Detection of donor-specific antibodies using HLA-coated microspheres: another tool for kidney transplant risk stratification*

Eric M. Gibney¹, Linda R. Cagle², Brian Freed², Stephanie E. Warnell², Larry Chan³ and Alexander C. Wiseman³

¹Division of Nephrology, Hume-Lee Transplant Center, Virginia Commonwealth University, Richmond, VA, ²Division of Allergy and Clinical Immunology and ³Division of Renal Diseases and Hypertension, University of Colorado Health Sciences Center, Denver, CO, USA

Abstract

Background. Sensitive techniques are able to detect low levels of circulating antibodies. For many newer techniques, the clinical consequences of these antibodies are unknown. We hoped to determine the significance of antibodies detected through the use of Luminex® microsphere-based assay.

Methods. Patients who received kidney transplants between March 2003 and May 2004 with negative anti-human globulin-augmented complement-dependent cytotoxicity (AHG-CDC) crossmatches were retested for pre-transplant panel reactive antibodies (PRA) using Luminex microspheres and stored sera. Patients were considered to have circulating antibodies if either class I or class II Luminex PRA was ≥15%. These patients were then analysed for pre-transplant donor-specific antibodies (DSA). Clinical outcomes were compared in patients with and without DSAs.

Results. Out of 136 patients who underwent transplantation, 55 had Luminex PRA ≥15%. Of these 55 patients, only 16 had a standard PRA ≥30% and 75% had a history of a sensitizing event. Twenty out of 55 patients were DSA+. Patients with DSA detected by Luminex had higher rates of primary non-function (PNF), delayed graft function, biopsy-proven acute rejection, and lower rates of graft survival at 6 months. A combined endpoint of immunological and clinical events was far more common in patients with DSA.

Conclusion. The detection of DSAs by Luminex microspheres was associated with significantly higher rates of graft dysfunction and immunological events. Conversely, the presence of antibodies but no DSA by Luminex was associated with excellent outcomes. In patients with negative AHG-CDC crossmatches, the occurrence of low-level DSA by Luminex could assist in identifying patients that require more aggressive immune monitoring or immunosuppressive strategies.

Keywords: donor-specific antibodies; flow beads; flow cytometry crossmatch; HLA beads; kidney transplantation; luminex®

Introduction

Since Patel and Terasaki [1] first reported the relevance of the pre-transplant lymphocyte crossmatch to post-transplant outcomes in 1969, the available test options for crossmatching potential transplant recipients have grown dramatically. The anti-human globulin-augmented complement-dependent cytotoxicity (AHG-CDC) crossmatch improved upon the sensitivity of the standard CDC crossmatch and thus became the preferred crossmatch prior to kidney transplantation [2]. Similarly, flow cytometry crossmatch (FCXM) again improved the sensitivity of screening for anti-donor antibodies and has replaced the AHG-CDC assay as the crossmatch of choice at many centres. Vigorous debate has accompanied the adoption of the FCXM. While studies have shown worse clinical and immunological outcomes in those with positive FCXM, some investigators have argued that routine FCXM is ‘too sensitive’ for unsensitized recipients and will exclude potentially acceptable donor/recipient pairs [3–6].

Even while clinicians struggle with uncertainty regarding the appropriate use of these established technologies, new antibody detection techniques continue to evolve. In 1998, a novel testing strategy was described that uses purified human leucocyte antigen (HLA) molecules coated on microspheres [7]. These microspheres are also coated with fluorochromes that can be detected by flow-cytometry-based techniques. Thus, recipient sera can be ‘crossmatched’ with the HLA-coated microspheres. Because of the
characteristics of the assay, both the percent panel-reactive antibodies (PRA) and the anti-HLA-specificity of antibodies can be determined. While advantages such as sensitive antibody detection and the lack of a need for donor cells are attractive, immunochemistry laboratories and transplant personnel lack published guidance on the relevance of these antibodies [8–15]. Therefore, we attempted to determine the clinical significance of circulating antibodies detected using the Luminex® microsphere-based assay. Specifically, we hypothesized that in patients with a negative AHG-CDC crossmatch, detection of pre-transplant donor-specific antibodies using the Luminex® platform would identify a subset of patients with higher immunological risk.

