Review: platelet matching for alloimmunized patients—room for improvement

S.T. Nance, S. Hsu, R.R. Vassallo, and S. Murphy

A Parable

A 40-year-old woman was admitted at midnight to HMO General Hospital for evaluation of profound fatigue. Her hemoglobin was found to be 2 g/dL. Her medical history included several pregnancies and transfusions. The intern had attended a lecture on transfusion medicine that day. He ordered a "six-pack," six units of random-donor RBCs. The technologist on call for the blood bank was a bacteriologist, cross-covering the transfusion service. The technologist removed six units of RBCs from the refrigerator, pooled them, and sent a liter of the mixture to the floor for transfusion, which was quickly followed by back pain and black urine for the patient.

The intern called the on-call transfusion service medical director, who was cross-covering from cardiology. The intern asked, "Can't you do immunologic testing or provide a matched product?"

The director responded, "No way! What was the 1-hour CCI? We insist upon two documented failures before we do an immunology workup. I'm sending the patient another pool."

"Oh no, not again!" said the patient. Shortly after transfusion, back pain and black urine recurred.

The resident called the medical director and said, "That's two, Doc!"

"OK. We'll phenotype the patient's RBCs and search for a good BX match in inventory. You know, Jk^a and Jk^b are crossreactive."

Once again the patient said, "Are you sure?" However, the transfusion was given and soon she was having back pain and black urine again.

In desperation, the medical director said, "I'll take random units off the shelf and crossmatch until I find a compatible unit." The unit was found and transfused and there was no back pain or black urine until 5 days later.

The next day, a new resident, Dr. Peter Petz-Garratty, came on service. He reviewed the chart and said, "Why don't you examine the patient's serum for antibodies and determine their antigenic specificities? Then, crossmatch products without those antigens."

The medical director said, "This man has a future."

Evolution of Strategies for RBC Matching

Practice was quite primitive when physicians first considered transfusing blood from one individual to another. They began transfusing whole blood between experimental animals, without any consideration whatsoever for RBC antigen-antibody compatibility. Interestingly, this lack of compatibility testing persists even today for the majority of platelet transfusions.

In an excellent historical review,[1] Myhre described a report by John Wilkins of a dog-to-dog transfusion of about 2 ounces of blood via syringe. At that time, it was not recognized that there was a possibility of naturally occurring alloantibodies (not present in dogs) or the relevance of a volume possibly too small to produce a reaction. Other investigators in London and France also experimented with animal-to-animal transfusions. This was done, of course, without compatibility testing.

Since animal-to-animal transfusions went quite well, soon the time came to try animal-to-human transfusions. Jean Baptiste Denys performed the first animal-to-human transfusion in mid-1667.[2] Nine ounces of lamb's blood was transfused to a 16-year-old patient who had been bled at least 20 times over 2 months. During the transfusion, it was reported that the patient experienced "very great heat along his arm." Again, no testing of the blood or the recipient was performed.

James Blundell was a major contributor to the early years of human-to-human blood transfusion. He reported a transfusion to a patient with obstinate vomiting, in 1819. The donors were several physicians;

12 to 24 ounces of blood was given by syringe.[2] This is reminiscent of the use of six packs for platelet transfusion today. As in the preceding two accounts, no laboratory testing was carried out on the donor or recipient blood. These early reports share relatively small transfused volumes and a lack of pretesting. One report remarked that animal blood was thought to be more pure, as the animal did not participate in some of the risky pursuits that humans did.

Fast forward to today, when involved parties' blood, both donor and recipient, are subjected to a large battery of tests. But how did the testing algorithm develop, and why? As mentioned previously, the first transfusions were given without testing of the donor or the recipient. Interest in transfusion waned in the late 19th century due to a high rate of severe adverse reactions to transfusion.[3] Landsteiner's experiments and direct transfusions by Carrel, described by Crile,[4] promoted a recurrence of interest. The subsequent determination of ABO groups gave rise to renewed interest in human-to-human blood transfusion.

In a publication by Ludwig Hektoen,[5] what would become the major and minor crossmatches were described in 1907. In the early days, the methods concentrated solely upon detection of room temperature IgM antibodies, as the "Coombs test" had not yet been described. Thus, these investigators were studying ABO compatibility. Ottenberg[6] and Epstein[7] studied a method for hemolysis and agglutination tests. Epstein promoted a pretransfusion crossmatch, using tests both for hemolysis and for agglutination. It was later recognized that when agglutination was present hemolysis might also be present, but in vitro hemolysis did not occur without agglutination.[8] With this information, agglutination tests could be used without tests for hemolysis. At this time, all testing still concentrated upon detection of IgM antibodies. Changes like the recommendation for testing at 37°C and concentration upon the major crossmatch followed. During World War I, Lee proposed that if type O blood were used, preliminary testing could be avoided.[9]

These red cell innovations in the early part of the 20th century were quite similar to the use of "blind" crossmatching to assess platelet compatibility today. The first practical method for platelet crossmatching was not described until 1988.[10] This will be discussed further in the next portion of this paper.

