 and 2012, pre-transplant sera were analyzed by Luminex screening assay to determine the presence of anti-human leukocyte antigen (HLA) antibodies and single-antigen bead assay [positive if mean fluorescence intensity (MFI) ≥ 1000] to assign anti-HLA specificities.

Results: Anti-HLA antibodies were present in 95 patients (20.6%), but only 40 (8.7%) had DSA. Antibody-mediated rejection (AMR) at 1-year was higher in patients with DSA (35.0%) than in those without them (0.9%) ($P < 0.001$). Only DSA with a MFI of >3000 were significantly associated with AMR occurrence. Receiver operator curves revealed that a MFI of >4900 in the highest DSA bead had a high sensitivity (85.7%) and that the sum of all DSA beads MFI > 11,000 had a high specificity (92.3%) for AMR prediction. Anti-thymocyte globulin versus basiliximab induction was more frequent in DSA+ AMR- (65.4%) versus DSA+ AMR+ (34.6%) patients ($P = 0.072$). Five-year censored graft survival was lower in DSA+ than in DSA- patients (respectively, 84.8% versus 94.9%, $P = 0.006$), although survival was only reduced in DSA+ AMR+ (68.8%) versus DSA+ AMR- (96.0%) patients ($P = 0.038$).

Conclusions: Preformed DSA is associated with kidney graft loss, in relation with AMR occurrence. DSA strength may be used to improve immunological risk stratification of sensitized patients and their clinical management.

6. De novo donor-specific antibodies in belatacept-treated vs cyclosporine-treated kidney-transplant recipients: Post hoc analyses of the randomized phase III BENEFIT and BENEFIT-EXT studies.

Bray RA, Gebel HM, Townsend R, *et al*.

Am J Transplant. 2018 Mar 6. doi: 10.1111/ajt.14721.

Abstract

Donor-specific antibodies (DSAs) are associated with an increased risk of antibody-mediated rejection and graft failure. In BENEFIT and BENEFIT-EXT, kidney-transplant recipients were randomized to receive belatacept more intense (MI)-based, belatacept less intense (LI)-based, or cyclosporine-based immunosuppression for up to 7 years (84 months). The presence/absence of HLA-specific antibodies was determined at baseline, at months 6, 12, 24, 36, 48, 60, and 84, and at the time of clinically suspected episodes of acute rejection, using solid-phase flow-cytometry screening. Samples from anti-HLA-positive patients were further tested with a single-antigen bead assay to determine antibody specificities, presence/absence of DSAs, and mean fluorescence intensity (MFI) of any DSAs present. In BENEFIT, de novo DSAs developed in 1.4%, 3.5%, and 12.1% of belatacept MI-treated, belatacept LI-treated, and cyclosporine-treated patients, respectively. The corresponding values in BENEFIT-EXT were 3.8%, 1.1%, and 11.2%. Per Kaplan-Meier analysis, de novo DSA incidence was significantly lower in belatacept-treated vs cyclosporine-treated patients over 7 years in both studies ($P < .01$). In patients who developed de novo DSAs, belatacept-based immunosuppression was associated with numerically lower MFI vs cyclosporine-based immunosuppression. Although derived post hoc, these data suggest that belatacept-based immunosuppression suppresses de novo DSA development more effectively than cyclosporine-based immunosuppression.

7. Characteristics of donor-specific anti-HLA antibodies and outcome in renal transplant patients treated with a standardized induction regimen.

Zecher D, Bach C, Staudner C, *et al.*

Nephrol Dial Transplant. 2017 Apr 1;32(4):730-737. doi: 10.1093/ndt/gfw445.

ABSTRACT

Background: Pre-transplant donor-specific anti-human leukocyte antigen (HLA) antibodies (DSA) have been associated with antibody-mediated rejection (AMR) and early kidney allograft loss. Uncertainties remain regarding the general applicability of these findings and the optimal induction therapy in DSA-positive patients.

Methods: Pre-transplant sera from 174 patients receiving a crossmatch-negative kidney transplant were retrospectively analysed for DSA using Luminex technology. DSA with mean-fluorescence intensity (MFI) values above 500 were considered positive. All recipients received basiliximab induction and tacrolimus-based maintenance immunosuppression. DSA were monitored post-transplantation in patients with pre-transplant DSA. Antibody results were correlated with the incidence of rejection and graft loss.

Results: In total, 61/174 patients had pre-transplant DSA. We found a strong correlation between the presence of DSA against class I and II HLA and DSA MFI greater than 10 000. Both DSA patterns independently predicted an increased risk of early AMR (odds ratio 4.24 and 4.75, respectively, $P < 0.05$). The risk for AMR in patients with intermediate MFI (3000-10 000) gradually increased with increasing MFI but group sizes were too small to allow for final conclusions. The risk for AMR was comparable to nonsensitized patients in patients with only class I or II HLA-DSA or MFI below 3000. 5-year allograft survival was lowest in patients with simultaneous presence of class I and II HLA-DSA and MFI above 10 000 (45%) but was comparable between patients with only HLA class I or II or no DSA (90.0, 90.0 and 88.1%, respectively). AMR was the only independent predictor of graft loss. Undetectable DSA 14 days post-transplant predicted excellent long-term outcome.

Conclusion: The favourable outcome in the majority of DSA-positive patients despite non-depleting antibody induction and the poor outcome in patients with class I and II HLA-DSA and high DSA strength call for a differentiated therapeutic approach in this patient population.

8. Preformed circulating HLA-specific memory B cells predict high risk of humoral rejection in kidney transplantation.

Lúcia M, Luque S, Crespo E, *et al.*

Kidney Int. 2015 Oct;88(4):874-87. doi: 10.1038/ki.2015.205. Epub 2015 Jul 15.

ABSTRACT

The accurate evaluation of donor-specific antibodies (DSAs) has allowed a precise identification of sensitized patients at risk of antibody-mediated rejection (ABMR). However, the scale of the humoral response is not always fully addressed, as it excludes the complete memory B-cell (mBC) pool such as that caused by antigen-specific mBC. Using a novel B-cell ELISpot assay approach, we assessed circulating mBC frequencies against class I and II HLA antigens in highly sensitized and nonsensitized patients in the waiting list for kidney transplantation. Also, kidney transplant patients undergoing ABMR were evaluated for the presence of donor-specific mBCs both at the time of rejection and before transplantation. For this purpose, 278 target HLA-sp antigens from 70 patients were studied and compared to circulating HLA-sp antibodies. Both class I and II HLA-sp mBC frequencies were identified in highly sensitized individuals but not in nonsensitized and healthy individuals, many years after first sensitization. Also, high donor-specific mBC responses were clearly found both during ABMR and before transplantation, regardless of circulating DSA. The higher the donor-specific mBC response, the more aggressive the allograft rejection. Thus, assessing donor-specific mBC frequencies may be relevant to better refine patient alloimmune-risk stratification, and provides new insight into the mechanisms of the adaptive humoral alloimmune response taking place in kidney transplantation.

9. A Comparison of Two Types of Rabbit Antithymocyte Globulin Induction Therapy in Immunological High-Risk Kidney Recipients: A Prospective Randomized Control Study.

Burkhalter F, Schaub S, Bucher C, *et al.*

PLoS One. 2016 Nov 17;11(11):e0165233. doi: 10.1371/journal.pone.0165233. eCollection 2016.

ABSTRACT

Background: Induction treatment with rabbit polyclonal antithymocyte globulins (ATGs) is frequent used in kidney transplant recipients with donorspecific HLA antibodies and shows acceptable outcomes. The two commonly used ATGs, Thymoglobulin and ATG-F have slightly different antigen profile and antibody concentrations. The two compounds have never been directly compared in a prospective trial in immunological high-risk recipients. Therefore we performed a prospective randomized controlled study comparing the two compounds in immunological high-risk kidney recipients in terms of safety and efficacy.

Methods: Immunological high-risk kidney recipients, defined as the presence of HLA DSA but negative CDC-B and T-cell crossmatches were randomized 1:1 to receive ATG-F or Thymoglobulin. Maintenance immunosuppressive therapy consisted of tacrolimus, mycophenolate mofetil and steroids.

Results: The per-protocol analysis included 35 patients. There was no immediate infusion reaction observed with both compounds. No PTLD or malignancy occurred during the follow-up in both groups. The incidence of viral and bacterial infections was similar in both groups (p = 0.62). The cumulative incidence of clinical and subclinical antibody mediated allograft rejection as well as T-cell mediated allograft rejection during the first year between ATG-F and Thymoglobulin was similar (35% versus 19%; p = 0.30 and 11% versus 18%; 0.54 respectively). The two-year graft function was similar with a median eGFR of 56 ml/min/1.73m2 (range 21-128) (ATG-F-group) and 51 ml/min/1.73m2 (range 22-132) (Thymo-group) (p = 0.69).

Conclusion: We found no significant differences between the compared study drugs for induction treatment in immunological high-risk patients regarding safety and efficacy during follow-up with good allograft function at 2 years after transplantation.

10. Diagnostic Contribution of Donor-Specific Antibody Characteristics to Uncover Late Silent Antibody-Mediated Rejection-Results of a Cross-Sectional Screening Study.

Eskandary F, Bond G, Kozakowski N, *et al.*

Transplantation. 2017 Mar;101(3):631-641. doi: 10.1097/TP.0000000000001195.

ABSTRACT

Background: Circulating donor-specific antibodies (DSA) detected on bead arrays may not inevitably indicate ongoing antibody-mediated rejection (AMR). Here, we investigated whether detection of complement-fixation, in parallel to IgG mean fluorescence intensity (MFI), allows for improved prediction of AMR.

Methods: Our study included 86 DSA+ kidney transplant recipients subjected to protocol biopsy, who were identified upon cross-sectional antibody screening of 741 recipients with stable graft function at 6 months or longer after transplantation. IgG MFI was analyzed after elimination of prozone effect, and complement-fixation was determined using C1q, C4d, or C3d assays.

Results: Among DSA+ study patients, 44 recipients (51%) had AMR, 24 of them showing C4d-positive rejection. Although DSA number or HLA class specificity were not different, patients with AMR or C4d + AMR showed significantly higher IgG, C1q, and C3d DSA MFI than nonrejecting or C4d-negative patients, respectively. Overall, the predictive value of DSA characteristics was moderate, whereby the highest accuracy was computed for peak IgG MFI (AMR, 0.73; C4d + AMR, 0.71). Combined analysis of antibody characteristics in multivariate models did not improve AMR prediction.

Conclusions: We estimate a 50% prevalence of silent AMR in DSA+ long-term recipients and conclude that assessment of IgG MFI may add predictive accuracy, without an independent diagnostic advantage of detecting complement-fixation.

11. De Novo Anti-HLA DSA Characteristics and Subclinical Antibody-Mediated Kidney Allograft Injury.

Yamamoto T, Watarai Y, Takeda A, *et al.*

Transplantation. 2016 Oct;100(10):2194-202. doi: 10.1097/TP.0000000000001012.

ABSTRACT

Background: It is unclear whether all donor-specific antibodies (DSA) can cause chronic antibody-mediated rejection (AMR). Subclinical stage before manifestation of renal dysfunction may be a critical period for reversing AMR. The aim of our study was to identify factors related to the development of subclinical AMR and to clarify the characteristics of de novo DSA.

Methods: Eight hundred ninety-nine renal transplants were screened for HLA antibody. De novo DSA were detected in 95 patients. Forty-three patients without renal dysfunction who underwent renal biopsies were enrolled in this study. Eighteen patients (41.9%) were diagnosed with biopsy-proven subclinical AMR and treated with plasmapheresis and rituximab-based therapy, whereas 25 showed no findings of AMR.

Results: Significant subclinical AMR-related factors were younger recipients, history of acute T cell-mediated rejection and DSA class II, especially DR-associated DSA. Mean fluorescence intensity (MFI) values of DR-DSA were significantly higher, whereas DQ-DSA was not different between subclinical AMR and no AMR. The ΔMFI (>50%), DSA-MFI values greater than 3000, and C1q binding DSA were also significant subclinical AMR-related factors ($P < 0.05$). Among 18 patients treated for subclinical AMR, 8 patients (44.4%) obtained over 50% reduction of DSA-MFI and/or improvement or no deterioration of pathological findings. In contrast, 25 patients without subclinical AMR did not show renal dysfunction clinically. Moreover, all of the 8 patients with rebiopsy after 2 years continued to demonstrate no AMR.

Conclusions: About 40% of patients with de novo DSA demonstrated biopsy-proven subclinical AMR, leading to progressive graft injury. To validate the intervention and treatment for de novo DSA-positive patients without renal dysfunction, further study is necessary.

12. Non-Complement-Binding De Novo Donor-Specific Anti-HLA Antibodies and Kidney Allograft Survival.

Guidicelli G, Guerville F, Lepreux S, *et al.*

J Am Soc Nephrol. 2016 Feb;27(2):615-25. doi: 10.1681/ASN.2014040326. Epub 2015 Jun 5.

