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REVIEW

Detection and clinical relevance of donor specific HLA antibodies: a matter of debate

Dave L. Roelen, Ilias I. N. Doxiadis and Frans H. J. Claas

Leiden University Medical Center, Department Immunohaematology and Blood Transfusion, Leiden, The Netherlands

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Correspondence
Frans H. J. Claas, Leiden University Medical Center, Dept.of Immunohematology and Blood Transfusion, Albinusdreef 2, building 1, L3-37, P.O.Box 9600, 2300 RC, Leiden, The Netherlands.
Tel.: +31715263802;
fax: +31715265267;
e-mail: fhjclaas@lumc.nl

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Summary

The introduction of new sensitive assays for the detection of HLA antibodies on basis of their binding to isolated HLA molecules has got an enormous impact on the decision-making process with respect to donor selection for sensitized patients. In the past, when only complement-dependent cytotoxicity was used as a tool to define HLA alloantibodies, the presence of donor specific antibodies (DSA) before transplantation was considered a contraindication for renal transplantation with that donor. The interpretation of the current DSA results is far more difficult and leads to a lot of discussions and controversy. The problems associated with the use of solid phase assays for clinical decision making and possible solutions are discussed.

Introduction

For many years, there was no doubt on the relevance of donor specific HLA antibodies (DSA) present in the serum of renal transplant recipients before transplantation. Antibodies, leading to a positive complement-dependent cytotoxicity (CDC) crossmatch, were associated with hyperacute or accelerated acute graft rejection [1,2]. The introduction of a serological crossmatch pre transplantation using this CDC method was meant to prevent the occurrence of immediate graft loss and a positive crossmatch was always considered a contraindication for transplantation. To prevent shipment of donor kidneys to patients with donor specific antibodies, sera from patients on the waiting list were regularly screened against a panel of HLA typed blood donors to determine the specificity of the antibodies using the same CDC assay. The presence of DSA as detected in a CDC antibody screening was considered a prediction of a positive CDC crossmatch and therefore also a contraindication for transplantation. Actual proof for a detrimental effect on early transplant outcome was only provided for HLA class I specific lgG antibodies in current sera [3,4] but a similar clinical impact was supposed for HLA class II specific antibodies, HLA class I specific antibodies only detectable in historical sera and lgM antibodies against HLA. Later studies revealed that DSA in historical sera were not always associated with poor results and especially if they were of the IgM type transplant results were excellent [4]. Hard evidence that HLA class II specific DSA are associated with hyperacute rejection is lacking while for HLA-C specific DSA only one or two case reports suggest that they may be associated with early rejection [5,6]. For these reasons, one can assume that many patients in the past were

denied a transplant although they could have been successfully transplanted in the presence of DSA. The interpretation of HLA antibody data and the evaluation of the relevance of DSA became even more complicated after the introduction of new and more sensitive antibody screening assays, which were based on the reactivity of antibodies with isolated HLA molecules on a solid phase. In the meantime it is clear that the 'dogma' that DSA are a contraindication for transplantation is not any more valid. The DSA should rather be considered a risk factor and the risk may vary from no risk at all to very high risk or contraindication. However, the problem is that the clinical relevance of DSA before transplantation is very difficult to estimate for an individual donor recipient combination.

The need for alternative HLA antibody screening methods

Although CDC has been the golden standard for many years, this assay is certainly not perfect and its use is associated with several problems. As the target in the CDC assay is a lymphocyte, not only HLA molecules but also other irrelevant cell membrane structures may be targets for antibody reactivity. Indeed, antibodies reactive with the patients' own lymphocytes (autoantibodies), immune complexes, and immunoglobulin allotypes have shown to interfere in this assay [7]. Furthermore, the assay is based on complement activation, which implies that HLA specific lgG antibodies, which are not able to fix complement such as lgG2 and lgG4, are not detected. This was the reason why more sensitive assays have been introduced such as the antiglobulin assay (detection of noncomplement activating HLA antibodies by addition of a complement fixing anti-human IgG antibody, which does fix complement) or indirect immunofluorescence (flow cytometry). These assays have indeed been proven to be more sensitive but still have the disadvantage that the target cell is a lymphocyte with many different (non-HLA) target molecules on its surface. To circumvent this problem solid phase assays using isolated HLA molecules as targets for antibody detection, have been developed. These assays, either based on ELISA or fluorescence can detect both complement fixing and noncomplement fixing IgG antibodies and a positive reaction is per definition due to reactivity with a HLA class I or class II molecule and not with another irrelevant cell membrane molecule.