Major changes to detect IgG antibodies followed the reports of enhancement of agglutination with bovine albumin[11] and antiglobulin testing.[12] Although other enhancement media and techniques (enzyme, LISS, PEG, and solid phase and column agglutination technology) have been described, the basic premise of the major crossmatch remains remarkably the same, ie., with testing of the patient's serum against donor RBCs.

At first, the minor crossmatch was also felt to be necessary, but a paradigm shift occurred. The minor crossmatch was eventually abandoned in favor of routine donor antibody screening. The need for complement-reactive antihuman globulin (AHG) sera has been eliminated because of the rare occurrence of clinically significant antibodies leaving only complement on the cell surface. The requirement for serum as a source of a test sample has been changed for the same reason.

Another change was the departure from room temperature testing to 37°C for detection of potentially significant antibodies. Most clinically significant antibodies are reactive at 37°C, and antibodies reactive solely at room temperature are generally not thought to have clinical significance.

More recently, AABB standards have allowed the use of immediate spin testing as long as the patient's antibody screen is negative. In some laboratories, electronic crossmatches have taken the place of the AHG crossmatch. Thus, few patients' samples are routinely subjected to an AHG crossmatch. When the antibody screen is positive, RBC units are selected that lack antigens to which the antibody is directed. This is quite similar to the evolution of antibody-based matching for platelets.

The number of RBC transfusions requiring a full crossmatch is proportionate to the number of platelet transfusions currently guided by crossmatch testing, but for vastly different reasons. In the case of potential RBC recipients, an AHG crossmatch will be required by the serologic determination of alloantibodies or autoantibodies. In the case of potential platelet recipients, however, crossmatching is ordered after several transfusions have not yielded expected increments. The present status of platelet crossmatching, then, parallels the state of RBC testing in the early 1900s.

Evaluation of Strategies for Platelet Matching

In current practice, platelet transfusion begins with randomly selected platelet products, either pooled whole-blood–derived platelets or platelets prepared by apheresis. This is analogous to the initial management

of the anemic woman in the parable. This approach continues until the patient is refractory, i.e., fails to show adequate increments after transfusion. To identify a patient as refractory, most clinicians require two or three failed transfusions. Again, this was the way the patient in the parable was managed. Commonly, increments are expressed as corrected count increments (CCI) where

$$CCI = \frac{\text{Plt. Ct. Increment (/}\mu\text{L)} \times \text{Body Surface Area (m}^2\text{)}}{\text{Plts. Transfused (} \times 10^{11}\text{)}}$$

Refractoriness is commonly defined as a CCI less than 4000 per μL per m^2 per 10^{11} platelets.[13] Thus, patients have to "earn" immunologic intervention by experiencing several therapeutic failures, as the woman in the parable did. This reactive policy contrasts with RBC transfusion, where patients are now screened for antibody before each transfusion, as suggested by "Dr. Petz-Garratty."

Most immunologic platelet refractoriness results from antibodies to HLA. It is important to emphasize that clinical refractoriness is often due not to alloimmunization but, rather, to clinical factors such as infection, splenomegaly, or disseminated intravascular coagulation.[14] Thus, it would make sense to perform one or more serologic tests to prove that alloimmunization was present. However, this approach was rare during the first 20 to 25 years of matched-platelet transfusions.[15,16]

The classical HLA approach to platelet matching began with the paper by Yankee et al.[15] that compared the use of platelets from HLA-identical siblings with randomly selected platelets in refractory patients. There were no increments with random products but excellent responses to the perfectly HLA-matched sibling donors.

It is generally not realistic to support refractory patients with sibling donors. Duquesnoy et al.[16] introduced the concept of supporting patients with HLA-matched platelets from the general population. However, they noted that a pool of 50,000 donors would be required to provide a 50 percent probability of finding ten perfectly matched donors for each of 100 potential recipients. This was also unrealistic. They proposed a method that we will call the CREG method. Cross-reactive groups (CREGs) of HLA antigens have been defined by serologic testing. Cross-reactivity among antigens within a CREG results from the sharing of one or more public epitopes. Patients do not make antibody to their own HLA antigens nor commonly to antigens within CREGs of their own HLA antigens.[16] Therefore, Duquesnoy et al.[16] defined matches between donor and patient as A, HLA identical; BU, (partially homozygous) donor antigens all present in the recipient; BX, all donor HLA antigens either identical to or within recipient CREGs; C & D, one or more donor antigens not present in recipient or in recipient CREGs. This is analogous to the phenotypic matching of RBCs.