ABSTRACT

C1q-binding ability may indicate the clinical relevance of de novo donor-specific anti-HLA antibodies (DSA). This study investigated the incidence and risk factors for the appearance of C1q-binding de novo DSA and their long-term impact. Using Luminex Single Antigen Flow Bead assays, 346 pretransplant nonsensitized kidney recipients were screened at 2 and 5 years after transplantation for de novo DSA, which was followed when positive by a C1q Luminex assay. At 2 and 5 years, 12 (3.5%) and eight (2.5%) patients, respectively, had C1q-binding de novo DSA. De novo DSA mean fluorescence intensity >6237 and >10,000 at 2 and 5 years, respectively, predicted C1q binding. HLA mismatches and cyclosporine A were independently associated with increased risk of C1q-binding de novo DSA. When de novo DSA were analyzed at 2 years, the 5-year death-censored graft survival was similar between patients with C1q-nonbinding de novo DSA and those without de novo DSA, but was lower for patients with C1q-binding de novo DSA (P=0.003). When de novo DSA were analyzed at 2 and 5 years, the 10-year death-censored graft survival was lower for patients with C1q-nonbinding de novo DSA detected at both 2 and 5 years (P<0.001) and for patients with C1q-binding de novo DSA (P=0.002) than for patients without de novo DSA. These results were partially confirmed in two validation cohorts. In conclusion, C1q-binding de novo DSA are associated with graft loss occurring quickly after their appearance. However, the long-term persistence of C1q-nonbinding de novo DSA could lead to lower graft survival.

13. Donor Specificity but Not Broadness of Sensitization Is Associated With Antibody-Mediated Rejection and Graft Loss in Renal Allograft Recipients.

Wehmeier C, Hönger G, Cun H, *et al.*

Am J Transplant. 2017 Aug;17(8):2092-2102. doi: 10.1111/ajt.14247. Epub 2017 Mar 27.

ABSTRACT

Panel-reactive antibodies are widely regarded as an important immunological risk factor for rejection and graft loss. The broadness of sensitization against HLA is most appropriately measured by the "calculated population-reactive antibodies" (cPRA) value. In this study, we investigated whether cPRA represent an immunological risk in times of sensitive and accurate determination of pretransplantation donor-specific HLA antibodies (DSA). Five hundred twenty-seven consecutive transplantations were divided into four groups: cPRA 0% (n = 250), cPRA 1-50% (n = 129), cPRA 51-100% (n = 43), and DSA (n = 105). Patients without DSA were considered as normal risk and received standard immunosuppression without T cell-depleting induction. Patients with DSA received an enhanced induction therapy and maintenance immunosuppression. Surveillance biopsies were performed at 3 and 6 months. Median follow-up was 5.7 years. Among the three cPRA groups, there were no differences regarding the 1-year incidence of ABMR (p = 0.16) and TCMR (p = 0.75). The 5-year allograft survival rates were similar and around 87% (p = 0.28). The estimated glomerular filtration rate at last follow-up was 50-53 mL/min (p = 0.45). On multivariable Cox proportional hazard analysis, the strongest independent predictor for ABMR and (death-censored) graft survival was pretransplantation DSA. cPRA were not predictive for ABMR, TCMR, or (death-censored) graft survival. We conclude that with current DSA assignment, the broadness of sensitization measured by cPRA does not imply an immunological risk.

14. 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.

Cioni M, Nocera A, Innocente A, *et al.*

J Immunol Res. 2017;2017:1747030. doi: 10.1155/2017/1747030. Epub 2017 Mar 6.

ABSTRACT

De novo posttransplant donor-specific HLA-antibody (dnDSA) detection is now recognized as a tool to identify patients at risk for antibody-mediated rejection (AMR) and graft loss. It is still unclear whether the time interval from transplant to DSA occurrence influences graft damage. Utilizing sera collected longitudinally, we evaluated 114 consecutive primary pediatric kidney recipients grafted between 2002 and 2013 for dnDSA occurrence by Luminex platform. dnDSAs occurred in 39 patients at a median time of 24.6 months. In 15 patients, dnDSAs developed within 1 year (early-onset group), while the other 24 seroconverted after the first posttransplant year (late-onset group). The two groups were comparable when considering patient- and transplant-related factors, as well as DSA biological properties, including C1q and C3d complement-binding ability. Only recipient age at transplant significantly differed in the two cohorts, with younger patients showing earlier dnDSA development. Late AMR was diagnosed in 47% of the early group and in 58% of the late group. Graft loss occurred in 3/15 (20%) and 4/24 (17%) patients in early- and late-onset groups, respectively (p = ns). In our pediatric kidney recipients, dnDSAs predict AMR and graft loss irrespective of the time elapsed between transplantation and antibody occurrence.

15. Preformed donor-specific antibodies and risk of antibody-mediated rejection in repeat renal transplantation.

Tsapepas DS, Vasilescu R, Tanriover B, *et al.*

Transplantation. 2014 Mar 27;97(6):642-7. doi: 10.1097/01.TP.0000440954.14510.6a.

ABSTRACT

Background: Allograft outcomes in patients undergoing repeat renal transplantation are inferior compared to first-time transplant recipient outcomes. Donor-specific antibodies detected by solid-phase assays (DSA-SPA) may contribute to the worse prognosis. The influence of DSA-SPA on repeat renal transplantation outcomes has not been previously studied in detail.

Design: This study reports the findings in 174 patients who underwent repeat renal transplantation between years 2007 and 2012. These included 62 patients with preformed DSA-SPA detected by Luminex at the time of transplantation. Patients received standard and consistent immunosuppression and were monitored closely for evidence of rejection. Recipients who underwent desensitization were excluded from this analysis. Endpoints included development of biopsy-proven acute rejection and analysis of graft survival and function.

Results: Patients in the DSA-SPA-positive and DSA-SPA-negative groups received similar immunosuppression, and a similar proportion of recipients had a peak panel reactive antibody greater than 20%; the two groups differed with respect to human leukocyte antigen mismatches (4.7 ± 1.1 vs. 4.1 ± 1.7, P=0.024). Recipients with preformed DSA-SPA had higher rejection rates (54.8% vs. 34.8%, P=0.01), including higher rates of antibody-mediated rejection (AMR) (32.3% vs. 7.1%, P<0.001). Recipients who were DSA-SPA-positive and flow cytometry crossmatch (FCXM)-positive had a higher incidence of both AMR (OR 4.6, P=0.009) and of acute rejection (OR 3.57, P=0.02) as compared to those who were DSA-SPA-positive and FCXM-negative. Overall allograft survival was similar in the DSA-SPA-positive and DSA-SPA-negative groups (log-rank test=0.63, P=0.428). Differences in allograft function were detectable after 2 years (32.8 ± 13.1 vs. 47 ± 20.2 mL/min/1.73 m(2), P=0.023) and may be reflective of more AMR among DSA-SPA-positive patients.

Conclusions: This analysis suggests that DSA-SPA increases the overall risk of acute rejection but does not appear to adversely impact allograft survival during the early follow-up period. Close monitoring of renal function and early biopsy for AMR detection appear to allow for satisfactory short-term allograft outcomes in repeat transplant recipients.

16. Impact of pretransplant donor-specific antibodies on kidney allograft recipients with negative flow cytometry crossmatches.

Kwon H, Kim YH, Choi JY, *et al.*

Clin Transplant. 2018 Apr 20:e13266. doi: 10.1111/ctr.13266.

ABSTRACT

The Luminex test can detect low levels of donor-specific antibody (DSA) that cannot be detected by flow-cytometric crossmatching (FCXM) in kidney transplantation (KT). This study evaluated the impact of DSA on clinical outcomes in KT recipients negative on FCXM. Of 575 consecutive patients who underwent living donor KT between January 2013 and July 2016, 494 (85.9%) were DSA-negative and 81 (14.1%) were DSA-positive. Although rates of acute cellular rejection (ACR) at 1 year were similar in the two groups (P = 0.54), the incidence of antibody-mediated rejection (ABMR) was significantly higher in the DSA-positive group (P < 0.01). There was no statistically significant association between rejection-free graft survival (RFGS) rates and pretransplant class I DSA. However, evaluation of pretransplant class II DSA showed that RFGS rates were significantly lower in patients with MFI >3000 than in patients with DSA negative (P < 0.01). On multivariate analyses, class II DSA MFI ≥5000 was a significant risk factor for acute rejection (hazard ratio, 7.48; P < 0.01). These findings suggested that pretransplant DSA alone did not affect graft survival in KT recipients without desensitization. However, class II DSA MFI >5000 was an independent predictor of acute rejection in DSA positive patients.

17. Pre-existing donor-specific antibodies are detrimental to kidney allograft only when persistent after transplantation.

Caillard S, Becmeur C, Gautier-Vargas G, *et al.*

Transpl Int. 2017 Jan;30(1):29-40. doi: 10.1111/tri.12864. Epub 2016 Oct 17.

ABSTRACT

Donor-specific antibodies (DSA) increase the risk of allograft rejection and graft failure. They may be present before transplant or develop de novo after transplantation. Here, we studied the evolution of preformed DSA and their impact on graft outcome in kidney transplant recipients. Using the Luminex Single Antigen assay, we analyzed the sera on the day of transplantation of 239 patients who received a kidney transplant. Thirty-seven patients (15.5%) had pre-existing DSA detected the day of transplantation. After 5 years, the pre-existing DSA disappeared in 22 patients whereas they persisted in 12. Variables associated with DSA persistence were age <50 years (P = 0.009), a history of previous transplantation (P = 0.039), the presence of class II DSA (P = 0.009), an MFI of preformed DSA >3500 (P < 0.001), and the presence of two or more DSA (P < 0.001). DSA persistence was associated with a higher risk of graft loss and antibody-mediated rejection. Previously undetected preformed DSA are deleterious to graft survival only when they persist after transplantation.

18. Donor-Specific Antibodies in Kidney Transplant Recipients.

Zhang R.

Clin J Am Soc Nephrol. 2018 Jan 6;13(1):182-192. doi: 10.2215/CJN.00700117. Epub 2017 Apr 26.

ABSTRACT

Donor-specific antibodies have become an established biomarker predicting antibody-mediated rejection. Antibody-mediated rejection is the leading cause of graft loss after kidney transplant. There are several phenotypes of antibody-mediated rejection along post-transplant course that are determined by the timing and extent of humoral response and the various characteristics of donor-specific antibodies, such as antigen classes, specificity, antibody strength, IgG subclasses, and complement binding capacity. Preformed donor-specific antibodies in sensitized patients can trigger hyperacute rejection, accelerated acute rejection, and early acute antibody-mediated rejection. De novo donor-specific antibodies are associated with late acute antibody-mediated rejection, chronic antibody-mediated rejection, and transplant glomerulopathy. The pathogeneses of antibody-mediated rejection include not only complement-dependent cytotoxicity, but also complement-independent pathways of antibody-mediated cellular cytotoxicity and direct endothelial activation and proliferation. The novel assay for complement binding capacity has improved our ability to predict antibody-mediated rejection phenotypes. C1q binding donor-specific antibodies are closely associated with acute antibody-mediated rejection, more severe graft injuries, and early graft failure, whereas C1q nonbinding donor-specific antibodies correlate with subclinical or chronic antibody-mediated rejection and late graft loss. IgG subclasses have various abilities to activate complement and recruit effector cells through the Fc receptor. Complement binding IgG3 donor-specific antibodies are frequently associated with acute antibody-mediated rejection and severe graft injury, whereas noncomplement binding IgG4 donor-specific antibodies are more correlated with subclinical or chronic antibody-mediated rejection and transplant glomerulopathy. Our in-depth knowledge of complex characteristics of donor-specific antibodies can stratify the patient's immunologic risk, can predict distinct phenotypes of antibody-mediated rejection, and hopefully, will guide our clinical practice to improve the transplant outcomes.

19. De novo donor-specific antibody following BK nephropathy: The incidence and association with antibody-mediated rejection.

Cheungpasitporn W, Kremers WK, Lorenz E, *et al.*

Clin Transplant. 2018 Mar;32(3):e13194. doi: 10.1111/ctr.13194. Epub 2018 Feb 11.

ABSTRACT

Background And Objectives: The risk of de novo donor-specific antibody (dnDSA) development following BK viremia (BKV) or nephropathy (BKN) after kidney transplant remains unclear. We aimed to evaluate the relationships among dnDSA, BKV (BK blood PCR > 15 000 copies), BKN, antibody-mediated rejection (AMR), and allograft loss.

Patients And Methods: We performed a retrospective cohort study of 904 solitary kidney transplant recipients transplanted between 10/2007 and 5/2014. Cox proportional hazards regression with time-dependent covariates were used to assess the relationships among BKN, isolated BKV, dnDSA, and the subsequent risk of AMR and allograft loss.

Results: In multivariate analysis, we observed that BKN, but not BKV was a risk factor for dnDSA (HR, 3.18, P = .008). Of the patients with BK nephropathy, 14.0% (6/43) developed dnDSA, which occurred within 14 months of BK diagnosis. DnDSA in this setting remains a risk factor for subsequent AMR (HR 4.75, P = .0001) and allograft loss (HR 2.63, P = .018).

