A Role of Complement Fixing DSA in Allograft Rejection – Evaluation of the Immucor LIFECODES® C3d Detection Assay

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Introduction

The presence of donor specific antibodies (DSA) is a major cause of allograft rejections[1, 2]. Although use of single antigen beads (SAB) on the Luminex platform allows better assessment of DSA in pre and post-transplant patients, not all DSAs identified using SAB cause allograft rejections or poor transplant outcomes[3]. The expanding body of evidence suggests a role of complement fixing DSA, as opposed to non-complement fixing DSA in allograft rejection[3-9].

There are two commercially available assays to detect complement fixing antibodies: the Immucor LIFECODES C3d Detection kit which detects complement component C3d, and the One Lambda C1q Screen Kit which detects complement component C1q. Both assays detect one of the complement components; however, there are fundamental differences between the two assays. One Lambda's C1q assay uses a recombinant C1q and detects C1q binding capacity of antibody bound to SAB. This assay measures the ability of antibody to bind to recombinant C1q rather than measuring the complement activation. Immucor's C3d assay uses sera as a source of complement; thus resulting in a natural complement component rather than recombinant complement. The C3d assay measures a physiologic complement activation that occurs when C1q binding triggers complement cascade.

This white paper highlights the recent findings and clinical relevance of C3d binding DSA in kidney, lung and heart transplant patients and the risk of antibody mediated rejection (AMR). Publications have indicated that:

- C3d-binding DSAs are better predictor of allograft rejection risk than C1q-binding DSAs
- C3d-binding DSAs are a strong risk factor for allograft loss
- The Immucor LIFECODES C3d Detection kit may add value in stratifying risk of allograft rejection

C3d-binding DSAs are a better predictor of allograft rejection risk than C1q-binding DSAs:

In 2013, Loupy et al. demonstrated a correlation between C1q binding DSA and risk of renal allograft loss during the first year after transplantation[10]. To confirm this finding, Sicard et al. evaluated the value of complement activation assays to stratify the risk of allograft loss using three methods: detection of C4d deposition in biopsied tissue, detection of C3d binding DSA using the Immucor LIFECODES C3d Detection kit and detection of C1q binding DSA using the One Lambda C1qScreen Kit[3]. Analysis of kidney graft survival at the time of rejection indicated that the presence of significant C4d deposition was not associated with higher risk of graft loss ($p = 0.33$) whereas the presence of C3d binding DSA at the time of rejection indicated a strong correlation with higher risk of graft loss ($p=0.0003$). Contrary to Loupy et al's findings, although patients with C1q positive DSA showed higher risk of graft loss, there was no statistical significance on the risk of allograft loss between C1q negative DSA and C1q positive DSA ($p=0.06$)[3].

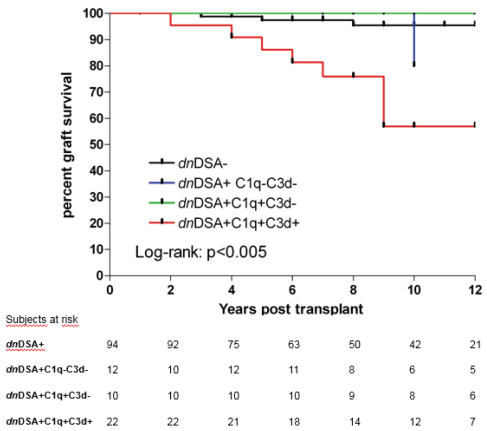

Figure 1: De novo donor-specific antibodies (dnDSAs) and risk of developing graft loss according to complement binding in 138 non sensitized pediatric kidney recipients. *The figure provided by courtesy of Dr. A. Nocera and Dr. F. Ginevri.*

A similar study was conducted by Comoli et al. in 2016. This study compared the outcomes of the two assays (the Immucor LIFECODES C3d Detection kit and the One Lambda C1q Screen Kit) for their predicative power on AMR development and graft loss in 114 pediatric non-sensitized recipients of first kidney allografts[4]. They observed overall poor survival of allograft in dnDSAs that bind to C3d and C1q. In addition, the results indicated that an addition of C3d assay stratified patients at risk of AMR more accurately and clearly discriminated patients at lower or higher risk for graft loss compared with C1q assay alone (Figure 1).

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In 2017, Kim et al. investigated the importance of testing complement binding capability of dnDSA to stratify patients with high risk of long term renal allograft rejection using the pediatric renal transplant recipient's cohort. They measured declining eGFR value, which indicates declining kidney function, between C1q+ and C1q- patients as well as C3d+ and C3d- patients. There was no difference between C1q+ and C1q- patients in declining eGFR value whereas C3d+ patients had a significantly faster decline of eGFR value compare to C3d- patients. In addition, C3d+ patients showed higher proportion of C4d deposition on biopsy ($p<0.05$).

