Transplant International

INVITED COMMENTARY

Tracing clinically relevant HLA antibodies prior to kidney transplantation: Commentary on "Pre-transplant HLA antibodies detected by single antigen bead assay are a risk factor for long-term kidney graft loss even in the absence of donor specific antibodies"

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Transplant International 2016; 29: 985–987

Received: 10 May 2016; Accepted: 11 May 2016

With the advent of the single antigen bead (SAB) assays in 2003 HLA antibody detection has been revolutionized [1]. Admittedly, other solid-phase immunoassays based on the ELISA principle to detect HLA-specific IgG antibodies have already been introduced to histocompatibility and immunogenetics (H&I) laboratories some years earlier to supplement the complement-dependent cytotoxicity test (CDC). However, the clear innovation of the SAB assays is the combination of the unparalleled sensitivity of the Luminex® technology and the use of single HLA molecules coated to individual beads to detect HLA antibodies (HLAab) to an unprecedented resolution. However, where much light as much potential shadow. Ever since the introduction of the SAB assays, H&I professionals and clinicians somewhat have been struggling with these assays. There are some technical issues inherent to the test that may lead to misinterpretation of results. A more detailed discussion of the advantages and disadvantages of the SAB assays in comparison with ELISA and CDC can be found elsewhere [2]. Briefly, the use of recombinantly produced instead of natural HLA and the increased sensitivity is two of the most profound arguments of critics, that is, contra SAB and pro ELISA for antibody monitoring prior to kidney transplantation. SAB assays were believed to detect several irrelevant HLAab that were not associated with inferior allograft survival but would prolong waiting time for recipients when considered as unacceptable antigen. Some first publications partly seemed to confirm these concerns [3]. Therefore, several H&I professionals still rely on ELISA as the pretransplant screening assay. In this issue, the article by Richter *et al.* [4] focused retrospectively on the comparison between ELISA and SAB assays to detect clinically relevant HLAab pretransplant. Briefly, the authors analyzed the 5-year death-censored allograft survival on a total of 197 kidney transplants performed with a negative CDC cross-match under consideration of unacceptable HLA class I antigens as defined by ELISA and CDC. The most proximate serum sample prior to transplantation

doi:10.1111/tri.12796

was reanalyzed by the SAB assay. As expected, the SAB assay detected a decent number of additional HLAab to the ELISA with striking clinical relevance as these resulted in a significantly reduced allograft survival of 46%. The most favorable graft survival probability was observed among patients without any HLAab as detected by the SAB assay (90%). Therefore, ELISA is definitely not capable of detecting all relevant HLAab prior to transplantation. As Richter *et al.* [4] clearly show in their supplemental figures, ELISA revealed a pronounced deficiency to detect HLA-C and DQ antibodies. More strikingly, ELISA even failed to detect certain HLA-A and B specificities commonly assigned using the SAB assay. This is probably an expression of the difference in sensitivity and panel composition between the two assays.

Nowadays, it is well acknowledged that IgG HLAab as detected by SAB are not necessarily a contraindication for kidney transplantation but may indicate an increased immunological risk for rejection and allograft loss [5]. However, not all pretransplant donor-specific HLAab (DSA) have a negative impact, especially low-level antibodies [6,7]. In their article, Richter *et al.* [4] could demonstrate that preformed DSA as detected by SAB exceeding 3000 mean fluorescence intensity (MFI) units represent an independent risk factor for allograft survival. As known from several post-transplant studies also non-DSA (NDSA) revealed a negative impact on graft survival which could also be confirmed here in the pretransplant setting. Most probably, NDSA *per se* are an indicator for high responsiveness of the recipient's immune system to alloantigens. That means that, on the one hand, these patients might have developed immunological memory against HLA in the setting of transfusions, pregnancies, or previous transplants but DSA are currently undetectable. On the other hand, these patients are believed to be more prone to develop *de novo* DSA post-transplant with the potential to deteriorate the allograft which, unfortunately, could not be confirmed in this study as post-transplant HLAab monitoring was not performed. Without doubt, a significantly increased proportion of patients with SAB HLAab pretransplant showed histological lesions of the allograft associated with antibody-mediated rejections. Admittedly, testing one serum most proximate to transplantation takes only a snapshot of the alloimmunization status at that particular time point and it is hard to draw any causative conclusions from it. However, the SAB assay helps identifying patients at an increased immunological risk for subsequent unfavorable allograft function. Richter *et al.* [4] nicely elaborated that patients preimmunized with SAB HLAab could potentially benefit from well-matched kidney allografts. Especially, the importance of matching for HLA-DR was eminent. The most obvious explanation is the DR-DQ linkage disequilibrium usually leading to DQ match in case of a DR match. In this study, HLA-DQ antibodies have been underestimated by ELISA testing, and thus were not considered as unacceptable antigens for transplantation. It can be estimated that a substantial proportion of patients have been transplanted across a DSA against HLA-DQ. The authors also addressed the ongoing discussion on a clinically relevant MFI cutoff. The analysis revealed that there was a 100% negative predictive value for HLAab lower than 1000 MFI. However, the cutoff of 3000 MFI turned out to be robust and compromise best between negative and positive prediction for adverse allograft outcome. Pretransplant risk stratification always aims at guiding early post-transplant patient management. Richter *et al.* [4] could nicely demonstrate that ELISA HLAab revealed no decreased graft survival probability but patients received intensified immunosuppression with antithymocyte globulin, tacrolimus, mycophenolate mofetil, and steroids. Thus, it can be speculated that patients at risk with pretransplant HLAab as detected by SAB could benefit from intensified immunosuppressive regimens.

Despite the retrospective nature of this analysis on an admittedly small cohort, it is a well-elaborated study on the clinical relevance of pretransplant HLAab among kidney transplants performed across SAB DSA. This study adds new perspectives to the discussion on proper pretransplant immunological risk assessment using SAB assays.

Funding

The authors have declared no funding.

Conflict of interest

The authors have declared no conflicts of interest.

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