Review

Defining Clinically Pathogenic HLA-Specific Antibodies - Granular Details in Characteristics in Pre and Early Time Following HLA-Antibody Incompatible Kidney Transplantation

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Abstract

Antibodies against donor HLA determine access to solid organ transplantation and in many cases the outcome of transplantation, but graft failure is not an inevitable consequence of their presence. Much research has been performed with two main aims – which antibodies represent the highest risk factor prior to transplantation, and second to understand how donor specific HLA antibodies behave after transplantation, with a long-term aim of being able to manipulate their production. HLA antibody incompatible kidney transplantation is the best model for examining antibody responses and this review looks at methods for interrogating the antibodies using 'traditional' snapshot techniques such as cytoxicity testing, and newer dissection techniques such as antibody subclass, complement binding and activity and affinity. Integral to the understanding of the large datasets generated is sophisticated mathematical analysis using techniques such as decision tree analysis and
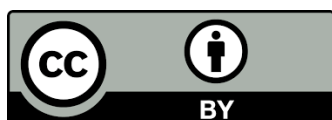
unsupervised machine learning. This review examines key aspects of this work, performed by us and others.

1. Introduction

Kidney transplantation is the optimal treatment for patients with end stage kidney disease but immunological barriers, especially HLA antibodies, are important factors limiting timely accessibility to transplantation. Assays detecting and defining HLA antibodies have evolved over the last few decades. This has allowed us to better understand humoral responses but has posed a major challenge in defining threshold at which we can safely transplant, as we are now detecting antibodies at very low levels with increasing sensitivity and specificity. This has redrawn the risk stratification in decision making but also increased complexities. Additionally, over the last two decades, transplantation across blood group and HLA antibody incompatibilities have been performed successfully in different parts of the world [1-7] using varied desensitisation therapies [8]. This is partly due to improvements in diagnostic techniques such as HLA tissue typing, identification of HLA-specific antibodies, crossmatch techniques [9] and advances in therapeutic approaches such as adsorption columns [10], removal of antibodies by plasmapheresis [11, 12], and newer biological therapies. Data from transplanted series tend to stratify results based on positive response in current assays, but none of the assays can accurately predict long-term clinical outcomes irrespective of desensitisation protocol utilised. Hence efforts have been made to characterise and analyse other properties of antibodies and the underlying regulatory mechanisms. This review summarises current literatures surrounding HLA-specific antibodies and outcome of HLA-incompatible kidney transplantation across the range of immunological risks.

2. Snapshot: Current Assays Used in Laboratories and Relation to Clinical Outcome

Three main assays are widely used to risk stratify donor specific antibodies (DSA) risks prior to HLA-incompatible kidney transplantation. Two of these are cellular assays (complement dependent cytotoxicity and flow cytometry), and the third one is solid phase luminex assay using microbeads coated with HLA proteins. The relationship between the three assays is shown in Figure 1. As the assays measure a different aspect of antibody binding, there is incomplete concordance between the results obtained. Microbead (luminex) assay is highly sensitive and is specific for HLA proteins. Low levels of HLA-specific antibodies are detected that may not give a positive signal on flow cytometry or CDC. Both flow cytometry and CDC assays use lymphocytes to study the binding of antibodies, but the principle differs from each other such that one studies complement fixing antibodies whereas other tests both complement fixing and non-complement fixing antibodies. Anti-human globulin (AHG) augmented CDC can detect non-complement fixing antibodies too, thus more sensitive compared to non-augmented CDC assay. However, the value

of a more sensitive CDC assay might be re-evaluated given the availability of flow cytometry and luminex bead assay and recent long term clinical outcome data.
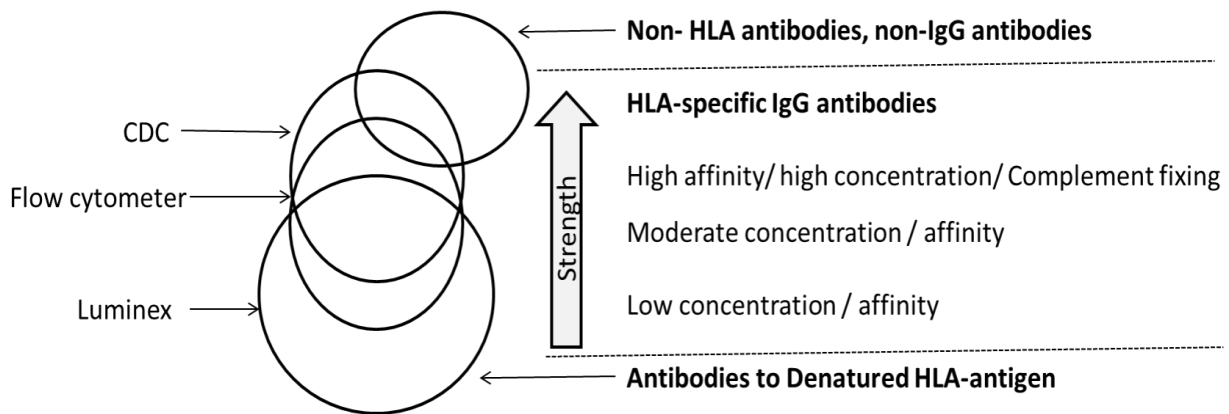

Figure 1 Overlap of current assays used in determining HLA-specific antibodies.

