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Tailoring Antibody Testing and How to Use it in the Calculated Panel Reactive Antibody Era: The Northwestern University Experience

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Abstract

Background—Patients with human leukocyte antigen antibodies constitute a significantly disadvantaged population among those awaiting renal transplantation. We speculated that more understanding of the patients' antibody makeup would allow a more "immunologic" evaluation of crossmatch data, facilitate the use of virtual crossmatch (XM), and lead to more transplantability of these patients.

Methods—We retrospectively compared the transplantability and transplant outcome of two consecutive patient populations transplanted in our center. Group I (n=374) was evaluated using solid-phase base testing for determination of percentage panel reactive antibody ("PRA screen") with limited antibody identification testing. Group II (n=333) was tested in a more comprehensive manner with major emphasis on antibody identification, antibody strength assignment, and the use of pronase for crossmatch.

Results—Given this approach, 49% (166/333) of the transplanted patients in group II were sensitized compared with 40% (150/374) of the recipients in group I; $P=0.012$. Transplant outcome at 1-year posttransplant was similar in both groups.

Conclusions—We conclude that comprehensive evaluation of human leukocyte antigen sensitization and application of immunologic in analyzing compatibility between donor and recipient can increase the transplantability of sensitized patients while maintaining similar outcome. Our approach is in line with United Network for Organ Sharing new guidelines for calculated panel reactive antibody and virtual XM analysis. We hope this report will prompt additional transplant programs to consider how they will use the new United Network for Organ Sharing algorithms.

Keywords

Highly-sensitized; CPRA; Transplantability

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Patients with human leukocyte antigen (HLA)-antibodies constitute a significantly disadvantaged population among those awaiting renal transplantation. According to the Organ Procurement and Transplant Network (OPTN)/Scientific Registry of Transplant Recipients (SRTR) report for 2006 (1), noncensored median time to transplant (TT) for all patients registered in the New Kidney Waiting List was 1198 days for patients registered during the year 2000 (we chose the year 2000 because median TT could not be calculated for sensitized patients registered later than that). During that year, nonsensitized patients had a median TT of 1118 days, that is, 3.06 years; patients with moderate sensitization—panel reactive antibody (PRA) levels of 10% to 79%—had a median TT of 1753, that is, 4.8 years; while the median TT for sensitized patients, with PRA more than 80%, could not be calculated, but certainly had a median TT of more than 6 years.

According to the 2006 OPTN/SRTR report (1) of all nonextended criteria deceased donors (DD), 22.1% of organs were transplanted in moderately sensitized patients and 12.6% were transplanted in highly sensitized patients. The balance of 65.3% was transplanted in nonsensitized patients. Highly sensitized patients are significantly disadvantaged also in their access to living donation. Among patients receiving living donor kidneys, an even greater percent of kidneys were transplanted into nonsensitized patients. Only 16% of the living kidneys were transplanted into the moderately sensitized group and a mere 5% were transplanted into highly sensitized patients.

In recognition of the above, sensitized patients with PRA more than 80% were awarded 4 points by the United Network for Organ Sharing (UNOS) renal allocation system, to increase their access to potentially compatible organs. During the last couple of years, though, with the introduction of solid phase based testing and the more sensitivity provided by these methods, the Kidney and Pancreas Transplantation Committee became concerned about the variability in testing methods and the UNOS Histocompatibility Committee was charged with reevaluating the PRA listing practices. The result of this investigation identified several problems with the then-existing system. Two main recommendations were put forth: (A) to use a system in which %PRA is calculated based on the HLA-antigen distribution of historic donors listed in the UNOS database; and (B) antibody testing should be performed using solid-phase based methodology. Importantly, the UNOS calculated PRA, known as calculated panel reactive antibody (CPRA), is based on calculating only those unacceptable HLA specificities entered for the individual patient in UNET (United Network for Organ Sharing secured internet-based transplant information database) (2, 3). The Histocompatibility Committee further recommended that each center should choose their own criteria to define which HLA antigens are to be considered as unacceptable based on their own contraindication for transplantation for each candidate.

