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REVIEW ARTICLE

Detection of anti-HLA antibodies by solid-phase assay in kidney transplantation: friend or foe?

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Abstract

Pre-formed and *de novo* anti-human leukocyte antigen (HLA) antibodies induce antibody-mediated rejection and are also involved in mechanisms leading to chronic graft nephropathy. The detection of anti-HLA antibodies by solid-phase assay (SPA) has revolutionized the management of immunized patients before and after kidney transplantation. Characterized by high sensitivity and specificity, the clinical relevance of anti-HLA antibodies by SPA has to be clarified. The presence of donor-specific antibody at the epitope level, their titer, and the use of different crossmatch technologies could help to determine which of the anti-HLA antibodies are friends and which are foes in kidney transplantation. In this review, we summarize the current state of the art on this debated topic, and give clinical guidelines for the management of antibody detection pre- and post-transplantation, based on these evidences and our own clinical expertise.

Introduction

Solid-organ transplantation (SOT) encounters various obstacles of genetic and immunological nature, e.g. ABO blood groups, major histocompatibility complex [MHC/human leukocyte antigen (HLA)], and minor histocompatibility antigens. In the majority of cases, ABO-compatible transplantation remains the rule, the main purpose being to prevent hyperacute humoral rejection even though living donor ABO-incompatible transplantation is possible with acceptable long-term results (1–3). Although HLA matching between donor and recipient is associated with improved long-term graft survival, the HLA barrier is no longer an impediment to successful SOT, thanks to immunosuppressive drugs.

Patient exposure to HLA molecules from a genetically unrelated individual can lead to the development of anti-HLA antibodies which occurs in three situations: blood transfusion, pregnancy, and previous transplantation (4, 5). Anti-HLA antibodies present before transplantation are referred to as 'pre-formed anti-HLA antibodies' and anti-HLA antibodies arising after transplantation as '*de novo* anti-HLA antibodies'. In kidney transplantation, the presence of pre-formed anti-HLA antibodies directed against the donor (donor-specific antibody, DSA) can induce hyperacute rejection or delay antibody-mediated rejection (AMR) (6, 7). In addition to AMR, anti-HLA antibodies play a crucial role in the pathophysiology of chronic rejection, leading to graft dysfunction (5). For decades, the method of detection based on complement-dependent cytotoxicity (CDC) was the only available technology to detect anti-HLA antibody (5, 8), and it allowed to prevent disastrous hyperacute rejections (8). However, this assay is neither particularly sensitive nor specific and the identification of specific antibodies to HLAs in highly sensitized patients was therefore a difficult task. Thanks to enzyme-linked immunosorbent assay (ELISA) and flow cytometry using fluorescent microspheres (Luminex® technology, Austin, TX), the detection of specific anti-HLA antibodies by solid-phase assay (SPA) is now more sensitive, accurate, and quite easy to perform in standardized protocols, in accordance with the requirements of the laboratory quality assurance system. However, the significance of the presence and strength of pre-formed or *de novo* anti-HLA antibodies detected by SPA is still debated in terms of their possible role in rejection, long-term graft survival, and with regard to the most useful therapeutic approach for their elimination. In this review, we will discuss the role and clinical relevance in

kidney transplantation of pre-formed and *de novo* HLA antibodies detected by SPA, as well as their relevance according to crossmatch techniques, based on the current state of the art literature and our own clinical experience.

Diagnosis and mechanisms of antibody-mediated and humoral rejection

Endothelial cells expressing MHC class I constitutively but MHC class II in response to inflammation are the targets of anti-HLA antibodies (9–11). These antibodies have direct and indirect cytotoxic effects mediated by the membrane attack complex of complement. This complex attracts inflammatory cells and activates phagocytosis. The damaged endothelial cells secrete von Willebrand factor and the exposed basal membrane induces aggregation and adhesion of platelets, leading to thrombosis and vascular occlusion. During hyperacute rejection, anti-ABO or anti-HLA antibodies in high concentrations lead to irreversible ischemic damage of the transplanted organ. The role of anti-HLA antibodies in acute and chronic rejections is a repetitive 'damage-repair-damage' process characterized by inflammation and proliferation of both endothelial and smooth muscle cells. Three factors influence the development of this process: (1) concentration of antibodies, (2) repair capacity of the tissue, and (3) intensity of the immunosuppressive therapy (4, 5, 12).

The current criteria for the diagnosis of kidney allograft rejection mediated by HLA antibodies (AMR), established at the Banff Conference in 1997 and reviewed in 2001 and 2007 (13, 14) are the following:

- On biopsy, morphologic evidence of acute tissue damage, such as (1) acute tubular damage, (2) presence of neutrophils and/or mononuclear cells in the peritubular capillaries and/or glomerules and/or capillary thrombosis, or (3) intimal arteritis/fibrinoid necrosis/transmural inflammation of arteries.
- Immunopathological evidence (immunohistochemistry) of antibody effects such as (1) C4d deposits and/or (in rare cases) immunoglobulins at the basal membranes of the peritubular capillaries or (2) presence of immunoglobulins and complement in arterial fibrinoid necrosis.
- Serological evidence of circulating antibodies directed against the donor (DSA) HLAs or against other endothelial antigens of the donor.

