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Using real data for a virtual crossmatch

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ABSTRACT

Virtual crossmatches have been performed for more than 40 years under the guise of unacceptable antigens. Today, solid-phase assays provide the opportunity for more accurate identification and more precise measurement of the strength of donor-specific antibodies. The process of performing a virtual crossmatch begins with establishing a correlation between the antibody testing assay and the results of actual crossmatches. We provide here data indicating that the identity and strength of DSA defined with solid-phase phenotype panels correlates significantly with the outcome of both cytotoxic (CDC; r = 0.83) and flow cytometric (r = 0.85) crossmatches. Based on the threshold established from these correlations, we were able to correctly predict the results of CDC and flow cytometric crossmatches in 92.8 and 92.4% of cases, respectively. The correlations with single antigen panels were substantially lower (82.6–47.9%) and may be caused by a variety of factors, including variability in the amount and condition of different antigens and extremely high sensitivity, which may make the test less robust. We demonstrate that adding additional information to the solid-phase results can increase the frequency of correct crossmatch prediction. We also present data demonstrating an additional use of the virtual crossmatch in posttransplant monitoring.

1. Introduction

Use of the virtual crossmatch is not new, although the term has only been applied in recent years [1,2]. Once the clinical significance of a positive crossmatch [3,4] was appreciated, two practices followed. The Southeastern Organ Procurement Foundation introduced regional organ procurement trays to provide an opportunity to perform a preliminary crossmatch before shipping a donor organ. Soon after, listing of unacceptable antigens was instituted, first in a voluntary matching program conducted by the Terasaki Laboratory at UCLA and later in a mandatory allocation program administered by the Organ Procurement and Transplantation Network, the United Network for Organ Sharing. The listing of unacceptable antigens is, in fact, equivalent to performing a virtual crossmatch. Initially, unacceptable antigens were defined by screening patients' sera in crossmatch tests using lymphocytes from a panel of donors selected to represent the maximum number of HLA possible. The antibodies identified were considered those that would yield a positive crossmatch and, thus, were unacceptable. The identification of unacceptable antigens today is complicated by the availability of highly sensitive and specific solid phase immunoassays and differences, among transplant centers, of what is unacceptable [5]. That is, centers may choose to avoid repeat mismatches in the presence of very low levels or no antibody to that mismatch, to avoid only those antigens that would yield positive cytotoxicity crossmatches (CDCXM), or to avoid those that would yield a negative cytotoxicity crossmatch but a positive flow cytometric crossmatch (FCXM). Centers with desensitization programs may have different levels of acceptable donor-specific antibody (DSA) than other programs [6].

The utility of the virtual crossmatch provides the opportunity of expanding the geographic area from which organs with limited permissible cold ischemia time can be recovered [7], reduces both waiting time and death on the waiting list [8,9], and is a good indicator of the risk of antibody-mediated rejection. There are numerous reports of the feasibility and utility of the virtual crossmatch determined from solid-phase immunoassays (SPI). Vaidya established SPI values that yielded a high correlation between predicted and actual crossmatches [10]. The basis of this was a correlation between the strength of antibodies and the reactivity strength determined by SPI. However, these correlations were optimized when the strength of DSA and not third-party-specific antibody was used for predicting crossmatch outcome [11,12]. It has been demonstrated by others that bead-based fluorometric assays are more sensitive than colorimetric enzyme-linked immunosorbent assays (ELISA; [13–15]). We have observed that the presence of DSA is detected by Luminex based testing nearly twice as often as by ELISA [16]. However, both assays have utility depending on the needs of the program. The strength of DSA correlates with the risk of antibody-mediated rejection. Thus, it is incumbent upon the histocompatibility laboratory to determine the correlation between strength of DSA and clinical outcome. This may be a simple correlation between SPI and crossmatch results or it may be more complicated when other factors, such as repeat mismatches

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or available treatment protocols, must be considered. The critical elements of a virtual crossmatch are a thorough analysis of antibody screening test results, an accurate determination of antibody specificity and strength, knowledge of the factors that may affect test outcome, and careful analysis of the factors that may influence crossmatch outcome. We present here data important in the interpretation of SPI test results and the application of those results in deriving a meaningful virtual crossmatch.

