

Pre-Transplant Assessment of Donor-Reactive, HLA-Specific Antibodies in Renal Transplantation: Contraindication vs. Risk

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Overview

In renal transplantation, a positive cytotoxic crossmatch between donor cells and recipient serum is associated with early rejection or graft loss and was the driving force behind the establishment of HLA laboratories. Initially, crossmatches were performed by relatively insensitive techniques [e.g. leukoagglutination and direct complement-dependent cytotoxicity (CDC) of target cells]. A negative result justified proceeding, while a positive crossmatch was considered a contraindication to renal transplantation. However, the underlying premises driving this practice, namely that (i) all positive reactions were the result of relevant (i.e. HLA) antibodies that could lead to allograft rejection; and (ii) all negative reactions predicted long-term graft survival were known to be incorrect. From its first clinical description, the simple complement-dependent assay was recognized as neither sufficiently specific nor sensitive to identify all relevant antibodies. Over time, more sensitive and specific lymphocyte crossmatch assays were developed that effectively decreased the incidence of early antibody-mediated rejection.

In recent years, advances in immunosuppressive therapy have led clinicians to ask whether antibodies identified by these more sophisticated crossmatch techniques represent a contraindication to transplantation. To answer this question, it is essential to prove (or disprove) that antibodies specific for donor HLA antigens are present in recipient sera. A critical analysis of the literature revealed that the majority of studies failed to provide sufficient evidence to ensure that positive (as well as negative) crossmatches were correctly assigned. Indeed, few investigators performed the labor-intensive studies necessary to document that positive crossmatches were the

result of antibodies specific for donor HLA antigens. Furthermore, the testing methodology used in those studies was relatively insensitive compared with current and emerging technologies. Given these limitations, we believe it is essential to re-examine the conclusions of studies that formed the basis of our current crossmatch paradigms.

Just as the clinical application of calcineurin inhibitors revolutionized transplant medicine, the recent development of HLA antigen specific solid-phase assays (i.e., ELISA and microparticle-based flow cytometric assays) has similarly revolutionized our ability to detect HLA antibodies. Specifically, by documenting whether patient sera possess donor-reactive HLA antibodies, a lymphocyte crossmatch can now be more reliably interpreted. Indeed, with solid-phase data, a positive lymphocyte crossmatch can now be categorized as (i) clinically irrelevant, (ii) a risk factor for rejection or graft loss, or (iii) a contraindication to renal transplantation. It is our position that only with accurate risk assessment can desensitization protocols [e.g., intravenous gamma globulin (IVIG) with or without plasmapheresis] be optimally applied.

Lymphocyte Crossmatch

In 1969, Patel and Terasaki published a landmark study in which they unequivocally demonstrated that the presence of recipient antibodies to antigens expressed on donor white cells were a major risk factor for immediate graft loss (1). The data were so compelling that the authors asserted '... the ethics of transplanting kidneys without the prior knowledge of the results of the lymphocyte crossmatch test ... can reasonably be expected to be questioned'. Since then, prospective crossmatching has been performed on a 24/7 basis. Consequently, although hyperacute allograft rejection has not been completely eliminated (2), its incidence has been significantly reduced, as patients with positive prospective lymphocytotoxic crossmatches against their prospective donors were not transplanted. The original complement-dependent crossmatch was a direct cytotoxicity assay referred to in many different terms, including; 'Basic Crossmatch', 'Standard Crossmatch', 'NIH Crossmatch' or the 'No-Wash Crossmatch'.

False-negative crossmatches

In their paper, Patel and Terasaki reported that early graft loss occurred in some patients despite a negative lymphocyte crossmatch (1). Concern that relevant antibodies were not being detected led to the development of more sensitive crossmatch techniques (3–17). These include the following:

- Extended CDC T cell: An extended incubation crossmatch, which simply lengthens the incubation times of both the serum plus donor cells and complement compared with the standard CDC, potentially augmenting test sensitivity. This same procedure can be applied to B cells.
- Amos (3-Wash) and Amos-modified (1-Wash) CDC T cell: Wash steps are added following the primary incubation and just before the addition of complement. The wash steps eliminate anticomplementary factors that promote false-negative CDC crossmatches (3). This procedure removes unbound serum from the lymphocyte suspension before the addition of complement. This same procedure can be applied to B cells.
- The assays listed below are 'indirect crossmatches', as second-step reagents are used to augment the detection of antibody in a complement dependent or independent fashion.
- AHG-CDC T-cell: An antihuman globulin (AHG) augmented assay, developed by Johnson et al. (4), identifies low titer antibodies as well as antibodies that do not fix complement *in vitro*, including the so-called cytotoxicity negative adsorption positive (CYNAP) antibodies (10). In this assay, a complement-fixing AHG (e.g. goat-antihuman light chain) is added before the addition of complement. This methodology provides increased sensitivity for antibody detection.
- AHG-CDC B cell: As B cells express surface immunoglobulin, it was thought that an AHG augmented assay to detect B-cell antibodies would not be feasible, as AHG would bind directly to surface Ig and fix complement independent of donor reactive antibodies. However, the AHG assay can be performed by a two-color immunofluorescence technique (11), wherein B cells are first labeled with a fluorochrome-conjugated anti-immunoglobulin. This effectively prevents the AHG from binding to surface immunoglobulin and permits B cells to be used as target cells.
- Flow crossmatch; T and B cell: The flow cytometric procedure detects donor-reactive antibodies independent of complement fixation (12). The flow cytometric crossmatch (FCXM) is performed by incubation of patient sera with donor lymphocytes that are then stained with a fluorochrome-conjugated secondary antibody (e.g. anti-IgG). Antibody is detected and measured by surface fluorescence of the lymphocytes. As originally reported by Garavoy et al. (12), the FCXM can be one to three logs more sensitive than the AHG-CDC assay (depending on the serum : cell combination). A major advantage of the FCXM is that antibody reactivity