All three criteria must be met to justify the diagnosis of AMR. If only two of these criteria are met, the correct diagnosis is suspicion of AMR.

Methods for detecting anti-HLA antibodies

It is important to determine the repertoire of specific anti-HLA antibodies before transplantation, as the presence of DSA has a direct impact on the management of organ allocation. In addition, the development of DSA after transplantation should prompt modification of the immunosuppressive treatment. An overview of the current techniques in use follows (for further details refer to the review by Tait et al. (15)):

- CDC assay: Historically, the first method for detecting anti-HLA antibodies was the CDC assay. It consists of testing the serum of the recipient for specific antibodies to lymphocytes. If antibodies are present, the classical complement pathway is activated, resulting in the lysis of donor cells by the membrane attack complex. A panel of 30–70 representative cells expressing common HLAs observed in the local population of potential donors are used as target cells. The percentage of wells (containing individual target cells) showing a positive reaction determines the percentage of 'panel-reactive antibody' (PRA). This method can also be applied to determine specificity of HLA alloantibodies (e.g. anti-HLA-A2). The advantage of the CDC assay is that it shows complement-fixing antibodies (i.e. cytotoxic antibodies), but it is not specific for the donor's HLA, as other antibodies to non-HLA lymphocyte antigens or auto-antibodies can also bind to complement and induce a positive reaction.

SPAs were developed thanks to purification of HLAs from transfected cells and their binding to different supports:

- ELISA: Specific HLAs immobilized on a plastic surface are incubated with the serum of the patient. Anti-HLA antibodies are showed after addition of an enzyme-linked anti-human IgG antibody directed against the Fc fragment of the antibodies. The amount of antibody is determined by spectrophotometry using substrate-converting enzymes (16).
- Fluorescent microspheres (flow PRA® or Luminex, One Lambda, CA): Specific HLAs immobilized on fluorescent microspheres are incubated with the serum of the patient. Anti-HLA antibody binding is showed by a second fluorescent anti-human IgG detected by flow cytometry (FACS). Using specific microspheres that match the different HLAs, the repertoire of anti-HLA antibodies of a given serum can be assessed with great accuracy (17, 18) (Figure 1A).

The strength or the titer of an anti-HLA antibody can be determined through the mean fluorescence intensity (MFI) (or the absolute molecular equivalent of fluorescence intensity, more accurate but more complicated to set up) (Figure 1B).

- Crossmatching methods: in addition to SPA, crossmatching methods continue to be useful in the detection of harmful DSA. As suggested by Taylor et al. (19), this allows differentiation between high and intermediate levels of immunological risk. From the CDC crossmatch developed 50 years ago (20) – still considered