Our center has been using solid-phase based methods to determine %PRA since the year 2000. Similar to concerns raised by UNOS members, we debated the role of more accurate antibody definition in interpreting crossmatch results and its affect on the center's transplantation practices. In mid 2004, in-depth antibody evaluation was added, tailoring the depth of work-up based on the patient's status and the prospect of living versus deceased donation. Antibody specificities were recorded as weak, moderate, and strong. More than 50% of transplanted kidneys in our center are from living donors, providing our team the

advantage of extra time to perform any required additional testing. Deceased donors, by contrast, undergo crossmatch at a central Organ Procurement Organization laboratory. The wait-list of our center is comprised of more sensitized patients compared with the mean presented on the national list (42.7% locally compared with 34.4% nationally [1]). We postulated that using more sophisticated immunologic approach to the crossmatch analysis was likely to increase the transplantability of our sensitized patients. We also proposed, based on experience published by other centers, that low-level donor-specific antibodies (DSA) could be overcome with the addition of pretransplant plasmapheresis or IVIg cycles, rituxan, and prolonged or delayed tapering of steroids compared with other, nonsensitized patients, transplanted at our center.

In retrospect, some of the procedures adopted by our center were similar to those later recommended by the UNOS Histocompatibility Committee. The present report details the algorithm chosen by our center to tailor antibody testing for specific patients awaiting renal transplantation. It also depicts how increased accuracy in detecting the specificity of HLA-directed antibodies can guide using the virtual crossmatch, promoting more efficient utilization of donor kidneys for sensitized recipients.

METHODS

Patients

Two patient populations are compared for this analysis. Group I consists of 374 consecutive patients transplanted between July 2002 and March 2004. Group II consists of 333 consecutive patients transplanted between July 2004 and March 2006. Overall, 707 patients are included in the “transplantability” evaluation. The two groups share similar demographic characteristics (Table 1). The differences between these two time periods are the approaches taken for identifying HLA-antibodies and for crossmatch analyses (see later).

Immunosuppression

Induction therapy for both patient groups entailed the use of alemtuzumab (Campath-1H, Genzyme Corp. Cambridge, MA) as a single dose of 30 mg over 2 hr intraoperatively through a peripheral intravenous catheter. Maintenance immunosuppression for all recipients included a 3-day course of corticosteroids (methylprednisolone: 500 mg in the operating room, 250 mg posttransplant day 1, and 125 mg post-transplant day 2). Oral prednisone was not used. Recipients also received tacrolimus (Prograf, Fujisawa Pharmaceuticals, Deerfield, IL) dosing adjusted to achieve 12-hr blood concentrations of approximately 6 to 8 ng/mL by immunoassay (IMX, Abbott Labs, IL) and mycophenolate mofetil (CellCept, Roche, Nutley, NJ), at a dose of 2000 to 2500 mg/day. This protocol was used for both patients' groups.

Histocompatibility Evaluation—Group I

Patients' sera was evaluated for the presence of HLA-antibodies using the FlowPRA Screening (Flow-Sc; an assay that provides information regarding the “breadth” of sensitization—%PRA—distinguishing between HLA class I versus HLA class II sensitization but does not identify the exact specificity of the antibodies within these two

groups) with minimal additional work-up using the FlowPRA Specificity (Flow-SP; a solid phase based test in which multiple HLA class I or class II antigens are attached to a single bead, similar to those present on a single cell. The assignment of antibody specificity is possible although not always readily determined, especially for the highly sensitized patients) assays (One Lambda, Inc., Canoga Park, CA) after the manufacturer's recommendations. Three-color flow cytometric crossmatch (FCXM) were always performed prospectively, after previously established methodologies (4). The decision to transplant was based mainly on the presentation of a negative T- and B-cell FCXM.