Clinical relevance of antibodies detectable in ELISA

The first assay which became available was an ELISA assay, in which HLA class I and HLA class II molecules were used as target molecules. Retrospective studies in patients transplanted in the presence of a negative CDC crossmatch showed that patients with both HLA class I and HLA class II antibodies before their first renal transplantation had a significantly poorer graft survival (76.5% vs. 87.5% at 2 years post-transplantation) compared with patients without HLA antibodies [8]. Patients with only HLA class I or only HLA class II antibodies did not experience a worse graft survival. The actual specificity of the antibodies involved was not determined in these first studies but indirect evidence was obtained that this effect was owing to donor specific antibodies as the impact on graft survival increased with the number of HLA mismatches between donor and recipient. More direct evidence for a clinical relevance of DSA detected in ELISA came from studies by Lefaucheur *et al.* [9,10], who found a significantly lower graft survival in patients with DSA compared with patients without DSA. Especially, when the presence of DSA was associated with the occurrence of antibody-mediated rejection (AMR), graft survival was worse in the DSA group. When DSA was not associated with AMR, graft survival in the DSA group was similar to that of patients without DSA (85% at 5 years post-transplantation). These data show that DSA detected in ELISA are a risk factor in a subgroup of patients but it is not possible to determine the risk factor for an individual patient before transplantation. Many patients with DSA have an excellent graft survival.

Clinical relevance of DSA detected in Luminex based assays

Assays based on antibody reactivity against HLA molecules attached to Luminex beads have become popular during the past years. They appear to be very sensitive and the availability of single HLA antigen beads facilitates the determination of the antibody specificity enormously compared to previous panel analyses. Many laboratories have introduced this method for the detection of DSA in their transplant recipients. The clinical relevance of these antibodies is a point of discussion at about every conference on histocompatibility testing and transplantation. In Table 1 an overview is given of the most relevant articles published on this topic. This table includes the total number of patients studied, number of DSA positive patients, type of crossmatch, type of donor, selection of patients and, if present, two possible outcomes of transplantation: AMR and graft survival [11–28].

Several retrospective studies show that the presence of DSA is associated with a significantly decreased graft survival, even in the case that no AMR takes place [18,19,23]. Furthermore, the clinical relevance of a positive B cell crossmatch was shown to be dependent on the presence of

Table 1. The clinical relevance of DSA varies amongst different studies.

Reference	*N*	DSA+ (*n*)	Type	Donor	Selection	AMR (C4d+)	Graft survival
Gibney *et al.* [11]	136	20	AHG-XM	DD/LD	No		↓
Patel *et al.* [12]	60	20	FCXM	LD	No	↑	↔
Gupta *et al.* [13]	121	16	CDCXM	DD	No		↓
Berg Loonen *et al.* [14]	34	13	CDCXM	DD	AM		↔
Billen *et al.* [15]	165	32	CDCXM	DD/LD	No		↓ I ↔ II
Eng *et al.* [16]	471	27	CDCXM	DD	BXM+	↑	↓
Vlad *et al.* [17]	325	27	CDCXM	DD	No	↑	↔
Amico *et al.* [18]	334	67	CDCXM	DD/LD	No	↑	↓
Wahrmann *et al.* [19]	338	39	CDCXM	DD/LD	No	↑	↓
Aubert *et al.* [20]	114	11	CDCXM	??	No	↔	↔
Phelan *et al.* [21]	64	12	CDCXM (AHG)	LD	No	↔	↔
Morris *et al.* [22]	149	15	CDC/FCXM	LD	No		↔
Lefaucheur *et al.* [23]	402	76	CDCXM	DD	No	↑	↓
Riethmüller *et al.* [24]	37	20	CDCXM	LD	Sens	↑ I ↔ II	↓
Bartel *et al.* [25]	68	51	CDCXM	DD	IA	↔	↔
Couzi *et al.* [26]	45	34	CDC/FCXM	DD	FCXM+	↑	↓
Couzi *et al.* [26]	45	11	CDC/FCXM	DD	FCXM−	↔	↔
Willicombe *et al.* [27]	480	45	CDC/FCXM	DD/LD	AL	↑	↓
Caro-Oleas *et al.* [28]	892	50	CDC	DD	No	↑	↓