It has generally agreed that selective responses of patient sera with above three assays can quantify strength of antibodies, with positive results for all three assays the most powerful and luminex assay alone positive at milder end of the spectrum. The microbead assay is also very important in defining positive CDC results that are not due to HLA antibodies and may not be clinically significant. Analysis of outcome from 114 HLA-incompatible kidney transplantation at our centre showed early antibody mediated rejection (within 30 days post transplantation) was higher in CDC (55%; N = 12/22) followed by flow positive group (50%; N=28/57) and least in luminex bead positive alone (23%; N=8/35). This was statistically significant (Figure 2A). The crossmatch status also influenced treatment success following an episode of early acute antibody mediated rejection (AAMR). At our centre, we have observed 100 % recovery of graft function at three months post episode of AAMR in non-CDC group (N=39/39) compared to 80% in CDC group (N=12/15), p = 0.4 [13].
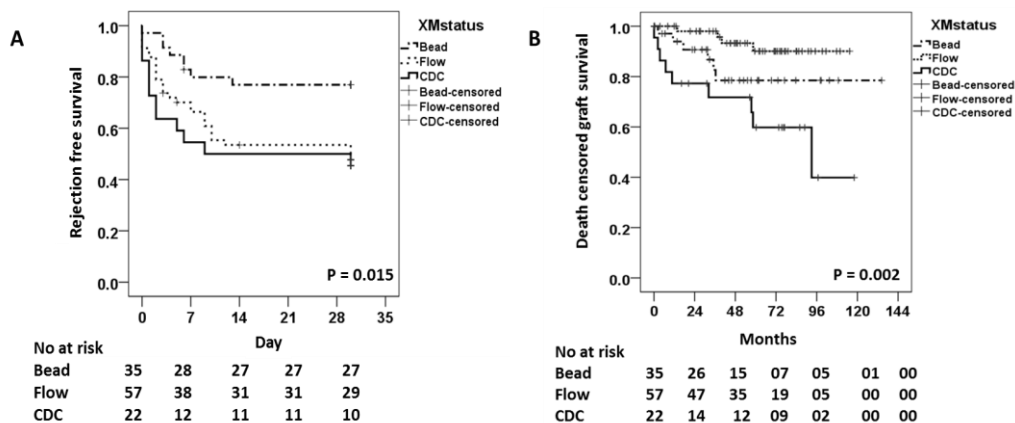

Figure 2 Outcomes following HLA-incompatible kidney transplantation in relation to current assays; A – early AAMR and B – death censored graft survival (Kaplan Meier survival analysis and statistics by log rank test).

Previously we have shown that five-year death censored graft survival following HLA-incompatible kidney transplantation are 78 %, 80 % and 54 % in groups with luminex bead assay alone positive, luminex and flow XM positive but CDC negative, and CDC (non-AHG) positive groups, respectively [2]. A recent analysis of 114 HLA-incompatible kidney transplantation at our centre revealed that patients with CDC positive continue to have worse graft survival, with death censored graft survival at eight-year reduced to 40%, whilst other two groups remained stable (Figure 2B). Similar findings were observed in a multicentre data evaluation in USA [7], which showed an unadjusted five-year graft survival of 80%, 71% and 60 % for group with luminex alone positive, luminex and flow XM positive but CDC negative and CDC (AHG) groups respectively.

Thus, many centres use risk stratification based on positive results obtained on the above three assays, with CDC positive at the highest risk and luminex microbead alone at lower risk and flow crossmatch at intermediate risk for poor outcome. In UK, living transplant pairs with higher titre CDC positive results on crossmatch are discouraged for direct transplantation, which is reserved for exceptional situations such as failing dialysis access or increasing recipient co-morbidities.

Although CDC positivity prior to transplantation is associated with poor outcome, this is not universal as one in two direct transplants across a positive CDC result has good outcome (both early antibody mediated rejection and longer-term graft survival), and low titre CDC positive transplants have good results in our series. On the other hand, pre-transplant flow cytometer crossmatch or luminex positive alone have equivalent outcomes as compatible kidney transplantation [2]. Patients with class I HLA-specific and multiple DSA antibodies giving CDC positive are associated with worse outcomes.

3. MFI Value of HLA-Specific Antibodies

With the advent of microbead luminex assay, laboratories are utilising this sensitive and specific assay for DSA risk stratification. Is MFI value-based stratification as good as CDC? Preliminary studies at our centre using multivariate analysis on pre-transplant variables such as IgG, IgG sub-classes and CDC showed that CDC was not an independent predictor of rejection or graft failure [14]. It should be noted that this outcome was obtained within our envelope for accepting risk, i.e., we transplanted some patients with a high titre of DQ or DP antibodies, but did not transplant patients with high CDC titres of Class 1 or DR antibodies (roughly 1:16 or greater). We have previously shown that a higher MFI value on luminex bead assay is correlated with worse graft survival (Figure 3) [2]. An MFI value (non-EDTA) of 3000 pre-transplantation is associated with a lower risk of AAMR; however early AAMR in patients who were CDC negative, pre-treatment does seem to be a treatable condition in our series, and 5-10 year outcomes are more important clinically [15].
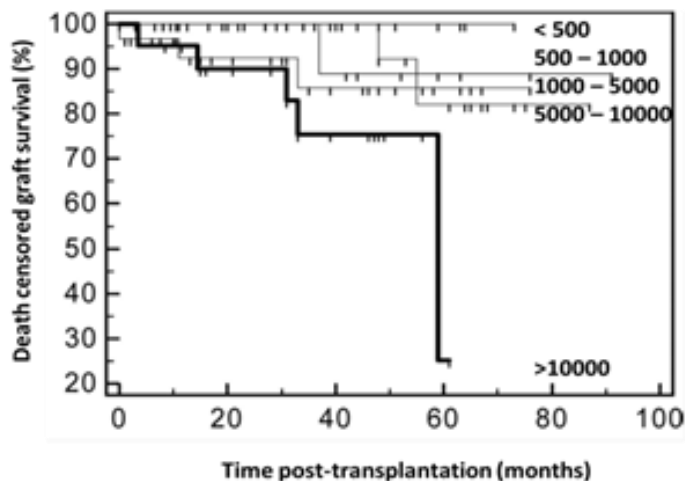
Figure 3 Death censored graft survival and MFI values of donor HLA-specific antibodies (adapted from Higgins et al [2]).