can be independently and simultaneously evaluated on donor T and B lymphocytes. In addition, because the FCXM is a semiquantitative measure of antibody binding, it can be less subjective than visual assessment of cell death in complement-dependent assays.

Currently, the AHG-CDC and flow cytometric techniques are widely accepted as being significantly more sensitive than direct CDC assays. However, in 2002, of the >150 labs participating in the ASHI-CAP class I crossmatch surveys (MX1-A, B, C), only 68–70% reported AHG augmented CDC and 47–52% flow-based crossmatches. While consensus (>90% agreement between centers) could be reached 75% of the time for direct CDC crossmatches, it occurred only 54% of the time for AHG and 58% of the time for flow crossmatch techniques. In part, significant variations in the methodology (e.g. sera dilution, number and type of target cells, number of washes, source of secondary reagents, etc.) contribute to the lack of consensus. Such variations were reported in the multicenter evaluation of the flow T-cell crossmatch published in 1997 by Scornik et al. who noted that the proportion of centers detecting antibodies in all sera expected to be positive was only 50–60% (16). Thus, while the enhanced sensitivity of the above assays helped to eliminate false-negative CDC crossmatch results from clinical practice, the collective data highlight the need to develop and implement objective approaches to limit the variability of crossmatch methodologies.

Irrelevant positive crossmatches

Historically, positive crossmatches were considered a contraindication to transplantation based on the presumption that recipient antibody reactivity against donor lymphocytes was HLA specific. However, multiple factors must be considered before interpreting a positive crossmatch as clinically relevant. For example, numerous studies have reported that autoreactive cytotoxic antibodies (IgM or IgG) can yield a positive crossmatch but are not a contraindication to transplantation (18–28). Thus, the performance of an auto-crossmatch aids interpretation of a positive allo-crossmatch.

Additional efforts to confirm that a positive crossmatch was the result of a donor-reactive HLA antibody frequently employed time-consuming techniques such as absorption of sera with autologous lymphocytes or platelets: if the postabsorption crossmatch remained positive, the original crossmatch was presumed to be the result of an HLA antibody. Unfortunately, assays relying on cellular absorption techniques are severely limited in their ability to reliably differentiate HLA from non-HLA antibodies.

The development and implementation of solid-phase assays utilizing purified HLA molecules as targets have greatly improved the identification of HLA antibodies (29–32). Enzyme-linked immunosorbent assays (ELISA) and

microparticle based technologies can be more sensitive and specific than cytotoxicity assays to identify antibodies to class I and class II HLA antigens (33). Improvements in these two attributes can clearly enhance the diagnostic accuracy of pretransplant HLA antibody analysis (i.e. PRA), thereby appropriately assigning clinical relevance to a positive crossmatch. However, it will be critical for each laboratory to validate and optimize the assays for their centers. Indeed, the performance of multiple assays should be considered, as no single test is without its limitation. For example, if a transplant was performed based exclusively on a negative direct CDC crossmatch, but the serum was then shown to contain donor-reactive HLA antibody by ELISA, the donor : recipient combination would have been inappropriately considered to have negligible risk. Therefore, while we recognize that CDC assays can provide useful information, we believe that (i) solid-phase assays provide additional clinically relevant information, and (ii) the final crossmatch assay should approximate the sensitivity of the solid-phase assay.

Potentially relevant crossmatches

The number of studies that investigated donor-reactive, HLA-specific, IgM antibodies is scarce, likely owing to the low incidence of these antibodies (22,34–36). Furthermore, none of these studies convincingly support or refute the clinical relevance of such IgM HLA antibodies. For example, in 1986, Chapman et al. (22) published that 3/7 patients with donor-reactive HLA-specific, IgM alloantibodies lost their grafts within 6 months post-transplant. In contrast, more recent data by McCalmon et al. (36) reported no significant impact of HLA-specific IgM antibodies in five renal transplant recipients. It should be emphasized that neither flow crossmatch nor solid-phase techniques were employed in those studies. Furthermore, the presence of underlying HLA-specific IgG antibodies was not determined. Despite the absence of definitive studies, general practice tends to disregard donor-reactive, HLA-specific, IgM antibodies.