The use of BX matches greatly expanded the number of potential donors. This was an advance because many BX matches provided good increments in the refractory patient. However, the predictive power of this approach was poor. Poor responses were frequent with BX matches and a surprising number of good responses were seen with C and D matches. The patient in the parable had a poor response to a "phenotypic match" at the *Kidd* locus.

The study of Duquesnoy et al.[16] can be criticized in retrospect because no serologic assessment was performed. They did not know if the patients had HLA antibodies and, if they did, what the specificities of the antibodies were. These determinations are always performed prior to a RBC transfusion, as was suggested by "Dr. Petz-Garratty."

The poor predictive power of the CREG method can be understood to some extent by examining the results of serologic studies in these patients. For many years, the standard serologic test for HLA antibodies has been a lymphocytotoxicity (LCT) assay, the antihuman globulin–augmented complement-dependent cytotoxicity test (AHG-CDC). In this assay, leukocytes from 50 to 100 donors with an appropriate heterogeneity of HLA types are incubated in wells with the patient's serum and complement. LCT is assessed microscopically. The presence of HLA antibody correlates with poor response to randomly selected platelets[17] and with improved response to HLA-selected patients.[18] On the other hand, if such antibodies are not present, poor responses are infrequently improved by HLA matching.[18–20]

In our laboratory, one half of patients referred for matched platelets have no antibody.[21] We discourage matching in such patients except in the rare circumstance that immunologic refractoriness is mediated solely by antibodies to human platelet antigens (HPAs). The results of the AHG-CDC assay determine not only the presence or absence of antibody, but also the breadth of immunization expressed as the percent reactive antibody (PRA), the percentage of wells in which lymphocytotoxicity is

seen. Furthermore, by analyzing the positive wells, one frequently can determine the specificities of the antibodies. When we analyze results with the LCT assay in refractory patients, the PRAs can range from 1 percent to 100 percent, so one can classify patients as mildly (1–40% PRA), moderately (40–70% PRA), and severely (PRA > 70%) immunized.

Furthermore, when the specificities are determined, frequently one can identify intra-CREG antibodies: antibodies against antigens within the patient's CREGs. With these facts in mind, one can understand why the CREG method has a relatively poor predictive capacity. When a BX match fails, there may be intra-CREG antibody. When a C or D match succeeds, the patient, although immunized, may have no antibody against the antigens present on the transfused platelets. There is a computer program available to better predict successful BX and C and D matches.[22,23] It is known as "HLA Matchmaker."

Furthermore, donor selection may be improved by "HLA Matchmaker." It is based upon the concept that HLA molecules express their antigenic determinants as amino acid triplets. These triplets are shared among otherwise unrelated HLA molecules. Antigens whose triplet complement is identical to ones present in the patient are highly likely to provide compatible products for an immunized patient. BX matches with fewer triplet mismatches may be more likely to succeed, as would triplet-identical C and D matches. This concept awaits validation in a clinical study.

A quite different approach, crossmatching of donor platelets with a patient's serum, was introduced in 1988 and, later, in 1997.[10,24] The most commonly used method, at least in the United States, has been the solid phase red cell adherence (SPRCA) assay, which is available commercially as Capture-P from Immucor, Inc. (Norcross, GA). The platelets of the potential donor are layered in microtiter wells and the patient's serum is added. The platelets are then washed and anti-IgG–coated indicator RBCs are added. If there is antibody to the platelets, the RBCs form a thin film in the well. If there is no antibody, the RBCs puddle in a button on the bottom of the well.

Many centers simply take platelets from inventory and perform "blind" crossmatching, with no preselection of the donors by identifying the antigens to which the patient has formed antibodies. This stands in contrast to RBC matching, in which cells are deliberately chosen for crossmatching that lack the antigens to which the patient has been sensitized.

The crossmatch method has the advantage of being fast, once the patient's serum is in hand. In addition, it is a form of screening for the presence of antibody. In our experience[21] comparing the SPRCA to the AHG-CDC assay, both tests detect antibody in 50 percent of referred patients, while all crossmatches are compatible in 30 percent. It is very rare for the SPRCA to be entirely negative (compatible) when the AHG-CDC is positive. Thus, if all crossmatches are compatible, it is very unlikely that the patient is refractory for immunologic reasons. On the other hand, 20 percent of these cases are SPRCA positive and AHG-CDC negative. The majority of such cases are seen in group O patients who have positive reactions to donors who are group A and/or B. In this situation, the positive crossmatch would be considered falsely positive since the platelets may be an excellent HLA match.