Subjects and methods

Luminex PRA

Stored sera from consecutive transplants performed between March 2003 and May 2004, that had negative pre-transplant AHG-CDC crossmatch on separated lymphocytes, were retested for PRA using the Luminex platform in conjunction with reagents from Tepnel Lifecodes. For each patient sample, a total of 25 μl of serum was mixed between two wells, each containing either HLA class I or class II coated microspheres. Sera and microspheres were incubated for 30 min on a 96-well membrane filter plate. The specimens were then washed three times using a vacuum manifold system. A phycoerythrin (PE)-conjugated anti-human IgG was then added to each well and incubated for 30 min. All incubations were performed at room temperature, in the dark, on a rotating platform. The Lifematch Fluoroanalyzer was used for bead and data acquisition. This instrument is a minidigital processing flow analyser that incorporates two lasers. When the fluid stream passes through the lasers, the first beam classifies the beads by HLA type. The second beam scans each bead for PE-labelled anti-human IgG bound to the HLA molecules. Luminex PRA was considered positive if ≥15% binding occurred for either class I or class II microspheres. Patients with elevated Luminex PRA were then analysed for the presence of donor-specific antibodies.

Donor-specific antibodies

Raw data were collected by the Lifecodes Fluoroanalyzer and exported to the LifeMatch software for analysis. This software processes the raw data and interprets the resultant bead patterns by ranking the beads in order from positive to negative. The antigens associated with each bead were listed along with whether or not the bead was positive or negative. The software then suggests which alleles/antigens to use for the final assignment of specificity. The reports generated by the software were then analysed by experienced technologists and compared with known donor HLA to determine if donor-specific antigen is present. Donors were previously HLA typed by either serological and/or molecular methods.

Clinical outcomes

Two transplant nephrologists collected clinical outcome data on the 55 kidney transplant recipients with elevated Luminex PRA. One patient was excluded because of transplant at another centre (data unavailable). Outcomes of interest included primary non-function (PNF), delayed graft function (DGF), biopsy-proven acute rejection (BPAR), presumed acute rejection (AR), renal dysfunction requiring biopsy, vascular rejection, 6-month graft survival and 6-month patient survival. PNF was defined as dialysis dependence from the time of transplant to the last follow-up. DGF was defined as the need for dialysis in the first post-transplant week. We also assessed for a combined endpoint including PNF, DGF, BPAR, presumed AR, vascular rejection, renal dysfunction requiring biopsy and graft loss. The events making up the combined endpoint were chosen to capture patients who experienced a clinically complicated post-transplant course. Each event in the combined endpoint was binary; the combined endpoint was ‘positive’ if any of the individual events occurred.

Clinical outcomes were compared in patients with and without the presence of donor-specific antibody using the chi-square test. All analyses used SAS version 8.2 (Cary, NC). The Colorado Multiple Institution Review Board approved this study and a Health Insurance Portability and Accountability Act (HIPAA) waiver of authorization was obtained.

Results

Out of 136 kidney transplants performed over the study period, 55 patients with Luminex class I or class II PRA ≥15% were identified. Baseline characteristics of these patients are found in Table 1. Sixty-two percent were female, and the mean age was 49 years. Seventy-one percent were Caucasian, 18% Hispanic and 9% were African-American. Primary diseases, induction therapies and maintenance immunosuppression generally reflected the diseases and practice patterns at our institution. Importantly, a history of a sensitizing event was documented in 75% of the study patients, with 25% having a prior transplant and the other 50% having either a previous pregnancy or blood transfusion. Eight of the 14 without a history of sensitizing events were men. The lack of a documented sensitizing event had no obvious influence on outcome, as two rejections occurred in these 14 patients.

Twenty patients (36%) with pre-transplant DSAs were identified. The antibodies identified were: class I DSA only in four patients, class II DSA only in six patients, and both class I and class II DSA in 10 patients. While differences in baseline variables were not statistically significant between the groups with and without DSA, African-Americans appeared over-represented in the DSA group (4/20 vs 1/35). Previous transplant had occurred in three patients in the DSA group and in nine in the non-DSA group. Induction therapies and type of transplant (deceased vs living donor) were not different between DSA groups.

The clinical outcomes stratified by the presence of DSAs are found in Table 2. BPAR also occurred more
Causes of graft loss and patient deaths are listed in Table 3.

| Donor specific antibody (DSA+) | Graft loss (5) | Death with functioning graft (2) |
| Patient death (2) | Primary non-function: arterial thrombosis |
| No donor specific antibody (DSA−) | Graft loss (2) | Death with functioning graft (2) |
| Patient death (2) | Refractory vascular rejection |

Table 3. Causes of graft loss and patient deaths

There were five cases of graft loss in the DSA group. Causes included two deaths with functioning grafts, two episodes of PNF and one episode of refractory vascular rejection. In those without DSA, there were two deaths with functioning grafts. Patient survival and renal dysfunction requiring biopsy were not statistically different between groups. The combined outcome of clinical and immunological events was far more common in patients with DSA (60 vs 26%, \( P = 0.01 \)).

