Posttransplant Human Leukocyte Antigen Antibodies in Stable Kidney Transplant Recipients

Gregor Bartel and Georg A. Böhmig

Division of Nephrology and Dialysis, Department of Medicine III, Medical University of Vienna, Vienna, Austria

Abstract

Detection of donor-specific antibodies represents a major criterion of antibody-mediated rejection. This type of rejection, a leading cause of long-term allograft failure, may be the consequence of a multistep process initiated by the formation of donor-specific antibodies, which may subsequently trigger microcirculation inflammation and tissue damage followed by graft dysfunction and loss. At first sight this concept is in strong support of implementing a per-protocol longitudinal antibody monitoring in all kidney transplant recipients. One may speculate that early detection of donor-specific antibody occurrence could guide timely anti-humoral treatment, preventing subsequent irreversible graft damage. However, recent studies have revealed controversial results. In contrast to recipients with graft dysfunction, a considerable proportion of patients with normal function at the time of antibody testing were shown to maintain excellent long-term survival despite detectable de novo donor-specific antibodies. Moreover, the persistence of detectable antibodies following desensitization in immunological high-risk patients was described to be not necessarily associated with inferior transplant performance. For donor-specific antibody positive stable patients, a role of transplant accommodation, a state of acquired resistance to immune injury, was speculated. The present review focuses on the still controversial issue of donor-specific antibody monitoring in kidney transplant recipients, putting a special focus on stable patients who present without clinical signs of ongoing rejection. (Trends in Transplant. 2014;8:3-9)

Corresponding author: Georg A. Böhmig, georg.boehmig@meduniwien.ac.at

Key words

Introduction

There is emerging evidence for a role of antibody-mediated rejection (AMR) as a leading cause of long-term kidney allograft injury and loss\(^1\text{-}^4\). In the last decade, distinct diagnostic criteria were established to classify and score the process of acute or chronic AMR. In the Banff classification and its amendments, detection of donor-specific antibodies (DSA) in sera of rejecting patients has been implemented as a major diagnostic criterion\(^5\text{-}^6\). Numerous studies have shown that in patients with clinical AMR, DSA detection may be closely associated with characteristic morphological changes, in particular, features of microcirculation inflammation and injury or C4d deposition, a footprint of antibody-triggered classical complement activation\(^2\text{-}^7\text{-}^8\).

While its role as a diagnostic marker of clinically overt rejection is now well established, the predictive value of DSA detection in patients with normal graft function is still a matter of debate. A major goal of a successful strategy of immune monitoring guiding targeted treatment may be the early detection of potentially deleterious (humoral) alloreactivity before detectable tissue inflammation and injury has occurred. Using bead array technology for sensitive, solid-phase human leukocyte antigen (HLA) antibody detection, one may argue that a timely identification of low levels of circulating DSA could provide a useful basis for the early implementation of therapeutic interventions, preventing the occurrence of refractory rejection and graft loss, respectively.

There is some line of experimental evidence that the development of chronic AMR may result from a sequence of changes, including DSA formation followed by the sequential occurrence of capillary complement deposition, morphological injury, and graft dysfunction and loss\(^8\). This concept would imply that DSA detection at an early stage, eventually in the context of protocol biopsy results, could serve as an early predictor of subsequent humoral graft injury and inferior graft performance. In clinical transplantation, however, only few studies have systematically analyzed the impact of DSA occurrence in kidney transplant patients presenting with normal graft function. Current data are controversial and there are some preliminary results suggesting that de novo DSA may not necessarily preclude excellent long-term outcomes\(^10\text{-}^13\). Moreover, when it comes to follow-up and treatment strategies once DSA have been detected, reliable data is even scarcer. This review will focus on the current literature analyzing the clinical impact of DSA in stable kidney allograft recipients and discuss the value of systematic longitudinal DSA monitoring in the context of targeted anti-humoral treatment.