Most recently, Cioni et al. investigated the efficacy of anti-humoral treatment for late AMR in unsensitized pediatric kidney recipients developing dnDSA[14]. They analyzed the role of C1q and C3d binding property of dnDSA on responsiveness to antibody removal treatment. In multivariable analysis, they observed that MFI > 10,000 and C3d-binding ability of dnDSA were independently associated with resistance to antibody removal ($p<0.05$) whereas C1q binding ability was not ($p=0.71$).

These studies indicated that the C3d assay is a better tool to stratify risk of graft loss after kidney transplant than the C1q assay[3,4,6,14].

C3d-binding DSA is a strong risk factor for allograft loss:

In 2018, Roux et al. evaluated correlation between complement activation by DSA and poor graft outcomes to indicate AMR diagnosis using Immucor's C3d assay in well characterized historic lung transplant cohorts[8]. The results revealed that DSA+ AMR- patients never had a strong C3d activation while DSA+ AMR+ patients had over 100 fold increase in C3d activation ($p<0.0001$). This indicated that DSA at the time of AMR has increased ability to activate complement pathway. Assessment of graft survival in respect to the C3d and IgG MFI cutoff also showed a strong correlation between positive cut off and poor graft survival[8].

In 2018, Zhang et al. demonstrated that the development of "newly detected DSA" (ndDSA), DSA detected post-transplant whether it is dnDSA or DSA developed in response to the previous exposure, was strongly associated with AMR and clinical significance of complement activating DSA in heart transplant patients[9]. Assessment of C3d and DSA characteristics as well as AMR status revealed that DSA+/C3d- patients had a 2.8 fold higher risk of developing AMR compared to DSA- patients. For patients with DSA+/C3d+, the risk of AMR increased 33 times compared with DSA- patients. Correlation between C3d positivity and C4d deposition in a graft exhibited a strong association in AMR+ biopsies suggests that C3d positive DSA is more pathogenic than C3d negative DSA[9]. In addition, five patients with C3d positive DSA with medium MFI (MFI <10,000) displayed graft rejection suggesting that the C3d assay may be a useful tool to identify patients at risk of AMR for DSA at low to medium MFI.

The Immucor LIFECODES C3d Detection kit may add value in stratifying risk of allograft rejection:

In 2017, Pelletier et al. analyzed clinical presentation and outcomes in 265 patients' sera with post-transplant DSA[5]. They stratified DSA class specificity, DSA IgG MFI, patient characteristics and clinical outcome as well as risk of renal allograft loss according to C3d binding capability. C3d binding was generally associated with high MFI for both class I and class II; however, five year renal allograft survival was still significantly lower with C3d binding DSA ($p<0.05$) with lower MFIs (< 7000). C4d deposition and vascular inflammation also showed a statistically higher percentage of high severity in C3d binding DSA. Based on these results, they concluded that the information obtained from the C3d assay may be useful to stratify patients' treatment options as well as recipient counseling regarding renal graft survival prognosis[5].

In 2018, Lan et al. evaluated the utility of the C3d assay to predict renal graft loss by comparing C3d binding capability of DSA[7]. Results indicated C3d positive patients exhibited poor graft function, inferior graft survival, and faster rate of graft loss after biopsy and higher C4d deposition compare to C3d negative patients. A strong correlation between DSA with high MFI and C3d binding was observed; however, analysis of a subset of recipients with different peak IgG MFI (< 7000 and < 5000) and sum MFI (< 20,000 and < 10,000) confirmed consistent inferior outcomes associated with C3d regardless of MFI values. Based on this study, they concluded that the ability of the C3d assay to discriminate DSA with low to moderate strength may add the utilization of this assay to patients where an allograft biopsy is not available [7].

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Discussion:

Correlation between the presence of post-transplant C3d binding DSA in patients with kidney, lung and heart transplant and higher risk of allograft rejection has been reported in recent publications[3-14]. It has been demonstrated that the presence of DSA is highly associated with allograft rejection, but not all DSA were shown to cause allograft rejection and an additional prognostic assay to assess individual risk was in high demand[3].

There are currently two commercial kits available for the detection of complement binding DSA: Immucor's LIFECODES C3d Detection kit which detects the complement components C3d, and One Lambda's C1q Screen kit from which detects C1q. Both assays detect one of the complement components; however, there is a fundamental difference between two assays. This white paper highlighted multiple studies that demonstrated the added prognostic values of Immucor's C3d assay for risk stratification of allograft rejection compare to One Lambda's C1q assay[3-6].

In addition to risk stratification of allograft rejection, another utility of the C3d binding assay may be the identification of patients who would benefit from immunosuppressive treatment using complement inhibitors such as Eculizumab[12, 13]. Though effective, due to very high cost of the treatment and adverse effects of the complement inhibitors, this treatment is not suitable for every patient diagnosed with AMR.

Additional clinical testing using the C3d assay is necessary to further prove clinical utility of C3d assay in stratifying the patients with high risk of allograft loss.

LIFECODES C3d Detection is for Research Use Only in the US and Canada.

LIFECODES C3d Detection is CE marked for In vitro diagnostic use.

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