2. Materials and methods

2.1. Crossmatch tests

Complement-dependent cytotoxicity (CDC) crossmatches were performed with AHG enhancement for T cells and with one wash step for B cells as described elsewhere [17]. The titer of CDC assays was given as the inverse of the highest dilution yielding a positive crossmatch. Flow cytometric crossmatches were performed as described previously [18]. The results of flow cytometric crossmatches were expressed either as the ratio of the median channel fluorescence of the test serum to that of the negative control (FCXM ratio) or as molecules of equivalent soluble fluorochrome (MESF).

2.2. SPI

Analyses to define antibody specificities were performed on the Luminex platform using HLA phenotype and single antigen panels as targets. The phenotype panels used were Lifematch class I and class II (Tepnel LifeCodes, Stamford, CT) and the single antigen panels were Single Antigen Beads (One Lambda, Inc., Canoga Park, CA). Tests were performed according to the manufacturer's instructions. Results of Luminex assays were expressed as mean fluorescence intensity (MFI). Specificity analysis was performed manually by two to three highly experienced individuals working independently.

2.3. Statistics

Correlation coefficients were determined from comparisons of crossmatch strength with that of the solid-phase values for donor-specific antibody. Chi-squared values were calculated for comparisons of actual and predicted crossmatch results. Sensitivity was determined dividing the number of correctly predicted positive crossmatches (*i.e.*, those in which both predicted and actual crossmatch were positive) by the actual number of positive crossmatches. Sensitivity was determined by dividing the number of correctly predicted negative crossmatches by the total number of negative crossmatches.

3. Results

3.1. Correlations between SPI values and crossmatch results

We determined SPI cutoff values for crossmatch prediction by examining the best fit for a threshold value defining positive and negative crossmatches (XM). For CDCXM we used titers established from doubling dilutions of the sera. Ratios and MESF values were used to assess FCXM strength. We compared ratios to MESFs for both T- and B-cell XMs and found them to be comparable, with $r \approx 0.93$ and 0.83 for T and B FCXM, respectively (data not shown).

When making comparisons between XM and phenotype results, we used the values of the highest ranking bead with reactivity resulting from DSA. For single antigen panels, we summed the values of the beads bearing donor antigens. For antigens represented by multiple alleles we used the value of the highest ranking allele. Sera containing autoantibodies were excluded from this study.

Fig. 1 shows the comparisons between T-cell XM results and MFI values from phenotype panels. Using MFI cutoff values of 10K and 6K for positive CDCXM and FCXM, respectively, we analyzed the correlations between those values and crossmatch outcome. The data for both T- and B-cell crossmatches are shown in Table 1. Correlations between SPI and crossmatch results were highly significant for all comparisons. The combined specificity and sensitivity for both T- and B-cell XMs predicted from phenotype panels were 89.9 and 94.6%, respectively, for CDCXM and FCXM taken together. The percentage of crossmatches that would be predicted correctly from the established SPI threshold values was 92.8% for CDCXM and 92.4% for FCXM, with an overall frequency of correct prediction of 91.7%. Importantly, the data were not skewed toward either positive or negative XMs.

It is necessary to determine the presence of DSA using single antigen panels when the DSA has reduced reactivity, has limited representation, or is masked by the presence of multiple antibodies on a phenotype panel. We compared XM results to the sum of SAB values for the DSAs present in the serum. Comparisons are shown in Fig. 2. The correlations between SPI and crossmatch results were low (−0.24) for T cells FCXM), as shown in the bottom half of Table 1. There were insufficient numbers of valid B-cell CDCXM to make a meaningful comparison. The specificity and sensitivity for all tests with single antigen panels were 47.9 and 77.4%, respectively. The percentage of correctly predicted XM was 82.6% for CDC, 47.9% for FCXM, and 56.2% for all XMs.