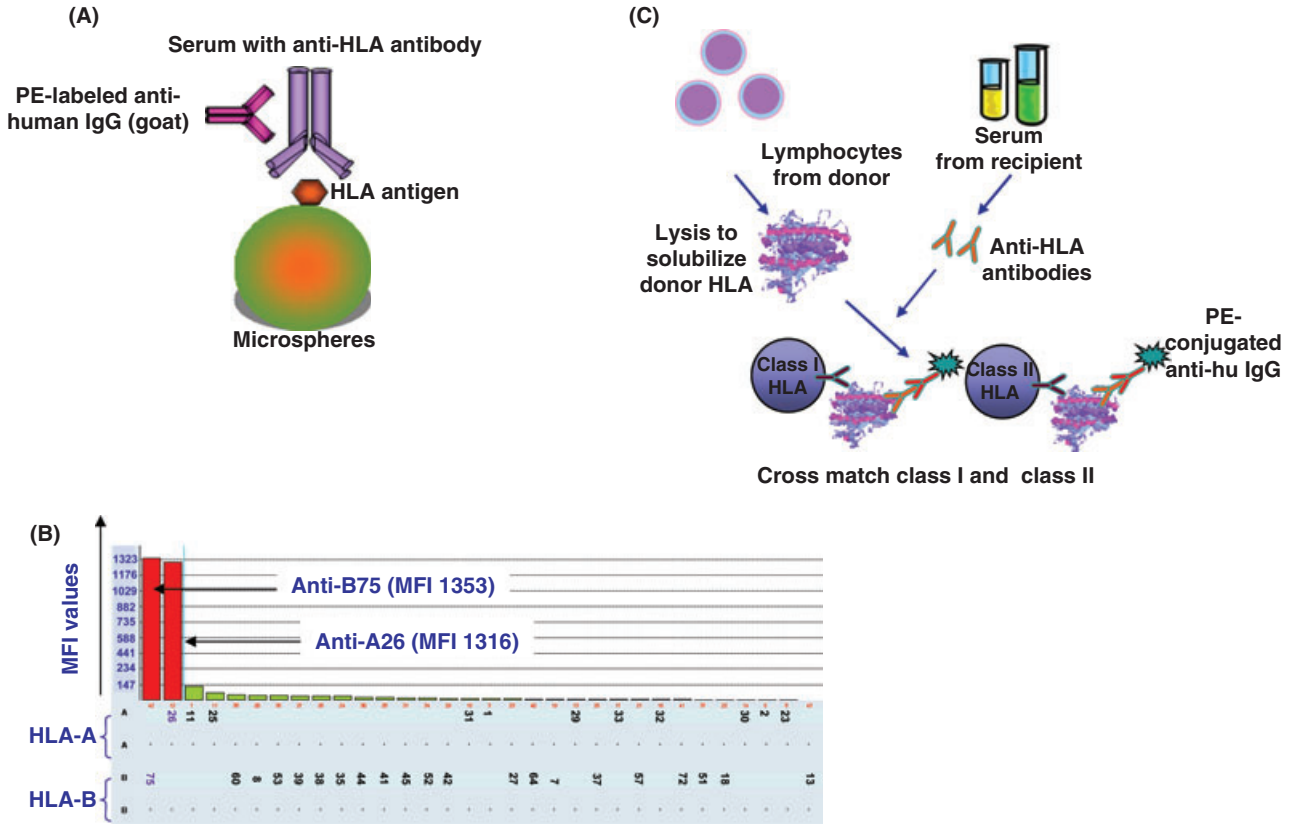

Figure 1 Luminex technology. (A) Patient serum is incubated with fluorescent beads coated with specific purified HLAs. Anti-HLA antibodies bound to individual HLA beads are targeted by goat anti-human IgG-PE. The first laser of the fluorometer (Luminex) excites the beads which are classified according to their fluorescence intensity, and the positive beads are also showed by IgG-PE staining. (B) Serum of a recipient positive for HLA-A26 and -B75 with MFI. (C) Schematic view of a crossmatch by solid-phase assay technology. Donor lymphocytes are lysed with lysis buffer and then incubated with anti-HLA class I or II specific beads, which capture donor HLAs. These donor HLA-loaded beads are then incubated with patient sera and anti-human PE-conjugated IgG is finally added for revelation (25). HLA, human leukocyte antigen; MFI, mean fluorescence intensity.

the 'gold standard' by most centers – technology has evolved to crossmatch by flow cytometry (FACS crossmatch), which is more sensitive but not specific for HLAs (21, 22). More recently, the SPA crossmatch (Luminex crossmatch) has provided interesting information (23–26). In this latter approach, donor cells are solubilized to extract cell membranes that express HLA, which are then captured by beads specific for anti-HLA class I or class II. The serum of the patient containing DSA binds to the cell membranes and the positive reaction is showed by a secondary anti-human-PE antibody (Figure 1C). This method has the advantage of involving similar technologies to that of SPA used for the detection and identification of anti-HLA antibody, but its clinical relevance needs to be assessed.

As these highly specific technologies are also very sensitive, the clinical relevance of anti-HLA antibodies with low and even with intermediate or high MFI is a matter of intense debate in the transplantation community (27, 28). The presence of such anti-HLA antibodies could result in denying a transplant to a patient on the basis of DSA that are clinically not relevant or, after transplantation, in over-treating a recipient who develops DSA.

Donor-specific and non-donor-specific anti-HLA antibodies

Donor-specific and non-donor-specific anti-HLA antibody (DSA and NDSA, respectively) are critical when considering clinical relevance of anti-HLA antibodies. DSA are deleterious while NDSA should not be. However, the situation is complicated by the fact that the high polymorphism of the MHC system is also characterized by sharing of epitopes between alleles not only of the same locus but also of different loci (29). Humoral sensitization is generally associated with anti-HLA antibodies specific for epitopes rather than antigens. A distinction between HLA epitopes and antigens is important for a better understanding of the humoral immune response to the sensitizing HLA mismatch, and even more importantly also for the determination of mismatch acceptability

of sensitized recipients. Epitopes can be defined as the physical area of an antigenic molecule that an antibody binds to in a specific way. In the case of proteins, epitopes are defined by the tertiary conformation of amino acid sequences. This means that the primary sequence of amino acids, i.e. the consecutive linear sequence of amino acids in the primary structure of a protein, does not necessarily define an epitope. Amino acids distant from each other in the primary structure can be close in the tertiary structure and may define amino acid epitopes. HLA epitopes can be structurally defined by HLAMATCHMAKER, an algorithm that considers eplets as critical elements of epitopes recognized by alloantibodies (30). An eplet represents a patch of amino acid residues within a radius of about 3 Å from a polymorphic residue on the HLA molecular surface (http://www.HLAMatchmaker.net). The concept of epitope matching should be considered when classifying a given anti-HLA antibody as DSA or NDSA, because antibodies NDSA at the antigen level can be DSA at the epitope level. The clinical relevance of the HLAMATCHMAKER approach is by now well established and should be used in clinical practice, as it allows optimization of organ allocation based on a functional algorithm. In this context, an important asset is represented by SPA which allows for the detection of a wide range of anti-HLA antibodies with high sensitivity, even in case of exposure to a limited number of HLAs (31, 32).