Histocompatibility Evaluation—Group II

Patients' sera were tested for the presence of HLA-antibodies using a comprehensive approach using solid-phase based testing, mainly the flow beads. Initial screen was performed using the Flow-Sc assay, followed by the Flow-SP and the FlowPRA Single Antigen (Flow-SA; in this assay each of the solid phase beads are coated with only one HLA antigen, for example HLA-B7, such that the amount of molecules of a specific HLA antigens available for binding the antibody is much larger, hence increasing the sensitivity of the detection) class I and II assays (One Lambda, Inc.) after the manufacturer's recommendations. If needed, additional testing was performed using the Lifecode ID (Tepnel, Inc., Stamford, CT) assays. The key point was to perform judicious, comprehensive, and cost-effective assessment of the patient's antibody profile. Antibody strength assessment was also performed based on fluorescent intensity of responses in relation to the negative control human serum provided with the kits. Specifically, initial studies correlated changes in individual bead fluorescence with actual titration (dilution) studies. In principle, the stronger the titer is, the more fluorescent the bead shift will be. However, because of inherent color compensation values of the flow cytometer, simple channel shift data are not sufficient for direct titration analysis. Additional means to evaluate antibody strength include molecular equivalent of soluble fluorescence or median fluorescent intensity. In our center, the determination of weak, moderate, strong antibodies was also based on correlation with FCXM obtained using the specific serum sample with a known cell carrying the relevant (positive) HLA antigens. It is important to state that each center should determine their own cutoff values for antibody strength based on the clinical practice and previous transplant outcome data in correlation to antibody titers.

Three-color FCXM were performed with the addition of pronase treatment of cells before use in assay, to minimize background fluorescence as previously described (5). Pronase is a proteolytic enzyme that cleaves Fc receptors from the cell membrane and minimizes the nonspecific binding of antibodies. It was added to routine crossmatch assays, improving especially the sensitivity and specificity of the B cell in flow cytometry crossmatch (5).

The decision whether the organ is immunologically compatible for transplant was based in most cases on virtual crossmatch, given the donors' HLA type and the recipients' comprehensive antibody analysis. At times, additional antibody evaluation was performed immediately before final decision to provide all necessary information for the virtual crossmatch.

Statistical Analysis

Fisher's exact test was used to compare categorical variables between the two groups and the independent sample *t* test was used to compare continuous variables.

RESULTS

Antibody Evaluation Flow Chart

Figure 1 details the decision flow chart regarding antibody work-up as patients' sera are evaluated. Patients were deemed nonsensitized if at least two consecutive serum samples, usually obtained 1 to 2 months apart, were tested using Flow Screen and no antibodies were detected. Reactivity on the Flow Screen assay, that is more than 0%PRA, was evaluated for specificity using the HLA class I or class II antibody Flow-SP kits, based on the initial screen results. In patients with relatively low levels of PRA, especially for class I antibodies, the Flow-SP assay was usually able to provide clear determination of the antibodies present. Using an in-house database of HLA-antigen relative frequencies (or more currently using the CPRA calculator by UNOS) %PRA detected by the Flow-Screen assay was compared with the %PRA calculated by the CPRA (or the in-house database) based on the specificities identified by the Flow-SP assay. If the calculated %PRA was lower than the Flow-Screen %PRA, it indicated that additional specificities, not identified by the Flow-SP, might contributed to the Flow-Screen %PRA and that additional testing need to be performed to identify these yet undetected specificities.

Most of the patients that were moderate to highly sensitized (PRA ≥ 50%) required additional testing. Because the Flow-SP was not able to provide a clear definition of the specificities present, or that the few specificities identified were not enough to explain the %PRA detected by the Flow-Screen assay. Additional testing using the Flow-SA assays most often indeed identified additional specificities. A few patients, for example those with allele level antibodies or those with antibodies against rare specificities, required additional work-up that included the use of different vendor reagents or additional reagents for the Flow assay.