3.2. Issues with single antigen panels

Crossmatch predictions based on data from phenotype panels were superior to those predicted from single antigen panel data. A factor that may affect the reliability of predictions from single antigen panels is differing amounts of antigen and/or native epitopes present on different beads. We have examined the results of sera tested in parallel on two different single antigen panels (data not shown). The reactivities can be grouped into three categories: (1) one of the two panels yields better results than does the other; (2) both panels provide very good results; and, (3) both panels yielded mediocre results. The categories appear to include serologically related antigens, suggesting that category 1 is more affected by the product than by the antigens themselves. We also examined differences in the reactivity of 21 sera yielding positive crossmatches based on either DR-specific or DQ-specific antibodies alone. The mean values of each that correlated with a positive FCXM were 4970 :± 4500 and 9974 :± 6652 for DR and DQ, respectively. Thus, simply adding the values of different single antigen beads is not sufficient for predicting crossmatches.

3.3. Predictions based on all available information

Many factors can influence the outcome of solid phase assays including certain therapeutic agents, substances intrinsic to the serum specimen, environmental conditions, and normal variability in the assays. We examined whether considering other factors, such as donor antigen homozygosity, donor antigen expression, and inherent differences in bead reactivity strength, along with SPI results, would improve the success rate of crossmatch predictions. Three experienced individuals, who were knowledgeable about the properties of the assays and proficient in identifying and characterizing HLA specific antibodies used SPI data along with information about the donor phenotype to predict CDCXM and FCXM results for 136 sera containing HLA-specific antibodies. The assays included single antigen and/or phenotype panels and the predictions were for positive and negative results for any (T or B) cell XM. They correctly predicted respective CDCXM and FCXM results 92.6 and 92.9% of the time (data not shown). These results indicate that crossmatch predictions incorporating all available data are accurate and more reliable than those based solely on results from SPI.

3.4. Application of the virtual crossmatch

In addition to crossmatch prediction, the virtual crossmatch can be applied to monitoring for response to desensitization and for