A second element that should be taken into consideration is the difference between the antigenicity of epitopes (i.e. the reactivity with anti-HLA antibody) and the immunogenicity of epitopes (i.e. the capacity of inducing anti-HLA antibody). Better characterization and understanding of epitope immunogenicity will be critical for designing new strategies to define permissible mismatches for sensitized and non-sensitized recipients.

Of note, false-positive results are also a matter of concern, mainly due to the partial denaturation of antigens coating some beads and that might be recognized by natural non-HLA antibodies (33).

Clinical relevance of pre-formed anti-HLA antibodies detected by SPA

Hyperacute rejection by high titers of pre-formed anti-HLA antibodies may be prevented by selecting a donor with a negative CDC crossmatch. Due to the high sensitivity of SPA, anti-HLA antibodies detected by this technique often fail to be associated with a positive pre-transplant CDC crossmatch, but could nevertheless contribute to AMR and long-term complications. A better understanding of the clinical relevance of pre-formed DSA is warranted to improve the criteria for kidney allocation. Several recent studies have addressed this crucial issue.

Gibney et al. (34) has compared retrospectively the results of anti-HLA antibody detection by CDC-PRA and by SPA (Luminex) in kidney transplant patients with negative CDC crossmatch. The study consisted of 136 patients with 55 patients presenting a CDC-PRA >15%, of whom 20 had DSA. At 6 months after transplantation, kidney dysfunction was manifest in 12/20 (60%) of PRA^+/DSA^+ patients, as compared to 9/35 (26%) of the PRA^+/DSA^- patients. Thus, two subgroups were described, one of high-risk patients (PRA^+/DSA^+) with a 25% rate of acute rejection and one of low-risk patients (PRA^+/DSA^-) whose rate of acute rejection was as low as 3%, with very good graft survival at 6 months (Table 1).

The clinical relevance of DSA detected by SPA has also been analyzed in subclinical AMR (SAMR). Loupy et al. (35) assessed SAMR in a cohort of 54 DSA-positive kidney transplant recipients from a deceased donor without pre-transplant desensitization treatment. SAMR is a frequent finding in patients with pre-formed DSA. This study showed that in addition to DSA, C4d deposition and typical histology are associated with poor clinical outcome. In this group, MHC class II DSA with high MFI has the worst outcome. This study highlights the importance to correlate DSA and histology status (35).

Amico et al. (36) analyzed the long-term survival of grafts and patients in a cohort of 67 with preformed DSA. The salient observation was that 37/67 patients with DSA detected by Luminex at the time of transplantation experienced clinical/SAMR (DSA^+ AMR^+) with 20% lower death-censored allograft survival at 5 years post-transplantation. However, the remaining 30/67 patients with DSA were free from AMR (DSA^+ AMR^-) and allograft survival rate was equal to that of patients without DSA. Interestingly, neither the number of DSA, HLA-classes (HLA-class I *vs* HLA-class II), and MFI nor the sensitizing events proved predictive for AMR. These results suggest that almost 50% of DSA as defined by SPA are clinically irrelevant and do not lead to AMR, emphasizing the difficulty of predicting their impact on graft survival (36).

In contrast to the above study, Lefaucheur et al. (37) found a correlation between the risk of AMR and graft loss with peak DSA titer (MFI). This study analyzed 402 kidney transplant patients and focused on 118 patients with DSA. They found that 8-year graft survival was significantly worse (61%) among patients with preexisting DSA at high MFI (>3000) compared with sensitized patients without DSA (93%) or with non-sensitized patients (84%). The peak HLA-DSA Luminex MFI (DSA-MFI) predicted AMR better than DSA-MFI in the current (at transplant) serum sample. The peak DSA-MFI was inversely correlated with graft survival and directly correlated with the risk of AMR. Patients with MFI >3000 had a more than 100-fold higher risk of AMR than patients with MFI <465.

Aubert et al. (38) analyzed a cohort of 113 patients of whom 11/113 (11%) had DSA, and reported that DSA with MFI below 2000 was not associated with short-term rejection events (cellular and humoral).