Conclusions: BKN is an independent risk factor for development of dnDSA. Improved understanding of the characteristics of patients with BKN who are at highest risk for development of dnDSA would be valuable to customize immunosuppression reduction in this population.

20. Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss.

Sicard A, Ducreux S, Rabeyrin M, *et al.*

J Am Soc Nephrol. 2015 Feb;26(2):457-67. doi: 10.1681/ASN.2013101144. Epub 2014 Aug 14.

ABSTRACT

Antibody-mediated rejection (AMR) is a major cause of kidney graft loss, yet assessment of individual risk at diagnosis is impeded by the lack of a reliable prognosis assay. Here, we tested whether the capacity of anti-HLA antibodies to bind complement components allows accurate risk stratification at the time of AMR diagnosis. Among 938 kidney transplant recipients for whom a graft biopsy was performed between 2004 and 2012 at the Lyon University Hospitals, 69 fulfilled the diagnosis criteria for AMR and were enrolled. Sera banked at the time of the biopsy were screened for the presence of donor-specific anti-HLA antibodies (DSAs) and their ability to bind C1q and C3d using flow bead assays. In contrast with C4d graft deposition, the presence of C3d-binding DSA was associated with a higher risk of graft loss (P<0.001). Despite similar trend, the difference did not reach significance with a C1q-binding assay (P=0.06). The prognostic value of a C3d-binding assay was further confirmed in an independent cohort of 39 patients with AMR (P=0.04). Patients with C3d-binding antibodies had worse eGFR and higher DSA mean fluorescence intensity. In a multivariate analysis, only eGFR <30 ml/min per 1.73 m(2) (hazard ratio [HR], 3.56; 95% confidence interval [CI], 1.46 to 8.70; P=0.005) and the presence of circulating C3d-binding DSA (HR, 2.80; 95% CI, 1.12 to 6.95; P=0.03) were independent predictors for allograft loss at AMR diagnosis. We conclude that assessment of the C3d-binding capacity of DSA at the time of AMR diagnosis allows for identification of patients at risk for allograft loss.

21. Assessing Antibody Strength: Comparison of MFI, C1q, and Titer Information.

Tambur AR, Herrera ND, Haarberg KM, *et al.*

Am J Transplant. 2015 Sep;15(9):2421-30. doi: 10.1111/ajt.13295. Epub 2015 Apr 30.

ABSTRACT

The presence of donor-specific HLA antibodies before or after transplantation may have different implications based on the antibody strength. Yet, current approaches do not provide information regarding the true antibody strength as defined by antigen-antibody dissociation rate. To assess currently available methods, we compared between neat mean fluorescence intensity (MFI) values, C1q MFI values, ethylenediaminetetraacetic acid (EDTA)-treated samples, as well as titration studies and peak MFI values of over 7000 Luminex-based single-antigen HLA antibody data points. Our results indicate that neat MFI values do not always accurately depict antibody strength. We further showed that EDTA treatment (6%) does not always remove all inhibitory factors compared with C1q or titration studies. In this study of patients presenting with multiple antibody specificities, a prozone effect was observed in 71% of the cohort (usually not affecting all antibody specificities within a single serum sample, though). Similar to titration studies, the C1q assay was able to address the issue of potential inhibition; however, its limitation is its low sensitivity and inability to detect the presence of weak antibodies. Titration studies are the only method among the approaches used in this study to provide information suggesting antigen-antibody dissociation rates and are, therefore, likely to provide better indication of true antibody strength.

22. Graft immunologic events in deceased donor kidney transplant recipients with preformed HLA-donor specific antibodies.

Ixtlapale-Carmona X, Arvizu A, De-Santiago A, *et al.*

Transpl Immunol. 2018 Feb;46:8-13. doi: 10.1016/j.trim.2017.09.006. Epub 2017 Sep 30.

ABSTRACT

Introduction: Pretransplant donor-specific HLA alloantibodies detected with the Single Antigen Bead (SAB) assay reflect an increased risk for acute antibody-mediated rejection (AMR). We herein report the incidence of both acute AMR and acute cellular rejection (ACR) during the first year posttransplantation, in a cohort of kidney transplant recipients (KTR) of deceased donor (DD) grafts, according to their DSA status. Pretransplant DSA do not preclude DD-KT in negative CDC-XM recipients at our center.

Patients And Methods: 246 KT were performed at our center between 01/2012 and 12/2015 and 100 KTR obtained from a DD were analyzed; 24% harbored DSA by SAB assay, MFI values >500 were considered positive. All recipients received thymoglobulin induction and generic tacrolimus-based maintenance therapy. Graft biopsies were performed by protocol on months 3 and 12 as well as per indication. The incidence of AMR and ACR was correlated with the existence of pretransplant DSA.

Results: Overall, 34% of patients developed an acute rejection episode, 54.2% in the DSA group versus 27.6% in the non-DSA group (p=0.032), and most of these events were detected as subclinical conditions in protocol biopsies. AMR events developed in 33.3% and 19.7% (p=0.176) in the DSA and the non-DSA groups, respectively. ACR events were found in 16.6% and 6.6% (p=0.127) in the DSA and non-DSA groups, respectively. Graft function was similar between groups at the end of the 1st year posttransplant and no immunological graft loss occurred.

Conclusion: Despite the use of depleting induction therapy and adequate tacrolimus trough levels along with MMF and steroids, a high rate of rejection events was observed during the first year post-transplantation.

23. Long-term Outcomes of Kidney Transplantation in Patients With High Levels of Preformed DSA: The Necker High-Risk Transplant Program.

Amrouche L, Aubert O, Suberbielle C, *et al.*

Transplantation. 2017 Oct;101(10):2440-2448. doi: 10.1097/TP.0000000000001650.

ABSTRACT

Background: There is an increasing number of anti-HLA sensitized and highly sensitized renal transplant candidates on waiting lists, and the presence of donor-specific alloantibodies (DSAs) at the time of transplantation leads to acute and chronic antibody-mediated rejection (AMR). Acceptable short-term outcomes have been described, notably because of desensitization protocols, but mid- and long-term data are still required.

Methods: Our high immunologic risk program included 95 patients with high peak or day 0 DSA levels (mean fluorescence intensity [MFI] > 3000) with a complement-dependent cytotoxicity-negative crossmatch, who received a posttransplant desensitization protocol starting at day 0 with high-dose intravenous immunoglobulin, plasma exchanges, and eventually rituximab. Their characteristics were compared with a control group including 39 patients with a lower immunologic risk (MFI between 500 and 3000 at day 0) who received the same posttransplant desensitization.

Results: The median MFI of the immunodominant class I or II DSA in the peak or day 0 serum was 9421 (interquartile range, 4959-12 610). An AMR occurred during the first posttransplant year in 31 patients (32.6%), and at one year, the rate of chronic AMR was 39.5%. The 1-, 3-, 5- and 7-year death-censored allograft survival rates were 98%, 91%, 86%, and 78%, respectively, with concomitant recipient survival rates of 97%, 93%, 85%, and 79%, respectively.

Conclusions: These results suggest that DSA-sensitized patients with high MFI levels can receive transplantation across the HLA-barrier, with the use of an intensified posttransplant immunosuppressive therapy starting at day 0 combined with close clinical, immunologic, and histologic monitoring.

24. Clinical Outcome of Patients With De Novo C1q-Binding Donor-Specific HLA Antibodies After Renal Transplantation.

Bamoulid J, Roodenburg A, Staeck O, *et al.*

Transplantation. 2017 Sep;101(9):2165-2174. doi: 10.1097/TP.0000000000001487.

ABSTRACT

Background: De novo donor specific anti-HLA antibodies (dnDSA) may cause graft loss in renal transplant recipients. The capability to bind the complement may help to stratify the risk for inferior outcomes associated with dnDSA. We developed a modified C1q-binding assay and hypothesized that C1q-binding dnDSA could differentiate between indolent and harmful dnDSA causing antibody-mediated rejection (AMR) and graft loss.

Methods: We retrospectively identified 59 renal transplant recipients who developed dnDSA and had serum available and complete follow-up. All patients were analyzed for C1q-binding dnDSA at the time of dnDSA detection, and 1-year later or at time of AMR. AMR-positive patients were also tested 6 to 12 months before the event if IgG dnDSA was present.

Results: Thirty-seven of 59 dnDSA patients developed AMR during 5.9 ± 3.1 years follow-up. AMR-positive patients had more dnDSA with a significant higher frequency of class I, a higher frequency and a higher mean fluorescence intensity value of C1q-dnDSA at all time-points. Death-censored AMR-free and allograft survivals were significantly lower in C1q-dnDSA patients. In multivariate analysis, C1q-dnDSA was an independent risk factor for AMR.

Conclusions: C1q-binding dnDSA is associated with inferior outcomes, yet not in all patients. Nevertheless, C1q-dnDSA was shown to be an independent risk factor of AMR and graft loss and may be a useful tool to stratify the immunological risk for AMR.

25. Risk factors associated with the development of histocompatibility leukocyte antigen sensitization.

Jordan SC, Choi J, Kim I, *et al.*

Curr Opin Organ Transplant. 2016 Aug;21(4):447-52. doi: 10.1097/MOT.0000000000000336.

ABSTRACT

Purpose Of Review: Despite excellent short-term kidney allograft survival rates, long-term outcomes have not improved. For years, the focus on improving these outcomes revolved around minimization or elimination of calcineurin toxicity. Despite our best efforts, approximately 5000 allografts are lost each year in the United States and results in a significant emotional burden for patients and financial burden for the healthcare system.

Recent Findings: Advancements in detection of donor-specific histocompatibility leukocyte antigen antibodies (DSAs) and improved assessment of allograft biopsy tissue have shown that the most common cause for graft failures is DSA-related antibody-mediated rejection. Sensitization is directly related to human tissue exposure prior to transplant. We now know that sensitization can occur in patients who are non compliant or poorly compliant with their calcineurin inhibitors. They develop de-novo DSAs, which are responsible for numerous allograft losses around the world.

Summary: Given the current evidence, it is imperative that all transplant physicians recognize the importance of encouraging medication adherence to prevent the consequences of DSA-induced graft failure. However, little progress has been made in this area. Other potential therapeutic approaches based on B-cell depletion or modulation early posttransplant may help to reduce the risk for de-novo DSA development.

26. Factors Predicting Risk for Antibody-mediated Rejection and Graft Loss in Highly Human Leukocyte Antigen Sensitized Patients Transplanted After Desensitization.

Vo AA, Sinha A, Haas M, *et al.*

Transplantation. 2015 Jul;99(7):1423-30. doi: 10.1097/TP.0000000000000525.

ABSTRACT

Background: Desensitization with intravenous immunoglobulin and rituximab (I+R) significantly improves transplant rates in highly sensitized patients, but antibody-mediated rejection (ABMR) remains a concern.

Patients And Methods: Between July 2006 and December 2012, 226 highly sensitized patients received transplants after desensitization. Most received alemtuzumab induction and standard immunosuppression. Two groups were examined: ABMR (n = 181) and ABMR (n = 45, 20%). Risk factors for ABMR, pathology, and outcomes were assessed.

Results: Significant risks for ABMR included previous transplants and pregnancies as sensitizing events, donor-specific antibody (DSA) relative intensity scores greater than 17, presence of both class I and II DSAs at transplant and time on waitlist. The ABMR showed a significant benefit for graft survival and glomerular filtration rate at 5 years (P < 0.0001). Banff pathology characteristics for ABMR patients with or without graft loss did not differ. C4d versus C4d ABMR did not predict graft loss (P = 0.086). Thrombotic microangiopathy (TMA) significantly predicted graft failure (P = 0.045). The ABMR episodes were treated with I+R (n = 25), or, in more severe ABMR, plasma exchange (PLEX)+I+R (n = 20). Graft survival for patients treated with I+R was superior (P = 0.028). Increased mortality was seen in ABMR patients experiencing graft loss after ABMR treatment (P = 0.004). The PLEX + Eculizumab improved graft survival for TMA patients (P = 0.036).

Conclusion: Patients desensitized with I+R who remain ABMR have long-term graft and patient survival. The ABMR patients have significantly reduced graft survival and glomerular filtration rate at 5 years, especially TMA. Severe ABMR episodes benefit from treatment with PLEX + Eculizumab. The DSA-relative intensity scores at transplant was a strong predictor of ABMR. Donor-specific antibody avoidance and reduction strategies before transplantation are critical to avoiding ABMR and improving long-term outcomes.

27. Impact of persistent and cleared preformed HLA DSA on kidney transplant outcomes.

Redondo-Pachón D, Pérez-Sáez MJ, Mir M, *et al.*

Hum Immunol. 2018 Jun;79(6):424-431. doi: 10.1016/j.humimm.2018.02.014. Epub 2018 Mar 7.