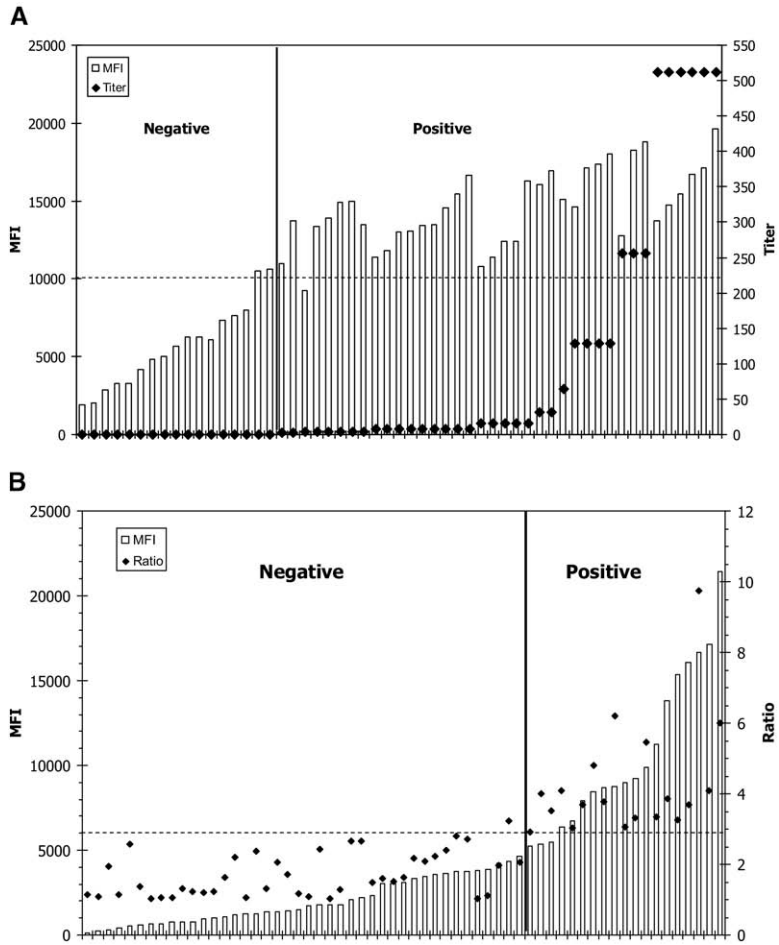

Fig. 1. Comparisons of crossmatch and phenotype panel solid-phase results. The MFI values for DSA obtained from tests using phenotype panels are compared with crossmatch results. Both comparisons are for T-cell crossmatches. Cytotoxicity and flow cytometric crossmatches are shown in a and b, respectively. The dashed lines indicate the thresholds from the phenotype panels for predicted positive crossmatch results. The correlation for cytotoxicity crossmatches is r = 0.83 and that for flow cytometric crossmatches is 0.85. Open bars are SPI results and filled diamonds are XM results.

onset of antibody-mediated rejection. Fig. 3 illustrates the case of a patient for whom we had predicted a moderately strong CDCXM based on the presence of two DSAs, both with MFI > 10K on phenotype panels. The patient's crossmatch, however, was negative because of reduced antigen expression in the donor. Based on the supposition that antigen expression would be increased on the donor organ at transplant due to the release of proinflammatory cytokines in response to the surgical trauma, we followed the antibodies of this patient using SPI. Fig. 3 shows the course of DSA during desensitization treatment occurring prior to and after transplant and illustrates a direct correlation between increases in antibody levels and the occurrence of antibody-mediated rejection. It

Table 1
Correlation between crossmatch and solid-phase immunoassay results

Target cells	Maximum MFI phenotype panels	CDC crossmatch				Maximum MFI phenotype panels	Flow cytometric crossmatch			
		Positive	Negative	r	r(T+B)		Positive	Negative	r	r(T+B)
T	0–10,000	1	15	0.87	0.83	0–6,000	3	41	0.90	0.85
	>10,000	37	2			>6,000	19	1		
B	0–10,000	4	15	0.8		0–6,000	3	18	0.74	
	>10,000	35	1			>6,000	31	4		

Target Cells	SAB Sum	CDC crossmatch			SAB sum	Flow cytometric crossmatch			
		Positive	Negative	r		Positive	Negative	r	
T	0–10,000	4	12	0.6	0–5,000	27	42	−0.24	−0.11
	>10,000	31	5		>5,000	14	7		
B	ND				0–5,000	50	20	0.06	
					>5,000	3	2		

			Specificity	Sensitivity
Phenotype panel:	Percent correct:	CDC = 92.8%; Flow = 92.4%	89.9%	93.7%
SAB sum panel:	Percent correct:	CDC = 82.6%; Flow = 47.9%	48.0%	77.4%

r = correlation coefficient; SAB = single antigen bead panels; MFI = mean/median fluorescence intensity.
There were insufficient valid B-cell CDC crossmatches for evaluation.
ND = not done.