Case Example

A 51-year-old black female renal patient (HLA A34, A66, B13, B58, DR8, DR13; Flow-Screen-PRA class I – 75% and class II – 16%) was evaluated for the antibody identification. Figure 2 illustrates the five positive responses observed in the Flow-SP assay. Four of these were against HLA-DR11. The fifth was against a bead coated with HLA-DR8, DR17, DRw52, DQ2, DQ6. Because no additional responses against DR17, DRw52, DQ2, or DQ6 were observed on any of the other beads, it is unlikely that they were the cause of the positive response. A Flow-SA assay was run to decipher the fifth response, confirming reactivity against DR11, but no other positive responses were observed to explain the fifth response. A closer look at the high-resolution typing of the antigens attached to the positive bead showed that this specific DR8 allele is DRB1*0803 whereas the other three DR8 coated beads that were part of the Flow-SP assay (and were negative) are DRB1*0801 or DRB1*0802 allele. The DR8 allele used in the Flow-SA kit (showed no reactivity) was also DRB1*0801. The patient was therefore high resolution typed—DRB1*0801 and the

antibody reactivity was defined as specific for an epitope carried by the DRB1*0803 allele. Interestingly, a later FCXM with a “6-antigen matched” deceased kidney donor resulted in a negative T-cell XM but a positive B-cell XM. Given the listing antibody history of this recipient, we passed on the organ offer. Retrospective high resolution typing confirmed that the donor indeed carried the DRB1*0803 allele, making the FCXM result a truly indicative of a DSA.

For patients with a potential live donor, the evaluation was tailored specifically to the presence or absence of DSA, and did not concentrate necessarily on identifying all HLA antibodies. If the patient was not sensitized, and the FCXM was negative, no additional work-up was required. If the patient was sensitized, initial testing were performed as for patients on the waiting list, but then additional testing concentrated on identifying potential DSA. More details are given in the Transplant Decision Flow Chart section (Fig. 3).

HLA Antibody Makeup

For this analysis, we evaluated a cross-section of our active transplant list at a fixed time point during early 2007 because these patients were routinely evaluated using the Flow-SP and Flow-SA assays. A total of 1476 patients were included in this analysis. Of those, 42.7% of patients (630/1476) had HLA-antibodies divided almost equally between the HLA-class I only group (44%) and both HLA-class I and class II group (42%). Less than 14% of the sensitized patients expressed HLA antibodies directed only against HLA-class II antigens. Similar composition of sensitized patients have been observed when antibody data was analyzed for previous years (range of sensitized patients 41% to 43%).

Among the patients who had HLA-class I antibodies, 73% carried antibodies to HLA-A antigens and 75% had antibodies to HLA-B antigens. Antibodies to HLA-Cw antigens were not tested routinely and therefore are not summarized here. Among patients who had HLA-class II antibodies, 84% expressed antibodies directed against HLA-DRB1 and 54% against HLA-DRB3/4/5. More than half (59%) had antibodies against HLA-DQ, and about a third (32%) had antibodies against HLA-DP antigens.

Determination of Antibody Strength

For antibody evaluation run by flow cytometry, the strength of antibodies was estimated based on the channel shift of a specific bead compared with the appropriate control for that bead (normal human serum). Antibody strength was arbitrarily categorized into three groups—weak, moderate, and strong (see Fig. 4, for one example)—based on correlations with actual titration studies.

For living donor recipients, when specific DSA were evaluated, antibody strength was determined by doubling-dilutions. A major concern in our center was the ability to follow-up on antibody strength through desensitization protocols and posttransplantation. Because our center uses Campath as induction therapy, cell-based assays cannot be performed reliably during the first few weeks posttransplant. The use of solid-phase based testing allowed for comparison between the pre- and post-transplant data regardless of the treatment the patient received.

Transplant Decision Flow Chart

Figure 3 represents the decision making tree as had been followed at our center for patients in group II and is now the main stay for decision making.

For unsensitized patients, that is, patients who were recently tested using solid phase assays as having 0% PRA, a result other than negative XM should be suspected to be false positive, or not because of HLA antibodies. A repeat FCXM in such events usually came up negative, indicating technical problems in the first assay (especially if pronase was not used appropriately).