AL, Induction with alemtuzumab; AHG-XM, complement dependent cytotoxicity crossmatch in the presence of anti-human-immunoglobulin; AMR antibody-mediated rejection; BXM, B cell crossmatch; CDC, complement-dependent cytotoxicity; CDCXM, crossmatch in complement dependent cytotoxicity; DD, deceased donor; DSA, donor specific HLA antibodies; FCXM, flow cytometric crossmatch; IA, immunoadsorption; LD, living donor.
↔ No difference.
↑ or ↓ Increased or decreased.

donor HLA class II specific antibodies in Luminex. Without DSA, a positive B cell crossmatch was not associated with a decreased graft survival [16]. However, other centers reported that DSA detected in Luminex are irrelevant in patients transplanted in the presence of a negative CDC crossmatch [20,21] or in the presence of a negative flow cytometric crossmatch [26]. These studies showed a similar incidence of rejection, similar serum creatinine levels and a similar graft survival in patients with and without DSA. A study in highly sensitized patients, transplanted in the presence of a negative CDC crossmatch via the acceptable mismatch program of Eurotransplant [29], showed an increased incidence of acute rejection but a similar graft survival in patients with and without DSA [14]. This observation was confirmed in a recent analysis of all Dutch patients transplanted via the acceptable mismatch program (Doxiadis *et al.*, manuscript in preparation). From Table 1 one can see that DSA detectable in Luminex can be a risk factor but it is unclear what the risk factor is for an individual patient. Many transplant centers use the results of the Luminex assay either not to transplant a patient or to desensitize a patient before transplantation but it may well be that for many patients-donor combinations transplantation is feasible, even without desensitization, this conclusion is supported by the CTS study of Süsal *et al.* [30], which showed no clinical impact of DSA that are detected in Luminex but not in ELISA.

Why is there no consensus on the relevance of DSA detected by Luminex?

So far, there is a lot of debate on the clinical relevance of DSA detectable in Luminex. Many reasons can be put forward for the controversial views of different transplantation centers. First of all, there is a problem with the assignment of positive and negative reactions. So far, no standardization of the interpretation of the results has been obtained. The MFI (mean fluorescence intensity) has been suggested to be a determinative factor but the MFI determining the border between positive and negative reactions differs between centers and amongst studies. It is clear that there is a need for standardization, which needs the input of tissue typers, clinicians, and the companies, which developed these products. The fact that the number of HLA molecules differs on every Luminex bead will make standardization difficult but feasible. Other determinative factors concern the antibody titer, which is often not reported and the immunoglobulin subclasses (IgG1, IgG2, IgG3, IgG4) causing the positive reactions. Especially the fact that some IgG subclasses are able to fix complement and others are not may be a determinative factor, especially if one considers the role of C4d deposition in the graft as a marker for humoral rejection [31,32]. For this reason, the Luminex-based assays have been adapted either by measuring complement deposition

