Donor Specific Antibody (DSA) Monitoring

Early Detection. Optimal Outcomes.
The Role of anti-HLA Antibodies in Transplantation

There are over 100,000 solid organ transplants performed annually worldwide, 29,000 of which are performed in the United States. Despite significant improvements in post-transplant care, long term graft function is less than optimal. In the United States, adjusted 10 year allograft survival rates for deceased and living donor kidney transplants are only 40.8% and 57.9%, respectively. Late stage graft failure, secondary to antibody mediated rejection (AMR) is a primary cause of poor graft survival.

Historically, anti-Human Leukocyte Antigen (HLA) antibodies were defined as preformed circulating antibodies present in the recipient’s immune system which were the result of a sensitization event pre-transplant (blood transfusion, previous transplant, or pregnancy). In more recent years, the concept of monitoring for the post-transplant development of clinically relevant antibodies directed against donor specific HLA class I and class II mismatches has been a significant area of interest within the transplant community. Whether detected pre- or post-transplant, the presence of antibodies directed against antigens expressed on donor organs, when not treated clinically, results in an immune attack on the transplanted organ, and increases risk of graft loss and/or rejection. DSA attacks the endothelium of the allograft, resulting in subsequent AMR. The progression of DSA development and the corresponding clinical events compound to damage the allograft, resulting in chronic changes over time that ultimately compromise graft function and survival.

(A, B) Acute antibody-mediated injury characterized by glomerular microthrombus, glomerular capillary endothelial cell activation (arrows) (A: Hematoxylin/Eosin, 400×; B: EM, 7500×).
(C, D) Chronic active antibody-mediated rejection showing glomerular endothelial cell swelling and early peritubular capillary basement membrane lamellation (arrows) (C: PAS, 400×; D: EM, 4200×).
(E, F) Chronic antibody-mediated rejection characterized by glomerular basement membrane duplication (arrows) (E: Jones, 600×; F: EM, 7500×).
Large cohort studies of over 5000 transplant recipients indicate that at any given time, approximately 25% of transplant recipients have antibody(s). Moreover, previous data in renal transplantation have shown that up to 96% of rejected allografts develop some level of detectable DSA.

While traditional markers can aid in diagnosing the clinical status of solid organ transplant recipients, they are generally non-specific and most often identifiable only after graft damage has occurred. There is strong evidence that anti-HLA antibodies contribute to the development of chronic renal failure, the leading cause of renal allograft failure. The early identification and subsequent removal of clinically harmful DSA associated with AMR, both pre- and post-transplant, may prevent allograft loss.

One Lambda, Inc. offers highly sensitive and specific solid-phase assays allowing for the accurate identification of DSA both pre- and post-transplant. This platform is currently being used as the standard-of-care diagnostic testing methodology in most transplant centers within the US.
The Spectrum of Antibody Mediated Rejection

Antibody mediated rejection can present as an early acute process, often resulting from an anamnestic response, or as a late and chronic process due to de novo antibody production. In the acute phase, it is often preformed antibodies that cause early rejection. De novo DSA can also develop in the early post-transplant period, resulting in acute rejection. Patients with preformed DSA are at significantly greater risk of having an acute AMR and have significantly lower graft survival. Chronic rejection is one of the leading causes of death-censored graft loss. Often described as a smoldering process, repeat cycles of alloantibody-mediated injury and repair result in distinct changes in the microvasculature of the allograft. Patients with preformed DSA and those who develop de novo DSA are at an increased risk of having chronic rejection.

Sub-Clinical Antibody Mediated Rejection

DSA may also develop without an immediate impairment of graft function. This type of rejection, or subclinical antibody mediated rejection (SAMR), has been reported to have an impact on long-term graft dysfunction and deterioration, instead of having an immediate impact. Loupy et al. demonstrated that the acute lesions were largely unabated, from 3 months to 3 years, whereas, the chronic lesions increased steadily and significantly over a 3 year period. Microcirculation inflammation is more common in late kidney biopsies after one year post-transplant, reflecting the high frequency of de novo DSA. SAMR is also a frequent finding in patients with preformed DSA, some of which the C4d stain is negative for SAMR. Typically, non-adherence to immunosuppressive therapy is associated with the aforementioned pathological findings.