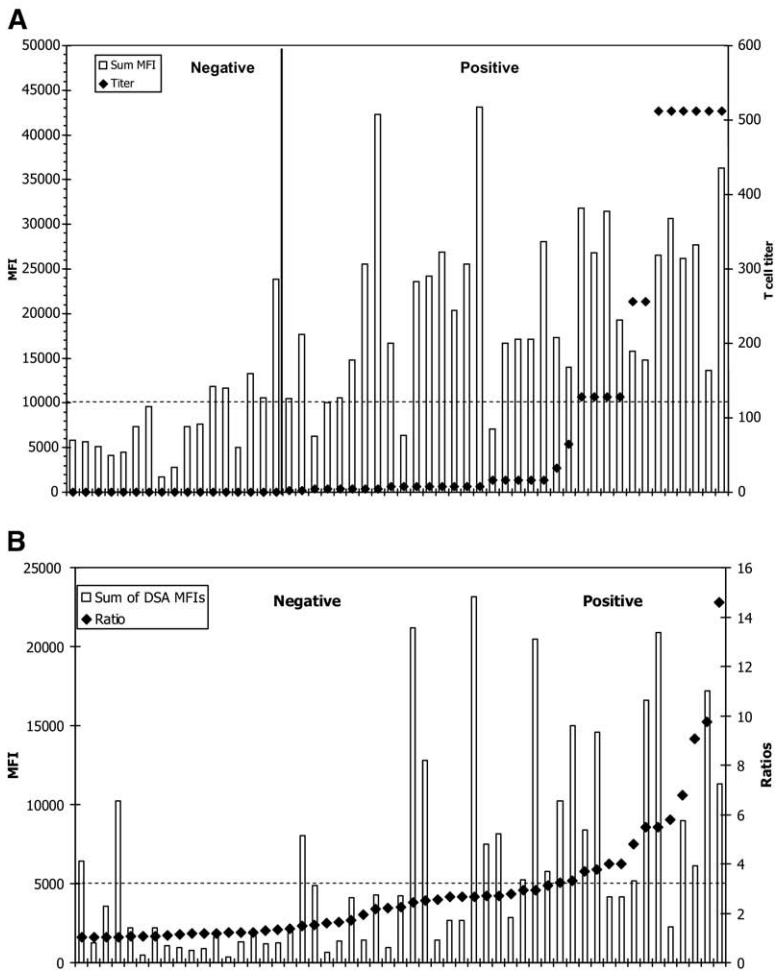

Fig. 2. Comparisons of crossmatch and single antigen panel results. The MFI values obtained for DSA obtained from tests using single antigen panels are compared with crossmatch results. The sum of the values from beads bearing donor class I antigens was used. For antigens represented by more than one allele, the allele giving the highest MFI was used. T-cell cytotoxicity crossmatches are shown in a with $r = 0.60$. T-cell flow cytometric crossmatches are shown in b with $r = -0.24$. The dashed lines indicate the thresholds from the single antigen panels for predicted positive crossmatch results. Open bars are SPI results and filled diamonds are XM results.

further shows that the results of tests against pooled antigens paralleled the course of DSA assessed on phenotype panels.

4. Discussion

We have described the process and shown the validity of performing a virtual crossmatch. The strength of DSA is directly proportional to the potential for damage to a transplanted organ and has traditionally been assessed with cell-based crossmatch tests. The data presented here demonstrate that the strength of DSA determined by crossmatch tests correlates significantly with that assessed by phenotype panel SPI. These data were from Luminex-based assays; however, we have also demonstrated [19] that reliable predictions (92.1% correct predictions) can be performed on an ELISA microchip assay (data not shown here). These strong correlations permit establishing threshold values that correctly predict crossmatch outcomes with high frequency and that translate into a virtual crossmatch.

However, predictions based on single antigen panels proved to be less accurate. Numerous reasons may account for this. It is not clear whether summing the values for antibodies of different specificities, as we have done here, is appropriate. For example, if several alleles of an antigen are serologically very similar, should all the alleles be counted even if the antibody of one might be sufficient to bind to all the antigens on the cell surface? The amount of either antigen or native epitope on a bead may vary and not be representative of the amount found on cells. The reaction strength of DQ-bearing beads is twice that of DR-bearing beads, on average, and yields different correlations with crossmatch results and there may be substantial variability between products from different sources. Idica *et al.*, have demonstrated reactions of antibodies with apparent nonnative or denatured epitopes that may arise as a result of distortion of HLA molecules when isolated or placed on a solid matrix [20]. Such variability is not limited to single antigen panels but also occurs with phenotype panels. We have observed that certain antigens tend to yield weaker or stronger results than others. It is difficult to determine from these observations whether the difference is in the stability of the target, the avidity of antibody, or the immunogenicity of different antigens [18]. Additionally, reactivity with some or all antigens or phenotypes can vary from lot to lot.