For sensitized patients, interpretation of the FCXM requires thorough evaluation of the potential presence of DSA. For example, a patient having class I antibodies with a positive T- and B-cell FCXM result but with no DSA to donor HLA-A or HLA-B antigen would be tested for the presence of antibodies to HLA-Cw antigens. Similarly, a patient having class II antibodies with a positive B-cell FCXM result but no DSA to donor HLA-DR or HLA-DQ antigens would be tested for the presence of antibodies to HLA-DP antigens. Our center was privileged in having the additional time required for such testing because more than 50% of our patients receive kidneys from living donors.

Once the presence of DSA was determined, the strength of the DSA and the number of different DSA present became a factor. Low levels DSA, if only against one or two antigens, usually did not result in contraindication to transplantation. These patients were treated according to our center protocols with rituxan, PP and IVIg pretransplant, and changes in antibody strength were monitored to determine whether additional intervention is required. Strong DSA to one donor antigen or weak to moderate DSA to multiple donor antigens were interpreted as contraindication to transplantation. The exact distinction between low, moderate, and strong antibodies should be center-specific based on correlation with FCXM results but moreover with patient outcome (specifically increased antibody levels posttransplant and antibody-mediated rejection).

Patients' Characteristics

Table 1 summarizes the demographic information regarding the two patient populations including gender, racial background, age at time of transplant, number of previous pregnancies, and number of previous transplants. Other than transplanting somewhat more black patients in the group II ($P=0.07$, NS) both groups are similar. The percent of recipients of DD organs was similar to that of recipients of living donors in both groups (70 or 18.7% of patients in Group I received DD compared with 69 or 20.7% of patients in group II, $P=0.51$). Among the sensitized patients in each group, the median % PRA as determined by Flow Screen for class I and class II was similar: 33% vs. 35% for class I and 30% vs. 31.5% for class II in groups I and II, respectively. Thus, similar to the other demographic characteristics, no significant differences in level of pretransplant sensitization were observed between the two groups, both for class I and class II. For comparison purposes, the overall proportion of sensitized patients awaiting renal transplantation on our "active list", as determined by Flow-Sc (%PRA), was approximately 42% for the first era and 43% for the second era.

Transplantability of Sensitized Patients

Increasing our ability to define the specificity of HLA-directed antibodies for patients in group II led to increasing the proportion of sensitized patients being transplanted during that period. Although the %PRA was determined using identical method—FlowPRA screening—for both time periods, and the level of sensitization was similar overall between the groups, patients in both groups differ by the methods used to evaluate the specificity of these antibodies. When analyzed retrospectively, it was clear that more of the patients in group II were transplantable. Specifically, among the 374 transplanted patients from group I, only 40% (150/374) had HLA-antibodies compared with 49% (163/333) of the transplanted patients in group II ($P=0.012$; Fig. 5). Similarly, analyzing for the number of patients with HLA class I antibodies, a significantly higher proportion of these patients were transplanted in group II, that is, 33% (125/374) in group I compared with 43% (142/333) in group II ($P<0.013$). No significant difference was observed in our ability to transplant patients with HLA class II directed antibodies (93/374 vs. 94/333, respectively; $P=0.35$).

Transplant Outcome for Sensitized Patients

Humoral and cellular rejection episodes were recorded according to the latest BANFF criteria. No major differences in immunosuppression protocols were noted between the two time periods. Humoral rejection episodes were observed in 2% of group I patients (3/150) and in 2.5% of patients from group II (4/163; $P=0.99$; NS). Cellular rejection was observed in 10 patients from group I (6.7%) and in 10 patients from group II (6.1%; $P=0.99$; NS). Graft loss occurred in 10.7% of patients in group I (16/150) compared with 6.7% (11/163) in group II ($P=0.31$; NS). Overall patient survival was similar in both time frames, being 6.0% (9/150) and 5.5% (9/163), respectively, $P=0.99$; NS). Serum creatinine levels were similar at 1 month (1.46 mg/dL and 1.44 mg/dL for patients in groups I and II, respectively) and 1 year posttransplant (1.65 mg/dL and 1.7 mg/dL, respectively). The number of patients with infection events requiring admission to the hospital was 12.9% (21/163) in group I compared with group II (18.7%, 28/150), $P=0.17$. The number of infection events was 42 in each group.