Recent results from Gelb and Leavitt[24] in 76 potentially immunized patients indicated a mean CCI of 1800 for randomly selected products and 9800 for crossmatch-compatible products. However, there were no compatible products for ten patients. Another mode of support based on HLA matching might have helped these ten patients. Furthermore, in the other 66 patients, the mean percentage of screened units that were compatible was 69 percent (range: 24–100%), suggesting that these patients were only mildly or moderately immunized. Overall, 59 percent of crossmatch-compatible products produced CCIs greater than 7500. On the other hand, 41 percent did not.

Crossmatching is an excellent way to begin support when a patient needs a product urgently, if appropriate technical staff are available (i.e., perhaps not on nights or weekends). It provides a quick answer to the question of whether or not the patient is alloimmunized. In our experience, it is generally satisfactory for mild and moderate degrees of alloimmunization but less helpful for the severely immunized. Furthermore, it may find a very good HLA match incompatible because of ABO antibodies in the patient. In our experience[21] with highly immunized patients, the majority of crossmatch-compatible products are not successful unless the degree of HLA compatibility is high.

A final approach was suggested by Dr. Peter Petz-Garratty in the parable. Why not examine the patient's serum for antibodies and determine their antigenic specificities? Then, provide antigen-negative products, perhaps with a final check by crossmatching. In fact, this approach was evaluated by Petz and Garratty et

al.[25] in a large number of alloimmunized patients. They called it the antibody specificity prediction (ASP) method. The specificities of the patient's antibodies were determined with the AHG-CDC method and antigen-negative platelets from an HLA-typed donor pool were selected for transfusion. This approach was compared to "blind" crossmatching. The increments were better with the ASP method, although not statistically so since there was a large range around the means for both methods. The authors also found that a perfect A or BU match produced the best result.

We studied the PRA of patients over time, up to a maximum of 16 months. Our results were similar to those of Lee and Schiffer.[26] PRAs tend to remain constant over time, even as the patients continue to be transfused. On the other hand, approximately 25 percent of immunized patients may lose their antibodies over a period of months, a phenomenon that we do not understand. In practice, this means that one can perform an AHG-CDC assay once monthly to monitor the patient's status without concern for major changes during the period.

A great advantage of the ASP method is that it increases the number of potential donors. Further, if one has identified the specificities of the patient's antibodies, one can distribute compatible products from inventory at any time without the need for testing. Our approach is outlined in Table 1.

It is clear that what Petz and Garratty et al.[25] are suggesting brings us to a method very similar to what we do with RBCs. We are using more complex methods but their cost is less than that of a failed matched-platelet product. With current methods, frequent failed platelet transfusions are accepted routinely. We would not tolerate this with RBCs, since morbidity from an incompatible RBC transfusion can be severe. However, there is morbidity from a failed platelet transfusion: a continuation of the patient's bleeding risk.

In addition, a new generation of technologies developed and used for organ transplantation can also be used for the platelet-refractory patient. These are discussed in detail in the next section. Briefly, new techniques for determining the specificity of antibodies take advantage of the ability to use isolated, single HLA molecules in ELISA or flow cytometric–based assays. This is in contrast to the AHG-CDC assay, in which two to four antigens are expressed on each target lymphocyte.

In addition, there are simple ELISA methods in which the target is a wide variety of HLA antigens. One could use these methods to screen patients for the presence of HLA antibodies, perhaps every 2 to 4 weeks. With this approach, one can be proactive and begin matching earlier. We could abandon the practice of forcing patients to "earn" their matched products by experiencing several therapeutic failures.

Table 1. Suggested patient management approach

A. **Patient Referral:**
1. Begin support from inventory by:
a. classic CREG method[16] using patient's HLA type
b. crossmatching of random units by SPRCA[24]
2. Particularly if using (a), strongly suggest HLA antibody screen
3. Urge clinicians to provide CCIs
B. **If:**
1. No HLA antibody is demonstrated, return to randomly selected products; consider testing for HPA antibody, especially if concomitant nonimmunologic platelet destruction is unlikely
2. Only ABO antibody is demonstrated, use platelets from ABO-identical or group O donors
3. HLA antibody is demonstrated but PRA < 70%, support from inventory using crossmatching[24] or antigen-negative platelets selected by antibody specificity prediction method[25]
C. **Monitor CCIs to judge response**
D. **If PRA > 70% and/or CCIs are poor:**
1. Crossmatch antigen-negative platelets[24,25]
2. Establish transfusion schedule and selectively recruit A, BU, or other very close matches

Platelet Matching: New Technology for the Future

For solid organ transplantation, the detection of circulating anti-HLA antibodies in allograft recipients correlates not only with hyperacute, acute, accelerated, and chronic graft rejection,[27-29] but also with reduced graft function and graft failure. Over the past 40 years, a plethora of papers has been published, discussing the merits of various antibody detection methodologies in relation to the clinical outcomes of solid organ transplants. In contrast, very little can be found in the literature relating these methods to platelet transfusion outcome. This may partly be due to the difficulty of such research due to the confounding effect of variables such as storage-related changes in platelet viability, concurrent recipient nonimmunologic plate-let destruction, and the difficulties of demonstrating clinically significant changes in outcome in the context of prophylactic platelet use. This meager literature is further confused by the use of less sensitive "gold standard" assays (unenhanced lymphocytotoxicity tests) for antibody screening in many comparative studies.[30]

The objective of this portion of the review is to discuss different testing methodologies currently in use for the detection of anti-HLA antibodies as they relate to platelet transfusion. We will also outline what we

believe to be the optimal approach to characterizing the HLA antibody profile of highly sensitized platelet-refractory patients.