Relevant positive crossmatches

Non-HLA antibodies (e.g., anti-phospholipid, anti-endothelial, organ specific) have been associated with allograft rejection. Such antibodies do not react with lymphocyte targets and are not the focus of this discussion. It is our position that 'relevant positive' lymphocyte crossmatches are those due to donor-reactive, HLA-specific, IgG antibodies. While the clinical significance of a positive direct cytotoxic crossmatch as a result of HLA antibodies is well accepted, there is debate as to the degree of clinical relevance of these antibodies under the following circumstances:

- Low titer and/or noncomplement fixing antibodies
- Antibodies against HLA class II antigens
- Donor-specific HLA antibodies detected in historic sera.

While each of these will be discussed, the following variations in methods and study design limit the extrapolation from historical data to current practice:

- Failure to confirm that a positive crossmatch was the result of HLA antibody.
- Failure to determine if the HLA antibody is directed against class I or class II HLA antigens (specifically for B-cell crossmatching).
- Failure to adhere to rigid criteria documenting the cause of graft rejection/loss. For example, performing pre- and post-transplant crossmatches and failing to correlate with histology consistent with antibody-mediated rejection (i.e. elution of donor-specific HLA antibody from the biopsy or at least C4d staining as a surrogate for complement activation).
- Failure to consider the relative impact of enhanced crossmatch techniques in the context of improved immunosuppressive therapies.

With these caveats in mind, the literature will be reviewed to address whether donor-directed, HLA-specific, IgG antibodies constitute a contraindication or a risk factor for transplant. Moreover, the data will be concurrently reviewed to assess if the conclusions can be equally applied to antibodies directed against class I and class II HLA antigens and to primary and regraft patients.

Clinical Significance of Low Titer and/or Noncomplement-Fixing Antibodies

The AHG-CDC crossmatch was developed in 1972 to detect low titer and/or noncomplement fixing antibodies (4). In 1983, the flow cytometric crossmatch described by Garavoy et al. (12) further enhanced the ability to detect these types of antibodies. Currently, the ASHI/CAP proficiency testing survey indicates that approximately 70% and 50% of participating laboratories perform the AHG-CDC and flow cytometric crossmatches, respectively. In the case of flow crossmatching, while instrumentation cost has been a limiting factor restricting its use, the principal reason for the lack of universal adoption has been the claim by some transplant programs that a crossmatch positive only by flow cytometry (1): does not reliably predict unacceptable clinical outcome (i.e. hyperacute or accelerated rejection) (2); would inappropriately deny an individual access to a transplant and (3) is 'too' sensitive (i.e. low titer or noncomplement fixing antibodies have limited impact). We believe that a positive FCXM should neither be considered an indisputable contraindication to transplantation nor be completely disregarded. Rather, the FCXM should be used to provide an assessment of risk for rejection and/or graft loss not predicted by a negative serologic crossmatch.

Despite the issue of standardization, investigators have reported fairly consistent rates of serologic negative, flow positive crossmatches (median = 15% for primary grafts

Table 1: Impact of HLA antibodies detected only by a flow cytometric crossmatch

Primary transplant	Pt (n)	CXM method	PRA method	Defn T+ FXCM ¹	% T+ FXCM	Early graft loss (<3 mo) FP vs. FN	Acute rejection FP vs. FN	Survival 1 yr FP vs. FN
Iwaki (38)	113	CDC	CDC	2 SD	16%	22% vs. 4%		
Cook (39)	196	CDC	CDC	10/256	18%	22% vs. 7%		
Kerman (43) ²	89	CDC	CDC	20/256	13%			83% vs. 78%
Mahoney (42)	67	AHG	AHG	40/1024	18%	33% vs. 7%		67% vs. 85%
Ogura (45)	841	CDC	CDC	50/1024	18%	20% vs. 7%		75% vs. 82%
Lefor (48)	214	AHG	AHG	50/1024	7%			75% vs. 86%
Pelletier (50) ²	102	Amos	Amos	40/1024	18%		67% vs. 51%	86% vs. 98%
Kimball (51)	157	Amos	Amos	40/1024	14%		51% vs. 25%	44% vs. 97%
Kerman (53) ² (Cadaveric)	97	AHG	AHG	20/256			44% vs. 40%	81% vs. 83%
Kerman (53) ² (Haplo LRD)	118	AHG	AHG	20/256			31% vs. 26%	89% vs. 92%
El Fettouh (54)	187			500 MESF	3%		80% vs. 30%	3 year 80% vs. 93%
Karpinski (56)	143	AHG	Flow class I and II	40/1024	13%	33% vs. 11%	OKT3 25% vs. 5%	