Table 1 Literature review over pre-formed anti-HLA antibodies, AMR, and kidney graft survival

Article	Technique studied	Sample size DSA (%)	Follow–up (days)[a]	AMR[b]	Graft survival[c] (months)
Gibney et al. (34)	CDC X-match T and B SPA	136 patients 20 DSA^+ (14)	180	$DSA^+ = 25\%$ $DSA^- = 3\%$	$DSA^+ = 75\%$ $DSA^- = 94\%$ (6)
Loupy et al. (35)	CDC X-match T and B SPA	137 patients 54 DSA^+ (39)	365	$DSA^+ = 10\%$ $DSA^- = 0\%$	$DSA^+ = 86.2\%$ $DSA^- = 96.2\%$ (48)
Amico et al. (36)	Current and historical CDC X-match T and B SPA	334 patients 67 DSA^+ (20)	200	$DSA^+ = 55\%$ $DSA^- = 6\%$	$DSA^+ AMR^+ = 68\%$ $DSA^+ AMR^- = 87\%$ $DSA^- = 89\%$ (60)
Lefaucheur et al. (37)	CDC X-match T and B SPA	402 patients 118 DSA^+ (30)	365	$DSA < 465 = 0.9\%$ $DSA < 3000 = 18.7\%$ $DSA > 3000 = 36\%$	$DSA < 465 = 82.5\%$ $DSA < 3000 = 78\%$ $DSA > 3000 = 60.6\%$ (96)
Aubert et al. (38)	CDC X-match T and B SPA	113 patients 11 DSA^+ (9)	365	$DSA^+ = 9\%$ $DSA^- = 0\%$	Not reported
Riethmuller et al. (26)	CDC X-match T and B FACS X-match T and B Luminex X-match T and B SPA	155 patients 20 DSA^+ (13)	365	$DSA^+ = 35\%$ $DSA^- = 6\%$	$DSA^+ = 85\%$ $DSA^- = 100\%$ (12)
Gloor et al. (40)	CDC X-match T and B FACS X-match T and B SPA	189 patients 119 DSA^+ (61)	540	$DSA^+ = 41\%$ $DSA^- = 1\%$	$DSA^+ > 5000 = 80\%$ $DSA^+ > 10,000 = 50\%$ $DSA^- = 100\%$ (72)

AMR, antibody-mediated rejection; CDC X-match, crossmatch by complement-dependent cytotoxicity; DSA, donor-specific antibody; HLA, human leukocyte antigen; SPA, solid-phase assay.

[a]Follow-up of the study with regard to AMR.

[b]Percent of AMR with regard to the presence or absence of DSA.

[c]Long-term graft survival reported with regard to the presence or absence of DSA.

These studies suggest that DSA detected by SPA alone are not sufficient to predict the risk of clinical events but that DSA with low MFI and those not correlated with positive CDC-PRA, positive CDC crossmatch, or FACS crossmatch could have a limited effect on graft survival in presence of sufficient immunosuppression.

Recently, we assessed the prognostic value of pre-transplant DSA detected by Luminex in association with crossmatch tests (26). Three generations of crossmatch tests were performed on 37 living donor kidney recipients that tested positive according to the anti-HLA antibody screening assay (20 patients DSA^+), but negative for T-cell CDC crossmatch. In 100% of DSA-positive patients – with MFI above 5000 – AMR occurred in the first year of transplantation. When the B-cell CDC crossmatch and, more importantly, the Luminex crossmatch for class I were positive, the prediction of AMR was associated with DSA at lower MFI ($\geq$900 MFI). Three patients with high levels of DSA (MFI $>10,000$) had major or irreversible AMR in the weeks following transplantation. Therefore, for sensitized recipients with a T-cell-negative CDC crossmatch, the most reliable prediction for AMR and consecutive graft function is provided either by DSA-class I alone at high strength or by DSA-class I at low strength added to a positive Luminex or CDC crossmatch.

Zachary et al. (39) have also reviewed the correlation between DSA defined by SPA and crossmatches. They showed that high MFI threshold values for DSA correlate with positive FACS (MFI $>$ 6000) and CDC crossmatches (MFI $>10,000$), but the study did not establish a correlation with the clinical outcome.

In a large cohort of recipients with DSA determined by single antigen beads (Luminex), Gloor et al. (40) assessed the risk of AMR in recipients with positive (119/189) or negative (70/189) crossmatch, in correlation with MFI. In this study, two generation of crossmatches, CDC and FACS, with two different channel shifts were performed. The level of DSA defined by MFI was compared to a positive AHG-CDC crossmatch, to a positive FACS crossmatch with channel shift $>$300 or $<$300, and to a negative crossmatch. The authors observed a significant correlation between DSA levels and sensitivity of the various crossmatches. Of note, every patient with a high level of DSA (MFI $>$ 10,000) had a positive AHG-CDC crossmatch.

Together, these studies suggest that pre-formed DSA at high levels (MFI $>$ 10,000) should be considered a contraindication to transplantation. DSA with low MFI ($<$1000 or $<$2000) are unlikely to have a deleterious effect on the graft (at least in the short term). For DSA with MFI between 2000 and 10,000, additional results of FACS crossmatch and/or Luminex crossmatch could help to consolidate a reasonable prediction of graft rejection events after transplantation. Table 1 summarizes the most relevant studies on pre-formed anti-HLA antibody analysis by SPA with regard to AMR and graft survival.