HLA Class I antigens are expressed on all nucleated cells, including platelets. HLA Class I antibodies are relevant not only to solid organ and tissue transplantation, but also to WBC and platelet transfusion. Class II antigens are expressed on B cells, monocytes, macrophages, dendritic cells, and activated T cells. HLA Class II antibodies are therefore unimportant in the context of platelet transfusion.

Within the relevant Class I HLA-A and HLA-B families (HLA-C being present on the platelet surface in a density too low to be clinically important), there are several CREGs, which express multiple public epitopes. These public epitopes are common antigenic determinants shared by different HLA antigens. *HLA-B* locus antigens have two mutually exclusive public epitopes, Bw4 and Bw6, as well as a number of others defining the CREGs, such as 5C, 7C, 22C, 27C, 8C, 12C, and 21C. Private epitopes refer to antigenic determinants specific for an individual antigen. In addition to "foreign" CREGs, patients can occasionally make antibodies to antigens within their own CREGs, either to private epitopes or to different public epitopes within a CREG.[31] The CREG antibody profile remains remarkably constant over time in patients.[31,32] Monthly fluctuations in PRA, if not due to new transfusion events or changes in assay technique or panel composition, usually reflect the waxing and waning of detectable specificities within the same CREG, or they can reflect the increasing strength or number of reactivities within the same CREG cluster.[30] In the transfusion setting, we have observed that greater than 50 percent of sera screened by flow cytometric methods contained both IgM and IgG antibodies.[33] These are mostly directed against public epitopes or high-frequency private epitopes present in transfused units. Additionally, some antibodies arise from an anamnestic response from prior sensitization to paternal antigens during pregnancy.[34,35]

Antibody screening provides information regarding the presence or absence of HLA antibodies, the PRA against HLA alloantigens, antibody specificities, the immunoglobulin isotypes produced, and even antibody titers. This information may in turn be used to predict products likely to provide successful patient platelet concentration increments.[21,25] The ideal screening method should be sensitive enough to detect the lowest level of clinically relevant IgM and/or IgG antibodies.

The original NIH two-stage complement-dependent cytotoxicity assay (CDC)[36] used for HLA phenotyping is illustrated in Figure 1,[30] which depicts the two steps of the basic CDC method with alternative steps (indicated by asterisks) included to illustrate (1) extended incubation to allow low avidity/affinity antibodies to bind cellular antigens; (2) a wash step to remove unbound serum and minimize "anticomplement factors," which may inhibit or weaken complement activity (Amos modified technique)[37]; and (3) goat AHG-CDC, which overrides inefficient C1q binding by single IgG molecules.[38,39] Many of the anti-public or "CREG" antibodies are not detectable by the standard CDC method due to inefficient complement activation by HLA antibodies. This is termed the cytotoxic-negative, adsorption-positive phenomenon (CYNAP).[40] The AHG-CDC method is the most sensitive of the four complement-dependent cytotoxicity tests. In some laboratories, the sensitivity of the AHG-CDC is comparable to the multiple-antigen-panel ELISA-based technique.

The major advantage of complement-dependent assays is the ability to detect both IgG and IgM antibodies. While IgM anti-HLA antibodies do not play a role in solid organ transplantation, this may not be the case in platelet transfusions. The disadvantages of complement-dependent assays include the detection of not only HLA-specific antibodies but also nonspecific specificities or autoantibodies. The latter can be