ABSTRACT

Preformed HLA donor-specific antibodies (DSA) only detected with Luminex have been associated with increased risk of antibody-mediated rejection (ABMR) and graft failure after kidney transplantation (KT). Their evolution after KT may modify this risk. We analyzed postransplant evolution of preformed DSA identified retrospectively and their impact on outcomes of 370 KT performed 2006-2014. Antibodies were monitored prospectively at 1-3-5 years after KT and if any dysfunction. Early acute ABMR was more frequent among patients with preformed DSA class-I or I + II than isolated class-II (29.4% vs 4.5%, p = 0.02). One year post-KT, 20 of 34 patients with functioning KT had persistent DSA. Preformed DSA class-II persisted more frequently than class-I/I + II (66.7% vs 33.3%; p = 0.031). The only risk factor independently associated with persistence was pretransplant MFI. Patients with de novo DSA had the highest risk of ABMR (HR 22.2 [CI 6.1-81.2]). Although recipients with persisting preformed DSA had significantly increased ABMR risk (HR 14.7 [CI 6.5-33.0]), those with cleared preformed DSA also had a higher risk than those without DSA (HR 7.01 [CI 2.2-21.8]). Preformed DSA are a very important risk factor for ABMR and graft loss. Patients who clear preformed DSA still show an increased risk of ABMR and graft loss after KT.

28. Impact of ABO Incompatibility on the Development of Acute Antibody-Mediated Rejection in Kidney Transplant Recipients Presensitized to HLA.

Chung BH, Joo YY, Lee J, *et al.*

PLoS One. 2015 Apr 21;10(4):e0123638. doi: 10.1371/journal.pone.0123638. eCollection 2015.

ABSTRACT

Whether the coexistence of anti-A/B antibody and donor specific anti-HLA antibody (HLA-DSA) has a synergistic impact on the development of acute antibody-mediated rejection (AAMR) in kidney transplant recipients (KTRs) is unclear. This study includes 92 KTRs who received a kidney from an ABO-incompatible (ABOi) donor or were presensitized to donor HLA (HLAs) and 292 controls (CONT). HLAs was defined as a crossmatch positivity or the presence of HLA-DSA. We compared the incidence of AAMR among ABOi (n = 58), ABOi+HLAs (n = 12), HLAs (n = 22), and CONT (n = 292) groups and evaluated the risk factors and antibody type (anti-A/B vs. HLA-DSA) responsible for AAMR. AAMR developed less frequently in ABOi and CONT than in the ABOi+HLAs or HLAs ($P < 0.05$ for all); however, there was no difference between the ABOi+HLAs and HLAs groups. AAMR developed more frequently with strong HLA-DSA at baseline; however, high baseline anti-A/B titer did not affect AAMR development. Strong baseline HLA-DSA was an independent predictor for AAMR, however the baseline anti-A/B titer was not. All four AAMR episodes in ABOi+HLAs were positive to HLA-DSA but not to anti-A/B. In conclusion, ABO incompatibility does not increase the risk for AAMR in HLAs KTRs.

29. Impact of Early Blood Transfusion After Kidney Transplantation on the Incidence of Donor-Specific Anti-HLA Antibodies.

Ferrandiz I, Congy-Jolivet N, Del Bello A, *et al.*

Am J Transplant. 2016 Sep;16(9):2661-9. doi: 10.1111/ajt.13795. Epub 2016 Apr 19.Author information

ABSTRACT

Little is known about the impact of posttransplant blood transfusion on the sensitization of anti-HLA antibodies and the formation of donor-specific antibodies (DSAs). The aims of our study were to determine the 1-year incidence of DSAs (assessed using a solid-phase assay) and antibody-mediated rejection (AMR) in kidney transplant patients who had or had not received a blood transfusion during the first year after transplantation. Included were 390 non-HLA-sensitized patients who had received an ABO-compatible kidney transplant and had not previously or simultaneously received a nonkidney transplant. Overall, 64% of patients received a red blood cell transfusion within the first year after transplantation, most within the first month. The overall 1-year incidence of DSAs was significantly higher in patients that had undergone transfusion (7.2% vs. 0.7% in patients with no transfusion, $p < 0.0001$). AMR occurred more often in the transfusion group (n = 15, 6%) compared with the nontransfusion group (n = 2, 1.4%; $p = 0.04$). Blood transfusion was an independent predictive factor for de novo DSA formation but not for AMR. Patients who had a transfusion and developed DSAs were more often treated with cyclosporin A (n = 10, 55.5%) rather than tacrolimus (n = 45, 19.4%; $p = 0.0001$). In conclusion, early posttransplant blood transfusion may increase immunological risk, especially in underimmunosuppressed patients.

30. Deleterious Impact of Donor-Specific Anti-HLA Antibodies Toward HLA-Cw and HLA-DP in Kidney Transplantation.

Bachelet T, Martinez C, Del Bello A, *et al.*

Transplantation. 2016 Jan;100(1):159-66. doi: 10.1097/TP.0000000000000821.

ABSTRACT

Background: It is widely accepted that HLA donor-specific antibodies (DSA) are associated with antibody-mediated rejection and graft loss. However, in many transplant programs, preformed anti-HLA-Cw and anti-HLA-DP DSA are not considered in organ allocation policies because their clinical relevance is still uncertain.

Methods: We analyzed the clinical impact of Cw/DP DSA through a retrospective study, comparing 48 patients transplanted with isolated preformed Cw/DP DSA (Cw/DP DSA group) with (i) 104 matched HLA-sensitized kidney transplant recipients with No DSA at D0 (No DSA group) and (ii) 47 kidney transplant recipients with preformed A, -B, -DR, -DQ DSA (A/B/DR/DQ DSA group).

Results: A positive flow cytometry crossmatch in the Cw/DP DSA group was more frequent than in the No DSA group and as frequent as in the A/B/DR/DQDSA group. Two years after transplantation, the biopsy-proven acute rejection-free survival was worse in the Cw/DP and A/B/DR/DQ DSA groups than in the No DSA group (65%, 84%, 93%, P = 0.001 and P = 0.05, respectively). Accordingly, graft survival was lower in the Cw/DP and the A/B/DR/DQ DSA groups than in the No DSA group (87%, 89%, 95%, P = 0.02 and P = 0.1, respectively).

Conclusions: These results suggest that preformed anti-HLA-Cw and anti-HLA-DP DSA are as deleterious as anti-HLA A/B/DR/DQ DSA. It justifies their inclusion in kidney allocation programs and in immunological risk stratification algorithms.

31. Complement-binding anti-HLA antibodies and kidney-allograft survival.

Loupy A, Lefaucheur C, Vernerey D, *et al.*

N Engl J Med. 2013 Sep 26;369(13):1215-26. doi: 10.1056/NEJMoa1302506.

ABSTRACT

Background: Anti-HLA antibodies hamper successful transplantation, and activation of the complement cascade is involved in antibody-mediated rejection. We investigated whether the complement-binding capacity of anti-HLA antibodies plays a role in kidney-allograft failure.

Methods: We enrolled patients who received kidney allografts at two transplantation centers in Paris between January 1, 2005, and January 1, 2011, in a population-based study. Patients were screened for the presence of circulating donor-specific anti-HLA antibodies and their complement-binding capacity. Graft injury phenotype and the time to kidney-allograft loss were assessed.

Results: The primary analysis included 1016 patients. Patients with complement-binding donor-specific anti-HLA antibodies after transplantation had the lowest 5-year rate of graft survival (54%), as compared with patients with non-complement-binding donor-specific anti-HLA antibodies (93%) and patients without donor-specific anti-HLA antibodies (94%) ($P<0.001$ for both comparisons). The presence of complement-binding donor-specific anti-HLA antibodies after transplantation was associated with a risk of graft loss that was more than quadrupled (hazard ratio, 4.78; 95% confidence interval [CI], 2.69 to 8.49) when adjusted for clinical, functional, histologic, and immunologic factors. These antibodies were also associated with an increased rate of antibody-mediated rejection, a more severe graft injury phenotype with more extensive microvascular inflammation, and increased deposition of complement fraction C4d within graft capillaries. Adding complement-binding donor-specific anti-HLA antibodies to a traditional risk model improved the stratification of patients at risk for graft failure (continuous net reclassification improvement, 0.75; 95% CI, 0.54 to 0.97).

Conclusions: Assessment of the complement-binding capacity of donor-specific anti-HLA antibodies appears to be useful in identifying patients at high risk for kidney-allograft loss.

32. mTOR inhibitors and risk of chronic antibody-mediated rejection after kidney transplantation: where are we now?

Grimbert P & Thaunat O.

Transpl Int. 2017 Jul;30(7):647-657. doi: 10.1111/tri.12975.

ABSTRACT

Antibody-mediated rejection (AMR) usually starts with generation of donor-specific anti-HLA antibodies (DSAs), arising from a B-cell response to antigen recognition. In vitro and preclinical data demonstrate that mammalian target of rapamycin (mTOR) inhibition attenuates the mTOR-mediated intracellular signaling pathway involved in AMR-related kidney damage. The limited available data from immunological studies in kidney transplant patients, however, have not shown such effects in vivo. In terms of clinical immunosuppression, the overriding influence on rates of de novo DSA (dnDSA) or AMR-regardless of the type of regimen-is patient adherence. To date, limited data from patients given mTOR inhibitor therapy with adequate concurrent immunosuppression, such as reduced-exposure calcineurin inhibitor (CNI) therapy, have not shown an adverse effect on the risk of dnDSA or AMR. Early switch to an mTOR inhibitor (<6-12 months post-transplant) in a CNI-free regimen, in contrast, can increase the risk of dnDSA, especially if adjunctive therapy is inadequate. Late conversion to CNI-free therapy with mTOR inhibition does not appear to affect the risk of dnDSA. More data, from prospective studies, are required to fully understand that association between use of mTOR inhibitors with different types of concomitant therapy and risk of dnDSA and AMR.

33. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts.

Everly MJ, Rebellato LM, Haisch CE, *et al.*

Transplantation. 2013 Feb 15;95(3):410-7. doi: 10.1097/TP.0b013e31827d62e3.

ABSTRACT

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.

34. HLA-DQ Mismatches and Rejection in Kidney Transplant Recipients.

Lim WH, Chapman JR, Coates PT, *et al.*

Clin J Am Soc Nephrol. 2016 May 6;11(5):875-83. doi: 10.2215/CJN.11641115. Epub 2016 Mar 31.

ABSTRACT

Background And Objectives: The current allocation algorithm for deceased donor kidney transplantation takes into consideration HLA mismatches at the ABDR loci but not HLA mismatches at other loci, including HLA-DQ. However, the independent effects of incompatibilities for the closely linked HLA-DQ antigens in the context of HLA-DR antigen matched and mismatched allografts are uncertain. We aimed to determine the effect of HLA-DQ mismatches on renal allograft outcomes.

Design, Setting, Participants, & Measurements: Using data from the Australia and New Zealand Dialysis and Transplant Registry, we examined the association between HLA-DQ mismatches and acute rejections in primary live and deceased donor kidney transplant recipients between 2004 and 2012 using adjusted Cox regression models.

Results: Of the 788 recipients followed for a median of 2.8 years (resulting in 2891 person-years), 321 (40.7%) and 467 (59.3%) received zero and one or two HLA-DQ mismatched kidneys, respectively. Compared with recipients who have received zero HLA-DQ mismatched kidneys, those who have received one or two HLA-DQ mismatched kidneys experienced greater numbers of any rejection (50 of 321 versus 117 of 467; P<0.01), late rejections (occurring >6 months post-transplant; 8 of 321 versus 27 of 467; P=0.03), and antibody-mediated rejections (AMRs; 12 of 321 versus 38 of 467; P=0.01). Compared with recipients of zero HLA-DQ mismatched kidneys, the adjusted hazard ratios for any and late rejections in recipients who had received one or two HLA-DQ mismatched kidneys were 1.54 (95% confidence interval [95% CI], 1.08 to 2.19) and 2.85 (95% CI, 1.05 to 7.75), respectively. HLA-DR was an effect modifier between HLA-DQ mismatches and AMR (P value for interaction =0.02), such that the association between HLA-DQ mismatches and AMR was statistically significant in those who have received one or two HLA-DR mismatched kidneys, with adjusted hazard ratio of 2.50 (95% CI, 1.05 to 5.94).

Conclusions: HLA-DQ mismatches are associated with acute rejection, independent of HLA-ABDR mismatches and initial immunosuppression. Clinicians should be aware of the potential importance of HLA-DQ matching in the assessment of immunologic risk in kidney transplant recipients.

35. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence.

Sellarés J, de Freitas DG, Mengel M, Reeve J, *et al.*

Am J Transplant. 2012 Feb;12(2):388-99. doi: 10.1111/j.1600-6143.2011.03840.x. Epub 2011 Nov 14.