Clinical relevance of *de novo* anti-HLA antibodies arising post-transplantation

The development of anti-HLA antibodies after kidney transplantation, as consequence of an immune response to allo-HLAs, can be observed in 10%–50% of the recipients (41, 42). The considerable variation in the reported percentages of this event is mainly due to different sensitivity of the methods used to detect anti-HLA antibodies (41, 42). According to previous work (34), the incidence of anti-HLA antibodies developing in patients 6 months after transplantation is roughly the same as after 10 years. In a prospective study, Terasaki et al. (43) assessed the survival of kidney grafts from living and deceased donors based on the presence or absence of anti-HLA antibodies. Anti-HLA antibodies were determined by CDC or SPA before transplantation and 6, 12, and 24 months post-transplantation. Of the 2231 patients analyzed, 478 (21.4%) were positive for anti-HLA antibodies 1 year post-transplantation. Of these, 6.2% underwent graft failure, in contrast to only 2.8% patients without anti-HLA antibodies ($P < 0.01$). Two years after transplantation, graft loss occurred in 15.1% of the patients with anti-HLA antibodies, whereas in recipients without anti-HLA antibodies the percentage was as low as 6.8% ($P < 0.02$). When the authors compared the patients who produced anti-HLA antibodies *de novo* after transplantation (233 patients) with those who had never developed anti-HLA antibodies (1331 patients), the rates of graft failure were 16.7% and 6.5% ($P < 0.01$), respectively (Table 2). This study clearly points out that the development of *de novo* anti-HLA antibodies increases the risk of graft failure (43). However, if these data are stratified according to the methods of anti-HLA antibody detection (CDC *vs* SPA), the predictive value of CDC for graft failure was higher than that of SPA.

A second study (44) consisted of 72 patients who had received their first kidney transplant from deceased donors. These 72 patients tested negative for anti-HLA antibodies by CDC and SPA before transplantation. All of them had undergone triple immunosuppressive therapy and were followed for at least 4 years. Sixteen of the 72 patients (22.2%) became positive for anti-HLA antibodies after transplantation, and in 12 of these 16 (75%) the detected antibodies were DSA. Ten of the 12 had acute or chronic rejection.

A statistically significant correlation was established between the appearance of anti-HLA antibodies and delayed graft function, acute rejection, chronic rejection, and graft loss due to immunological causes (44). Patients with high titers of *de novo* anti-HLA antibodies experienced more severe acute rejection and early graft loss, whereas those with lower titers tended to develop chronic rejection. This suggests that titration and monitoring of alloantibodies could be useful in evaluating the risk of rejection (44).

Lachmann et al. (45) showed that in a cohort of 1014 kidney transplant recipients from deceased donors, monitored in a cross-sectional manner for the development of anti-HLA

antibodies using SPA (Luminex), 29% of the recipients tested positive. DSAs were found in 31% of these antibody-positive patients (9% of the 1014 patients). The presence of DSA was associated with a significantly lower graft survival of 49% compared to 83% in the anti-HLA antibody-negative subjects. Interestingly, at late stage of graft failure (low GFR), the presence of anti-HLA antibody without donor specificity (NDSA) also had an adverse effect on graft survival (70%), similar to DSA. Concomitant DSA absorbed by the graft or NDSA related to mismatched can be explained by this intriguing finding. Shared epitope analysis was not performed in this study.

In a prospective 5-year longitudinal analysis of 54 patients without anti-HLA antibodies, graft survival was 86% as opposed to 14% in patients who developed *de novo* anti-HLA antibodies in the 5 years after the first testing ($P = 0.05$). Interestingly, those with anti-HLA antibodies not directed against the donor (NDSA) also had an impaired graft survival (53%). No information as to antibody titer was provided by the authors (46).

In summary, the presence of *de novo* DSA is clearly deleterious to the graft. It leads to AMR and/or contributes to chronic graft nephropathy that in turn reduces long-term graft survival. The development of DSA *de novo* could be viewed as a condition of insufficient immunosuppression, and we therefore believe that any reduction in the dosage of immunosuppressant should be followed by the repeated determination of anti-HLA antibody in the subsequent weeks. Table 2 summarizes the most relevant studies on *de novo* anti-HLA antibody analysis by SPA with regard to AMR and graft survival.

Management of DSA before and after transplantation

The management of patients with anti-HLA antibodies requires a multidisciplinary approach involving close collaboration between HLA laboratory and clinicians.

Before transplantation, careful evaluation of the anti-HLA antibody repertoire with SPA is strongly recommended. Due to cost-effectiveness considerations, anti-HLA determination by SPA should be repeated once a year, except in the case of an immunization event. Indeed, the relevance of the 'natural' fluctuation of anti-HLA levels (MFI) is not known. Virtual PRA (or calculated PRA) and virtual crossmatch approaches (47, 48) or the acceptable mismatch system (49) will be more and more based on anti-HLA antibodies detected by SPA only. Therefore, there is a risk that transplant candidates with anti-HLA antibodies based on DSA identified by SPA only could be discriminated in that they are denied a transplant.

The combination of DSA detected by SPA and crossmatch (by FACS and Luminex) with high sensitivity seems to be an interesting option. Easy to implement in living donor transplantation, where it can be part of the patient/donor evaluation before transplantation, it would probably be more difficult in cases of deceased donor transplants, especially for the HLA laboratory, which should be able to perform FACS and/or Luminex crossmatches on a 24-h basis.

