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Special Feature

Calculated PRA (CPRA): The New Measure of Sensitization for Transplant Candidates

J. M. Cecka

UCLA Immunogenetics Center, Los Angeles, CA
Corresponding author: J. Michael Cecka, mcecka@ucla.edu

The ways we measure whether a patient is sensitized to HLA antigens and to what extent sensitization affects access to transplantation have changed remarkably during the past decade. What we mean by sensitized and broadly sensitized today is heavily dependent upon the sensitivity of the test that is used to measure antibodies. Because we provide additional allocation points for broadly sensitized patients in the United States kidney allocation system in an effort to compensate for their biological disadvantage, some consistency and accountability are required. The calculated panel-reactive antibody, which provides an estimate of the percentage of deceased organ donors that will be crossmatch incompatible for a candidate provides both consistency and accountability.

Key words: Access to transplantation, anti-HLA antibodies, crossmatching, donor-specific antibodies, histocompatibility, PRA

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Sensitization remains a formidable barrier to transplantation. Patients who have preformed antibodies against HLA antigens are at risk for hyperacute rejection, accelerated acute rejection, antibody-mediated rejection, delayed graft function and longer term complications when transplanted from a donor expressing the target HLA antigens. We avoid donor-specific antibodies by crossmatching all potential donors and recipients before transplantation, and as a result, sensitized transplant candidates have limited access to transplantation in proportion to how broadly their anti-HLA antibodies react with the potential donor population. In the case of renal transplant candidates, those who are broadly sensitized (80+% panel-reactive antibody; PRA) received additional points in the UNOS allocation system to compensate for their biological disadvantage. This is a strategy that did not work very effectively because over time, the most broadly sensitized patients with a combination of long accumulated waiting time and four additional sensitization points appeared in the same order at the top of the match run for each blood group compatible donor. Although preliminary crossmatches eliminated most of these patients from consideration, many laboratories used a more sensitive test for their final than their preliminary crossmatch and positive final crossmatches were common among the broadly sensitized patients. To facilitate timely placement of organs, a limited number of broadly sensitized patients would be crossmatched and those patients would be the most likely to be crossmatch incompatible. UNOS implemented a new strategy on October 1, 2009, using unacceptable HLA antigens and a calculated PRA (CPRA) to award sensitization points that fundamentally changes how sensitized renal candidates are ranked for kidney offers.

PRA has been the measure of sensitization since the recognition that catastrophic hyperacute rejection was associated with anti-donor HLA antibodies in the mid-1960s (1). This landmark paper by Patel and Terasaki also described a simple surrogate test that could identify sensitized patients and estimate their likelihood of finding a crossmatch-compatible donor using a panel of normal blood donors as representative of the potential local organ donor pool. PRA was simply the percentage of this pool of donors to which a patient had reactive antibodies. A patient with 80% PRA would be crossmatch incompatible with 80% of donors.

The crossmatch tests used today often are more sensitive than those that were used in the past. Even more importantly, the technologies available for identifying and measuring anti-HLA antibodies have undergone remarkable changes in the past decade and particularly in the past 5 years since the introduction of solid-phase tests using single HLA antigens produced by recombinant DNA technologies. The diversity of HLA antibody tests being performed by HLA laboratories has increased as these newer, more sensitive and more precise technologies have become available. Lacking organized guidelines for laboratories to indicate which PRA should be reported, many labs and transplant centers chose the highest PRA value among their test platforms because broadly sensitized patients receive four extra points. With diverse test platforms and sensitivities now many fold higher than previous lymphocytotoxicity tests permitted (2), sensitization estimates can

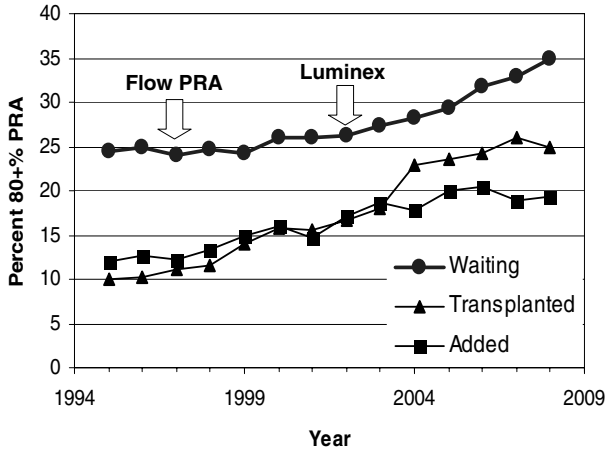

Figure 1: Escalating sensitization levels and correlation with more sensitive test platforms. The percentage of sensitized patients who had 80+% PRA added to the UNOS waitlist, remaining on the waitlist at the end of each year and transplanted each year increased because the introduction of microparticle solid-phase tests using purified or recombinant HLA antigens, most notably following the introduction of Luminex technology for HLA antibody identification in 2002 (based on OPTN waitlist data as of July 3, 2009 and OPTN recipient histocompatibility data as of July 3, 2009).