ABSTRACT

We prospectively studied kidney transplants that progressed to failure after a biopsy for clinical indications, aiming to assign a cause to every failure. We followed 315 allograft recipients who underwent indication biopsies at 6 days to 32 years posttransplant. Sixty kidneys progressed to failure in the follow-up period (median 31.4 months). Failure was rare after T-cell-mediated rejection and acute kidney injury and common after antibody-mediated rejection or glomerulonephritis. We developed rules for using biopsy diagnoses, HLA antibody and clinical data to explain each failure. Excluding four with missing information, 56 failures were attributed to four causes: rejection 36 (64%), glomerulonephritis 10 (18%), polyoma virus nephropathy 4 (7%) and intercurrent events 6 (11%). Every rejection loss had evidence of antibody-mediated rejection by the time of failure. Among rejection losses, 17 of 36 (47%) had been independently identified as nonadherent by attending clinicians. Nonadherence was more frequent in patients who progressed to failure (32%) versus those who survived (3%). Pure T-cell-mediated rejection, acute kidney injury, drug toxicity and unexplained progressive fibrosis were not causes of loss. This prospective cohort indicates that many actual failures after indication biopsies manifest phenotypic features of antibody-mediated or mixed rejection and also underscores the major role of nonadherence.

36. Clinically-relevant threshold of preformed donor-specific anti-HLA antibodies in kidney transplantation.

Salvadé I, Aubert V, Venetz JP, *et al.*

Hum Immunol. 2016 Jun;77(6):483-9. doi: 10.1016/j.humimm.2016.04.010. Epub 2016 Apr 13.

ABSTRACT

Background: Pretransplant anti-HLA donor-specific antibodies (DSA) are recognized as a risk factor for acute antibody-mediated rejection (AMR) in kidney transplantation. The predictive value of C4d-fixing capability by DSA or of IgG DSA subclasses for acute AMR in the pretransplant setting has been recently studied. In addition DSA strength assessed by mean fluorescence intensity (MFI) may improve risk stratification. We aimed to analyze the relevance of preformed DSA and of DSA MFI values.

Methods: 280 consecutive patients with negative complement-dependent cytotoxicity crossmatches received a kidney transplant between 01/2008 and 03/2014. Sera were screened for the presence of DSA with a solid-phase assays on a Luminex flow analyzer, and the results were correlated with biopsy-proven acute AMR in the first year and survival.

Results: Pretransplant anti-HLA antibodies were present in 72 patients (25.7%) and 24 (8.6%) had DSA. There were 46 (16.4%) acute rejection episodes, 32 (11.4%) being cellular and 14 (5.0%) AMR. The incidence of acute AMR was higher in patients with pretransplant DSA (41.7%) than in those without (1.6%) ($p<0.001$). The median cumulative MFI (cMFI) of the group DSA+/AMR+ was 5680 vs 2208 in DSA+/AMR- ($p=0.058$). With univariate logistic regression a threshold value of 5280 cMFI was predictive for acute AMR. DSA cMFI's ability to predict AMR was also explored by ROC analysis. AUC was 0.728 and the best threshold was a cMFI of 4340. Importantly pretransplant DSA>5280 cMFI had a detrimental effect on 5-year graft survival.

Conclusions: Preformed DSA cMFI values were clinically-relevant for the prediction of acute AMR and graft survival in kidney transplantation. A threshold of 4300-5300 cMFI was a significant outcome predictor.

37. Deceased donor kidney transplantation across donor-specific antibody barriers: predictors of antibody-mediated rejection.

Schwaiger E, Eskandary F, Kozakowski N, *et al.*

Nephrol Dial Transplant. 2016 Aug;31(8):1342-51. doi: 10.1093/ndt/gfw027. Epub 2016 Mar 24.

ABSTRACT

Background: Apheresis-based desensitization allows for successful transplantation across major immunological barriers. For donor-specific antibody (DSA)- and/or crossmatch-positive transplantation, however, it has been shown that even intense immunomodulation may not completely prevent antibody-mediated rejection (ABMR).

Methods: In this study, we evaluated transplant outcomes in 101 DSA+ deceased donor kidney transplant recipients (transplantation between 2009 and 2013; median follow-up: 24 months) who were subjected to immunoadsorption (IA)-based desensitization. Treatment included a single pre-transplant IA session, followed by anti-lymphocyte antibody and serial post-transplant IA. In 27 cases, a positive complement-dependent cytotoxicity crossmatch (CDCXM) was rendered negative immediately before transplantation. Seventy-four of the DSA+ recipients had a negative CDCXM already before IA.

Results: Three-year death-censored graft survival in DSA+ patients was significantly worse than in 513 DSA- recipients transplanted during the same period (79 versus 88%, P = 0.008). Thirty-three DSA+ recipients (33%) had ABMR. While a positive baseline CDCXM showed only a trend towards higher ABMR rates (41 versus 30% in CDCXM- recipients, P = 0.2), DSA mean fluorescence intensity (MFI) in single bead assays significantly associated with rejection, showing 20 versus 71% ABMR rates at <5000 versus >15 000 peak DSA MFI. The predictive value of MFI was moderate, with the highest accuracy at a median of 13 300 MFI (after cross-validation: 0.72). Other baseline variables, including CDC assay results, human leukocyte antigen mismatch, prior transplantation or type of induction treatment, did not add independent predictive information.

Conclusions: IA-based desensitization failed to prevent ABMR in a considerable number of DSA+ recipients. Assessing DSA MFI may help stratify risk of rejection, supporting its use as a guide to organ allocation and individualized treatment.

38. The Impact of Donor-Specific Anti-Human Leukocyte Antigen (HLA) Antibody Rebound on the Risk of Antibody Mediated Rejection in Sensitized Kidney Transplant Recipients.

Lee KW, Park JB, Cho CW, *et al.*

Ann Transplant. 2017 Mar 28;22:166-176.

ABSTRACT

Background: Donor-specific anti-HLA antibody (DSA) detected on Luminex-based single antigen assay (LSA) has become the subject of desensitization based upon the results of previous studies. We retrospectively investigated the impact of preoperative DSA on the incidence of antibody mediated rejection (AMR) in patients desensitized using a protocol based on rituximab and rabbit antithymocyte globulin (rATG).

Material And Methods: Nine patients (Group 1, 9/327, 2.8%) were complement dependent cytotoxicity crossmatch (CDC-XM) positive and underwent desensitization with rituximab (375 mg/m^2), intravenous immunoglobulin (IVIG; 400 mg/kg), plasmapheresis, and rATG. Twenty-two patients (Group 2, 22/327, 6.7%) were CDC-XM negative but DSA positive on LSA and had received desensitization with rituximab and rATG, while 55 patients (Group 3, 55/327, 16.8%) were CDC-XM and DSA negative with a calculated panel reactive antibody (cPRA) ≥50%. Another 241 patients (Group 4, 241/327, 73.7%) were CDC-XM and DSA negative with a cPRA <50%.

Results: Recipients with DSA (Group 2) experienced more AMR than other groups ($p<0.01$). More de novo DSAs also developed in Group 2 ($p<0.001$). The mean fluorescence intensity (MFI) of DSA of patients with AMR tended to rebound ($p=0.01$).

Conclusions: Patients who were CDC-XM negative but DSA positive status were at a higher risk of developing AMR even though they had received desensitization with rATG and rituximab. A more intense desensitization protocol is needed for these recipients. Patients with MFI rebound of DSA should be carefully monitored for the risk of AMR.

39. Relationship between Mean Fluorescence Intensity and C1q/C3d-fixing capacities of anti-HLA antibodies.

Claisse G, Absi L, Cognasse F, *et al.*

Hum Immunol. 2017 Apr;78(4):336-341. doi: 10.1016/j.humimm.2017.02.003. Epub 2017 Feb 9.

ABSTRACT

Background: Complement-binding assays are proposed to better stratify the risk of antibody-mediated rejection associated-graft failure. Despite promising clinical results, some have suggested that the MFI of anti-HLA antibodies may influence these tests.

Methods: We investigated the impact of Abs MFI reduction, induced by plasmapheresis, on C1q- and C3d-binding assays. Sera provided from 7 sensitized kidney transplant patients were analyzed.

Results: Four hundreds and thirty-three SABs were analyzed. Before plasmapheresis, when compared to C1q- SABs, C1q+ SABs had a higher median MFI [17397 (IQR: 14851-18794) vs. 2745 (IQR: 1125-6476), p<0.01]. SABs that remained C1q+ after plasmapheresis had a higher median MFI. Regarding the C3d assay, results were strictly comparable. MFI value was a powerful predictor of both C1q and C3d positivity [AUC 0.97 (CI95% 0.95-0.99) and 0.96, (CI95% 0.93-0.98), respectively].

Conclusion: Our data suggest that both C1q- and C3d-binding assays are intimately linked to the MFI of anti-HLA Abs.

40. Revisiting traditional risk factors for rejection and graft loss after kidney transplantation.

Dunn TB1, Noreen H, Gillingham K, *et al.*

Am J Transplant. 2011 Oct;11(10):2132-43. doi: 10.1111/j.1600-6143.2011.03640.x. Epub 2011 Aug 3.

ABSTRACT

Single-antigen bead (SAB) testing permits reassessment of immunologic risk for kidney transplantation. Traditionally, high panel reactive antibody (PRA), retransplant and deceased donor (DD) grafts have been associated with increased risk. We hypothesized that this risk was likely mediated by (unrecognized) donor-specific antibody (DSA). We grouped 587 kidney transplants using clinical history and single-antigen bead (SAB) testing of day of transplant serum as (1) unsensitized; PRA = 0 (n = 178), (2) third-party sensitized; no DSA (n = 363) or (3) donor sensitized; with DSA (n = 46), and studied rejection rates, death-censored graft survival (DCGS) and risk factors for rejection. Antibody-mediated rejection (AMR) rates were increased with DSA ($p < 0.0001$), but not with panel reactive antibody (PRA) in the absence of DSA. Cell-mediated rejection (CMR) rates were increased with DSA ($p < 0.005$); with a trend to increased rates when PRA>0 in the absence of DSA ($p = 0.08$). Multivariate analyses showed risk factors for AMR were DSA, worse HLA matching, and female gender; for CMR: DSA, PRA>0 and worse HLA matching. AMR and CMR were associated with decreased DCGS. The presence of DSA is an important predictor of rejection risk, in contrast to traditional risk factors. Further development of immunosuppressive protocols will be facilitated by stratification of rejection risk by donor sensitization.

41. Risk of antibody-mediated rejection in kidney transplant recipients with anti-HLA-C donor-specific antibodies.

Aubert O, Bories MC, Suberbielle C, *et al.*

Am J Transplant. 2014 Jun;14(6):1439-45. doi: 10.1111/ajt.12709. Epub 2014 May 7.

ABSTRACT

Anti-HLA donor-specific antibodies (DSAs) cause acute and chronic antibody-mediated rejection (AMR). However, the clinical relevance of anti-HLA-C antibodies remains unclear. We evaluated the clinical relevance of the presence of anti-HLA-C DSA at day 0 in renal transplant recipients. In this retrospective, case-controlled study, 608 patients who underwent kidney transplantation between August 2008 and March 2012 were screened for the presence of isolated anti-HLA-C DSA at day 0. A total of 22 renal transplant recipients were selected and followed for a period of 1 year. AMR was classified according to the Banff classification. The 22 patients were compared with 88 immunized patients. Acute AMR was diagnosed in six patients (27.3%). The median level of DSA at day 0 was 1179 (530-17,941). The mean fluorescence intensity in the anti-C group was 4966 (978-17,941) in the AMR group and 981 (530-8012) in the group of patients without AMR. Acute AMR was diagnosed less frequently in the 88 immunized individuals (9.1%) than in the DSA anti-C group ($p = 0.033$). The level of DSA at day 0 was predictive for AMR ($p = 0.017$). Patients with a high level of pretransplant anti-HLA-C DSAs are likely to develop acute AMR during the first year after transplantation.

42. Outcomes and risk stratification for late antibody-mediated rejection in recipients of ABO-incompatible kidney transplants: a retrospective study.

Lonze BE, Bae S, Kraus ES, *et al.*

Transpl Int. 2017 Sep;30(9):874-883. doi: 10.1111/tri.12969. Epub 2017 Jun 2.

ABSTRACT

The required intensity of monitoring for antibody-mediated rejection (AMR) after of ABO-incompatible (ABOi) kidney transplantation is not clearly formulized. We retrospectively evaluated a single-center cohort of 115 ABO-incompatible (ABOi) kidney transplant recipients, of which 32% were also HLA incompatible (ABOi/HLAi) with their donors. We used an adjusted negative binomial model to evaluate risk factors for late AMR. Using this model, we risk-stratified patients into high- and low-risk groups for the development of late AMR; 26% of patients had at least one AMR episode; 49% of AMR episodes occurred within 30-days after transplant and were considered early AMR. Patients with an early AMR episode had a 5.5-fold greater incidence of developing late AMR [IRR = 5.5, (95% CI: 1.5-19.3), P = 0.01]. ABOi/HLAi recipients trended toward increased late AMR risk [IRR = 1.9, (95% CI: 0.5-6.6), P = 0.3]. High-risk recipients (those with an early AMR or those who were ABOi/HLAi) had a sixfold increased incidence of late AMR [IRR = 6.3, (95% CI: 1.6-24.6), P = 0.008] versus low-risk recipients. The overall incidence of late AMR was 20.8% vs. 1.5% in low-risk recipients. Changes in anti-A/B titer did not correlate with late AMR (IRR = 0.9 per log titer increase, P = 0.7). This risk-stratification scheme uses information available within 30 days of ABOi transplantation to determine risk for late AMR and can help direct longitudinal follow-up for individual patients.