on the beads, either C4d [19,33] or C1Q [34,35] or by using secondary antibodies detecting IgG subclasses [36,37]. Some studies show indeed that complement fixing DSA are more relevant than noncomplement fixing DSA, [38]. Some patients with noncomplement fixing DSA (IgG2/IgG4) before transplant have excellent graft survival without rejection [39]. However, other studies showed a similar incidence of AMR in patients with weak DSA irrespective of their ability to fix complement [40]. Furthermore, IgG2 and IgG4 and IgA DSA are detected in sera from patients, who rejected a donor kidney [36] and even in eluates of rejected grafts [41] suggestive for a role of these antibodies in graft rejection. There are also technical problems with assays based on the detection of DSA using HLA molecules attached to a solid phase. A study by Morales Buenrostro *et al.* [42] showed that HLA antibodies are frequently observed in sera from healthy males with no history of allosensitization. These sera contained antibodies react with both HLA class I and HLA class II molecules on Luminex beads. Further studies revealed that the Luminex beads not only accommodate intact HLA molecules but also denatured HLA molecules. The latter molecules lack beta-2 microglobulin and the respective peptide leading to a different conformation and the expression of different antibody epitopes. The 'natural' HLA antibodies present in nonimmunized males react preferentially with these denatured HLA molecules. Similar reactions may occur with sera from potential transplant recipients. A study by Cai *et al.* [43] showed that antibodies reactive with these denatured HLA molecules are clinically irrelevant. Graft survival in patients with this type of antibodies is the same as that of patients with no DSA. The presence of such 'natural' HLA antibodies may also affect studies on the relevance of DSA detected in solid phase assays. In a proof of principle study Zoet *et al.* [44] could demonstrate that binding of solubilized class I molecules on beads may alter the tertiary structure of the antigen leading to false negative and false positive reactions when human monoclonal antibodies directed against class I molecules were used as detecting reagents. Recently, the company has introduced so called clean beads, which are supposed to carry only intact HLA molecules. Future validation should also include this type of beads. On the other hand, false negative reactions despite the presence of DSA of the IgG type have been reported to be due to the interference of IgM binding to the HLA target [45] or C1q binding to the bound IgG molecule [46] hampering the reactivity of the labeled anti-IgG antibody used as a read-out. Various suggestions have been put forward by these latter studies to circumvent/overcome these interference problems. These adaptations include dilution of the sera (1:10), pretreatment with DTT, EDTA, or C1 inhibitor and heat inactivation, of which pretreatment with EDTA was the suggested method of choice. Another important factor related to the clinical relevance of DSA before transplantation is the ability of the recipient to produce a memory response after transplantation [47,48].However, at the moment no predictive parameter for a memory response is available.

Finally, the differences reported on the clinical relevance of DSA in different centers may be due to different immunosuppressive regimes. It is clear that there are many reasons for the controversial results on the relevance of DSA in different centers. Finally, there is a marked difference in the reactivity of antibodies in the assays from the two current providers, making the interpretation of the results complicated. Therefore, comparison of the results from different centers is virtually impossible owing to many reasons, both related to differences in antibody detection (what is positive, what is negative, which IgG subclasses are involved, what are the strength and the titer of the antibodies and which immunosuppressive regimen has been used?).

Similar discrepant data are obtained when one compares CDC antibody screening data from different centers. Actually, the EPT (external proficiency testing) scheme of Eurotransplant showed that the concordance between different laboratories analyzing sera by Luminex is slightly

Table 2. External proficiency testing organized by the Eurotransplant Reference laboratory (ETRL) in 2011.

		Concordant (*N*)	False negative (*N*)	False positive (*N*)
Method	Participants (*N*)	Per serum	Per serum	Per serum
CDC	53	1.4	0.2	0.2
LUM SA	45	11.8	0.8	1.3
Factor		8.4	4	6.5

CDC, complement-dependent cytotoxicity; LUM SA, luminex single antigen beads
Participants received 12 sera from multipara women. The rate of the missing discrepancy was calculated on the basis of the 75% consensus, i.e., when 75% of the participants reported a specificity this specificity is regarded as recognized by the serum. For the additional reporting of specificities the 95% consensus was used, i.e., when 95% of the participants report specificity as negative, then the serum does not contain antibodies against this specificity.

higher than obtained with CDC (Table 2). However, many more specificities are detected with Luminex, and as a consequence the number of false negative and false positive HLA specificities within a serum was higher for Luminex compared with CDC.

It is clear that DSA detectable in solid phase assays are not a contraindication for transplantation but they should be considered a risk factor. So far, the risk has been established in individual centers and there is no consensus between centers on the clinical relevance. The latter hampers the application of these solid phase assays for the allocation of organs between centers in organ allocation organizations. This is the reason why for the time being, the results of the CDC tests are still leading in the allocation of organs to sensitized patients within Eurotransplant [29].

The use of the results of antibody screening for organ allocation to sensitized and highly sensitized patients, nowadays called a virtual crossmatch, is a policy which is used by Eurotransplant and UK Transplant already for many years [49,50]. Especially, the prediction of a negative crossmatch which is the basis of the Acceptable Mismatch program of Eurotransplant [29], enables transplantation of highly sensitized recipients who are otherwise severely disadvantaged to be prioritized in organ allocation schemes. Prediction of a positive crossmatch to avoid inappropriate shipment of organs to sensitized incompatible patients has recently also been promoted by the United Network for Organ Sharing [51]. Sensitive techniques such as Luminex PRA and single-antigen beads have been evaluated as a suitable basis for a virtual crossmatch by predicting flow crossmatch outcomes in highly sensitized patients [52–54].