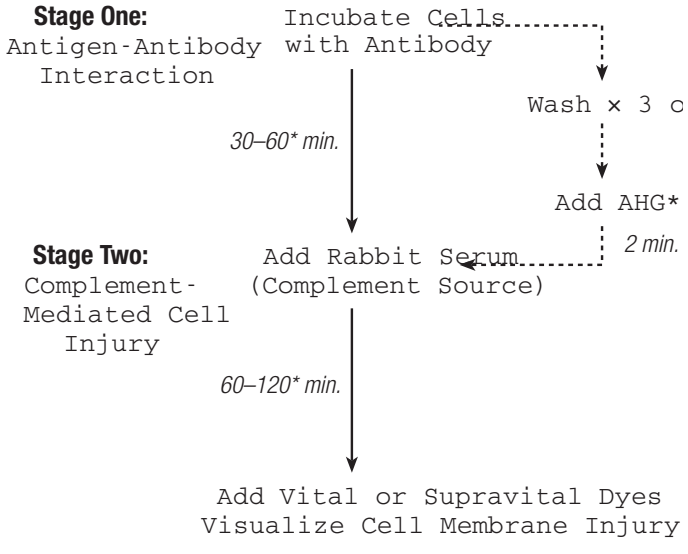

Fig. 1. The original NIH two-stage complement-dependent cytotoxicity assay (CDC) and its modifications *(see text).

eliminated by heating the serum at 63°C for 10 minutes, treating the serum with DTT, or performing an autocrossmatch in parallel. CDC-determined PRAs may fluctuate due to changes in panel cell composition or poor viability of frozen cells. Without prior adsorption, sera obtained from patients receiving OKT3 or anti-T cell immunoglobulins cannot be tested by CDC methods.

There are two forms of solid phase–based methods: the ELISA[41,42] and flow cytometric assays.[43–45] These methods employ purified soluble HLA antigens affixed to solid phase matrices (plastic trays for ELISA or beads for flow cytometry) as targets for the binding of HLA antibodies instead of the live cells employed in CDC assays.

The advantages of solid phase–based methods are the ability to distinguish between IgG and IgM isotypes, better detection of HLA-specific antibodies, objective end-result determination by instrument optical density readings, the lack of assay interference by OKT3 treatment, and faster turnaround time.

Currently, ELISA assays are available in two or three formats. The first is the screening test for the presence or absence of HLA antibodies. A pool of HLA antigens is affixed to a single well for this purpose. Positive sera are further defined using a panel of HLA antigens purified from EBV cell lines or platelets to allow the determination of PRA and antibody specificity. The sensitivity and specificity of ELISA using heterozygous antigen panels (i.e., each well coated with multiple antigens) is comparable to that of the AHG-CDC method. A third type of ELISA tray is the high-definition (HD) or single-antigen tray. Each well in the tray is coated with a single HLA Class I–soluble antigen derived from recombinant DNA technology.[46] This high-definition antigen panel provides the ideal tool to characterize antibody specificities contained in high-PRA sera. Both the sensitivity and the specificity of screening sera using high-definition ELISA trays are increased when compared with the AHG-CDC method.[47] In general, the ELISA method is not sensitive for the detection of IgM HLA antibodies.

Similar to the variety of ELISA methods, there are also three kinds of flow beads available: polyspecific beads, useful as a screening tool; beads coated with heterozygous soluble Class I antigens to determine PRA; and antibody specificity or high-definition flow beads. Currently the high definition beads consist of 34 individual recombinant *A* locus antigens, 57 *B* locus antigens, and 19 *C* locus antigens.

Recently, another flow cytometric platform was developed, based upon Luminex Microsphere (bead) technology. This technology combines two of the most powerful detection and identification tools, the exquisite binding specificity between biotin and streptavidin and the versatility of the flow cytometer. By the precise blending of different fluorescent intensities of the two dyes, 100 unique color codes are created. When the colored beads are coated with purified Class I antigens, they can be used for screening and identification of HLA antibody specificities. Although a number of large parallel studies between standard and Luminex flow methods are still in progress, data published thus far indicate that they are likely to be comparable to each other.[47]

It is well established that flow cytometry methods are more sensitive than AHG-CDC and ELISA. Better clinical correlations are obtained using flow methods not only for antibody screening but also for transplantation crossmatches. Flow methods can detect extremely low-level antibodies undetectable by either AHG-CDC or ELISA methods.[48–50]

Considering reported sensitivities and specificities together with our own comparison studies of AHG-CDC versus ELISA or flow methods,[33] our ranking of preferred antibody elucidation methods for platelet-refractory patients is: flow cytometry (HD panel) > ELISA (HD panel) > AHG-CDC.

The optimal approach to characterizing the HLA antibody profiles of highly sensitized patients, in our opinion, is to HLA type potential platelet recipients and to perform a thorough determination of the antibody specificities using either ELISA or flow methods on high-definition antigen or bead panels. Knowledge of the recipient's HLA type assists in analyzing the antibody profiles. Patients can only produce HLA antibodies against mismatched public and private epitopes. Patients are less likely to be sensitized by antigens belonging to their own CREG groups, although intra-CREG antibody formation is not uncommon.

Screening high-PRA individuals using high definition trays sometimes results in near-100 percent panel reactivities. This is mostly due to the presence of high-titer antibodies. We now use high initial optical density readings (O.D. > 2.0) from the screening assay for the presence of HLA antibodies as a guide to dilute these high-titer PRA sera 1:10 or 1:20 for subsequent screening on high-definition antigen or bead panels. The few antigens to which these individuals are not sensitized may be confirmed with compatible lymphocyte crossmatches from products negative for

the corresponding antigens.