43. From Humoral Theory to Performant Risk Stratification in Kidney Transplantation.

Lefaucheur C, Viglietti D, Mangiola M, *et al.*

J Immunol Res. 2017;2017:5201098. doi: 10.1155/2017/5201098. Epub 2017 Jan 2.

ABSTRACT

The purpose of the present review is to describe how we improve the model for risk stratification of transplant outcomes in kidney transplantation by incorporating the novel insights of donor-specific anti-HLA antibody (DSA) characteristics. The detection of anti-HLA DSA is widely used for the assessment of pre- and posttransplant risks of rejection and allograft loss; however, not all anti-HLA DSA carry the same risk for transplant outcomes. These antibodies have been shown to cause a wide spectrum of effects on allografts, ranging from the absence of injury to indolent or full-blown acute antibody-mediated rejection. Consequently, the presence of circulating anti-HLA DSA does not provide a sufficient level of accuracy for the risk stratification of allograft outcomes. Enhancing the predictive performance of anti-HLA DSA is currently one of the most pressing unmet needs for facilitating individualized treatment choices that may improve outcomes. Recent advancements in the assessment of anti-HLA DSA properties, including their strength, complement-binding capacity, and IgG subclass composition, significantly improved the risk stratification model to predict allograft injury and failure. Although risk stratification based on anti-HLA DSA properties appears promising, further specific studies that address immunological risk stratification in large and unselected populations are required to define the benefits and cost-effectiveness of such comprehensive assessment prior to clinical implementation.

44. Pretransplant immunologic risk assessment of kidney transplant recipients with donor-specific anti-human leukocyte antigen antibodies.

Marfo K, Ajaimy M, Colovai A, *et al.*

Transplantation. 2014 Nov 27;98(10):1082-8. doi: 10.1097/TP.0000000000000191.

ABSTRACT

Background: Patients with pretransplantation strong donor-specific anti-human leukocyte antigen (HLA) antibodies (DSA) are at higher risk for rejection. We aimed to study the safety of kidney transplantation in patients with lower strength DSAs in a prospective cohort study.

Methods: Three hundred and seventy-three consecutive adult kidney transplant recipients with (DSA+; n=66) and without (DSA-; n=307) DSA were evaluated. Anti-HLA antibodies with mean fluorescence intensity values over 5,000 for HLA-A, HLA-B, and HLA-DR and more than 10,000 for HLA-DQ were reported as unacceptable antigens. Patients received transplant if flow cytometry T-cell and B-cell cross-match channel shift values were less than 150 and 250, respectively, with antithymocyte globulin and intravenous immunoglobulin induction treatment.

Results: Patients had a mean number of 1.6 ± 0.8 DSAs with a mean fluorescence intensity value of 2,815 ± 2,550. Twenty-seven percent were flow cytometry cross-match positive with T-cell and B-cell channel shift values of 129 ± 49 and 159 ± 52, respectively. During a median follow-up of 24 months (range, 6-50), there were no statistically significant differences in patient (99% vs. 95%) and graft survival (88% vs. 90%) rates between DSA+ and DSA- groups, respectively. Cumulative acute rejection rates of 11% in the DSA+ group and 12% in the DSA- group were similar. Two DSA+ (3%) and five DSA- (2%) patients developed chronic antibody-mediated rejection (3%). The mean serum creatinine levels were identical between the two groups (1.4 ± 0.6 mg/dL).

Conclusion: Similar patient and graft survival, and acute rejection rates can be achieved in DSA+ patients compared to DSA- patients with pretransplantation immunologic risk assessment.

45. Rituximab as induction therapy after renal transplantation: a randomized, double-blind, placebo-controlled study of efficacy and safety.

van den Hoogen MW, Kamburova EG, Baas MC, *et al*.

Am J Transplant. 2015 Feb;15(2):407-16. doi: 10.1111/ajt.13052.

ABSTRACT

We evaluated the efficacy and safety of rituximab as induction therapy in renal transplant patients. In a double-blind, placebo-controlled study, 280 adult renal transplant patients were randomized between a single dose of rituximab (375 mg/m(2)) or placebo during transplant surgery. Patients were stratified according to panel-reactive antibody (PRA) value and rank number of transplantation. Maintenance immunosuppression consisted of tacrolimus, mycophenolate mofetil and steroids. The primary endpoint was the incidence of biopsy proven acute rejection (BPAR) within 6 months after transplantation. The incidence of BPAR was comparable between rituximab-treated (23/138, 16.7%) and placebo-treated patients (30/142, 21.2%, $p = 0.25$). Immunologically high-risk patients (PRA >6% or re-transplant) not receiving rituximab had a significantly higher incidence of rejection (13/34, 38.2%) compared to other treatment groups (rituximab-treated immunologically high-risk patients, and rituximab- or placebo-treated immunologically low-risk (PRA ≤ 6% or first transplant) patients (17.9%, 16.4% and 15.7%, $p = 0.004$). Neutropenia ($<1.5 \times 10(9)$ /L) occurred more frequently in rituximab-treated patients (24.3% vs. 2.2%, $p < 0.001$). After 24 months, the cumulative incidence of infections and malignancies was comparable. A single dose of rituximab as induction therapy did not reduce the overall incidence of BPAR, but might be beneficial in immunologically high-risk patients. Treatment with rituximab was safe.

46. Rituximab prevents an anamnestic response in patients with cryptic sensitization to HLA.

Zachary AA, Lucas DP, Montgomery RA, Leffell MS.

Transplantation. 2013 Mar 15;95(5):701-4. doi: 10.1097/TP.0b013e31827be3c1.

ABSTRACT

Background: Some patients sensitized to HLA antigens do not have antibody present in serum specimens that are available before transplantation. However, such patients are at risk for an anamnestic response resulting from a proinflammatory response to the trauma of transplant surgery. Quantifying HLA-specific B cells provides a way to identify these patients and provide treatment to prevent an anamnestic response.

Methods: B cells were isolated before transplantation from 59 patients, 20 of whom were treated with rituximab at the time of transplantation. Ninety-nine tests were performed to quantify HLA-specific B cells by staining with HLA tetramers. Patients were considered sensitized or nonsensitized based on the frequencies of HLA-specific B cells. Pretransplantation and posttransplantation sera were tested for the detection of antibody specific for the tetramer antigen.

Results: Of the 24 cases where patients were considered sensitized to HLA antigens but did not have antibody before transplantation, no posttransplantation antibody to the tetramer antigen was detected in 10 cases when patients were treated with rituximab, but antibody was detected in 13 of 16 cases when there was no rituximab treatment (P=0.00006). The mean frequencies of B cells specific for HLA-B7 were the same in rituximab-treated patients who did not make antibody and in nontreated patients who did make antibody (6.0% vs. 5.7%; P=0.8).

Conclusions: Elimination of peripheral HLA-specific B cells in patients who are sensitized to HLA antigens but lacking detectable antibody abrogates an anamnestic response.

47. Clinical Significance of HLA-DQ Antibodies in the Development of Chronic Antibody-Mediated Rejection and Allograft Failure in Kidney Transplant Recipients.

Lee H, Min JW, Kim JI, *et al.*

Medicine (Baltimore). 2016 Mar;95(11):e3094. doi: 10.1097/MD.0000000000003094.

ABSTRACT

With the development of the single antigen beads assay, the role of donor specific alloantibody (DSA) against human leukocyte antigens in kidney transplantation (KT) has been highlighted. This study aimed to investigate the clinical significance of DQ-DSA detected at renal allograft biopsy. We evaluated 263 KT recipients who underwent allograft biopsy and DSA detection at the same time. Among them, 155 patients who were nonsensitized before transplantation were selected to investigate the role of de-novo DQ-DSA. Both the total and nonsensitized subgroup was categorized into 4 groups each according to DSA results as: DQ only, DQ+non-DQ, non-DQ, and no DSA. In the total patient group, post-KT DSA was positive in 79 (30.0%) patients and DQ-DSA was most prevalent (64.6%). In the nonsensitized subgroup, de-novo DSAs were detected in 45 (29.0%) patients and DQ-DSA was also most prevalent (73.3%). The DQ only group showed a significantly longer post-KT duration compared to the other groups ($P<0.05$). The overall incidence of antibody-mediated rejection (AMR) was 17.9%. B-DSA, DR-DSA, and DQ-DSA were associated with AMR ($P<0.05$), but in the analysis for chronic AMR, only DQ-DSA showed significance in both the total and the nonsensitized subgroup ($P<0.05$). On comparison of Banff scores among groups, those representing humoral immunity were significantly dominant in all DSA positive groups compared to the no DSA group ($P<0.05$), and higher scores of markers representing chronic tissue injury were more frequently detected in the groups with DQ-DSA. The worst postbiopsy survival was seen in the DQ+non-DQ group of the total patient group, and patients with de-novo DQ-DSA showed poorer graft survival in the nonsensitized subgroup compared to the no DSA group ($P<0.05$). In the multivariate analysis, de-novo DQ-DSA was the only significant risk factor associated with late allograft failure ($P<0.05$). Our study is the first to demonstrate the association of DQ-DSA with detailed histological findings representing chronic AMR. These findings suggest that the detection of DQ-DSA in nonsensitized patients is significantly associated with the development of chronic AMR and late allograft failure. Therefore monitoring of DQ-DSA not only in sensitized patients, but also nonsensitized patients may be necessary to improve long-term allograft outcomes.

48. IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury.

Lefaucheur C, Viglietti D, Bentlejewski C, *et al.*

J Am Soc Nephrol. 2016 Jan;27(1):293-304. doi: 10.1681/ASN.2014111120. Epub 2015 Aug 20.

ABSTRACT

Antibodies may have different pathogenicities according to IgG subclass. We investigated the association between IgG subclasses of circulating anti-human HLA antibodies and antibody-mediated kidney allograft injury. Among 635 consecutive kidney transplantations performed between 2008 and 2010, we enrolled 125 patients with donor-specific anti-human HLA antibodies (DSA) detected in the first year post-transplant. We assessed DSA characteristics, including specificity, HLA class specificity, mean fluorescence intensity (MFI), C1q-binding, and IgG subclass, and graft injury phenotype at the time of sera evaluation. Overall, 51 (40.8%) patients had acute antibody-mediated rejection (aABMR), 36 (28.8%) patients had subclinical ABMR (sABMR), and 38 (30.4%) patients were ABMR-free. The MFI of the immunodominant DSA (iDSA, the DSA with the highest MFI level) was 6724±464, and 41.6% of patients had iDSA showing C1q positivity. The distribution of iDSA IgG1-4 subclasses among the population was 75.2%, 44.0%, 28.0%, and 26.4%, respectively. An unsupervised principal component analysis integrating iDSA IgG subclasses revealed aABMR was mainly driven by IgG3 iDSA, whereas sABMR was driven by IgG4 iDSA. IgG3 iDSA was associated with a shorter time to rejection ($P<0.001$), increased microcirculation injury ($P=0.002$), and C4d capillary deposition ($P<0.001$). IgG4 iDSA was associated with later allograft injury with increased allograft glomerulopathy and interstitial fibrosis/tubular atrophy lesions ($P<0.001$ for all comparisons). Integrating iDSA HLA class specificity, MFI level, C1q-binding status, and IgG subclasses in a Cox survival model revealed IgG3 iDSA and C1q-binding iDSA were strongly and independently associated with allograft failure. These results suggest IgG iDSA subclasses identify distinct phenotypes of kidney allograft antibody-mediated injury.

49. Kidney Transplant With Low Levels of DSA or Low Positive B-Flow Crossmatch: An Underappreciated Option for Highly Sensitized Transplant Candidates.

Schinstock CA, Gandhi M, Cheungpasitporn W, *et al.*

Transplantation. 2017 Oct;101(10):2429-2439. doi: 10.1097/TP.0000000000001619.

ABSTRACT

Background: Avoiding donor-specific antibody (DSA) is difficult for sensitized patients. Improved understanding of the risk of low level DSA is needed.

Methods: We retrospectively compared the outcomes of 954 patients transplanted with varied levels of baseline DSA detected by single antigen beads and B flow cytometric crossmatch (XM). Patients were grouped as follows: -DSA/-XM, +DSA/-XM, +DSA/low +XM, +DSA/high +XM, and -DSA/+XM and followed up for a mean of 4.1 ± 1.9 years (similar among groups, P = 0.49).

Results: Death-censored allograft survival was similar in all groups except the +DSA/high +XM group, which was lower at 79.1% versus 96.2% in the -DSA/-XM group (P < 0.01). The incidence of chronic antibody-mediated rejection (CAMR) based on surveillance biopsy was higher with increasing DSA (8.2% -DSA/-XM, 17.0% +DSA/-XM, 30.6% +DSA/low +XM, and 51.2% +DSA/high +XM, P < 0.01), but similar in groups without baseline DSA (8.1% -DSA/-XM vs 15.4% -DSA/+XM, P = 0.19). Having a calculated panel-reactive antibody (cPRA) of 80% or greater was independently associated with CAMR (hazard ratio, 5.2; P = 0.03) even when DSA was undetected at baseline. By 2 years posttransplant, the incidence of CAMR was 19.4% in patients with cPRA of 80% or greater and undetected DSA and negative XM at baseline.