Although antibody specificities detected by solid phase assays should not be the basis of organ allocation, transplant patients can certainly benefit from the outcome of these assays [55]. In case there are indications of sensitization these tests could be useful to determine the exact specificities. In addition, one can use these tests to look for 'holes in the B cell repertoire' i.e., acceptable mismatches toward which no antibodies are found, which is especially of benefit for (highly) sensitized patients. For retransplant patients the luminex test can also be of benefit as shown by the detection of additional specificities after graft removal [56,57].

To be able to use solid phase assays more efficiently, standardization is essential. This is only possible by a close collaboration between HLA laboratories, clinicians and the companies providing these assays. Wet workshops using patient sera and relating the results to clinical consequences such as incidence and severity of rejection, graft function, and graft survival should help the transplant community to implement the results of these assays in a more reliable and reproducible way in clinical decision making.

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References

1. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969; **280**: 735.
2. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O. Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. *Lancet* 1966; **2**: 662.
3. Chapman JR, Taylor CJ, Ting A, Morris PJ. Immunoglobulin class and specificity of antibodies causing positive T cell crossmatches. Relationship to renal transplant outcome. *Transplantation* 1986; **42**: 608.
4. Taylor CJ, Chapman JR, Ting A, Morris PJ. Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation. Relationship to primary and regraft outcome. *Transplantation* 1989; **48**: 953.
5. Chapman JR, Taylor C, Ting A, Morris PJ. Hyperacute rejection of a renal allograft in the presence of anti-HLA-Cw5 antibody. *Transplantation* 1986; **42**: 91.
6. Bachelet T, Couzi L, Guidicelli G, *et al.* Anti-Cw donor-specific alloantibodies can lead to positive flow cytometry crossmatch and irreversible acute antibody-mediated rejection. *Am J Transplant* 2011; **11**: 1543.
7. Ting A, Morris PJ. Successful transplantation with a positive T and B cell crossmatch due to autoreactive antibodies. *Tissue Antigens* 1983; **21**: 219.
8. Susal C, Dohler B, Opelz G. Presensitized kidney graft recipients with HLA class I and II antibodies are at increased risk for graft failure: a Collaborative Transplant Study report. *Hum Immunol* 2009; **70**: 569.
9. Lefaucheur C, Suberbielle-Boissel C, Hill GS, *et al.* Clinical relevance of preformed HLA donor-specific antibodies in kidney transplantation. *Contrib Nephrol* 2009; **162**: 1.
10. Loupy A, Suberbielle-Boissel C, Hill GS, *et al.* Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. *Am J Transplant* 2009; **9**: 2561.
11. Gibney EM, Cagle LR, Freed B, Warnell SE, Chan L, Wiseman AC. Detection of donor-specific antibodies using HLA-coated microspheres: another tool for kidney transplant risk stratification. *Nephrol Dial Transplant* 2006; **21**: 2625.
12. Patel AM, Pancoska C, Mulgaonkar S, Weng FL. Renal transplantation in patients with pre-transplant donor-specific antibodies and negative flow cytometry crossmatches. *Am J Transplant* 2007; **7**: 2371.