Schemes of risk assessment according to the presence of anti-HLA antibody and crossmatch should help the discussion between laboratory and clinicians and could also be of great interest to develop a consensus between laboratories from different centers (19).

Figure 2 illustrates the current strategy in our institution to stratify the risk of AMR with regard to the different technologies of anti-HLA antibody detection. We recommend that the determination of anti-HLA antibody should be carried out by CDC and SPA before transplantation. The CDC crossmatch remains the gold standard but the FACS and the Luminex crossmatch should be performed whenever possible. In living donation, FACS and Luminex crossmatches can be easily implemented before transplantation to assess the risk of AMR and early graft loss. For cadaveric donation, FACS and Luminex crossmatch are not performed on a routine basis before transplantation since it can be carried out in the next 24–48 h.

The decision to transplantation will be taken according to this risk, knowing that in presence of DSA, a CDC-positive T-cell crossmatch T remains an absolute counter-indication to transplantation. The other options leave space for desensitization protocols in selected cases but in principle a positive FACS crossmatch and high MFI (>10,000) DSA are also considered a counter-indication to transplantation. Further studies with regard to the relevance of DSA by SPA will fine-tune the decision.

Before transplantation, the presence of anti-HLA should be extensively studied by both CDC and SPA methods (Figure 3A).

After transplantation, the development of anti-HLA antibody by SPA (or CDC) should be carefully followed and lead to early kidney biopsy (Figure 3B). Such a strategy needs to be validated by prospective studies.

To ensure successful transplantation, several approaches of desensitization (50) to remove anti-HLA antibodies have been reported on a limited number of patients with significant success in terms of short- and long-term graft survival (6, 51, 52). The long-term graft survival remains to be determined but the main deficiencies of such approaches are due to the limited number of control studies. However, we believe that such approaches should be intended for patients with very limited chances of receiving a transplant without DSA, i.e. those with large numbers of anti-HLA antibodies (Figure 3).

Acute AMR requires rapid lowering of circulating DSA by plasmapheresis in addition to the standard treatment consisting of steroids, anti-thymoglobulin, and anti-CD20 antibody (53–56) (Figure 3B). However, despite the reversal

Table 2 Literature review over *de novo* anti-HLA antibodies and kidney graft survival

Article	Technique studied	Sample size Abs (%)	Follow-up (years)[a]	AMR[b]	Graft failure[c] (months)
Terasaki et al. (43)	CDC X-match T and B ELISA SPA	1564 patients *De novo* Abs (15)	2	—	*De novo* Abs = 16.7% Abs^- = 6.5% (24)
Mihaylova et al. (44)	CDC X-match T and B FACS X-match T and B SPA	72 patients *De novo* Abs (22.2)	1–5	*De novo* Abs = 37.5% Abs^- = 3.6%	*De novo* Abs = 50% Abs^- = 68.2% (60)
Lachmann et al. (45)	CDC X-match T and B ELISA LSM SPA	1014 patients *De novo* Abs (29) DSA^+ = 9% $NDSA^+$ = 20%	Mean 5.5	—	DSA^+ = 49% $NDSA^+$ = 70% DSA^- = 83% (66)
Mao et al. (46)	CDC X-match T and B LSM SPA	54 patients *De novo* Abs (59) DSA^+ = 28% $NDSA^+$ = 31%	5	—	DSA^+ = 86% $NDSA^+$ = 47% DSA^- = 14% (60)

Abs, *de novo* antibodies development; AMR, antibody-mediated rejection; CDC X-match, crossmatch by complement-dependent cytotoxicity; DSA, donor-specific antibody; ELISA, enzyme-linked immunosorbent assay; HLA, human leukocyte antigen; LSM, LABScreen Mixed; NDSA, non-DSA; SPA, solid-phase assay.

[a]Follow-up of the study after transplantation.

[b]Percent of AMR with regard to the presence or absence of DSA.

[c]Graft failure reported with regard to the presence or absence of *de novo* Abs or DSA.

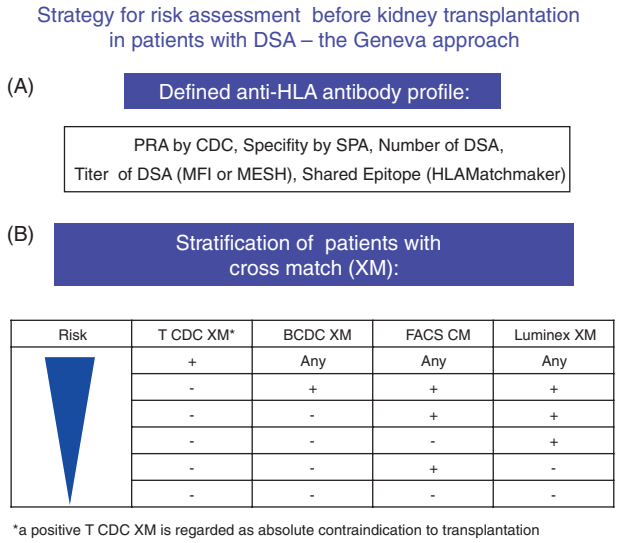

Figure 2 Strategy for risk assessment before kidney transplantation in patients with DSA – the Geneva approach. The determination of the presence of anti-human leukocyte antigen antibodies is performed by CDC and SPA (A). Crossmatch performed by CDC, FACS, and Luminex is used to stratify the risk of AMR before transplantation, and decision to transplantation is based on these results (B). AMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity; DSA, donor-specific antibody; SPA, solid-phase assay.