A genuinely low value of MFI pre-transplantation can give false reassurance, and amnestic response following HLA-incompatible kidney transplantation is seen associated with AAMR. We have shown that the MFI values can go low as time goes by, at times below the clinical threshold and on re-stimulation of epitope/antigen can trigger rapid rise of antibody levels and this may vary on initial sensitisation event, with pregnancy related sensitisation more important than prior transplantation or blood transfusion [16]. There is a higher proportion of cases with highest percentage rise in MFI values compared to baseline in patients undergoing HLA-incompatible kidney transplantation from pregnancy related sensitisation as a spouse or child donor compared to other sensitisation events (Figure 4). Various approaches are proposed to reduce sensitisation such eplet matching strategies [17] judicious use of blood products in patients with kidney disease and, avoiding graft nephrectomy and maintaining low dose immunosuppressive medications after transplant failure [18].
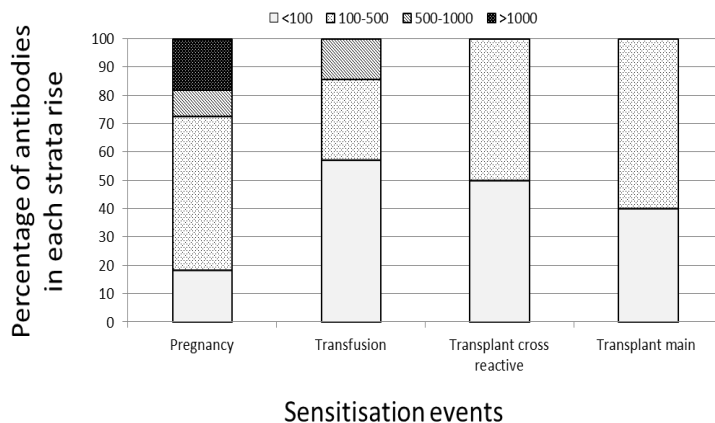

Figure 4 Percentage rise of HLA-specific antibodies following HLA-incompatible kidney transplantation related to sensitisation events. Rise in MFI values are divided in four strata of rise - <100 %, 100 to 500 %, 500 to 1000% and > 1000% rise from baseline.

MFI values obtained from the binding of HLA-specific antibodies on luminex assay are sometimes used quantitatively, but the assay readout is a composite of both concentration and affinity of the HLA-specific antibodies [19]. The binding of antibodies to the solid phase assays is influenced by the affinity of the antibodies. Hence, low affinity antibodies could be washed away in multi-wash steps during the procedure to get rid of non-specific adsorption to the beads. Also, if the antibodies are tested on single antigen beads, then the antibodies bind to a number of specific beads due to shared epitope and if present in low concentration, could give false negative result against the 'primary' target HLA but a crossmatch can still be positive [20]. Using purified human monoclonal HLA-specific antibodies, we have shown that MFI values are affected by both the affinity and concentration of the antibodies and thus a product of two distinct characteristics - the higher affinity suggesting further steps in humoral maturation [19, 21].

A very high concentration of antibodies can sometimes give a false low binding response due to competition for binding space; this is called hook effect [22], or prozone effect. This can be overcome by repeating the test with a dilution of sample, or treatment with EDTA [23]. This interference could be due to the presence of C1q molecule interfering binding of fluorescence conjugated detecting antibodies [24]. Although most laboratories are now using EDTA, correlation with long term outcomes is not yet fully defined with this assay. It is also important to note that MFI values and Luminex testing have intra and inter-centre variation [25] which limits comparisons of outcome studies. Studies have demonstrated certain risk factors that are associated with poor outcome following antibody incompatible transplantation (Table 1) but it is important that these are not good in evaluating risks precisely.

Table 1 Risk factors for rejection and graft loss in HLA-incompatible kidney transplantation (adapted from Montgomery et al [26] and others such as * Higgins et al [15], ** Higgins et al [16], *** Vo et al [27], **** Senev et al [28]).