Detection of donor-specific antibodies in stable kidney transplant recipients

In a recent study, our study group evaluated a cohort of 164 recipients of a deceased-donor kidney allograft, applying serial serological testing using FlowPRA technology to detect HLA reactivity patterns\(^10\). We focused on a subgroup of 34 kidney transplant recipients showing excellent graft function within the first year (estimated glomerular filtration rate \(\geq 60\) ml/min, 24-hour urinary protein excretion \(\leq 0.5\) g, no episode of graft dysfunction, no desensitization, and no rejection treatment). An unexpected finding was that in the stable group, proportions of HLA- and DSA-positive patients were not significantly lower than those detected for the other 130 recipients. Remarkably, within this subgroup, long-term allograft function did not differ between HLA-positive and -negative patients and no graft loss was noted for alloantibody-positive patients within a median follow of 5.4 years. These data suggested that DSA positivity in absence of graft dysfunction in the first posttransplant year may not necessarily be related to inferior graft performance\(^10\).
In another single-centre analysis, Lachmann, et al.\textsuperscript{13} studied a total of 1,014 renal transplant recipients with functioning allografts at a median of five years posttransplantation. In a cross-sectional design, patients were tested for circulating HLA antibodies using Luminex-based bead array technology and followed for another 5.5 years. Within the different time frames of six months to two years \((n = 211)\), two to four years \((n = 139)\), four to eight years \((n = 128)\), and beyond eight years after transplantation \((n = 259)\), 24, 38, 35, and 50\% of the patients, respectively, were classified DSA-positive. Overall, DSA detection was associated with a markedly higher graft loss rate, and as many as 44\% of the patients with failed allografts showed detectable DSA. On the other hand, the authors noted a cohort of 200 patients who maintained good allograft function despite the presence of HLA antibodies. Remarkably, in 24\% of these patients, HLA antibodies were directed against donor antigens. There was no difference in alloantigen specificity, antibody strength, number of previous transplants, or pretransplant HLA antibody status between patients who lost their graft and recipients who maintained graft function. However, a remarkable finding was that kidney function at the time of antibody testing differed significantly: DSA-positive subjects who lost their graft upon follow-up had significantly worse allograft function at the time of antibody testing\textsuperscript{13}.

Similar results were earlier reported by Terasaki, et al.\textsuperscript{12}. In a multicentre analysis, kidney transplant recipients were subjected to a single snapshot of HLA antibody testing after \(\geq 6\) months posttransplantation. At a median of two years posttransplantation, 16.7\% of the 233 allografts from patients with \textit{de novo} HLA antibodies failed compared to 6.5\% of the 1,331 antibody-negative patients. Analyzing transplant outcomes in relation to serum creatinine levels measured at the time of antibody testing, the authors found no effect of circulating HLA antibodies on graft survival among patients with a serum creatinine \(< 2\) mg/dl. In contrast, for patients with a serum creatinine between 2-4 mg/dl, marked differences were observed\textsuperscript{12}.

The three studies mentioned above point to a particular relevance of documenting allograft function at the time of antibody testing when it comes to interpretation of the clinical significance of DSA. Nevertheless, in interpreting study results several inherent limitations have to be pointed out. One may argue that subclinical subtle morphological changes and a very slow deterioration of graft function late after transplantation has been missed due to limited follow-up periods and a lack of protocol biopsies.

In a more recent study, which also included an evaluation of a considerable number of protocol and indication biopsies, Wiebe, et al.\textsuperscript{11} systematically analyzed the results of a comprehensive serial antibody monitoring over a long-term follow-up in a cohort of 315 low-risk patients (mainly recipients of a first transplant; no detectable pre-sensitization). Applying FlowPRA technology, \textit{de novo} DSA were detected in 15\% of the patients after a median of 4.6 years posttransplantation. Major risk factors for DSA occurrence were nonadherence, mismatch in HLA-DR, and the detection of cellular rejection in six-month protocol biopsies. Overall, patients with \textit{de novo} DSA had a worse 10-year graft survival compared to DSA-negative patients (59 vs. 96\%). In a subsequent subanalysis, the authors evaluated the cohort of DSA-positive patients, building three different subgroups: (i) patients with acute dysfunction at the time of \textit{de novo} DSA occurrence showing 100\% graft loss upon follow-up \((n = 14)\), (ii) an “indolent” group of 15 patients where \textit{de novo} DSA preceded a rise in proteinuria and serum creatinine levels; in this group graft loss occurred in 40\% of the patients, and (iii) a stable group \((n = 18)\) where no proteinuria or an increase in serum creatinine and no graft loss was documented.
within a median follow-up of 19 months after \textit{de novo} DSA detection. Interestingly, 10 of the 14 protocol biopsies in stable kidney transplant patients with \textit{de novo} DSA showed evidence of microvascular inflammation, which was significantly more frequent than in stable DSA-negative patients. Moreover, three of four repeat biopsies showed histological progression despite augmentation in immunosuppression. These data provide evidence that at least some of the stable patients with \textit{de novo} DSA may show morphological features of antibody-mediated graft injury. The authors speculated that subclinical AMR could culminate in dysfunction late after transplantation, beyond the documented median follow-up of 19 months\textsuperscript{11}.