13. Gupta A, Iveson V, Varagunam M, Bodger S, Sinnott P, Thuraisingham RC. Pretransplant donor-specific antibodies in cytotoxic negative crossmatch kidney transplants: are they relevant? *Transplantation* 2008; **85**: 1200.
14. van den Berg-Loonen EM, Billen EV, Voorter CE, *et al.* Clinical relevance of pretransplant donor-directed antibodies detected by single antigen beads in highly sensitized renal transplant patients. *Transplantation* 2008; **85**: 1086.
15. Billen EV, Christiaans MH, van denBerg-Loonen EM. Clinical relevance of Luminex donor-specific crossmatches: data from 165 renal transplants. *Tissue Antigens* 2009; **74**: 205.
16. Eng HS, Bennett G, Tsiopelas E, *et al.* Anti-HLA donor-specific antibodies detected in positive B-cell crossmatches by Luminex predict late graft loss. *Am J Transplant* 2008; **8**: 2335.
17. Vlad G, Ho EK, Vasilescu ER, *et al.* Relevance of different antibody detection methods for the prediction of antibody-mediated rejection and deceased-donor kidney allograft survival. *Hum Immunol* 2009; **70**: 589.
18. Amico P, Hönger G, Mayr M, Steiger J, Hopfer H, Schaub S. Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads. *Transplantation* 2009; **87**: 1681.
19. Wahrmann M, Bartel G, Exner M, *et al.* Clinical relevance of preformed C4d-fixing and non-C4d-fixing HLA single antigen reactivity in renal allograft recipients. *Transpl Int* 2009; **22**: 982.
20. Aubert V, Venetz JP, Pantaleo G, Pascual M. Are all donor-specific antibodies detected by solid-phase assay before transplantation clinically relevant? *Transplantation* 2009; **87**: 1897.
21. Phelan D, Mohanakumar T, Ramachandran S, Jendrisak MD. Living donor renal transplantation in the presence of donor-specific human leukocyte antigen antibody detected by solid-phase assay. *Hum Immunol* 2009; **70**: 584.
22. Morris GP, Phelan DL, Jendrisak MD, Mohanakumar T. Virtual crossmatch by identification of donor-specific anti-human leukocyte antigen antibodies by solid-phase immunoassay: a 30-month analysis in living donor kidney transplantation. *Hum Immunol* 2010; **71**: 268.
23. Lefaucheur C, Loupy A, Hill GS, *et al.* Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J Am Soc Nephrol* 2010; **21**: 1398.
24. Riethmüller S, Ferrari-Lacraz S, Müller MK, *et al.* Donor-specific antibody levels and three generations of crossmatches to predict antibody-mediated rejection in kidney transplantation. *Transplantation* 2010; **90**: 160.
25. Bartel G, Wahrmann M, Regele H, *et al.* Peritransplant immunoadsorption for positive crossmatch deceased donor kidney transplantation. *Am J Transplant* 2010; **10**: 2033.
26. Couzi L, Araujo C, Guidicelli G, *et al.* Interpretation of positive flow cytometric crossmatch in the era of the singleantigen bead assay. *Transplantation* 2011; **91**: 527.
27. Willicombe M, Roufosse C, Brookes P, *et al.* Antibody-mediated rejection after alemtuzumab induction: incidence, risk factors, and predictors of poor outcome. *Transplantation* 2011; **92**: 176.
28. Caro-Oleas JL, González-Escribano MF, González-Roncero FM, *et al.* Clinical relevance of HLA donor-specific antibodies detected by single antigen assay in kidney transplantation. *Nephrol Dial Transplant* 2012; **27**: 1231.
29. Claas FH, Doxiadis II . Human leukocyte antigen antibody detection and kidney allocation within Eurotransplant. *Hum Immunol* 2009; **70**: 636.
30. Süsal C, Ovens J, Mahmoud K, *et al.* No association of kidney graft loss with human leukocyte antigen antibodies detected exclusively by sensitive Luminex single-antigen testing: a Collaborative Transplant Study report. *Transplantation* 2011; **91**: 883.
31. Feucht HE, Schneeberger H, Hillebrand G, *et al.* Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int* 1993; **43**: 1333.
32. Feucht HE. Complement C4d in graft capillaries – the missing link in the recognition of humoral alloreactivity. *Am J Transplant* 2003; **3**: 646.
33. Wahrmann M, Exner M, Schillinger M, *et al.* Pivotal role of complement-fixing HLA alloantibodies in presensitized kidney allograft recipients. *Am J Transplant* 2006; **6**: 1033.
34. Yabu JM, Higgins JP, Chen G, Sequeira F, Busque S, Tyan DB. C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. *Transplantation* 2011; **91**: 342.
35. Chin C, Chen G, Sequeria F, *et al.* Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. *J Heart Lung Transplant* 2011; **30**: 158.
36. Arnold ML, Dechant M, Doxiadis II, Spriewald BM. Prevalence and specificity of immunoglobulin G and immunoglobulin A non-complement-binding anti-HLA alloantibodies in retransplant candidates. *Tissue Antigens* 2008; **72**: 60.
37. Honger G, Hopfer H, Arnold ML, Spriewald BM, Schaub S, Amico P. Pretransplant IgG subclasses of donor-specific human leukocyte antigen antibodies and development of antibody-mediated rejection. *Transplantation* 2011; **92**: 41.
38. Smith JD, Hamour IM, Banner NR, Rose ML. C4d fixing, Luminex binding antibodies- a new tool for prediction of graft failure after heart transplantation. *Am J Transplant* 2007; **7**: 2809.
39. Lobashevsky A, Rosner K, Goggins W, Higgins N. Subtypes of immunoglobulin (Ig)-G antibodies against donor class II HLA and cross-match results in three kidney transplant candidates. *Transpl Immunol* 2010; **23**: 81.
40. Honger G, Wahrmann M, Amico P, Hopfer H, Bohmig GA, Schaub S. C4d-fixing capability of low-level donor-specific HLA antibodies is not predictive for early antibody-mediated rejection. *Transplantation* 2010; **89**: 1471.