Primary and regrafts	Pt (n)	CXM method	PRA method	Defn T+ FXCM ¹	% T+ FXCM	Early graft loss (<3 mo) FP vs. FN	Acute rejection FP vs. FN	Survival 1 yr FP vs. FN
Thistlethwaite (37)	80	AHG	AHG	20/256	28%	27% vs. 2%	55% vs. 45%	
Talbot (41)	68	CDC	CDC	3 SD	18%	25% vs. 8%	OKT3/ATG 30% vs. 6%	
Berteli (44)	154	CDC	CDC	1 SD	20%	35% vs. 11%	94% vs. 67%	58% vs. 85%
Christiaans (46) ²	114	CDC	CDC	3 SD	13%		53% vs. 41%	80% vs. 87%
Utzig (49)	38	CDC	CDC	2 SD	18%		71% vs. 26%	
O'Rourke (51)	100	AHG	AHG	10/256	29%		69% vs. 45%	76% vs. 83%

Regrafts	Pt (n)	CXM method	PRA method	Defn T+ FXCM ¹	% T+ FXCM	Early graft loss (<3 mo) FP vs. FN	Acute rejection FP vs. FN	Survival 1 yr FP vs. FN
Iwaki (38)	23	CDC	CDC	2 SD	26%	67% vs. 12%		
Cook (39)	35	CDC	CDC	10/256	18%	56% vs. 21%		
Mahoney (42)	23	AHG	AHG	40/1024	43%	60% vs. 0%		40% vs. 77%
Kerman (43)	47	CDC	CDC	20/256	62%		76% vs. 44%	48% vs. 83%
Mahoney (47)	103	AHG	AHG	40/1024	34%	34% vs. 13%		66% vs. 85%
Bryan (52)								
AHG-ve	174	AHG	AHG			32%		70%
Flow-ve	106			2 SD		8%		88%

¹Defn T + CXM: Center definition as to what constitutes a positive flow crossmatch: values are expressed as standard deviation (SD) from normal sera or channel shift/scale.

²Studies showed no significant difference compared with flow-negative cross-match recipients.

CXM = crossmatch, FXCM = flow crossmatch, FP = flow positive, FN = flow negative.

and 34% for regrafts (37–56) (Table 1). A majority of groups comparing direct CDC techniques to flow cytometry found that early graft loss (i.e. <3 months) occurred in approximately 20% of primary grafts and 60% of regrafts when the flow crossmatch was positive compared with 5% and 15%, respectively, when the flow crossmatch was negative (Table 1). This trend is evident even when comparing AHG-CDC crossmatches to flow cytometry. Furthermore, recent papers by Karpinski et al. and Kerman et al. report that patients with pretransplant HLA antibodies detectable only by flow microparticles have significantly more episodes of acute rejection compared with patients with no detectable antibody, as well as graft loss if patients also have a positive pretransplant flow cytometric cross-

match (56–58). The data by Kerman et al. are particularly informative because they are in direct opposition to their earlier publication, where crossmatch data was interpreted without the benefit of solid-phase methodology to confirm (or deny) that HLA antibodies were present (57,58). Finally, in a recent report by Cho and Cecka, multivariate analysis of UNOS data on 86 447 renal transplants from 1991 to 2000, found that transplants performed across a flow positive vs. flow negative crossmatch conferred an odds ratio (OR) of 1.54 (1.22, 1.93), $p < 0.001$, for graft loss in the first year (59). The study underscores how few transplants have actually been performed across AHG or flow T-cell-positive crossmatches: it would appear that in practice transplant centers are actively avoiding these cases.

Table 2: Post-transplant donor specific HLA antibody and renal allograft outcome

Study (Pre-Tx CXM -ve)	n	Time post-transplant sera collected	Pre/Post CXM or Ab	Post-Tx PRA class	Acute rejection	Early graft loss (<3 mo)	Graft survival
Martin (60) (CDC)	171	0 – 5 yr	Neg/Neg	I (50%) II (11%) I/II (36%)			5 year 76%
	63		Neg/Pos				12%
Suciu-Foca (62) (CDC)	28	0 – 12 mo	Neg/Neg				4 year 71%
	30		Neg/Pos				45%
Halloran (63) (CDC)	51	0 – 3 mo	Neg/Neg	I (13)	41%	4%	
	13		Neg/Pos		100%	38%	
Lobo (65) (AHG-CDC) Stable	47	0 – 12 mo	Neg/Neg			0%	1 year 100%
	63		Neg/Neg			10%	72%
Rejection	17		Neg/Pos			76%	24%
Christiaans (71) (CDC)	113	0 – 6 mo	Flow Neg/Neg		35%		5 year 60%
	14		Neg/Pos		80%		35%
Muller-Steinhardt (74) (CDC)	59	0 – 12 mo		Flow Neg (50)	39%		1 year 92%
				I (5)	40%		60%
				II (15)	50%		25%
Supon (75) (AHG-CDC)	176	1wk – 11 yr		ELISA Neg (160)	49%		1 year 86%
				I (1)	100%		100%
				II (15)	60%		80%
Piazza (76) (Flow-CXM)	91	0 – 12 mo	Neg/Neg	Flow Neg (91)	13%		2 year 99%
	29		Neg/Pos	I (7)	62%		66%
				I + II (11)			
				II (2)			
Pelletier (77) (Flow-CXM)	229	1 mo – 9.5 yr (mean 2.6 yr)		Flow Neg (178)	4%		8 year 97%
				I (5)	44%		58%
				II (34)			
				I+II (11)			

CXM = crossmatch.