Taken together, there is no doubt that circulating DSA may pose an increased risk of immunological graft failure. However, it has also become clear that at an individual level it may not be possible to reliably predict whether and when after DSA occurrence graft dysfunction will occur or worsen, respectively.

\textbf{Donor-specific antibodies after recipient desensitization}

There is growing evidence that the finding of circulating DSA has to be interpreted differently in the context of desensitization. For example, studies by our group have shown limited value of serological alloantibody monitoring in this specific context\textsuperscript{14,15}, and we reported a cohort of 68 deceased-donor renal allograft recipients who were subjected to a protocol of peritransplant immunoadsorption. Treatment consisted of a single session of immediate pretransplant immunoadsorption (protein A) followed by posttransplant immunoadsorption and anti-lymphocyte antibody therapy. For 52 patients, posttransplant serum samples collected 3-9 months after transplantation were available for HLA antibody testing. After transplantation, 23 patients still had alloreactivity against donor HLA antigens. Within a median follow-up of nine years, detection of persistent or \textit{de novo} DSA after transplantation was not associated with adverse death-censored graft survival as compared to patients without posttransplant DSA. There was also no difference regarding rates of acute and/or chronic C4d-positive AMR, cellular rejection, delayed graft function, serum creatinine or protein excretion. Among the four patients with \textit{de novo} DSA, one developed acute C4d-positive graft dysfunction (graft loss after 18 months due to non-biopsied chronic dysfunction). The remaining three patients, however, had no rejection episode and maintained stable graft function. Analysis of indication biopsies revealed no difference between groups regarding active humoral lesions.

Kraus, \textit{et al.}\textsuperscript{16} presented the results of 116 protocol biopsies obtained in the first year posttransplantation and serum antibody measurement from 50 stable crossmatch-positive recipients having received desensitization treatment including intravenous immunoglobulin (IVIg), plasmapheresis (PP) and in some cases anti-CD20 therapy or splenectomy. Also in this study, DSA detection posttransplantation was not associated with histological features of microvascular injury. Moreover, subclinical cellular rejection was detected in 27.3 and 25.8% of the biopsies in the presence or absence of DSA, respectively.

Taken together, these studies suggest that the predictive value of DSA for microvascular damage and graft survival might be limited in the particular context of pre- and/or peritransplant antibody removal.

\textbf{Donor-specific antibodies in operational tolerance}

In a very recent study, Brouard, \textit{et al.}\textsuperscript{17} described a cohort of long-term kidney transplant recipients who were in a state of operational
During a median follow-up time of 10 years without immunosuppression, 30% (8 out of 25) of the subjects developed anti-HLA antibodies, four of them with antibodies directed towards donor antigens. Of these eight patients showing post-weaning de novo immunization, six kept stable graft function (median of seven years post-weaning), whereas two of the patients lost their graft six months and four years after the appearance of HLA antibodies, respectively. These data suggest that DSA may occasionally occur in the context of operational tolerance. In some patients, de novo DSA may predict antibody-mediated allograft damage.17

**Accommodation**

A variety of studies have shown that de novo DSA may not inevitably trigger graft injury. One possible explanation for this phenomenon may be the establishment of transplant accommodation, a putative state of acquired resistance of a transplanted organ to antibody-mediated injury. The concept of accommodation has its origin in observations made in ABO-incompatible transplantation and xenotransplant models.18,19 On a molecular level, several patho-mechanisms have been discussed, with subtle differences depending on the nature of the involved antigens, such as ABO versus HLA antigens.