Wide breadth of HLA reactivity
Previous transplants***
Previous early graft losses
Sustained antibody production to all or most previous mismatched antigens
Multiple repeat mismatches (spreading specificities during rejection)
Eplet mismatch load, particularly at HLA-DQ****
Multiple donor specific antibodies *
Multiple sensitising events
High risk combinations (husband-to-wife or child-to-mother transplants) ** & ***

4. Granular Characterisation of HLA-Specific Antibodies: Reasons for Varied Clinical Outcome and Newer Approaches for Risk Stratification

We have observed varied graft responses in the presence of donor HLA-specific antibodies (Figure 5): in some instances causing acute rejection, and in other instances existing with perfectly functioning grafts or the resolution of rejection in the presence of high levels of antibodies [29, 30].
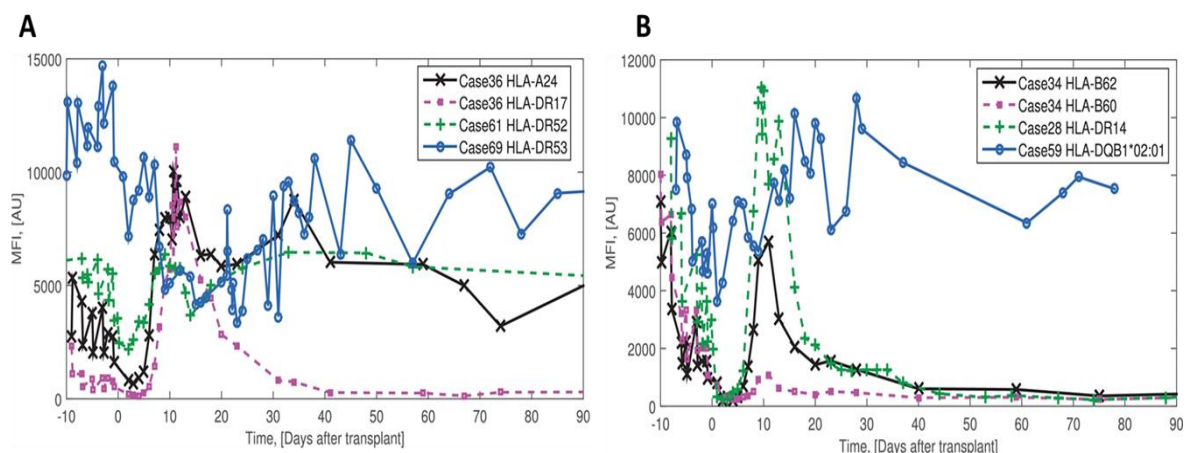
Figure 5 Variable graft response to donor HLA-specific antibody (DSA). (A) Cases who developed graft rejection accompanied with the rise of DSA, then a resolution of rejection and fall in DSA and (B) Cases whose graft functioned perfectly normally in the period of observation despite a steep rise and falls, in some with plateau of DSA (despite plasmapheresis as seen in dip of levels). All cases with AMR received Anti-thymoglobulin (ATG) treatment but otherwise cases in both groups treated similarly. (adapted from Zhang et al. [30]).

These variable responses could be due to antibody-specific factors, donor kidney and/or host-specific responses; therapy is also a possible factor. Although the mechanisms underlying these variable responses are complex, it is important to note that the mere presence or absence of antibodies does not determine adverse events. There is a change either in the immunological milieu or graft environment, or in the characteristics of these antibodies that determine immunological response and graft fate. Various parameters of antibodies have been studied by different groups, and the findings are summarised below.

4.1 Complement Activation Ability

Complement activation following the binding of antibodies to the HLA antigens on endothelial cells is considered one of the most potent mechanisms for antibody mediated rejection. This may occur through cytotoxicity generated directly or with the help of effector cells, including neutrophils, macrophages and T cells. Though the benefit of reducing AAMR by complement blockade at the time of transplant was limited to complement activating DSA and not from non-complement activating antibodies [31].

Data from CDC assay suggest complement fixation is associated with rejection, but CDC is not a specific assay for HLA-specific antibodies. Studies using a C1q binding assay protocol showed that the presence of C1q fixing antibodies is associated with worse graft function [32, 33]. Study by Loupy et al has shown that C1q positivity, in addition to DSA specificity, is associated with graft outcome [32]. Additionally, they showed that conversion of C1q negative DSA to positive post-transplantation was associated with worse outcomes. Additional testing of C1q binding ability may stratify the DSA risk further. However, the usefulness of C1q binding has not been uniformly reproduced in other studies [34-38]. The limitations of the C1q assay is probably related to its

correlation with IgG DSA MFI as the C1q positivity is a mere reflection of higher IgG MFI levels [39]. Also, C1q positivity could be due to the binding of IgM antibodies. The complement activating properties of HLA-specific antibodies can also be assessed using complement C3 split product, C3d, which is further downstream in the complement pathway, and this may overcome some of the limitations of C1q assay [40]. In our multicentre cohort, 139 highly sensitized patients with positive crossmatch who underwent HLA antibody incompatible renal transplantation were studied for C3d binding. As depicted in Figures 6a and 6b, presence of C3d-positive DSA in pre-transplant and post-transplant periods were associated with worse renal allograft survival [41].
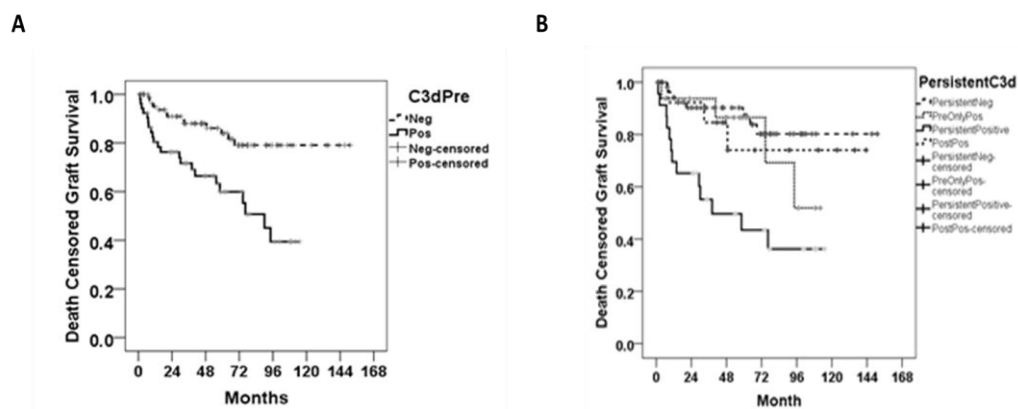

Figure 6 Kaplan Meier survival analysis: death censored graft survival was significantly worse in patients with pre-transplant C3d positive DSA (A) and with persistent C3d positive DSA compared to C3d negative pre- and post-transplant patients (B) (adapted from [41]).