Although the ability to predict accurately XM outcome from solid phase data alone is substantial, there remains the possibility of further improvement. The recognition and application of the factors mentioned above, as well as others such as high background, must be taken into account. We have shown elsewhere that the occurrence of high negative control values and/or low positive control values in SPI may reduce the strength of HLA-specific antibodies and also affect the ability to recognize the presence of DSA using phenotype panels. We have also shown that, in many cases, the background can be eliminated, restoring the rec-

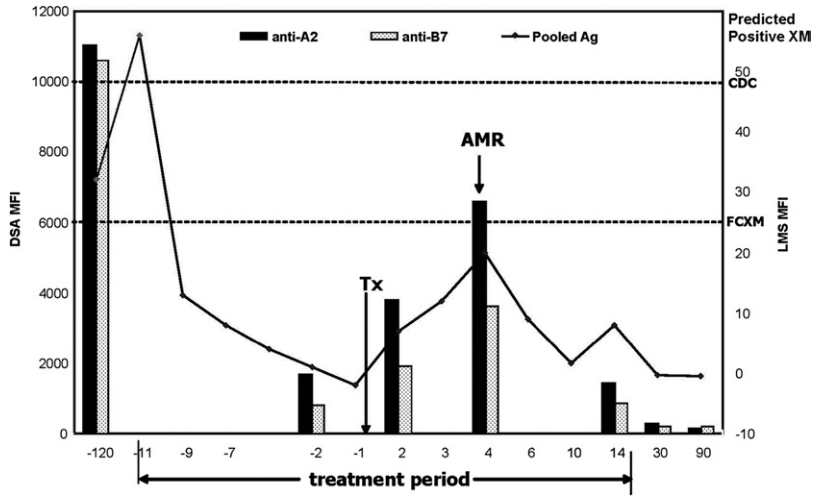

Fig. 3. Correlation between solid phase results and clinical course and the clinical course of a patient who had an antibody mediated rejection (AMR) while undergoing desensitization. Valid crossmatches could not be obtained and DSA was tracked using SPI phenotype panels. The graph illustrates the strengths of the patient's two donor-specific antibodies, anti-HLA-A2 (unfilled bars) and HLA-B7 (filled bars), as well as the values obtained from the pooled antigen screen. The dashed lines indicate the thresholds for predicted positive crossmatch results for CDCXM and FCXM, respectively. Increases in DSA predicted the development of the AMR.

ognition of DSA and increasing its strength [21]. Others have demonstrated that normalizing single antigen panels for the amount of antigen improves the accuracy of the assessment of strength [22]. Our experiment of having two or three individuals make predictions based on an analysis that includes some of these additional factors improved the accuracy of crossmatch predictions. Interestingly, the collective evaluation of the individuals was better than that of any one individual, which may reflect differences in familiarity with the SPIs and amount of experience.

Use of the virtual crossmatch (VXM) provides numerous benefits [5–13,23–25]. The most common use of the virtual crossmatch is for deceased-donor transplants. Identifying and listing unacceptable antigens based on correlations between solid phase immunoassays and levels of antibody considered permissible for transplantation reduces the number of unanticipated positive crossmatches and permits expansion of the area from which donors can be recovered. However, the virtual crossmatch is also valuable for living-donor transplantation when crossmatch tests are not feasible because the patient has received cytotoxic therapeutic agents [12]. Notably, SPI are useful for monitoring the response to desensitization treatments and for early detection of posttransplant antibody-mediated rejection [26,27]. Following transplantation, the virtual crossmatch permits rapid identification of increases in the strength of existing DSA or the development of new DSA, which in turn provides an opportunity for preemptively treating a developing antibody-mediated rejection and may avoid the need for a biopsy.

Given achievable accuracies of at least 93%, it is debatable whether a virtual crossmatch can replace an actual crossmatch. The risk of error is greater for sensitized than for nonsensitized patients and the consequences of error are potentially greater for false-negative results than for false-positive ones. The likelihood of the virtual crossmatch being disparate with the actual crossmatch can be affected by several factors, including the age of the SPI data and the accuracy of the antibody characterization. If the SPI analyses are not current, the possibility of a sensitizing event that increases the strength or breadth of antibody increases. Locke *et al.* have demonstrated that among sensitized patients proinflammatory events such as infection and trauma can nonspecifically provoke increases in HLA-specific antibodies [28]. Such factors could result in falsely negative crossmatch predictions when analyses of current antibody are not available. Certainly, the virtual crossmatch may be the only pretransplant crossmatch possible for organs with short permissible ischemia times, but in many cases, actual crossmatches can be performed without adding to ischemia time because of the time required for other pretransplant testing or procedures. Considering all of the above, we believe that the question is not whether an actual crossmatch is necessary, but rather whether the benefit to be gained from omitting that crossmatch is worth the possible consequences.