It was shown that crosslinking of HLA class I molecules with antibody activates the Akt/PI3K/mTOR pathway, leading to upregulation of survival genes Bcl-2 and Bcl-xL.20,21 The intracellular events initiated by HLA class I ligation were influenced by the concentration of the anti-HLA antibody, with the lowest tested concentrations of antibody stimulating the highest level of Bcl expression. These results suggest that exposure of the graft endothelium to low concentrations of anti-HLA antibodies may promote cell survival by transducing signals, resulting in upregulation of cell-survival genes. Cardiac allografts in a mouse model treated with anti-donor class I antibody for 15 days showed high levels of Akt phosphorylation and Bcl-2 expression, consistent with an accommodation phenotype. In contrast, grafts recovered on day 30 showed phosphorylation of proteins involved in cell proliferation.22 These data suggest a critical role of the duration of antibody exposure contributing to differences in transplant outcome. Short-term exposure may promote activation of proteins involved in accommodation, whereas long-term exposure might activate proteins associated with cell proliferation and chronic rejection.

In accordance with these in vitro studies, Salama, et al.23 examined renal biopsies of HLA-sensitized patients subjected to desensitization with immunoadsorption before renal transplantation. In three of four cases where alloantibodies had returned without evidence of hyperacute rejection, the authors observed an upregulation of Bcl-xL in glomerular and peritubular capillaries. In contrast, in eight control subjects (patients in whom antibody did not return following desensitization and transplantation, normal kidneys and diseased kidneys from non-transplant patients) Bcl-xL expression was not increased. These preliminary clinical data may suggest a role of accommodation also in the context of renal allotransplantation. It can be speculated that this protective state could at least in part explain the fact that some recipients maintain stable graft function despite testing DSA-positive.

**Treatment in stable patients with donor-specific antibodies or subclinical antibody-mediated rejection?**

Treatment of clinically overt chronic AMR has turned out to be a major challenge. Some uncontrolled studies and anecdotal reports
have suggested improvement or stabilization of allograft function in subgroups of patients treated with specific anti-humoral measures, such as IVIg in combination with rituximab or bortezomib. However, no systematic studies are available to prove the efficiency of this and other treatments in this particular context. One may argue that implementation of treatment at a stage of irreversible chronic injury may be too late to counteract ongoing tissue damage and prevent graft loss.

However, as detailed above, it has become evident that early detection of circulating alloantibodies in patients with normal graft function may not necessarily predict inferior allograft performance. These data warrant careful interpretation of AMR features detected in stable patients, and the potential benefits of pre-emptive anti-humoral therapy for the prevention of graft injury remain speculative. One may argue that early treatment on the basis of systematic HLA antibody monitoring in patients with normal or near normal graft function may result in overtreatment in a considerable proportion of cases.

Currently, there are few studies analyzing early treatment of subclinical AMR either in desensitized high-risk recipients or in standard-risk patients. However, due to small sample sizes and heterogeneous treatment protocols it is difficult to draw general conclusions from these data.

Haas, et al. have retrospectively reviewed data from 83 patients who received HLA antibody incompatible renal allografts following desensitization to remove DSA. Ten patients had an allograft biopsy showing subclinical AMR during the first year after transplantation. The mean increase in chronic damage from those biopsies showing subclinical AMR to follow-up biopsies one year later was significantly greater than in 24 recipients of HLA-incompatible grafts with no subclinical AMR over a similar interval. Three patients with subclinical AMR were treated with PP and IVlg, one had his daily dosages of mycophenolate and tacrolimus increased, and six received no additional therapy. In total, three patients (one treated with PP/CMV Ig) subsequently showed an increase in serum creatinine.

In another study, Gloor, et al. reported four patients with subclinical AMR who were subjected to crossmatch conversion using PP, IVIg, rituximab, and splenectomy. They all responded to treatment with methylprednisolone, PP, and low-dose IVIg with resolution of histologic abnormalities. Moreover, treated patients had stable graft function 8-24 months later, although three of them had mildly elevated serum creatinine levels.

Wiebe, et al. described four patients with stable graft function and de novo DSA who showed morphological evidence of AMR in renal biopsy. All patients were treated with IVIg and steroids. However, despite treatment, three patients showed histologic progression in control biopsies.

Summary and conclusion

Antibody-mediated rejection represents a major cause of allograft failure in the long-term and the detection of DSA posttransplantation was associated with inferior allograft outcomes. The occurrence of DSA in the context of normal or near normal allograft function, uncovered by systematic serial HLA antibody monitoring, however, may not necessarily imply inferior graft performance in the long-term. In contrast, graft dysfunction at the time of antibody testing may be a strong predictor of graft failure. Accordingly, a careful interpretation of the results of serial DSA monitoring in transplant populations is necessary, especially in the context of targeted anti-humoral treatment implemented early before clinically overt graft injury has occurred.
References


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