In this multicentre cohort study [41], C3d assay was able to clearly define groups with better outcome among moderate to high immunological risk (flow and CDC positive) cases. Cases with pre-treatment C3d-negative DSAs are likely to have a good result at five years, whether the reactions become C3d-positive or not at around day 14. The best survival is seen in patients with C3d-negative DSA at both pre-treatment and day 14.

A single centre study of 68 highly sensitized patients that included 21 CDC crossmatch positive patients looked at pre-treatment risk stratification using in-vitro C4d deposition on SAB. Presences of pre-transplant C4d+DSA were associated with acute AMR. One, three and five-year death censored graft survival was also significantly lower in the C4d positive DSA patients than in C4d negative DSA patients [42]. Other studies have shown that in crossmatch negative sensitised patients, DSAs with complement activating potential, measured by C1q binding at the time of transplantation and/or post-transplantation, are also associated with poor renal allograft survival [32, 43, 44]. A large study by Kamburova et al concluded that the presence of pre-transplant C3d-positive DSAs was associated with reduced renal allograft survival but did not reach statistical significance [45]. Post-transplant development of complement activating DSA has also been shown to be a risk factor in paediatric renal transplantation [46, 47].

Two comparable studies have indicated that testing for complement activating DSA at the time of AMR predicts graft survival using C1q binding and C3d activating assays, respectively [32, 40]. Similarly, a recently published study from the deteriorating kidney allograft function (DeKAF)

investigators, in a cohort of standard renal transplants, the results of a C3d assay performed at the time of development of DSA and graft dysfunction, predicted a higher risk of graft failure in C3d-positive DSA group compared to C3d negative DSA group [48].

4.2 Correlation of Complement-Fixing/Activating Antibodies with Crossmatch

It is established that complement activating antibodies, as detected by complement-dependent cytotoxicity crossmatch, are associated with worse graft survival [9]. However, the CDC assay is not always specific and can identify other non-HLA complement activating antibodies [49, 50], so current practice is to regard a positive CDC test as relevant when microbead testing confirms the presence of DSA. The availability of viable donor cells still limits the CDC assay, and it is not always practical to perform this assay on multiple occasions in the pre-transplant and post-transplant periods. In recently published single-centre studies, positive C3d assay was shown to correlate with high specificity, and a positive predictive value with a positive FC crossmatch, although sensitivity and negative predictive value were low [51, 52]. A negative C3d assay had a higher negative predictive value for CDC; this could be a useful surrogate marker for risk stratification as a negative CDC crossmatch is generally required at the time of transplantation. The flow cytometry crossmatches, although more sensitive than CDC, have similar limitations. Typically, a positive pre-transplantation crossmatch is discouraged due to the high risk of rejection [53] and reduced graft survival [2, 54]. In the subgroup analysis of FC crossmatch patients, pre-treatment C3d-positive DSA was associated with lower graft survival. This finding is not entirely surprising as in one of the earlier studies that looked at the utility of C4d-positive DSA in a cohort of highly sensitized patients, the presence of pretransplant C4d positive DSA in CDC crossmatch patients were associated with worse graft survival compared to the C4d-negative cohort [55].

4.3 Class and Subclass Switching

As the immune response progresses with time, there is a class switch whereby the Fc portion of the antibodies are altered, resulting in structural and functional change. IgG HLA-specific antibodies can be of different sub-classes (IgG1-4), and the current assay gives a composite readout at the IgG level. We have shown significant heterogeneity of IgG subclasses in cases with pre-formed HLA-specific antibodies [56]. Different IgG subclass differs in its ability to bind to complement and hence complement activations; IgG1 and IgG3 are strong complement activating followed by IgG2. Hence many studies have explored the subclass profiles and related them to the outcome of HLA-incompatible kidney transplantation.

Earlier studies on pre-formed HLA-specific antibodies had contradictory findings [57, 58]. Recently, we have shown that IgG4 sub-class presence prior to transplantation is independently associated with poor outcome, namely antibody mediated rejection within first 30 day post-transplantation and medium term graft survival [59]. Similarly, a study from Paris showed IgG3 sub-class presence was associated with clinical biopsy-proven acute rejection and IgG4 was associated with sub-clinical rejection on protocol biopsies [60].

Other classes of antibodies particularly IgM, despite being strongly complement-fixing, is not considered harmful [61-63]. However, recent case reports and small case series suggest they may modulate immune responses and are associated with rejection [64-66] and hence IgM class of HLA-specific antibodies warrants a detailed study, especially in relation to long term outcomes

rather than simply AAMR. In the setting of de novo HLA-specific antibody evolution, a recent study observed that the presence of IgM itself was not harmful but a co-existing IgG3 HLA-specific antibodies predicted poor graft survival [67].