Conclusions: Kidney transplantation with low-level DSA with or without a low positive XM is a reasonable option for highly sensitized patients and may be advantageous compared with waiting for a negative XM deceased donor. The risk for CAMR is low in patients with no DSA even if the XM is positive. Patients with cPRA of 80% or greater are at risk for CAMR even if no DSA is detected.

50. Renal transplant outcomes and de novo donor-specific anti-human leukocyte antigen antibodies: a systematic review.

Sharma A, Lewis JR, Lim WH, *et al.*

Nephrol Dial Transplant. 2018 Apr 11. doi: 10.1093/ndt/gfy077.

ABSTRACT

Background: Pre-transplant donor-specific anti-human leukocyte antigen antibodies (DSAs) are known risk factors for acute rejection and reduced graft survival after kidney transplantation. DSAs may also develop de novo DSAs (dnDSAs) after transplantation but the clinical implications of these antibodies remain uncertain.

Methods: We undertook a systematic review of observational studies that examined the association between dnDSAs and graft and patient outcomes (through August 2017) with the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system of reporting used to assess the quality of evidence available.

Results: Thirty-six studies involving 10 535 transplant recipients were eligible. There was moderate quality evidence that transplant recipients who developed dnDSAs had increased risks of acute antibody-mediated rejection (AMR) [relative risk (RR) 9.66; 95% confidence interval (CI) 6.79-13.73, 16 studies, n = 4174]. For all other outcomes, the evidence was low to very low due to moderate-high heterogeneity and low study quality (acute cellular rejection, RR 2.92; 95% CI 2.16-3.94, 22 studies, n = 4991, low-quality evidence; chronic AMR and transplant glomerulopathy RR 6.78; 95% CI 4.31-10.66, 3 studies, n = 1617, very low-quality evidence; and graft loss RR 4.95; 95% CI 3.81-6.43, 19 studies, n = 5473, low-quality evidence). Meta-regression indicated that deceased kidney donation ($R2 = 1.00$, $P < 0.001$) and region of study conduction ($R2 = 0.50$, $P = 0.005$) modified associations between dnDSAs and outcomes.

Conclusions: dnDSAs are associated with increased risks of adverse graft and patient outcomes after kidney transplantation, but estimation uncertainty of the augmented risks exist due to limitations such as heterogeneity within the existing literature. Therapeutic interventions targeted to eliminate or prevent these antibodies evaluated in randomized controlled trials are needed to establish whether dnDSAs are causal to transplantation outcomes.

51. Clinical Utility of Complement Dependent Assays in Kidney Transplantation.

Lan JH, Tinckam K.

Transplantation. 2018 Jan;102(1S Suppl 1):S14-S22. doi: 10.1097/TP.0000000000001819.

ABSTRACT

Formation of antibodies against polymorphic HLA molecules on donor endothelium is central to the pathogenesis of antibody-mediated rejection, the dominant cause of long-term kidney allograft loss. Although introduction of the single-antigen bead assay has greatly facilitated the immune risk assessment of transplant recipients, it is recognized that not all IgG HLA antibodies detected using this method are equally relevant. In recent years, novel assays (C4d, C1q, C3d) have been developed to interrogate the complement-activating potential of anti-HLA antibodies in vitro, with the hypothesis that complement-fixing antibodies are more immediately injurious to the graft compared with noncomplement-binding antibodies. Although initial studies demonstrated the potential of these assays to risk-stratify antibodies beyond the conventional limited metric of mean fluorescence intensity values, new data from recent analyses challenge some of these early findings. In this review, we examine the technical aspects of these assays and key studies that evaluated the discriminant capacity of these tests to predict numerous outcomes in kidney transplantation. We discuss conflicting data and emerging controversies in the context of recent experimental evidence which offer new insights into the major factors that influence complement activation. Finally, we provide our perspective on the current role and utility of complement diagnostic assays as 1 variable in the multifactorial risk assessment and management of kidney transplant recipients.

52. High Intrapatient Variability of Tacrolimus Concentrations Predicts Accelerated Progression of Chronic Histologic Lesions in Renal Recipients.

Vanhove T, Vermeulen T, Annaert P, *et al.*

Am J Transplant. 2016 Oct;16(10):2954-2963. doi: 10.1111/ajt.13803. Epub 2016 Apr 21.

ABSTRACT

High intrapatient variability (IPV) of tacrolimus concentrations is increasingly recognized as a predictor of poor outcome in solid organ recipients. How it relates to evolution of histology has not been explored. We analyzed tacrolimus IPV using the coefficient of variability (CV) from months 6-12 after transplantation in a cohort of 220 renal recipients for whom paired protocol biopsies at 3 mo and 2 years were available. Recipients in the highest CV tertile had an increased risk of moderate to severe fibrosis and tubular atrophy by 2 years compared with the low-IPV tertile (odds ratio [OR] 2.47, 95% confidence interval [CI] 1.09-5.60, p = 0.031; and OR 2.40, 95% CI 1.03-5.60, p = 0.043, respectively). Other predictors were donor age, severity of chronic lesions at 3 mo, and presence of borderline or subclinical rejection at 3 mo. Chronicity score increased significantly more in the high CV tertile group than in the middle and low tertiles (mean increase 1.97 ± 2.03 vs. 1.18 ± 2.44 and 1.12 ± 1.80, respectively; $p < 0.05$). CV did not predict evolution of renal function, which did not deteriorate within the 2-year follow-up period. These results indicate that high IPV is related to accelerated progression of chronic histologic lesions before any evidence of renal dysfunction.

53. Within-Patient Variability in Tacrolimus Blood Levels Predicts Kidney Graft Loss and Donor-Specific Antibody Development.

Rodrigo E, Segundo DS, Fernández-Fresnedo G, *et al.*

Transplantation. 2016 Nov;100(11):2479-2485.

ABSTRACT

Background: Lack of adherence to immunosuppressive drugs is a risk factor for development of de novo donor-specific antibodies (dnDSA) and can contribute to antibody-mediated rejection and graft loss. Moreover, nonadherence is the main determinant of immunosuppressive drug level variability. High intrapatient variability of tacrolimus relates to a worse outcome in transplant recipients through unknown mechanisms. We hypothesized that a high within-patient variability of tacrolimus could increase the rate of dnDSA development and contribute to further death-censored graft loss (DCGL).

Methods: We included 310 adult renal transplants receiving twice-daily tacrolimus throughout their first posttransplant year, with (1) at least 3 blood trough levels available to calculate coefficient of variation (CV) from month 4 to 12, (2) graft survival longer than 1 year, and (3) absence of pretransplant DSA. The dnDSA were analyzed in sera at 1, 3, and 5 years and around 6 month before the last follow-up visit or graft loss by single-antigen beads.

Results: During the follow-up, 53 patients lost their graft excluding death. A total of 116 patients (37.4%) had a CV greater than 30% and 39 (12.6%) developed dnDSA. Coefficient of variation greater than 30% (hazards ratio, 2.613; 95% confidence interval, 1.361-5.016; P = 0.004) independently related to DCGL. Acute rejection, re-transplant and CV greater than 30% (hazards ratio, 2.925; 95% confidence interval, 1.473-5.807; P = 0.002) were the only variables related to dnDSA development by Cox regression analysis.

Conclusions: Tacrolimus level variability is a strong risk factor for dnDSA development and DCGL. Variability must be added to the current monitoring of kidney transplant recipients due to its relationship with adherence and to graft outcome.

54. Immunologic outcome in elderly kidney transplant recipients: is it time for HLA-DR matching?

Halleck F, Khadzhynov D, Liefeldt L, *et al.*

Nephrol Dial Transplant. 2016 Dec;31(12):2143-2149. Epub 2016 Jul 1.

ABSTRACT

Background: The Eurotransplant Senior Program (ESP) neglects HLA matching for elderly (≥65 years) kidney transplant recipients (KTR). Few data regarding the influence of DR matching on clinical and immunologic outcome in elderly KTR exist.

Methods: This retrospective long-term observational study included 244 elderly out of n = 972 adult KTR between 2004 and 2014. Data analysis included patient and graft survival, biopsy-proven rejections [T-cell-mediated rejections (TCMR) and antibody-mediated rejections] and development of de novo donor-specific HLA antibodies (DSA). Outcome data were assessed over a maximum period of 10 years.

Results: Due to the nature of the ESP, elderly KTR showed significantly more HLA mismatches, shorter time on dialysis and shorter cold ischaemia time. Elderly KTR had significantly worse graft and patient survival, and after 7 years, the rate of de novo DSA (33 versus 25%, P = 0.034) and TCMR (39 versus 27%, P < 0.001) was significantly higher compared with younger KTR. Multivariate analysis identified donor age, delayed graft function and HLA-DR mismatches as independent risk factors for TCMR. Within the group of elderly KTR, HLA-DR mismatches were associated with a significantly higher incidence of TCMR and development of de novo DSA. Occurrence of TCMR and de novo DSA in elderly KTR resulted in significantly worse graft survival.

Conclusions: In elderly KTR, HLA-DR mismatches are independent risk factors for TCMR and the development of all classes of de novo DSA, both of which significantly impair graft survival. Introduction of HLA-DR matching in elderly KTR might significantly improve immunologic and overall outcome.

55. Posttransplant peripheral blood donor-specific interferon-γ enzyme-linked immune spot assay differentiates risk of subclinical rejection and de novo donor-specific alloantibodies in kidney transplant recipients.

Crespo E, Cravedi P, Martorell J, *et al.*

Kidney Int. 2017 Jul;92(1):201-213. doi: 10.1016/j.kint.2016.12.024. Epub 2017 Mar 6.

ABSTRACT

Noninvasive diagnosis of kidney allograft inflammation in transplant recipients with stable graft function (subclinical rejection) could permit more effective therapy and prevent later development of de novo anti-donor HLA antibodies and/or graft dysfunction. Here we tested whether quantifying posttransplant donor-specific alloreactive T-cells by IFN-γ ELISPOT assay noninvasively detects subclinical T-cell mediated rejection and/or predicts development of anti-donor HLA antibodies. Using an initial cross-sectional cohort of 60 kidney transplant patients with six-month surveillance biopsies, we found that negative donor-specific IFN-γ ELISPOT assays accurately ruled out the presence of subclinical T-cell mediated rejection. These results were validated using a distinct prospective cohort of 101 patients where donor-specific IFN-γ ELISPOT results at both three- and six-months posttransplant significantly differentiated patients with subclinical T-cell mediated rejection at six months, independent of other clinical variables (odds ratio 0.072, 95% confidence interval 0.008-0.653). The posttransplant donor-specific IFN-γ ELISPOT results independently associated with subsequent development of significant anti-donor HLA antibodies (0.085, 0.008-0.862) and with significantly worse two-year function (estimated glomerular filtration rate) compared to patients with a negative test. Thus, posttransplant immune monitoring by donor-specific IFN-γ ELISPOT can assess risk for developing subclinical T-cell mediated rejection and anti-donor HLA antibodies, potentially limiting the need for surveillance biopsies. Our study provides a guide for individualizing immunosuppression to improve posttransplant outcomes.

56. Decreased chronic cellular and antibody-mediated injury in the kidney following simultaneous liver-kidney transplantation.

Taner T, Heimbach JK, Rosen CB, *et al.*

Kidney Int. 2016 Apr;89(4):909-17. doi: 10.1016/j.kint.2015.10.016. Epub 2016 Jan 23.

ABSTRACT

In simultaneous liver-kidney transplantation (SLK), the liver can protect the kidney from hyperacute rejection and may also decrease acute cellular rejection rates. Whether the liver protects against chronic injury is unknown. To answer this we studied renal allograft surveillance biopsies in 68 consecutive SLK recipients (14 with donor-specific alloantibodies at transplantation [DSA+], 54 with low or no DSA, [DSA-]). These were compared with biopsies of a matched cohort of kidney transplant alone (KTA) recipients (28 DSA+, 108 DSA-). Overall 5-year patient and graft survival was not different: 93.8% and 91.2% in SLK, and 91.9% and 77.1% in KTA. In DSA+ recipients, KTA had a significantly higher incidence of acute antibody-mediated rejection (46.4% vs. 7.1%) and chronic transplant glomerulopathy (53.6% vs. 0%). In DSA- recipients at 5 years, KTA had a significantly higher cumulative incidence of T cell-mediated rejection (clinical plus subclinical, 30.6% vs. 7.4%). By 5 years, DSA+ KTA had a 44% decline in mean GFR while DSA+SLK had stable GFR. In DSA- KTA, the incidence of a combined endpoint of renal allograft loss or over a 50% decline in GFR was significantly higher (20.4% vs. 7.4%). Simultaneously transplanted liver allograft was the most predictive factor for a significantly lower incidence of cellular (odds ratio 0.13, 95% confidence interval 0.06-0.27) and antibody-mediated injury (odds ratio 0.11, confidence interval 0.03-0.32), as well as graft functional decline (odds ratio 0.22, confidence interval 0.06-0.59). Thus, SLK is associated with reduced chronic cellular and antibody-mediated alloimmune injury in the kidney allograft.