The ability to predict crossmatches accurately is a boon for transplantation. However, solid phase immunoassays provide assessment of antibody strength over a large range measured on a continuous, rather than discreet, scale. When these finely measured levels of antibody are correlated with clinical outcomes, these data, along with assessment of the patient's history as a high or low responder, identification of mismatches repeated in the current donor, and mismatch homozygosity and immunogenicity, can provide an assessment of risk of antibody-mediated rejection rather than a simple crossmatch outcome. For this reason, we believe it is more important to standardize tests between a laboratory and the transplant program(s) it serves rather than among laboratories. We note that although all or nearly all laboratories perform cytotoxicity testing, there are multiple varieties of the procedure in use and laboratories performing flow cytometric crossmatches vary in the way the data are presented and in their values for positivity. Therefore, precedent indicates that this variability should be permissible for the virtual crossmatch as well. Both crossmatch prediction and assessment of risk require thorough and accurate analysis of solid phase data, which requires an awareness of the strengths and shortcomings of these assays, ongoing quality control to improve the evaluation of the data, ongoing assessment of the correlation between tests for measuring antibody strength, and the ability to assess which test is providing the most accurate information when crossmatch and SPI outcomes cannot be reconciled. Importantly, the data presented here also demonstrate the value of having both single antigen and phenotype panels available.

References

[1] Mickey MR, Ayoub G, Terasaki PI. Prediction of negative cross match: an aid for cost-effective kidney sharing. Transplant Proc 1982;14:279–81.
[2] Oldfather JW, Anderson CB, Phelan DL, Cross DE, Luger AM, Rodey GE. Prediction of cross match outcome in highly sensitized dialysis patients based on the identification of serum HLA antibodies. Transplantation 1986; 42:267–70.