In our cohort of ninety-two highly sensitised patients, we studied for the presence of IgM donor specific antibodies at pre-transplant and at days 7, 14 and 30 post-transplant. IgM DSA were detected using Luminex-SAB kits (One lambda, CA). Detailed methods of IgM detection is described in previous publication [68]. We showed that over and above of current assays, a pre-transplant IgM DSA is not helpful for the prediction of early antibody mediated rejection and renal allograft survival [68]. Presence of IgM DSA in the early post-transplant period was associated with poor renal allograft survival (Figure 7) in Kaplan Meier analysis. This suggests, further de novo responses or epitope spreading adds to the immunogenicity and thus is damaging. A new IgM DSA response against HLA that also has IgG pre-formed response may be new epitope-specific that antigen level assays cannot unleash.
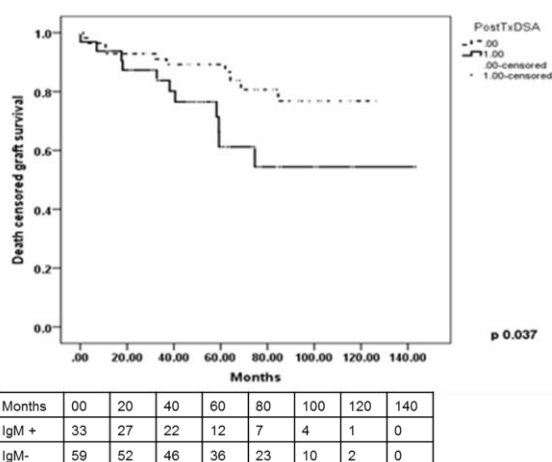

Months	00	20	40	60	80	100	120	140
IgM +	33	27	22	12	7	4	1	0
IgM-	59	52	46	36	23	10	2	0

Figure 7 Kaplan Meier survival graph showing lower renal allograft survival in the presence of post-transplant (day-14) IgM DSA (p = 0.037) (unpublished data).

4.4 Affinity Maturation

Binding of antibodies to the corresponding antigen is the initial event followed by activation of various effector mechanisms. The strength of binding of these interactions is called affinity and can vary between individuals and within individuals for different antibody specificities and over time. Studies have shown that profiles of antibodies demonstrate heterogeneous affinity across the immune response and that higher affinity antibodies cause increased pathogenic responses [69-73]. There are no studies looked at this directly in context of kidney transplantation. A previous study speculated that higher binding strength of the interaction between the HLA epitope and HLA-specific antibody results in higher binding energy release, which brings conformational change to the immunoglobulin molecule, particularly to the complement fixing Fc portion. Thus it has been speculated that complement fixation could serve as a surrogate marker for higher affinity interactions [74]. The authors have further postulated that the reactivity and strength depend on antibody paratope interaction with eplets and other residues within 15

angstrom of the eplet in the structural epitopes. The larger area of interactions will depend on the difference between the given allele and the immunizing allele. The larger the area of interaction will give rise to stronger binding force/affinity. We have shown human monoclonal HLA-specific antibodies with the same concentration can have different affinities that influence binding on luminex assay [19]. Preliminary studies at our centre have shown that affinity of polyclonal HLA-specific antibodies can differ widely (from E-03 to E-024 M), and different antibodies from the same patients have different affinities (Figure 8) [75]. The main challenge has been a high throughput assay minimising the non-specific bindings [21].
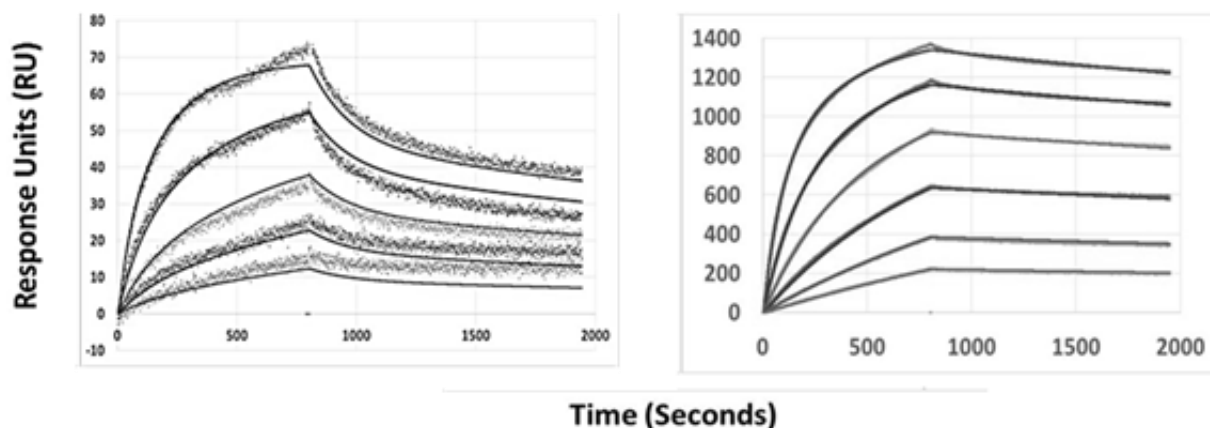

Figure 8 Binding kinetics of polyclonal HLA-specific antibodies from two different patients against HLA B40:02 proteins studied by surface plasmon resonance. Sensorgram shows binding response curve at reducing the concentration of antibodies; the solid line represents a mathematical fit to data using a heterogeneous analyte model (unpublished data and methodology- http://wrap.warwick.ac.uk/70964/).