Further support for the pathogenic potential of donor-reactive HLA antibodies detectable only by AHG-CDC or flow cytometry is derived from studies of post-transplant antibody monitoring (60–77). Early reports, transplanting on the basis of a negative direct CDC crossmatch, found a prevalence of donor-reactive HLA IgG antibody post-transplant ranging from 20 to 52% (Table 2). These antibodies, classified as *de novo*, correlated with severe acute rejection and graft loss. By comparison recent studies transplanting on the basis of a negative AHG-CDC or flow crossmatch have noted a *de novo* prevalence of only 9–22% (Table 2). There are at least two interpretations for these data. First, immunosuppressive therapies have been significantly improved in their ability to suppress *de novo* antibody production. Alternatively, enhanced sensitivity of newer methods has allowed a more accurate assessment

of pretransplant donor-reactive HLA antibody. In support of the latter explanation, Campbell et al. recently re-evaluated their earlier data which described '*de novo*' post-transplant antibody production in patients with direct CDC negative crossmatches. They now report that two thirds of their reported cases had pretransplant antibody as detected by more sensitive techniques including flow cytometry (78; <http://cnserver0.nkf.med.ualberta.ca/Banff/2001/>).

The contention that antibodies detected exclusively by flow cytometry are irrelevant since they are incapable of complement fixation *in vitro* has been specifically addressed in only one study. Karuppan et al. reported that among 14 patients, who experienced acute rejection within two weeks post-transplant, 11 had pretransplant HLA antibodies detectable by flow crossmatch (79). Interestingly,

Table 3: Impact of a T-cell-negative, B-cell-positive crossmatch

Positive B-cell crossmatch	Pt (n) positive B CXM	Antibody specificity or isotype	CXM method	Induction therapy	Maintenance drugs	Early graft loss (<3 mo) BP vs. BN	Survival 1 year BP vs. BN
Ettenger (80) ¹	7		CDC			0%	
Ting (81) ¹	11		CDC			27% vs. 17%	
Sirchia (82)	21 (1°)	Class II IgG	CDC				40% vs. 82%
Jeannett (85) ¹	16		CDC			26% vs. 21%	
Lazda (86) ¹	20		CDC	ALS	AZA/Pred		40% vs. 56%
Lazda (86) ¹	12		CDC	No	CsA/Pred		83% vs. 76%
Russ (87)	36 [24 (1°)]		CDC			40% vs. 14%	48% vs. 75% (42% vs. 79%)
Noreen (88)	49		AHG	ALG (Cadaveric)	CsA/Pred ±AZA		71% vs. 88%
Phelan (89)	10	Class I IgG	AHG			40%	
	4	Class II IgG				0%	
Taylor (24)	6 (1°)						
	10 (2°)		CDC	No	CsA/Pred ± AZA		100%
Karuppan (90)	18	Class I IgG	CDC	No	CsA/Pred ± AZA		30%
	3	Class I/II IgG					50%
	2	Class II IgG					67%
							100%
Mahoney (42) (AHG -ve)	3 (1°)		Flow	No	CsA/AZA Pred	33% vs. 6%	
	7 (2°)					71% vs. 0%	
Scornik (91)	2 (1°)	Class II IgG	AHG			100%	0%
	2 (2°)	Class II IgG				100%	0%
ten Hoor (92)	3 (2°)	Class I IgG	CDC	No	AZA/Pred ± CsA	33%	67%
	4 (1°)	Class II IgG				50%	25%
	9 (2°)	Class II IgG				44%	33%
Ladza (93)	23 (1°)		Flow %				65% vs. 90%
	12 (2°)						58% vs. 92%
LeFor (48)	13		Flow	No	CsA/AZA Pred		69% vs. 87%
Bittencourt (95)	13		Flow	ATG	CsA/AZA Pred		4 year 62% vs. 79%
Mahoney (97) (UNOS '94-'95)	93 (1°)	PRA >10%	CDC	NA	NA	12% vs. 8%	84% vs. 87%
	52 (2°)	AHG	Flow			20% vs. 12%	75% vs. 84%

¹Studies showed no significant effect or difference compared to negative B-cell crossmatch transplants.

(1°) = 1st transplant, (2°) = regraft.