[3] Williams GM, Hume DM, Hudson RP Jr, Morris PJ, Kano K, Milgrom F. "Hyperacute" renal-homograft rejection in man. N Engl J Med 1968;279:611–8.
[4] Patel R, Terasaki PI. Significance of the positive cross match test in kidney transplantation. N Engl J Med 1969;280:735–9.
[5] Zachary AA, Montgomery RA, Leffell MS. Defining unacceptable HLA antigens. Curr Opin Organ Transplant 2008;13:405–10.
[6] Zachary AA, Leffell MS. Detecting and monitoring human leukocyte antigen-specific antibodies. Human Immunol 2008;69:591–604.
[7] Appel JZ III, Hartwig MG, Cantu E III, Palmer SM, Reinsmoen NL, Davis RD. Role of flow cytometry to define unacceptable HLA antigens in lung transplant recipients with HLA-specific antibodies. Transplantation 2006;81: 1049–57.
[8] Zangwill SD, Ellis TM, Zlotocha J, Jaquiss RD, Tweddell JS, Mussatto KA, Berger S. The virtual cross match—a screening tool for sensitized pediatric heart transplant recipients. Pediatr Transplant 2006;10:38–41.
[9] Zangwill S, Ellis T, Stendahl G, Zahn A, Berger S, Tweddell J. Practical application of the virtual cross match. Pediatr Transplant 2007;11:650–4.
[10] Vaidya S. Clinical importance of antihuman leukocyte antigen-specific antibody concentration in performing calculated panel reactive antibody and virtual cross matches. Transplantation 2008;85:1046–50.
[11] Vaidya S, Partlow D, Susskind B, Noor M, Barnes T, Gugliuzza K. Prediction of cross match outcome of highly sensitized patients by single and/or multiple antigen bead luminex assay. Transplantation 2006;82:1524–8.
[12] Tambur AR, Leventhal J, Kaufman DB, Friedewald J, Miller J, Abecassis MM. Tailoring antibody testing and how to use it in the calculated panel reactive antibody era: the Northwestern University experience. Transplantation 2008; 86:1052–9.
[13] Yang J, Schall C, Smith D, Kreuser L, Zamberlan M, King K, Gajarski R. HLA sensitization in pediatric pre-transplant cardiac patients supported by mechanical assist devices: the utility of luminex. J Heart Lung Transplant 2009; 28:123–9.
[14] Lee PC, Ozawa M, Hung CJ, Lin YJ, Chang SS, Chou TC. Reappraisal of HLA antibody analysis and cross matching in kidney transplantation. Transplant Proc 2009;41:95–8.
[15] Muro M, Llorente S, MarÎn L, Moya-Quiles MR, Gonzalez-Soriano MJ, Prieto A, et al. Acute vascular rejection mediated by HLA antibodies in a cadaveric kidney recipient: discrepancies between FlowPRA, ELISA and CDC vs luminex screening. Nephrol Dial Transplant 2005;20:223–6.
[16] Zachary AA, Montgomery RA, Simpkins CE, Warren D, Leffell MS. Impact of persistent, low levels of donor specific antibody on renal transplants. Am J Transplant 2005;5(S11):245.
[17] Hopkins KA. The basic lymphocyte microcytotoxicity tests: standard and AHG enhancement. In: Hahn AB, Land GA, Strothman RM, eds. The ASHI laboratory manual, 4th ed. Mt. Laurel, NJ: The American Society for Histocompatibility and Immunogenetics; 2000, p 1.C.1.1–1.C.1.5.
[18] Zachary AA, Delaney NL, Lucas DP, Leffell MS. Characterization of HLA class I specific antibodies by ELISA using solubilized antigen targets. I. Evaluation of the GTI QuikID assay and analysis of antibody patterns. Human Immunol 2001;62:228–35.
[19] Vega R, Trujillo O, Sikorski P, Houp J, Sholander J, Zachary AA, et al. A new fully-automated solid-phase assay for HLA-specific antibodies. Hum Immunol 2008;68:S78.
[20] Idica AK, Kaneku HK, Sasaki N, Rebellato LM, Terasaki PI. Denatured and intact HLA specific antibody in transplantation. Abstract accepted for poster presentation, American Transplant Congress, May 31, 2009. (http://www.abstracts2view.com/atc/view). Accessed April 6, 2009.
[21] Zachary AA, Lucas DP, Detrick B, Leffell MS. Naturally occurring interference in luminex® assays for HLA-specific antibodies: characteristics and resolution. Human Immunol 2009 April 11 [Epub ahead of print].
[22] Warner PR, Youngs D, Nelson KA. HLA density on microparticles versus antibody detection sensitivity: implications for cross match prediction. Hum Immunol 2007;68:S28.
[23] Bielmann D, Honger G, Lutz D, et al. Pretransplant risk assessment in renal allograft recipients using virtual cross matching. Am J Transplant 2007;7:626–32.
[24] Bingaman AW, Murphey CL, Palma-Vargas J, Wright F. A virtual cross match protocol significantly increases access of highly sensitized patients to deceased donor kidney transplantation. Transplantation 2008;86:1864–8.
[25] Tait BD, Hudson F, Cantwell L, Brewin G, Holdsworth R, Bennet G, Jose M. Luminex technology for HLA antibody detection in organ transplantation. Nephrology 2009;14:247–54.
[26] Reinsmoen NL, Lai C, Vo A, et al. Acceptable donor-specific antibody levels allowing for successful deceased and living donor kidney transplantation after desensitization therapy. Transplantation 2008;86:820–5.
[27] Leffell MS, Zachary AA. The role of the histocompatibility laboratory in desensitization for transplantation. Curr Opin Organ Transplant 2009 May 4 [Epub ahead of print].
[28] Locke JE, Zachary AA, Warren DS, Segev DL, Houp JA, Montgomery RA, et al. Proinflammatory events are associated with significant increases in breadth and strength of HLA-specific antibody. Am J Transplant 2009;in press.