DISCUSSION

The development of solid-phase based assays that use solubilized HLA antigens as targets have greatly increased the ability to detect and identify HLA-specific antibodies (6–8). Using these newer solid phase based techniques, several centers have reported different algorithms to improve the prediction of a negative final crossmatch and therefore to better organ allocation. Bray et al. (9) reported on “the Emory algorithm” by which a comparable 5-year graft survival was achieved for their unsensitized, moderately sensitized (PRA <30%) and highly sensitized (referred to as PRA more than 30%) patients—70%, 69%, and 66%, respectively. Other groups have used virtual crossmatch results to stratify risk and modify immunosuppression protocols (10); the HLA-matchmaker to identify suitable crossmatch negative donors (11); virtual crossmatch for heart transplantation in pediatric candidates (12); or luminex analysis to predict negative cross-match results (13).

We report here on our center's experience with risk stratification of patients based on antibody work-up and on a virtual XM. As indicated in the Transplant Decision Flow Chart, patients were transplanted across a positive cross-match if the absence of DSA was proven. In fact, the ability to identify false-positive FCXM was a significant contributor to our ability to transplant more sensitized patients. These patients had a gratifying graft outcome. Another portion of the sensitized patients were transplanted across low levels of DSA, with modified immunosuppression and frequent monitoring of changes in DSA levels that dictated additional changes in immunosuppression as prophylaxis. These also had an excellent graft outcome. It is conceivable that during the first era patients were transplanted across a low levels DSA in the face of a negative FCXM, however, because this fact was not known to the transplant team at the time of transplant, no monitoring took place and these patients went on to develop antibody-related complications early posttransplant. Using this approach, we were able to increase the likelihood of identifying an immunologically compatible kidney donor, leading to more sensitized patients being transplanted in group II ($P=0.012$) while maintaining similar 1-year patient and graft outcome results.

A significant question nowadays in our field is the role of low-level antibodies. Can we consider "weak" antibodies (cytotoxic XM negative, FCXM positive crossmatch) as no risk to transplant and only "strong" antibodies (cytotoxic XM positive) should remain a contraindication? It has been shown before that even low levels of antibodies are markers for the presence of memory B and plasma cells that are not naïve to the potential donor HLA type. These cells, once re-exposed to the sensitizing agent will quickly mount a humoral immune response. Karpinski et al. (14) have shown that the presence of low level HLA class I-DSA, as detected only by flow crossmatch led to significant acute antibody mediated rejection. Mizutani et al. (15) reported similar data regarding the presence of HLA class II-DSA detected by FCXM. Similar results were reported by Vasilescu et al. (16), Pollinger et al. (17) and others. In our center, the knowledge of the pretransplant DSA strength dictates the use of prophylactic protocols (rituximab, IVIg, PP) to control B lineage-cell responses and frequent monitoring for DSA levels posttransplant.

As of the beginning of 2008, transplant centers are also facing the question of which HLA specificities should be listed as unacceptable antigens. Because only the unacceptable antigens are considered in the determination of CPRA, there is an incentive to list as many unacceptable antigens—to increase the likelihood of a patient to receive additional points for being highly sensitized (CPRA more than 80%). On the other hand, any specificity that is listed as unacceptable will exclude the patient from being considered as a potential recipient for an organ carrying that specificity.

The results of our study had prepared us for the use of CPRA. For example, we have seen the significant distribution of antibodies against HLA-DQ and HLA-DP (18–20). Our data further suggest that the lack of DSA should be confirmed by longitudinal and in-depth assessment of the antibody makeup. For example, the fact that a single specificity is not listed as unacceptable antigen does not necessary mean that no DSA are present. In our center, we list unacceptable specificities only for those antigens that, regardless of the FCXM result, will not be considered acceptable for a specific patient. Antibody specificities with low strength are not considered contraindication to transplantation and therefore will

not appear as unacceptable antigens. Such a patient would still be considered as a potential recipient and the final decision as to whether to transplant or not will be based on the number and strength of DSAs to that donor. Posttransplant antibody monitoring and immunosuppression protocols will be adjusted accordingly.