41. Heinemann FM, Roth I, Rebmann V, Arnold ML, Spriewald BM, Grosse-Wilde H. Characterization of anti-HLA antibodies eluted from explanted renal allografts. *Clin Transpl* 2006; 371.
42. Morales-Buenrostro LE, Terasaki PI, Marino-Vazquez LA, Lee JH, El-Awar N, Alberu J. ''Natural'' human leukocyte antigen antibodies found in nonalloimmunized healthy males. *Transplantation* 2008; **86**: 1111.
43. Cai J, Terasaki PI, Anderson N, Lachmann N, Schonemann C. Intact HLA not beta2m-free heavy chain-specific HLA class I antibodies are predictive of graft failure. *Transplantation* 2009; **88**: 226.
44. Zoet YM, Brand-Schaaf SH, Roelen DL, Mulder A, Claas FH, Doxiadis II . Challenging the golden standard in defining donor-specific antibodies: does the solid phase assay meet the expectations? *Tissue Antigens* 2011; **77**: 225.
45. Kosmoliaptsis V, Bradley JA, Peacock S, Chaudhry AN, Taylor CJ. Detection of immunoglobulin G human leukocyte antigen-specific alloantibodies in renal transplant patients using single-antigen-beads is compromised by the presence of immunoglobulin M human leukocyte antigen-specific alloantibodies. *Transplantation* 2009; **87**: 813.
46. Schnaidt M, Weinstock C, Jurisic M, Schmid-Horch B, Ender A, Wernet D. HLA antibody specification using single-antigen beads–a technical solution for the prozone effect. *Transplantation* 2011; **92**: 510.
47. Burns JM, Cornell LD, Perry DK, *et al.* Alloantibody levels and acute humoral rejection early after positive crossmatch kidney transplantation. *Am J Transplant* 2008; **8**: 2684.
48. Kimball PM, Baker MA, Wagner MB, King A. Surveillance of alloantibodies after transplantation identifies the risk of chronic rejection. *Kidney Int* 2011; **79**: 1131.
49. Claas FH, Witvliet MD, Duquesnoy RJ, Persijn GG, Doxiadis II. The acceptable mismatch program as a fast tool for highly sensitized patients awaiting a cadaveric kidney transplantation: short waiting time and excellent graft outcome. *Transplantation* 2004; **78**: 190.
50. Fuggle SV, Martin S. Toward performing transplantation in highly sensitized patients. *Transplantation* 2004; **78**: 186.
51. Cecka JM. Calculated PRA (CPRA): the new measure of sensitization for transplant candidates. *Am J Transplant* 2010; **10**: 26.
52. Vaidya S, Partlow D, Susskind B, Noor M, Barnes T, Gugliuzza K. Prediction of crossmatch outcome of highly sensitized patients by single and/or multiple antigen bead luminex assay. *Transplantation* 2006; **82**: 1524.
53. Ho EK, Vasilescu ER, Colovai AI, *et al.* Sensitivity, specificity and clinical relevance of different cross-matching assays in deceased donor renal transplantation. *Transplant Immunol* 2008; **20**: 61.
54. Bingaman AW, Murphey CL, Palma-Vargas J, Wright F. A virtual crossmatch protocol significantly increases access of highly sensitized patients to deceased donor kidney transplantation. *Transplantation* 2008; **86**: 1864.
55. Cecka JM. Current methodologies for detection sensitization to HLA antigens. *Curr Opin Organ Transplant* 2011; **16**: 398.
56. Marrari M, Duquesnoy RJ. Detection of donor-specific HLA antibodies before and after removal of a rejected kidney transplant. *Tranplant Immunology* 2010; **22**: 105.
57. Süsal C, Morath C. Current approaches to the management of highly sensitized kidney transplant patients. *Tissue Antigens* 2011; **77**: 177.