CXM = crossmatch, BP = B-cell positive, BN = B-cell negative.

only 1/11 had HLA antibodies that were limited to a non-complement fixing subclass (i.e. IgG4). More recently, Karpinski et al. reported several cases of accelerated rejection/loss in which patients displayed C4d staining in the peritubular capillaries (56). Each patient exhibited pretransplant donor-reactive HLA antibodies detectable only by flow cytometry. This was the first study to clearly demonstrate the ability of such donor-reactive HLA antibodies to fix complement *in vivo* even though complement activation could not be demonstrated *in vitro*. Possible explanations for such an occurrence are (i) *in vivo* complement activation is more efficient compared with *in vitro*, or (ii) the location of donor HLA acts to concentrate low titer antibodies to a sufficient density to allow effective complement activation *in vivo*. Regardless of the mechanism, these studies demonstrate that complement directed cytotoxicity is an effector pathway by which these low titer antibodies are capable of mediating endothelial injury. These data would suggest that *in vitro* CDC assays do not detect all relevant complement fixing antibodies. The collective data supports the ar-

gument that low titer donor-reactive HLA antibodies, only detectable by enhanced crossmatch techniques such as flow cytometry can have significant clinical consequence. While not necessarily a contraindication to transplantation, these antibodies appear to represent a significant risk factor that should be integrated into the patient assessment algorithm.

Clinical Significance of Antibodies to Class II Antigens

In the 1970s and 1980s, several centers implemented B-cell crossmatches in an effort to detect antibodies to donor class II HLA antigens (80–85). With the exception of Sirchia et al. (82), the majority of early studies exploring the significance of an isolated B-cell-positive crossmatch reported no effect (Table 3). In contrast, studies since 1987 report that 1-year graft survival rates range from 42 to 100% for primary grafts and 30–58% for re-grafts (Table 3; 86–98). While these studies suggest that an

isolated positive B-cell crossmatch is a risk factor they do not address the issue of antibody class. The presumption was that B-cell reactivity equated with antibodies to class II HLA antigens. Unfortunately, there is a relative paucity of studies that address this issue. Nevertheless there are compelling cases of hyperacute/accelerated rejection attributed to class II-directed antibodies (reviewed in 99,100). Moreover, in one study, antibodies directed against class II antigens of the donor were eluted from the kidney at the time of graft loss (91). In another, the investigators reported early graft loss rates of 50% for primary grafts and 44% for regrafts with accompanying 1-year graft survival rates of 25% and 33%, respectively, in the presence of donor-reactive antibodies to class II antigens (92). It is important to mention here that the detection of HLA antibodies directed against class II antigens has been quite difficult. Most direct cytotoxic methods can detect only high titer antibody and for the most part, many laboratories do not even perform routine screening for class II-reactive antibodies (unpublished data from SEOPF Proficiency Testing Program). If a patient's serum contained antibodies against HLA class I, it was extremely difficult to determine if antibodies specific for class II antigens were also present. As a result, few laboratories attempted to test for these antibodies. This aspect has been somewhat rectified with the development of solid-phase assays (ELISA and microparticles) that can independently measure antibodies against class I and/or class II HLA antigens. A recent study using solid-phase assays demonstrated that >80% of patients with cytotoxic class I-directed HLA antibodies also possess underlying antibodies to class II antigens (101). Clearly, the lack of appropriate assessment tools compromised our ability to understand the contribution of antibodies to class II HLA antigens.

Another consideration frequently overlooked is that B cells also express class I antigens (perhaps at even higher levels than those found on T cells) (102). Thus, a T-cell negative, B-cell positive crossmatch could be the result exclusively of low levels of class I-directed antibodies that could limit allograft survival. In fact, several groups clearly identifying T-cell-negative, B-cell-positive crossmatches as resulting from low titer antibodies to donor class I antigens reported early graft loss of 33–40% and 1-year graft survival of 50–67% (Table 3). Interestingly, the magnitude of risk is indistinguishable between positive B-cell crossmatches resulting from class I- vs. class II-directed antibodies. Collectively, the literature supports the concept that T-cell-negative, B-cell-positive crossmatches predict poor clinical outcome.

Despite the aforementioned data, there remains widespread debate regarding the value of the B-cell crossmatch. That a T-cell-negative, B-cell-positive crossmatch could be the result of nonspecific antibodies was originally recognized by those few centers that performed absorption assays. Two recent studies using flow bead technology reveal that >75% of T-cell-negative,

B-cell-positive crossmatches were not the result of HLA antibodies (56–103). These reports highlight the fact that B cells are challenging targets resulting from their expression of Fc receptors, which bind IgG nonspecifically. Such nonspecific binding leads to an irrelevant positive crossmatch. Vaidya et al. recently overcame this problem using pronase to cleave Fc receptors from the B-cell surface (104). Clearly, to optimize interpretation of a B-cell crossmatch, key elements will be: (i) evaluation of patient sera with HLA-specific solid-phase targets, (ii) reduction of nonspecific binding of B-cell targets (e.g. pronase), and (iii) inclusion of autologous cells for crossmatching. It is only with these approaches that the value of a B-cell crossmatch will be fully understood.