Our ultimate goal is to rapidly identify individuals with HLA Class I antibodies and attempt to provide so-called antigen-negative platelet products, even to highly sensitized patients, akin to the traditional approach outlined above for RBC transfusions. While this approach may be somewhat expensive compared with the current practice of therapeutic trial and error, the savings engendered by early identification of alloimmunization and consequent avoidance of failed transfusion, prolonged thrombocytopenia, and associated morbidity and costs may be justified in the long run.

References

1. Myhre, BA. The first recorded blood transfusions: 1656–1688. Transfusion 1990;30:358-62.
2. Myhre BA. James Blundell—pioneer transfusionist. Transfusion 1995;35:74-8.
3. Oberman HA. The crossmatch. A brief historical perspective. Transfusion 1981;21:645-51.
4. Crile GW. Hemorrhage and Transfusion. New York: D. Appleton & Co., 1909:318.
5. Hektoen L. Isoagglutination of human corpuscles. JAMA 1907;48:1739.
6. Ottenberg R. Transfusion and arterial anastamosis. Am J Surg 1908;47:486.
7. Epstein AA, Ottenberg R. A method for hemolysis and agglutinin tests. Arch Intern Med 1909;3:286.
8. Moss, WL. Studies on isoagglutination and isohemolysis. Bull J Hopk Hosp 1910;21:63.
9. Lee RI. A simple and rapid method for the selection of suitable donors for transfusion by the determination of blood groups. Br Med J 1917; 2:684.
10. Rachel JM, Summers TC, Sinor LT, Plapp FV. Use of a solid phase red blood cell adherence method for pretranfusion platelet compatibility testing, Am J Clin Pathol 1988;90:63-8.
11. Diamond LK, Denton RL. Rh agglutination in various media with particular reference to the value of albumin. J Lab Clin Med 1945;30:821.
12. Coombs RR, Mourant AE, Race R. A new test for the detection of weak and "incomplete" Rh agglutinins. Br J Exp Pathol 1945;26:255-66.
13. Bishop JF, Matthews JP, Yuen K, et al. The definition of refractoriness to platelet transfusions. Transfus Med 1992;2:35-41.
14. Bishop JF, McGrath K, Wolf MM, et al. Clinical factors influencing the efficacy of pooled platelet transfusions. Blood 1988;71:383-7.
15. Yankee RA, Grumet FC, Rogentine GN. Platelet transfusion. The selection of compatible platelet donors for refractory patients by lymphocyte HL-A typing. N Engl J Med 1969;281:1208-12.
16. Duquesnoy RJ, Filip DJ, Rodey GE, et al. Successful transfusion of platelets "mismatched" for HLA antigens to alloimmunized thrombocytopenic patients. Am J Hematol 1977;2:219-26.
17. Hogge DE, Dutcher JP, Aisner J, Schiffer CA. Lymphocytotoxic antibody is a predictor of response to random donor platelet transfusion. Am J Hematol 1983;14:363-9.
18. McFarland JG, Anderson AJ, Slichter SJ. Factors influencing the transfusion response to HLA-selected apheresis donor platelets in patients refractory to random platelet concentrates. Br J Haematol 1989;73:380-6.
19. Phekoo KJ, Hambley H, Schey SA, et al. Audit of practice in platelet refractoriness. Vox Sang 1997;73:81-6.
20. Engelfriet CP, Reesink HW, Aster RH, et al. Management of alloimmunized, refractory patients in need of platelet transfusions. Vox Sang 1997;73:191-8.
21. Murphy S, Varma M. Selecting platelets for transfusion of the alloimmunized patient: A review. Immunohematology 1998;14:117-23.
22. Duquesnoy RJ. HLA Matchmaker: A molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. Hum Immunol 2002;63:339-52.
23. Duquesnoy RJ, Marrari M. HLA Matchmaker: A molecularly based algorithm for histocompatibility determination. II. Verification of the algorithm and determination of the relative immunogenicity of amino acid triplet-defined epitopes. Hum Immunol 2002;63:353-63.
24. Gelb AB, Leavitt AD. Crossmatch-compatible platelets improve corrected count increments in patients who are refractory to randomly selected platelets. Transfusion 1997;37:624-30.
25. Petz LD, Garratty G, Calhoun L, et al. Selecting donors of platelets for refractory patients on the basis of HLA antibody specificity. Transfusion 2000;40:1446-56.
26. Lee EJ, Schiffer CA. Serial measurement of lymphocytotoxic antibody and response to nonmatched platelet transfusions in alloimmunized patients. Blood 1987;70:1727-9.