4.5 Other Characteristics

Function and the effector response of antibodies could be altered by other properties such as glycosylation pattern of HLA-specific antibodies, polymorphisms of Fc gamma receptor on immune cells and interaction of Fc portion of HLA-specific antibodies [76]. Glycosylation status dictates flexibility of antibody molecule and thus may influence the interaction with antigen on a cell surface [77]. Similarly, Fc portion interactions with effector cells may differ depending on the Fc receptor's polymorphism on these cells and the affinity of the interactions. In a study of 1682 patients, authors analysed the possible mechanisms of graft failure in 135 patients who experienced AMR. Seventy-three (54%) patients had complement activating antibodies (C3d-positive DSA) as cause of graft failure. In remaining sixty-two (46%) patients, cause of worse graft survival was attributed to enhanced recruitment of Natural Killer (NK) cells by non-complement activating DSA due to missing self HLA I antigens. Proposed mechanism for chronic graft rejection include absence of inhibitory signals to NK cells due to missing HLA I antigens on the endothelial cells [78].

5. Novel Machine Learning Applications

Methods that can analyse complex datasets independent of operator bias are potentially very important; especially now centres and registries are generating 10–15-year outcome data, which is surely going to be the gold standard for judging clinical success and inferring biologically important mechanisms. Therefore, in our cohort, we have used mathematical approaches to interrogate such clinical datasets, in particular, decision trees and random forests for risk prediction of antibody mediated rejection and graft survival. Using random forests, HLA mismatches, IgG MFI values and IgG4 subclass were shown to be associated with the highest risk for graft failure (Figure 9) [79]
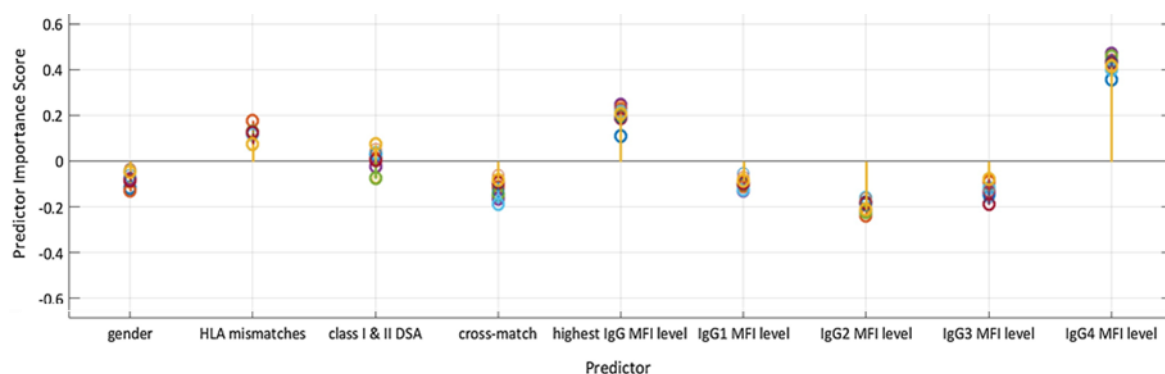

Figure 9 Based on 10 random forests, HLA mismatches, IgG MFI and IgG4 were associated with worse graft survival (adapted from Shaikhina et al. [79]).

6. Dynamicity of HLA-Antibody Responses Following Transplantation

In addition to individual characteristics of antibodies, dynamic behaviour after antigen exposure (transplantation) holds key to the underlying immune response and mechanism for injury and outcome. The limiting factor is the cost in such intensive monitoring of MFI values of HLA-specific antibodies following HLA-incompatible kidney transplantation. We have shown previously varied trends of MFI value. In some cases, there is a rapid rise followed by a rapid fall to an undetectable level. Dynamics of the DSA in the post-transplant period may help to predict antibody mediated rejection or graft survival later. In our cohort, we studied the falls of the DSA using mathematical models and found differences in the groups with AMR and no AMR [30]. We noticed that the falling MFI dynamics of HLA DSA after the peak value could not be described by a simple exponential law or, indeed, by a superposition of several exponential functions. Therefore, we have developed a generic form of a model that was able to capture the common patterns in all DSAs. Namely, we have described the DSA falls using 3rd order linear differential equations and found that the DSA decay times, as well as the general dynamic trends, were different in the two groups, i.e. with and without AMR. Three dimensional mathematical models (Figure 10) showed dissipations rates of DSAs faster in cases with AMR (Figure 10a). These models are novel ways to study dynamics of DSA behaviour and association with longer term outcomes are required prior to testing clinical application.
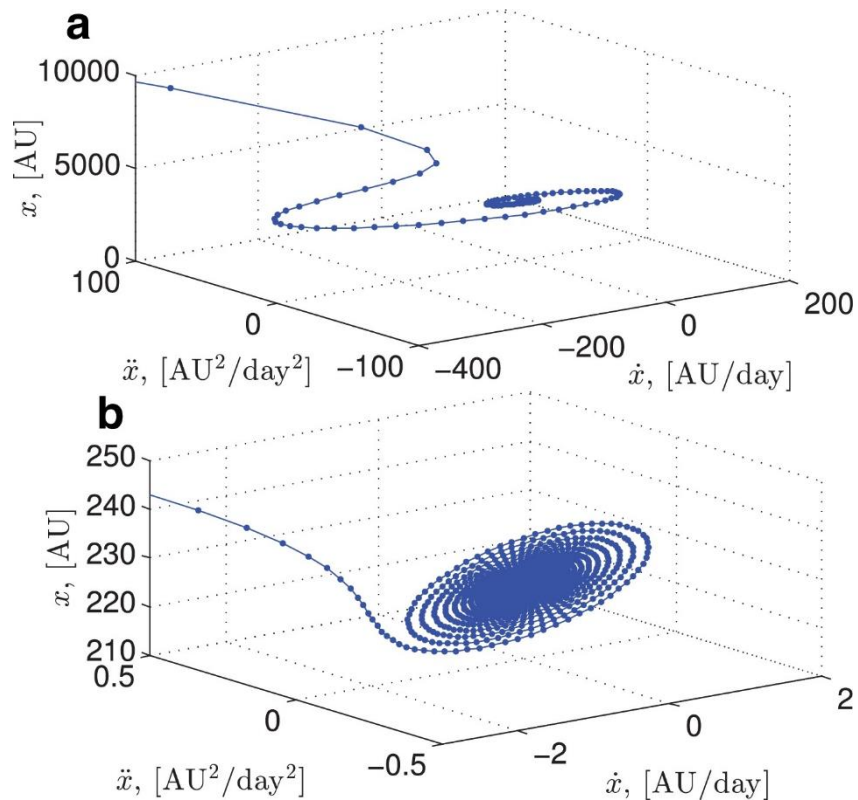
Figure 10 Phase portraits of the three-dimensional system for two DSA time series, (a) from a patient in the AMR group and (b) from a patient in the no-AMR group. [30]; x is the function of time that describes MFI dynamics; $\dot{x}$ and $\ddot{x}$ are first and second derivatives of x, correspondingly. (adapted from [30]).