In the non-DSA group there was one cellular rejection (Ia). In the DSA group, there were two cellular rejections (Ia) and three vascular rejections (IIa, IIb and III). C4d staining was not routinely performed at our institution at the time of the study. The type of antibody appeared to have an influence on outcome. There were 10 patients with both class I and class II DSA (nine females, one male, one with previous transplant). In this group, there were four rejections (two vascular, two cellular), two graft losses due to PNF (thrombosis, vascular rejection), and two deaths (thrombotic microangiopathy, sudden death). Of the four patients with class I DSA only, there was one vascular rejection that led to graft loss. Patients with class II DSA only had very few clinical events: two cases of DGF occurred in deceased donor kidney transplants in patients with previous transplants, but none of the six patients with only class II antibodies developed AR, dysfunction requiring biopsy, graft loss or death. While the numbers of patients in each group are not great enough to make conclusions, the results may reflect the importance of anti-class I antibodies over anti-class II antibodies in predicting outcome.

### Discussion

For detection of anti-HLA antibodies, newer immunological techniques have outpaced clinical information on outcomes associated with these antibodies. Our study takes an important step at identifying higher risks associated with DSAs detected by a microsphere-based assay. Importantly, this study is the first to our knowledge that demonstrates the clinical utility of antibody detection by microsphere flow beads. Specifically, adverse outcomes related to sensitization,
such as AR, DGF, PNF and graft loss are likely to be more frequent in patients who produce even a small level of DSA, even with a negative AHG-CDC crossmatch. Perhaps just as importantly, our study appears to define a low-risk subgroup in patients with elevated flow-based PRA, a group that has previously been shown to have adverse outcomes [16]. In our study, elevated Luminex PRA in the absence of DSA was associated with a very low rate of AR (3%) and excellent 6-month patient and graft survival. Thus, Luminex PRA and analysis for DSA appears to be a valuable test to characterize clinical and immunological risks prior to transplant.

An important corollary to these results are the findings that, while the presence of DSAs clearly impact AR rates and graft survival, DSAs detected by flow cytometry-based methodology do not universally portend poor outcomes and should not be used unilaterally to discourage transplantation. In fact, given that the AR rate and 6-month graft survival rate within the DSA+ group were 25 and 75%, respectively, it is evident that many patients producing DSAs can have good short-term outcomes.

The strength of the current study was the ability to use stored sera to retest for DSAs. Thus, we had a relatively large group of patients available and clinical management was not influenced by the detection of DSA. Retrospective analysis of pre-transplant sera was extremely helpful, as this study is unlikely to be replicated prospectively. For example, when DSA is detected, other donors may be chosen or recipients may be treated (plasmapheresis, intravenous immune globulin, rituximab) to modify the antibody or lower immunological risk. Limitations of the study are the single-centre design, modest numbers of patients and the inability to compare and confirm the results with FCXM. This was attempted but was unsuccessful due to the lack of viable frozen donor cells. Also, it is possible that DSAs that were not well represented in the Luminex assay were present and were not identified.

Another question is the source of antibodies in the patients without documented sensitizing events. Since most were men, it is likely that they were either unreported transfusions or artifact. For women, it may have been undocumented transfusions, pregnancy terminations or artifact. However, outcomes in these patients were not different than those with documented sensitization. Finally, routine and reliable testing of biopsies for C4d was not uniform at our centre during the study period, but could have given additional information about the likelihood of antibody involvement in the pathogenesis of clinical events. While vascular rejections were likely humoral in origin, we may have identified additional humoral events with the addition of C4d testing.

An important question has still not been answered: how should transplant clinicians logically use the tests available to them for pre-transplant crossmatching? While there are many possible approaches, we believe that a reasonable approach would be to use a flow cytometry PRA (e.g. Luminex) to screen patients for circulating anti-HLA antibodies. If this test is negative (i.e. anti-HLA antibodies are not present), an AHG-CDC crossmatch can probably be performed. This may potentially eliminate many FCXMs and reduce false-positive tests. If the flow-based PRA is positive, FCXM and DSA testing (using Luminex or other assays) can be used to determine pre-transplant immunological risk. For the small number of patients for whom FCXM and DSA testing are discordant, the transplant community needs more information and prospective study, beyond current published anecdotes [12].

Conclusion

Detection of anti-HLA antibodies prior to kidney transplantation is an evolving science. Our study showed that patients with a negative AHG-CDC crossmatch can be further stratified into higher risk subgroups by testing for elevated PRA and DSA using the Luminex microsphere-based assay. Adverse immunological and clinical outcomes appear more common in those with low levels of DSA. Conversely, elevated Luminex PRA without DSA was associated with low rates of AR and excellent graft survival.

Conflict of interest statement. None declared.

References


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