57. The prevalence and clinical significance of C1q-binding donor-specific anti-HLA antibodies early and late after kidney transplantation.

Calp-Inal S, Ajaimy M, Melamed ML, *et al.*

Kidney Int. 2016 Jan;89(1):209-16. doi: 10.1038/ki.2015.275. Epub 2016 Jan 4.

ABSTRACT

We aimed to determine the prevalence and clinical significance of complement-binding donor-specific antibodies (DSA) detected up to 30 years after kidney transplantation. Group 1 patients included 284 consecutive DSA negative patients who underwent kidney transplantation after 1 May 2009. Group 2 included 405 patients transplanted before this date and followed at our center with functioning allografts. DSA were tested using Luminex Single Antigen and the C1q assay. In Group 1 patients, who were monitored prospectively, 31 (11%) developed de novo DSA during a median follow-up of 2.5 (1.9, 3.6) years. Of these, 11 (4%) had C1q+ and 20 (7%) had C1q negative DSA. In Group 2 patients, 77 (19%) displayed DSA. Among these, 33 (8%) had C1q+ and 44 (11%) had C1q negative DSA. The incidence of acute antibody-mediated rejection (AMR) was significantly higher in C1q+DSA patients in both Group 1 (45%) and Group 2 (15%) compared with C1q negative DSA (5% and 2%) and DSA negative patients (1% and 3%; $P < 0.001$ and $P = 0.001$). The incidence of chronic AMR was 36% (Group 1) and 51% (Group 2) in patients with C1q+DSA. In contrast, chronic AMR occurred in 5% and 25% of C1q negative DSA, and 2% and 6% of DSA negative Group 1 and 2 patients, respectively ($P < 0.001$). Although the graft survival was lower in Group 1 C1q+DSA patients (73%) compared with C1q negative DSA (95%) and DSA negative (94%) patients, the difference was not statistically significant by Kaplan-Meier survival analysis ($P = 0.21$). Our results indicated that the presence of C1q+ DSA was associated with acute and chronic AMR.

58. A report of the epidemiology of de novo donor-specific anti-HLA antibodies (DSA) in "low-risk" renal transplant recipients.

Rebellato LM1, Everly MJ, Haisch CE, *et al.*

Clin Transpl. 2011:337-40.

ABSTRACT

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

59. Fibrosis progression according to epithelialmesenchymal transition profile: a randomized trial of everolimus versus CsA.

Rostaing L, Hertig A, Albano L, *et al.*

Am J Transplant. 2015 May;15(5):1303-12. doi: 10.1111/ajt.13132. Epub 2015 Mar 23.

ABSTRACT

Markers of epithelial-mesenchymal transition (EMT) may identify patients at high risk of graft fibrogenesis who could benefit from early calcineurin inhibitor (CNI) withdrawal. In a randomized, open-label, 12-month trial, de novo kidney transplant patients received cyclosporine, enteric-coated mycophenolate sodium (EC-MPS) and steroids to month 3. Patients were stratified as EMT+ or EMTbased on month 3 biopsy, then randomized to start everolimus with half-dose EC-MPS (720 mg/day) and cyclosporine withdrawal (CNI-free) or continue cyclosporine with standard EC-MPS (CNI). The primary endpoint was progression of graft fibrosis (interstitial fibrosis/tubular atrophy [IF/TA] grade increase ≥1 between months 3-12) in EMT+ patients. 194 patients were randomized (96 CNI-free, 98 CNI); 153 (69 CNI-free, 84 CNI) were included in histological analyses. Fibrosis progression occurred in 46.2% (12/26) CNI-free EMT+ patients versus 51.6% (16/31) CNI EMT+ patients ($p = 0.68$). Biopsyproven acute rejection (BPAR, including subclinical events) occurred in 25.0% and 5.1% of CNI-free and CNI patients, respectively ($p < 0.001$). In conclusion, early CNI withdrawal with everolimus initiation does not prevent interstitial fibrosis. Using this CNI-free protocol, in which everolimus exposure was relatively low and administered with half-dose EC-MPS, CNI-free patients were overwhelmingly under-immunosuppressed and experienced an increased risk of BPAR.

60. Combining sensitive cross-match assays with donor/recipient HLA Eplet matching predicts Living-donor Kidney Transplant outcome.

Meneghini M, Melilli E, Martorell J, *et al.*

Kidney Int Rep (2018) 3, 926–938.

ABSTRACT

Introduction: Despite the different assays available for immune-risk stratification before living-donor kidney transplantation (LDKT), the precise type and number of tests to perform remain uncertain.

Methods: We retrospectively analyzed in a cohort of 330 consecutive LDKT, all CDC-crossmatch negative, the impact on main clinical outcomes of most sensitive immunoassays [CDC-PRA, flow-cytometry cross-match (FC-XM), donor-specific antibodies (DSA) and their complement-binding capacity (DSAC3d)], together with donor/recipient HLA-eplet matching. Mean follow-up was 67 months (range: 24-190).

Results: Out of 330 patients, 35 (11%) showed a CDC-PRA>20%; 17 (5%) FC-XM+; 30 (9%) DSA+, 18(5%) DSAC3d+, with low overlapping results (10 patients positive in all donor-specific tests). Unlike HLA allele compatibility, mean number of HLAclass-II eplet-mismatches was higher within LDKT with positive baseline tests.

DSAC3d+ showed higher MFI-DSA, with a cut-off MFI of 6192 accurately predicting complement fixation (AUC=0.85, p=0.008). While all assays associated with acute rejection (AR), only DSAC3d+ (OR=6.64, p=0.038) or high MFI-DSA (OR=7.54, p=0.038) independently predicted AR. Likewise, poorly HLA-class-II eplet matched patients were at higher risk of AR, particularly in patients with negative baseline tests (OR=1.14, p=0.019). Finally, previous AR and FCXM+/DSA+, regardless C3d positivity, independently predicted graft loss.

Conclusion: Combining FC-XM and solid-phase assays with the evaluation of donor/recipient HLA-eplet mismatches, are most accurate tools for immune-risk stratification prior LDKT.

61. Longitudinal profile of circulating T follicular helper lymphocytes parallels anti-HLA sensitization in renal transplant recipients.

Cano-Romero FL, Laguna Goya R, Utrero-Rico A, *et al.*

Am J Transplant. 2018 Jun 27. doi: 10.1111/ajt.14987.

ABSTRACT

Antibody-mediated rejection is responsible for 30%-50% of renal graft failures. Differentiation of B cells into antibody-producing plasmablasts depends on the collaboration of follicular helper T cells (Tfh). We analyzed circulating Tfh (cTfh) in kidney recipients and studied cTfh relationship with anti-HLA antibody production and graft outcome. cTfh were longitudinally analyzed in a prospective cohort of patients (n = 206), pre- and posttransplantation. Clinical data, HLA sensitization, and cTfh function were recorded. Both pretransplant and 6-month posttransplant cTfh were able to derive IgG-producing plasmablasts. Pretransplant cTfh was decreased in patients, especially in those who received dialysis. However, these cells were increased in patients with previous allograft or transfusions and in HLA-sensitized recipients. After transplantation cTfh expanded, significantly more in patients who developed de novo anti-HLA antibodies than in patients who remained unsensitized. Augmented pretransplant cTfh positively correlated with higher intensity of pretransplant anti-HLA class I and with de novo anti-HLA class I and anti-HLA class II antibodies. Consistently, pretransplantation cTfh were higher in patients who experienced acute rejection (HR = 1.14 [1.04-1.25]). Thus, we show a role for Tfh in anti-HLA sensitization and rejection. Multicenter studies with additional patient cohorts are needed to validate these results. Immunosuppressive drugs targeting Tfh could be useful to improve outcomes.

62. Value of Monitoring Circulating Donor-Reactive Memory B Cells to Characterize Antibody-Mediated Rejection after Kidney Transplantation.

Luque S, Lúcia M, Melilli E, *et al.*

Am J Transplant. 2018 Aug 7.

ABSTRACT

Antibody-mediated rejection (ABMR) is defined by specific histopathological lesions and evidence of circulating donor-specific antibodies (DSA). While DSA are not always detectable, monitoring donor-reactive memory B-cells (mBC) could recognize at-risk patients of developing ABMR. Peripheral donor-reactive mBC using a novel HLA B-cell ELISpot assay, serum DSA and numbers of different B-cell subsets were assessed in 175 consecutive kidney transplants undergoing either for-cause or 6 and 24-month surveillance biopsies for their association with main histological lesions of ABMR and impact on allograft outcome. In 85 incident for-cause biopsies, high frequencies of donor-reactive mBC were detected in all 16(100%) acute ABMR/DSA+ and most chronic ABMR, with or without DSA (24/30[80%] and 21/29[72.4%], respectively). In a longitudinal cohort of 90 non-sensitized patients, a progressively higher expansion of donor-reactive mBC than de novo DSA was observed at 6 and 24 months (8.8% vs 7.7% and 15.5% vs 11.1%, respectively) and accurately identified patients with on-going subclinical ABMR (AUC=0.917 and AUC=0.809, respectively). An unsupervised hierarchical cluster analysis revealed a strong association between donor-reactive mBC with main fundamental allograft lesions associated to ABMR and conferred a significant deleterious impact on graft outcome.

63. Anti-HLA Donor-Specific IgG Subclasses and C1q-binding Evolution in Posttransplant Monitoring.

Von Glehn Ponsirenas R, Cazarote HB, de Almeida Araújo S, *et al.*

Transplantation Direct 2018;4: e385; doi: 10.1097/TXD.0000000000000823. Published online 22 August, 2018.

ABSTRACT

Background: The identification of low-level antibodies by single-antigen bead methodology has brought advancements to risk evaluation of kidney transplant recipients. However, the use of mean fluorescence intensity (MFI) to quantify antibodies and to guide therapy is not enough. Notably, immunoglobulin G (IgG) subclass switching is hypothesized to follow a programmed sequence after an emergency signal from the germinal center. In transplantation this process is not clear yet. In the present study, we sequentially evaluate anti-HLA donor specific antibody (DSA) subclasses, their profile changes, and C1q-binding ability and the influence of those characteristics on antibody mediated rejection (AMR) occurrence and allograft function.

Methods: A total of 30 DSA-positive patients were tested for IgG subclass content and C1q-binding in sequential serum samples.

Results: Twenty-one patients were DSA-positive before transplant; patients sensitized only by transfusion or pregnancies had IgG1 and/or IgG3, and patients sensitized by both transfusion and pregnancies or previous transplant showed a broader range of IgG subclasses. C1q binding was detected in high MFI made up of IgG1 or multiple IgG subclasses. Only 4 patients were positive for C1q posttransplantation and 3 of these showed an increase in MFI, changes in subclasses patterns, AMR, and allograft dysfunction.

Conclusions: Posttransplant evaluation of DSA subclasses and the ability to bind C1q may be informative for both AMR occurrence and allograft dysfunction. Monitoring these events may help to better define risk and interventional time points.

64. Donor-derived Cell-free DNA Identifies Antibody-mediated Rejection in Donor Specific Antibody Positive Kidney Transplant Recipients.

Jordan SC, Bunnapradist S, Bromberg JS, *et al.*

Transplantation Direct 2018;4: e379; doi: 10.1097/TXD.0000000000000821. Published online 20 August, 2018.

ABSTRACT

Background: Elevated levels of donor-derived cell-free DNA (dd-cfDNA) in the plasma of renal allograft recipients indicates organ injury and an increased probability of active rejection. Donor-specific antibodies (DSA) to HLA antigens are associated with risk of antibody-mediated rejection (ABMR). This study assessed the combined use of dd-cfDNA and DSA testing to diagnose active ABMR.

Methods: Donor-derived cell-free DNA was assayed in 90 blood samples with paired DSA and clinically indicated biopsies from 87 kidney transplant patients. Sixteen cases met criteria for active ABMR. Performance characteristics of dd-cfDNA for diagnosis of active ABMR were determined for samples with prior or current positive DSA (DSA+, n = 33).

Results: The median level of dd-cfDNA (2.9%) in DSA+ patients with active ABMR was significantly higher than the median level (0.34%) in DSA+ patients without ABMR ($P < 0.001$). The median level of dd-cfDNA in DSA− patients was 0.29%. The positive predictive value of dd-cfDNA (at 1%) to detect active ABMR in DSA+ patients was 81%, whereas the negative predictive value was 83%. The positive predictive value for DSA+ alone was 48%.

Conclusions: The combined use of dd-cfDNA and DSA testingmay improve the noninvasive diagnosis of active ABMR in kidney transplant patients. Patients with dd-cfDNA+/ DSA+ results have a high probability of active ABMR.