De Novo DSA in Post-Transplant Solid Organ Transplant Recipients

The development of de novo donor-specific HLA antibodies (dnDSA) post-transplantation has been associated with higher graft failure rates. The range of dnDSA is 24% to 62% with the highest presence in post liver transplant recipients.
Proposed model for patients developing de novo donor-specific antibodies as they evolve from transplantation to graft failure. IFTA, interstitial fibrosis and tubular atrophy; TG, transplant glomerulopathy. Adapted from reference.20

DE NOVO RESPONSE IN CARDIAC ALLOGRAFTS21

- Development of de novo antibodies during the first year post-transplant has been shown to result in 15-year graft survival at 52%.
- Development after 1 year post-transplant results in worse outcomes, with 15 year graft survival at 40%.
- Strong association between Grade 3, worse ACR and subsequent development of de novo antibodies after 1 year has been observed.

De Novo Antibody Production34

Chronic rejection in patients (n=14) who did not have preformed HLA antibodies prior to transplantation (Ab-) and who were examined for antibodies at 6-month intervals. All the patients developed antibodies (Ab+) (red bars). Antibodies persisted for a period of 6 months to 8 years before the kidney was rejected.20

Several prospective and retrospective studies have demonstrated that de novo DSA development in renal transplant patients, predominately directed at Class II donor HLA mismatches, is associated with poor outcomes, and is often detectable well before graft failure.13

As clinicians gain a more thorough understanding of the role of post-transplant de novo DSA development, it has become clear that this phenomenon has been misunderstood or underestimated in the past, likely due to its delayed clinical manifestation post-transplant.14

De novo antibody production may cause sub-clinical AMR that is associated with long-term graft dysfunction.

• Negative outcomes can take several years to occur after de novo antibody production.
**DSA Monitoring**

**DSA is Predictive of Lower Graft Survival**

Over time, the risk of developing chronic rejection leading to AMR increases with the presence of anti-HLA-Class II antibodies. Several studies have demonstrated that the presence of HLA antibodies predicts kidney graft loss.

**Effect of DSA on Renal Allograft Survival**

(A) The graft survival of patients with *de novo* donor-specific antibodies (dnDSA) versus those without.

(B) The graft survival of pre-transplant human leukocyte antigen (HLA) antibodies, post-transplant *de novo* HLA antibodies, or no antibodies compared to patients with dnDSA.

**The Effect of Transplant Glomerulopathy (TG) on Renal Allograft Survival**

Risk of developing TG according to anti-HLA-II levels. The patients groups include: no anti-HLA-II antibodies in pre-transplant sera; anti-HLA-II antibodies and NV>2000 (n=40); NV 2001-10,000 (n=42); and NV>10,000 (n=39).

Graft Survival in patients: without TG (n=530), patients with C4d-negative TG (n=52), and patients with C4d positive TG (n=16).
DSA is Predictive of Lower Patient Survival

In addition to rejection and graft loss, a significant association between the presence of anti-HLA antibody and patient survival has been demonstrated. A recent study by Dunn et al. found that actuarial survival was significantly decreased at 5-years post-transplant in DSA positive patients compared to DSA negative patients (p=0.0006, HR = 10.0).  

Smith et al. observed that persistent, de novo DSA was an independent predictor of poor patient survival (p < 0.0001, HR = 4.763).

Liver Transplantation

Early AMR in liver transplants is often difficult to identify as an immunological event. This is due to the lack of clear clinical, histological, and immunohistological criteria for diagnosing AMR. Other factors that may contribute to misdiagnosing AMR include the lack of crossmatch results at the time of organ implant, positive crossmatch results at the time of transplant, and the nonspecific clinical and pathological findings of cholestasis that are similar to AMR. The recipients that maintain a positive crossmatch after transplantation are at high risk of AMR. Recent data suggest that the diagnosis of AMR in liver transplant recipients may be based on the presence of DSA in the recipient serum.

O’Leary et al. demonstrated for the first time the association of DSA and chronic rejection after liver transplantation. This group evaluated the total MFI, (Mean Fluorescence Intensity, defined as the sum of distinct DSA), and determined that preformed class I antibodies were more detrimental to graft survival than class II antibodies. The presence of de novo DSA within the first year of liver transplants was more common in patients that presented with a chronic rejection; 92% had detectable DSA before chronic-rejection-induced graft failure occurred.
DSA Is Associated With Bile Duct Injury in C4d+ Liver Transplant Recipients\textsuperscript{15}

In liver transplant patients, DSA is associated with negative outcomes, indicating that AMR may contribute to interlobular bile duct injury and loss.

- 40% of patients had significant circulating DSA associated with diffuse portal C4d deposition.
- 70% of patients with ductopenia had DSA.
- 60% of ductopenia cases had DSA associated with diffuse portal C4d.
- 53.6% of ACR (acute cellular rejection) cases had evidence of concurrent humoral alloreactivity based on detection of circulating DSA with diffuse portal C4d.
- DSA+/diffuse C4d+ patients had a significantly higher frequency of ACR (88% vs. 50%), \( p=0.02 \).
- DSA+/diffuse C4d+ patients had a significantly higher frequency of steroid resistant rejection (41% vs. 19%), \( p=0.03 \).