Clinical Significance of HLA Antibodies Detectable Only in Historic Sera

Since the routine application of the prospective crossmatch, the question of which patient sera to include in the pretransplant crossmatch has been paramount. Standard laboratory practice is to collect and evaluate routine (e.g. monthly) serum samples on patients awaiting renal transplantation. This approach provides an 'immunologic history' of a potential recipient to be drawn upon when that individual is considered for transplantation. Throughout the 1970s, standard practice was to test all historical patient sera against potential donors. If any of the sera tested positive, the recommendation was to avoid transplantation. This practice was challenged in 1982 by Cardella et al. who reported no difference in the 1-year graft survival of recipients transplanted with current negative, historic positive (CNHP) CDC T-cell crossmatches (67% vs. 75%) (105). In rapid succession, other groups reported similar findings (106–111), leading many centers to rely exclusively on 'current' patient serum for crossmatching against donor cells. These studies documented the ability to transplant CNHP patients without risk of hyperacute rejection. However, what is infrequently cited is that in Cardella's original report, 40% of CNHP patients experienced antibody-mediated rejection within 3 months post-transplant. Thus, it was not surprising when subsequent studies (24,56,92,112–117) reported a higher incidence of early graft loss among CNHP patients (Table 4).

We speculate that, had more sensitive crossmatch techniques been available, donor-reactive HLA antibody would have been detectable at the time of transplant in at least some of the CNHP transplant recipients with poor outcomes. Indeed, in the Cardella study, 27% of CNHP patients received transplants despite current positive B-cell crossmatches (105). Among this group, 75% of the patients lost their grafts within 16 months. While failure to detect HLA antibodies in current sera may, in part, explain the risk associated with CNHP crossmatches, it has been recently reported that early graft loss still occurs in CNHP patients with no detectable antibodies in current sera using flow cytometric approaches (56). Thus, while the

Table 4: Impact of a current negative, historic positive T-cell crossmatch

Remote positive crossmatch	Pt (n) CNHP	Graft number	CXM method	Induction therapy	Maintenance drugs	Early graft loss (<3 mo) CNHP vs. CNHN	1-year graft survival CNHP vs. CNHN
¹ Cardella (105)	15	60% Re graft	CDC	ATG	AZA/Pred		67% vs. 75%
¹ Matas (107)	5	60% Re graft	Amos		AZA/Pred	40% vs. NA	60% vs. 55%
¹ Sanfilippo (106)	2	Primary					50% vs. 66%
	4	Re graft					100% vs. 45%
¹ Norman (109)	6	Primary	AHG	No	AZA/Pred	0% vs. NA	100% vs. NA
¹ Falk (110)	21	Primary	CDC	ATG	AZA/Pred		71% vs. 73%
	40	Re graft					58% vs. 60%
¹ Goeken (111)	109	Primary					69% vs. NA
(ASHI survey)	107	Re graft					53% vs. NA
¹ Rosenthal (108)	12	67% Re graft	NIH	No	CsA/Pred	17% vs. NA	83% vs. NA
Kerman (112)	14	Primary	NIH	No	CsA/Pred		86% vs. 81%
	4	Re graft					0% vs. 75%
Reed (118)	20	20% Re graft	Extend CDC			50% vs. NA	
Turka (113)	41	Primary	AHG		CsA/Pred		68% vs. 86%
	29	Re graft					51% vs. 79%
Taylor (24)	1	Primary	CDC	No	CsA/Pred	100% vs. NA	0% vs. NA
	8	Re graft			± AZA	87% vs. NA	13% vs. NA
ten Hoor (92)	7	Primary	CDC	No	AZA/Pred ± CsA		29% vs. NA
Bryan (114)	19	Primary	AHG			37% vs. 9%	
	20	Re graft				45% vs. 16%	
Avlonitis (115)	14	50% Re graft	CDC	29% ATG 7% OKT3	CsA/Pred ± AZA	43% vs. NA	57% vs. 90%
(current T-cell flow CXM-ve)							
Karpinski (56)	7	Primary	Flow	No	CsA/Pred AZA or MMF	29% vs. 11%	
(current T- and B-cell flow CXM-ve)							
Noreen (117)	38	Primary	AHG		CsA based		74% vs. 87%
	19	Re graft					74% vs. 93%

¹Studies showed no significant effect or difference compared to historic negative crossmatch recipients. CXM = crossmatch, NA = not available.

cumulative data suggest that a historic positive crossmatch is not necessarily a contraindication to transplant, it is clearly a risk factor.

We cannot ignore the fact that significant proportions of CNHP transplants were successful. While the mechanism(s) fostering this outcome is unclear, advances in immunosuppressive therapy may be involved. Alternatively, studies by Reed et al. suggested that, in some CNHP patients, long-term graft survival is the result of the presence of anti-idiotypic antibodies in the patient's current sera, which inhibited historical donor-reactive HLA antibodies (118). While many laboratories implemented testing for such anti-idiotypic antibodies soon after the publication of this report, few, if any, continue this practice today. However, the emergence of IVIG therapy and the search for its mode of action has rekindled the interest in anti-idiotypic antibodies.