While promoting the use of virtual crossmatching, we are cautious in considering the assignment of HLA-antibody specificity and the statement of “no DSA”. Obviously, for the nonsensitized patients, a virtual crossmatch is trivial. For the less privileged patients who were exposed to alloantigens and had developed HLA-specific antibodies, one must assure the absence of DSA for all 12 HLA antigens (two sets of HLA-A, -B, -Cw, -DR, -DQ, -DP; assuming the recipient has antibodies to both HLA class I and class II antigens) to assign or predict a negative virtual crossmatch. This raises the notion of obtaining the complete (12 antigens) HLA type for the potential donor. Currently, donors are not typed to that extent. To complicate things even further, many laboratories have detected antibodies against some alleles of certain HLA antigens, but no antibodies against others. An example of such a case is presented in this article. Because the current reporting system does not allow for allele level antibody assignment, nor are potential donors typed at the allele level, one may anticipate significant problems with a virtual crossmatch. Another important consideration is to provide the transplant center with a list of antigens used to determine antibody specificities. We have seen cases where a sensitized patient had a positive XM result with no apparent DSA just because the antigens in question were never tested for (but were actually present when the appropriate reagents were used).

The approach presented in this report is in line with the recommendations by KARS, ASHI, and UNOS. We have delineated some of our successes but also concerns about the accuracy of antibody identification and strength assessment. Clearly, further studies and validation by other centers are needed to advance this field.

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FIGURE 1.

Antibody work-up flow chart, as used by the transplant immunology laboratory at Northwestern University.

FIGURE 2.

Flow-SP analysis of HLA class II antibodies. Each “dot” represents a single bead to which HLA-DR and HLA-DQ antigens are attached. The dividing line is placed such that beads that shift to the right of it are considered positive. The “+” sign represents an interpretation of a positive results for the individual bead. Five beads were considered positive in this assay (one of which is weak positive, labeled±; tube 3). This figure presents only three of the four tubes usually run in this kind of assay. All DR11 positive beads have shifted to the right of the dividing line. An additional bead—coated with DR8, DR17, DR52, DQ2, DQ6 (tube 1)—was also positive, raising the question whether other antibodies are present in the tested serum sample.

FIGURE 3.

Transplant decision flow chart, as used by the transplant immunology laboratory at Northwestern University.

FIGURE 4.

An example of antibody strength determination using Flow Cytometry basic view. Antibodies to A1, A3, are strong; antibodies to A25 are on the boarder between moderate and strong; Antibodies to A30 and B49 are considered moderate; antibodies to A26 and A29 are weak and there are no antibodies to A2. The exact cutoff between the three strength levels should be determined by the individual centers based on their own correlation between florescence data (whether molecular equivalent of soluble florescence, median florescent intensity) and titration studies, corresponding FCXM or AHG XM results and ultimately patient outcome data.

FIGURE 5. Makeup of patients transplanted in the two study periods indicates that group II had more sensitized patients transplanted, $P<0.012$.

TABLE 1

Demographic characteristics of the two study groups

	Group I	Group II	P
N	374	333	
Male (%)	57.5	55.3	NS
Race (W/B/H) (%)	64/19/12	58/24/11	0.07
Age at TX (median±SD)	47.2±12.7	47.5±12.4	NS
Pregnancies (median±SD)	1.2±1.9	1.4±1.9	NS
Previous TX (median±SD)	1.2±0.6	1.3±0.6	NS
CIT (median±SD, DD only)	21.1±7.5	21.2±5.9	NS
LD/DD (%)	57/43	55/45	NS

Nonsensitized patients defined as patients with no HLA-directed antibodies as measured using flow screen PRA.

W, white; B, black; H, Hispanic; TX, transplant; CIT, cold ischemic time; LD, living donor; DD, deceased donor.