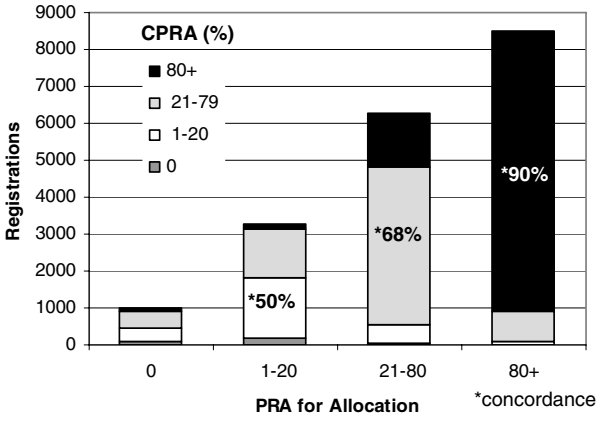

Figure 2: Correlation between PRA and CPRA. Among 19,046 active registrations on the UNOS Kidney waiting list with a CPRA value, this figure shows the distribution of CPRA values calculated within each PRA group. In the group with 1–20% PRA, 50% also had 1–20% CPRA. Concordance was 68% for the 21–79% group and 90% for the 80+% PRA group. In the lower PRA groups, CPRA tended to be higher, whereas some patients in the 80+% PRA group did not have sufficient unacceptable antigens reported to warrant this CPRA level. CPRA was rounded to zero when only unacceptable antigens with a frequency less that 1% were listed (based on OPTN data as of June 12, 2009 and reported to the UNOS Histocompatibility Committee at its July 15 meeting).

vary widely depending upon the method used to identify antibodies.

Figure 1 shows that levels of sensitization reported to UNOS have escalated during the past 15 years. The percentage of sensitized patients with 80+% PRA who were added to the UNOS waitlist each year, who were on the waitlist at the end of each year or who were transplanted each year all increased by about 10% during this period. There was a clear rise in the percentage of broadly sensitized waitlist candidates and transplant recipients beginning in 2002, the year when solid-phase antibody tests using purified HLA antigens on the luminex platform were introduced. Although some centers may have become more adroit at transplanting their broadly sensitized patients through desensitization or transplantation in the face of a positive crossmatch (3–5), it seems more likely that the 2002 introduction and widespread use of solid-phase tests was a contributing factor in escalating PRA levels.

The UNOS Histocompatibility Committee crafted a proposal to bring some accountability to PRA reporting and, at the same time, to take advantage of the new and evolving technologies. The calculated CPRA is based upon unacceptable HLA antigens to which the patient has been sensitized and which, if present in a donor, would represent an unacceptable risk for the candidate or the transplant program. The CPRA is computed from HLA antigen frequencies among approximately 12,000 kidney donors in the United States between 2003 and 2005 and thus represents the percentage of actual organ donors that express one or more of those unacceptable HLA antigens. What adds accountability is that entering an unacceptable antigen for a patient means that kidneys from donors expressing that antigen will not be offered for that patient. The higher the CPRA, the fewer offers would be received. The proposal was approved by the UNOS Board of Directors and the initial phase was implemented in December 2007. During the first phase, the CPRA value appeared on the UNET waitlist form together with the traditional peak and current PRA values determined by the laboratories. At least one unacceptable antigen had to be entered for a patient to receive PRA points based on the traditional PRA. In the second phase, which began on October 1, 2009, the CPRA replaced peak and current PRA and sensitization points are now awarded based upon the CPRA.

The UNOS Histocompatibility Committee monitored the first phase of CPRA during the past year and noted rapid acceptance. By March 2009, only 13 of the 256 U.S. kidney transplant programs—most small—had not entered unacceptable antigens for any of their patients. Figure 2 shows a comparison between the PRA value programs had designated for use in allocation with the patient's CPRA. Concordance was high, with 90% of active renal candidates with a PRA 80% or higher having a CPRA in the same range. At the time of the analysis, about 12% of candidates who would have received points for 80+% PRA would not have gotten any points based upon their CPRA. The actual

number of patients at risk of losing sensitization points was presented during Fall 2009 at each of the UNOS Regional meetings (tailored for each Region) to increase awareness and to encourage communication between transplant programs and their laboratories on the assignment of unacceptable HLA antigens. It may be that these patients had an inflated PRA that could not be justified based on the frequencies of the antigens to which they were sensitized. On the other hand, nearly 20% of active candidates whose PRA was 21–79% would receive points based on their CPRA. In fact, concordance was generally lower among the lower PRA groups due to underestimation of these patients' sensitization levels using traditional PRA. CPRA provides a more accurate estimate of sensitization because it includes both class I and class II HLA specificities in the calculation, a major departure from traditional PRA, where class I and class II specificities are measured separately. Even B-cell panels, which express both class I and class II antigens are generally constructed to cover the class II HLA antigens and are not representative of HLA distributions in the general population.