Pre-Transplant Assessment of Donor-Reactive HLA Antibodies in Renal Transplantation

The preceding literature review supports the position that any level of detectable donor-reactive, HLA-specific, IgG

antibody pretransplant is, at the very least, a risk to transplantation. More importantly, we contend that there are critical laboratory evaluations that must be carried out in order to confirm that 'donor-reactive, HLA-specific, IgG antibody' is present (see [I] below). Once such antibody is confirmed, donor-specific risk assessment must be performed before transplantation to optimally guide clinical management. Indeed, for highly sensitized patients, who wait longer and are transplanted at a lower rate, exhaustive definition of HLA IgG antibody specificity is critical. With this information, it should be possible to transplant highly sensitized patients with a higher frequency (and lower risk) than is currently achieved, in part by simply avoiding unacceptable mismatches, and identifying acceptable mismatches.

In contrast to the above position are the recent proposals that suggest foregoing a pretransplant crossmatch for nonsensitized recipients (119–121). While clinically appealing, the above-presented information would argue that this practice is not risk free because it is predicated on the assumption that the patient is devoid of donor-reactive HLA antibodies. An accurate patient history, confirmed by the most sensitive method, is required to define individuals as nonsensitized (33 122). For example, when a study

Table 5: Risk assignment based on crossmatch interpretation

Crossmatch Donor-reactive, HLA-specific, IgG antibody	Risk categories		
	High	Intermediate	Negligible ³
Current positive direct CDC	X		
Current positive AHG-CDC	X		
Current positive flow crossmatch only	X		
Historic positive direct CDC		X ¹	
Historic positive AHG-CDC		X ¹	
Historic positive flow crossmatch only		X	
Current and historic negative direct CDC			X ^{1,2}
Current and historic negative AHG-CDC			X
Current and historic negative flow crossmatch			X

¹High risk if current flow crossmatch is positive.

²Intermediate risk if remote flow crossmatch is positive.

³Negligible risk if accurate sensitization history can be ensured.

CDC = complement-dependent cytotoxicity.

exploring this issue was performed using flow microparticle technology, the data revealed that 20% of 'nonsensitized' patients, defined by serologic techniques, actually had detectable HLA antibodies (33). Moreover, many patients dialyze at locations remote to the transplant center and, as a result, their sensitization histories are often incomplete or inaccurate.

Evaluation for donor-reactive, HLA-specific, IgG antibodies

The following recommendations are intended to optimally define if a donor-reactive, HLA-specific, IgG antibody is present. Each recommendation is based on data from the literature and assigned a level of evidence as follows:

- *Level A:* Excellent data to support recommendation.
- *Level B:* Good data to support recommendation.

Recommendations to define the 'non-sensitized' patient:

- Validate patient history for the lack of sensitizing events.
- Confirm that a patient is nonsensitized using a solid-phase assay documented to be more sensitive than CDC assays (*Level A*).

Recommendations to evaluate the 'sensitized' patient:

- To optimize detection of low titer HLA antibodies, monitoring should be performed using sensitive solid-phase assays (*Level A*).
- Monitoring should include evaluation for both antibodies to class I and class II HLA antigens (*Level A*).
- A crossmatch test must be performed before transplantation using, as a minimum, an enhanced CDC technique (*Level A*).
- The final crossmatch technique should be of equal sensitivity to the solid-phase assay used to screen for the presence of HLA antibody (*Level A*).

- A B-cell crossmatch should be included in the final crossmatch (*Level A*).
- Peak sera should be included in the final crossmatch (*Level B*).
- Auto-crossmatches should be utilized to aid in the interpretation of allo-crossmatches (*Level B*).

Risk assignment

Pre-formed donor-reactive, HLA-specific, IgG antibodies have been, are and will continue to be a challenge to renal transplantation. Whether an antibody is a contraindication or risk factor must always be individually assessed (e.g. recipient age, time to/nature of previous graft loss, repeat HLA mismatches). In addition, the transplant center's ability to support the pre- and postoperative management of such patients, as well as to provide post-transplant diagnostic monitoring must also be considered. Based on the data obtained in [1], broad risk categories for a given donor-recipient combination are assigned (Table 5). In this paper, 'risk' refers to the potential for antibody-mediated rejection or early graft loss.

- High risk: Clinical programs may consider this category a contraindication to transplantation. If transplanted, patients require major pretransplant intervention (IVIG + plasmapheresis to modify risk) and post-treatment/transplant monitoring.
- Intermediate risk: Clinical program may consider this category a contraindication to transplantation. If transplanted, patients may require augmented immunosuppression and post-transplant monitoring.
- Negligible risk: Clinical programs may proceed to transplant with no change in their normal practice.

We recognize that the above risk categories are broad generalizations. For example, a multiparous female, re-graft patients (with or without early graft loss), patients receiving repeat mismatches, and recipients of husband-to-wife or child-to-mother transplants all of whom have no detectable HLA antibodies may still be at increased risk for rejection

and/or graft failure for the following reasons: (i) antibody was in fact present in historical serum but no sample was available to test, (ii) antibody is present but at less than current detection levels, or (iii) antibody is absent but memory B cells/T cells are present (123). These risk categories are only intended as a guide for transplant professionals in their decision making process. Obviously, these categories cannot capture all the subtleties encountered in an individual case.

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