The disappearance of antibodies from the serum in some patients is much quicker than the half-life of IgG molecule, and this observation suggests some active immune-modulating mechanism [80]. This could be due to the immune complex formation of HLA-specific antibodies to the soluble HLA molecule released from the transplanted organ [81], but current assays are unable to detect such complexes. Preliminary results from our centre have suggested lower HLA-specific antibodies levels in cases with donor specific soluble HLA in the circulation following transplantation [82]. Another mechanism of neutralisation of HLA-specific antibodies could be by anti-idiotypic antibodies [83, 84].

7. Shortcomings of Current Approaches

All these studies have shown that understanding the role of HLA antibodies in graft outcome is more complex than previously thought, with a series of relatively weak clinical associations, and the understandable, but erroneous, concentration on AAMR as a surrogate for adverse clinical outcome. Some studies have suggested the long-term outcome following early episode of AAMR is not always detrimental [85, 86] and thus studies relating characteristics with graft survival are important as studies using AAMR as an endpoint may be misleading. Hopefully, specific immunological assays will predict and explain the key immunological pathologies in transplantation. What is notable is that the recent long-term studies have not weakened the significance of the CDC assay – indeed have strengthened its significance. Although the assay was

designed to predict hyperacute rejection in our patients it is the best predictor, of the pre-transplant tests available, of 10-year graft survival, given that our results show low levels of CDC positivity are tolerated. Perhaps some insights might arise from examination of what aspects of this venerable assay are particularly significant, developed by Terasaki in 1968 [9].

Finally, however good our observation of antibody dynamics becomes, this is only part of the system that determines transplant outcome. We may be like the anthropologist trying to reconstruct an entire hominid species from a single tooth. Even if we can assemble a skeleton (the antibodies), that does not inevitably lead to an understanding of the soft tissues that support and command the skeleton (T and B lymphocytes, plasma cells, for example).

8. Summary

Advent of newer diagnostic and therapeutic modality has allowed safe transplantation in the presence of donor HLA-specific antibodies, up to low titre CDC positivity. However, the clinical outcome remains unpredictable with current assays, which take snap shots, and hence detailed analysis of other characteristics of antibodies are carried out by many centres (dissection analysis). This effort has been mainly focused on studying complement-fixing properties and recently on the analysis of sub-classes in relation to clinical outcome. Determination of the affinity of HLA-specific antibodies is only just undertaken, whilst other characteristics such as glycosylation, enhanced recruitment of innate cells in the absence of HLA I antigens and Fc portion binding to effector cells are being studied. Also, in recent times there is greater recognition and understanding the role antibodies directed against minor histocompatibility and non–HLA antigens. Developing better assays and studying detailed characteristics of antibody function and biophysical properties may give better composite biomarkers to predict rejection and graft failure in the sensitised patients and thus allow safer HLA-incompatible organ transplantation and will require unbiased machine learning analysis of large datasets.

Acknowledgments

Author Contributions

SD wrote the first draft which was edited by RH; further addition by AB and NK. All authors did final editing and agreed the content.

Competing Interests

Both Dr Daga and Dr Higgins are part of a team that collaborates with Pure Transplant LLC at Oklahoma that has provided soluble HLA for research work. The company or any members of the company did not directly influence our research work or preparation of this manuscript.

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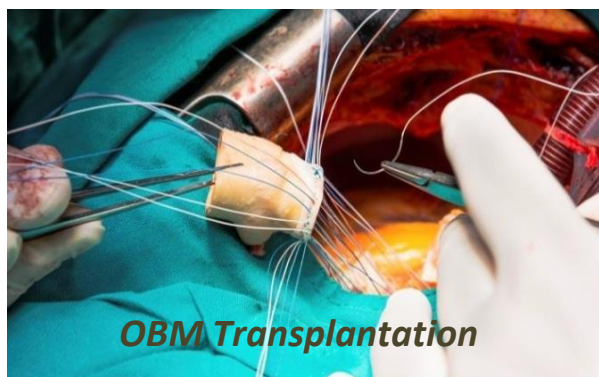
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