Pancreas Transplantation

Recent studies in pancreas allograft recipients suggest that the development of post-transplant DSA is significantly associated with worse outcomes, including increased incidence of rejection and decreased graft survival.

Cantarovich et al. reported that long-term pancreas graft survival is inferior to survival of other solid organ transplants because the diagnosis of pancreas graft rejection is biologically and clinically complex. However, identifying markers such as post-transplant DSA may be utilized as an independent risk factor for graft survival.\textsuperscript{25}
Islet Cell and Multi-Visceral Transplantation

Current data in pancreas and islet cell transplantation suggest that the presence of pre-transplant DSA is associated with poor outcomes after transplant. Multi-visceral transplantation (MVT) is the concurrent transplantation of the small bowel and liver with 1 or more of the following organs: stomach, pancreaticoduodenal complex, jejunum, ileum and/or colon. Rejection after small bowel and MVT transplant is a serious complication affecting both patient and graft survival. The presence of DSA indicates that the patient is at a higher risk of rejection. Early diagnosis of acute rejection in small bowel transplant is essential to control the rejection process.

The role of DSA monitoring in pancreas, multi-visceral and small bowel transplants is a valuable diagnostic tool in early diagnosis and treatment of acute rejection.

(A) Distribution of acute rejection (AR) in small bowel allograft biopsies. In this study, total 291 biopsy samples were obtained and the distribution was: 80 no rejection (27.79%), 148 grade indeterminate (50.86%), 44 mild grade (15.12%), 2 moderate grade (0.69%), and 17 severe grade (5.84%).

(B) Distribution of rejection severity in biopsy samples showing AR with and without donor-specific antibody (DSA). In presence of DSA, percentage of severe rejection is significantly higher (43.33 vs. 12.12%, P=0.009)
**Lung Transplantation**

**Patient Survival in Lung Transplant BOS-Free Cumulative Survival and Antibody Presence in Lung Transplantation**

Bronchiolitis Obliterans Syndrome (BOS) is a complication in lung transplantation. Due to the high prevalence of BOS and the poor prognosis and shorter graft survivals in patients with BOS, it is imperative to detect DSA sooner.

Anti-HLA antibodies are associated with a higher prevalence of BOS than absence of anti-HLA antibodies – 71% vs. 24%.

In one study, the detection of anti-HLA antibodies preceded diagnosis of BOS by approximately 1.1 years.  

![Graph showing BOS-free cumulative survival](image)

BOS-free cumulative survival was significantly lower in patients with HLA-specific antibodies (Ab) versus no HLA-specific antibodies (No Ab).

**Cardiac Transplantation**

Cardiac allograft vasculopathy (CAV) is the primary cause of death and re-transplant in long-term heart transplant survivors.

Circulating antibody can result in complement activation and fixation on the graft endothelium, thereby predisposing the patient to graft loss, accelerated CAV, and death.

In a study reported by Kazmerek et al., freedom from CAV in DSA(+) patients was 94.4%, 81.0%, 41.2% and 10.3% at 1, 5, 10 and 15 years post-transplant compared with 96.2%, 83.4%, 67.3% and 34.7% in DSA(-) patients.  

![Graph showing freedom from CAV](image)

Freedom from CAV: Patients +/- DSA
The Future of DSA Monitoring: Effect of C1q DSA on Allograft Survival

The detection of anti-HLA antibody in transplant recipients allows the clinician to better predict AMR in the post-transplant patient. Since not all DSAs are complement fixing, and may not be clinically relevant to organ rejection, it is imperative to identify those that are complement fixing in order to treat transplant patients properly. A novel C1q assay designed to detect immunoglobulin G (IgG) antibodies capable of fixing complement has been studied in the post-transplant setting in combination with existing methodologies utilized to define a patient’s immunologic profile. In a recent study of pediatric heart transplant recipients, all patients with C1q (+) DSA after transplant had AMR at the next biopsy, suggesting DSA monitoring is effective in predicting AMR.

The role of complement fixation, as detected by C4d staining, in predicting AMR is not as widely accepted now as it had been in the past for the post-transplant monitoring of solid organ transplant recipients. Additionally, the proper identification of recipient HLA antibody specificity is critical in the assessment of those antibodies that can activate the classical complement pathway. Research has suggested that the evaluation of the C1q assay provides a further layer of immunologic antibody information that may prove to be an adjunct in optimizing post-transplant care.