27. Terasaki PI, Marchioro TL, Starzl TE. Sero-typing of human lymphocyte antigens: preliminary trials on long-term kidney homograft survivors. In: Histocompatibility Testing. Report of a Conference and Workshop. Washington DC: National Academy of Sciences—National Research Council 1965: 83-96.
28. Kissmeyer-Nielsen F, Olsen S, Peterson VP, Fjeldborg O. Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. Lancet 1966;2(7465):662-5.
29. Terasaki PI. Humoral theory of transplantation. Am J Transplant 2003;3:665-73.
30. Rodey G. HLA beyond tears: introduction to human histocompatibility. 2nd ed. Durango, CO: De Novo, Inc., 2000: Chapters 4, 8, 9 and 10.
31. Rodey GE, Revels K, Fuller TC. Epitope specificity of HLA Class I alloantibodies. II. Stability of cross-reactive group antibody patterns over extended time periods. Transplantation 1997;63:885-93.
32. Rodey GE, Neylan JF, Whelchel JD, et al. Epitope specificity of HLA Class I alloantibodies. I. Frequency analysis of antibodies to private versus public specificities in potential transplant recipients. Hum Immunol 1994;39:272-80.
33. Meny GM, Hsu S, Murphy S, Pei R. ELISA and flow cytometry provide advantages over lympho-cytotoxicity in detecting HLA antibodies. (In preparation) 2004.
34. Scornik JC, Ireland JE, Howard RJ, Pfaff WW. Assessment of the risk for broad sensitization by blood transfusions. Transplantation 1984;37: 249-53.
35. Braun, W.E. Donor-specific antibodies. Clinical relevance of antibodies detected in lymphocyte crossmatches. Clin Lab Med 1991:11:571-602.
36. Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. Nature 1964;204: 998-1000.
37. Amos DB, Bashir H, Boyle W, et al. A simple micro cytotoxicity test. Transplantation 1969;7:220-3.
38. Johnson AH, Rossen RD, Butler WT. Detection of alloantibodies using a sensitive antiglobulin microcytotoxicity test: identification of low levels of pre-formed antibodies in accelerated allograft rejection. Tissue Antigens 1972;2:215-26.
39. Fuller TC, Fuller AA, Golden M, Rodey GE. HLA alloantibodies and the mechanism of the antiglobulin-augmented lymphocytotoxicity procedure. Hum Immunol 1997;56:94-105.
40. Yunis EJ, Ward FE, Amos DB. Observations on the CYNAP phenomenon. In: Terasaki PI, ed. Histocompatibility 1970. Copenhagen: Munksgaard, 1970:351-6.
41. Kao KJ, Scornik JC, Small SJ. Enzyme-linked immunoassay for anti-HLA antibodies—an alternative to panel studies by lymphocytotoxicity. Transplantation 1993;55:192-6.
42. Wang G, Tarsitani C, Denham S, Chen M, Lee J-H. A new micro-ELISA for anti-Class I and Class II antibody detection. Human Immunol 1998:59(1): 133.
43. Garovoy MR, Rheinschmidt MA, Bigos M, et al. Flow cytometry analysis: A high technology crossmatch technique facilitating transplantation. Transplant Proc 1983;15:1939-44.
44. Pei R, Wang G, Tarsitani C, et al. Simultaneous HLA Class I and Class II antibodies screening with flow cytometry. Human Immunol 1998;59:313-22.
45. Pei R, Lee J, Chen T, et al. Flow cytometric detection of HLA antibodies using a spectrum of microbeads. Hum Immunol 1999;60:1293-1302.
46. Pei R, Lee JH, Shih NJ, et al. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. Transplantation 2003;75:43-9.
47. Visuals of the Clinical Histocompatibility Workshop, Cancun, Mexico 2002, Hawaii 2003, sponsored by One Lambda, Inc. Paul Terasaki, Ed.
48. Tambur AR, Bray RA, Takeomoto SK, et al. Flow cytometric detection of HLA-specific antibodies as a predictor of heart allograft rejection. Transplantation 2000;70:1055-9.
49. Gebel HM, Bray RA. Sensitization and sensitivity: defining the unsensitized patient. Transplantation 2000;69:1370-4.
50. Gebel HM, Bray RA, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: contraindication vs. risk. Am J Transplant 2003;3: 1488-1500.

Sandra Taddie Nance, MS, MT(ASCP)SBB, Director, Technical Services; Susan Hsu, PhD, Director, Histocompatibility/Molecular Genetics; Ralph R. Vassallo, Jr., MD, Senior Medical Officer; Scott Murphy, MD, Chief Medical Officer, American Red Cross Blood Services, Penn-Jersey Region, 700 Spring Garden Street, Philadelphia, PA 19123.