The new accountability built into the CPRA calculation requires a change in how we regard sensitization. A high traditional PRA value meant a high probability of a positive crossmatch, but because CPRA is based on unacceptable antigens that will prevent offers from those donors to which the patient is most highly sensitized, an offer for a patient with a high CPRA value should mean a high probability of a negative crossmatch. Previously, the same highly sensitized patients would appear at the top of each match run for donors with their blood group, and OPOs and centers were reluctant to set up final crossmatches for more than a few highly sensitized patients for fear of not placing the kidneys. The order of sensitized patients on the match run now is dictated by the donor's HLA type and different sensitized patients will be ranked first for different donors, increasing their chances for a transplant. Indeed, several programs have reported a higher rate of transplantation for sensitized patients using the 'virtual' preliminary crossmatch (6–8). OPOs and centers that avoid broadly sensitized patients should abandon the practice of limiting final crossmatches for sensitized patients.

Defining unacceptable antigens was left to the transplant programs and their laboratories. Some programs may be more aggressive and willing to assume the risks associated with donor-specific HLA antibodies, while others may not have the experience or resources to provide the more intensive and aggressive treatments for these patients. Communication between histocompatibility laboratories and the transplant programs they serve is a critical element for the success of this new method for assessing sensitization levels.

The lack of standards for identifying anti-HLA antibodies and defining unacceptable antigens initially raised some concerns. A meeting between Laboratory Directors and Transplant Physicians was held in Chicago in March 2008 to identify the problems of using solid-phase testing and to develop some solutions. There was general agreement that laboratories were quite good at defining strong antibodies—evidence from proficiency testing revealed excellent concordance among laboratories in identifying specific antibodies, even in complex antisera, that were present in high quantities. There was less agreement among laboratories for weaker antibodies and a major issue was a lack of data on the clinical relevance of antibodies that could only be detected by the very sensitive solid-phase tests. The participants identified several strategies to improve and monitor uniformity in solid-phase antibody testing that have been or are about to be implemented, including comparisons of raw test results as well as interpretations among several laboratories using the same test specimens and collection of raw data by providers of proficiency testing in which all accredited laboratories must participate. Many laboratories had already begun to correlate the solid-phase test results with their crossmatch results and were able to eliminate their preliminary crossmatch tests with a 'virtual' preliminary crossmatch based on antibody strength and specificity to define what was unacceptable. Since that meeting, a number of laboratories have reported more detailed strategies to define unacceptable antigens and to avoid predictably positive crossmatches (9–14). Weak anti-HLA antibodies appear to have little clinical importance (15–17). Some have suggested that it is important that laboratories use multiple tests on different platforms when initially assigning unacceptable antigens to reduce the potential pitfalls of anomalous results from a single test (10,14). The solid phase tests have very fine sensitivity and not every antibody that can be detected is important. Although listing every HLA antigen to which a patient has detectable antibody as unacceptable will eliminate positive crossmatches, it may also eliminate all potential donor offers. The best strategy may be to begin predicting very strong positive crossmatches and tighten the thresholds to reduce the incidence of unacceptable crossmatches.

There are some remaining problems with predicting crossmatches based upon antibody strength and specificities. We do not know whether multiple weak antibodies may have additive or synergystic effects on crossmatches or transplants. The single HLA antigen solid-phase tests also allow for detection of antibodies that react with antigens that are not always typed in deceased donors. Antibodies to the HLA-Cw, the DQ alpha chain and DP antigens may prevent accurate crossmatch prediction (18) for some patients. Extensive data on the role of these antibody specificities in transplantation are not available yet, but the antibodies may cause positive crossmatches when the target antigen is present in the donor (19,20). These antigens do not contribute to the CPRA calculation at present, because too few donors had been typed for these antigens to estimate their frequencies. The C-locus antigens are being revisited in the current update of frequency tables, but

donors typed for DQ alpha chains and DP antigens are still extremely rare. Allele-specific antibodies are sometimes detected in the single antigen tests, which may cause problems because donor HLA alleles are rarely typed and because when an antibody detected against an allele of the patient's own HLA antigen is detected, it cannot be listed as unacceptable.

Despite these limitations, laboratories can accurately predict many incompatible crossmatches, streamlining allocation and providing more options for broadly sensitized patients. Like PRA, CPRA is a tool for characterizing and monitoring sensitization. Unlike PRA, the CPRA provides a meaningful estimate of transplantability for most patients, because it is calculated from unacceptable HLA antigens that will preclude offers from predictably crossmatch incompatible donors.

The change to CPRA represents a paradigm shift in many ways. Although the concepts of unacceptable antigens and virtual crossmatches have been with us for many years, their widespread use and formal incorporation into the system for kidney allocation in the United States is unprecedented. As our ability to predict crossmatches and compatibility of donors for sensitized patients improves, it should be possible to encourage wider geographical sharing of deceased donor kidneys for this disadvantaged group beyond the current practice of sharing zero-HLA mismatched kidneys for sensitized patients. Accurate crossmatch prediction is a critical aspect of paired living-donor kidney exchanges as these increasingly involve patients and donors at different transplant centers. Finally, although the current focus is on their role in renal transplant allocation, unacceptable antigens and CPRA are also important tools for many thoracic programs that have begun using virtual crossmatches for distant donors to broaden the opportunities for their sensitized patients.

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