In a study reported by Yabu et al., kidney transplant recipients with C1q (+) DSA were nearly 6 times more likely to lose their grafts than those with C1q (-) DSA.

Chin et al. found that heart transplant patients with C1q (+) DSA after transplant had AMR at the next biopsy, demonstrating that complement binding DSA is predictive of AMR.

The presence of C1q (+) DSA was associated with a significantly greater incidence of acute allograft rejection, and patients with C1q (+) DSA were significantly more likely to have C4d deposition found on biopsy. The C1q assay preferentially detects antibodies that have the capacity to fix and activate complement.
Studies have shown that when DSA is identified early through routine monitoring, the initiation of anti-humoral therapy may begin sooner, allowing for DSA to clear and improving overall survival rates.

Antibody-Directed Therapy After Lung Transplantation

Survival was significantly worse in recipients who had persistent DSA than in those who had cleared the DSA.

Reduction of Donor Specific Antibody Levels in AMR Prevents Renal Allograft Loss

Death-censored allograft survival stratified by percent reduction in immunodominant DSA at 14 days post-biopsy

Renal allograft survival stratified on ability to yield antibody reduction
Why Monitor With Single-Antigen Beads?

Luminex®-based single-antigen beads (SAB) allow for a precise, highly sensitive determination of a patient's antibody profile. This makes discrimination between donor-specific and non-donor-specific antibodies possible.

The use of Luminex® SABs as part of a comprehensive monitoring program provides a number of advantages, including:

- The presence of low-level DSA can put patients at risk for rejection
- Changes in DSA or the appearance of new DSA specificities may reveal an early rejection process

Why Monitor DSA Post-Transplant?

- Ten-year renal allograft survival in early antibody developers (<1 year) was 27% vs. 80% in the late antibody developers.33
- More than 40% of kidney transplant recipients with AMR developed transplant glomerulopathy.34
- Solid organ transplant recipients diagnosed with transplant glomerulopathy experienced 5-year graft survival rates less than 50% from the time of diagnosis.35
- 15-year graft survival in early antibody developers (<1 year) was 52% vs. 40% in late developers.20
- De novo DSA production in heart transplant recipients was strongly associated with decreased patient survival (HR = 3.198) and worse for those with persistent DSA (HR = 4.351).17
- Many post-transplant solid organ transplant recipients developed de novo DSA secondary to non-adherence to immunosuppressive therapy, particularly in the pediatric population.10
- Insufficient immunosuppression may contribute to the development of de novo DSA and AMR secondary to misclassifications based on C4d negativity.29
Optimizing DSA Monitoring Strategies

While implementation of routine post-transplant monitoring becomes increasingly recognized as a standard-of-care practice, the frequency of testing is highly variable. The frequency of post-transplant monitoring will be patient-specific. Choosing a monitoring frequency based on a patient’s individual risk of developing AMR post-transplant will be the most efficient and clinically relevant strategy.

Serial monitoring of DSA is more valuable than single-point testing, particularly in the post-transplant setting, and is crucial in optimizing patient outcomes. Kimball et al. performed quarterly monitoring of patients for 3 years post-transplant and reported that 65% of patients eliminated antibody within the first year of transplant due to early detection and subsequent treatment. In those patients that exhibited pre-transplant DSA against Class I and II HLA, antibody levels declined by 90% at 6 months and were undetectable at 1 year with effective treatment.

Conclusion

Since the first solid organ transplant in 1954, the field of transplantation has witnessed several milestones that have significantly modified the solid organ transplant recipient treatment paradigm and positively impacted both graft and patient survival. Surgical innovation and advancement has been complemented by the introduction and optimization of novel immunosuppressive and infectious disease regimens. For immunologic monitoring, cytotoxic assays have given way to solid-phase methodologies with higher levels of sensitivity and specificity. Having evolved over time, emerging technologies are now available for the detection of the presence of anti-HLA antibodies, and more importantly, the identification of specific anti-HLA DSA.

Historically, the immunologic profile obtained from the assays conducted in the histocompatibility laboratory was utilized strictly in the pre-transplant setting, as a means to minimize or avoid post-transplant rejection. More recently, data has been reported, indicating that the identification and subsequent treatment of DSA in the post-transplant setting, for all solid organ types, may be an important consideration in the long-term treatment of the transplant patient. Reported data demonstrate the potential benefit of routine DSA monitoring in post-transplant patient outcomes. The routine monitoring of DSA in the post-transplant setting offers promise in long term graft and patient survival.
For additional information on DSA monitoring please visit www.onelamba.com
References

References