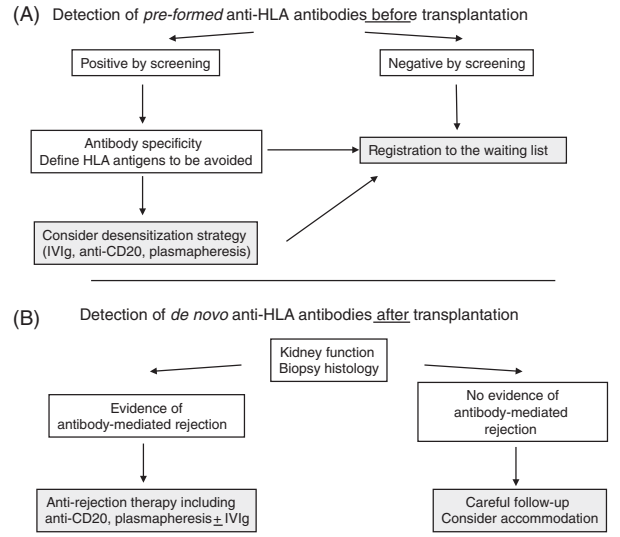

Figure 3 Clinical strategies according to the patient anti-human leukocyte antigen (HLA) antibody status before or after transplantation. Possible HLA antibody testing results (white boxes) and resulting clinical strategies (gray boxes) before transplantation (A). After transplantation, clinical strategies (gray boxes) after detection of *de novo* anti-HLA antibodies should be based on biopsy results (white boxes) (B).

of acute renal dysfunction, this treatment does not deplete antibody-secreting plasma cells in spleen and bone marrow, and circulating DSA commonly remains detectable in peripheral blood. DSA induce microvascular endothelial lesions that can lead to chronic AMR. Long-term exposure to anti-HLA antibodies is also associated with shortened allograft survival and transplant glomerulopathy, even in the absence of documented acute AMR. Although effective treatment is available for acute AMR, allografts remain at risk for chronic AMR and shortened survival (57).

Conclusion

New methods for detecting anti-HLA antibodies such as SPA provide a valuable tool in anticipating AMR or graft dysfunction before transplantation. These methods have proved a 'friend' to physicians in charge of immunized patients before and after transplantation. Results of anti-HLA antibody detection by SPA should be integrated into the global decision of organ allocation, ensuring both short- and long-term benefits to the selected recipient. We also believe that before transplantation, CDC crossmatch should remain mandatory for patients with anti-HLA antibodies. FACS crossmatch or the new SPA crossmatch method (Luminex crossmatch) may also be an excellent means of improving the decision algorithm and should be implemented in HLA laboratories. More studies are necessary to optimize SPA results for anti-HLA antibody titers, such as their predictive value (sensitivity and specificity) for the risk of AMR, as well as short- and long-term graft survival. A better interpretation of these SPA results would increase the values of this technique, its widespread use, and the resulting benefits for the patients.

Conflict of interest

The authors have declared no conflicting interests.

References

1. Crew RJ, Ratner LE. ABO-incompatible kidney transplantation: current practice and the decade ahead. *Curr Opin Organ Transplant* 2010: **15**: 526–30.
2. Montgomery RA, Locke JE, King KE et al. ABO incompatible renal transplantation: a paradigm ready for broad implementation. *Transplantation* 2009: **87**: 1246–55.
3. Genberg H, Kumlien G, Wennberg L, Berg U, Tyden G. ABO-incompatible kidney transplantation using antigen-specific immunoadsorption and rituximab: a 3-year follow-up. *Transplantation* 2008: **85**: 1745–54.
4. Halloran PF. The clinical importance of alloantibody-mediated rejection. *Am J Transplant* 2003: **3**: 639–40.
5. Terasaki PI, Cai J. Humoral theory of transplantation: further evidence. *Curr Opin Immunol* 2005: **17**: 541–5.
6. Lefaucheur C, Suberbielle-Boissel C, Hill GS et al. Clinical relevance of preformed HLA donor-specific antibodies in kidney transplantation. *Contrib Nephrol* 2009: **162**: 1–12.
7. 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**: 2684–94.

8. Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. *Nature* 1964: **204**: 998–1000.
9. Zhang X, Reed EF. Effect of antibodies on endothelium. *Am J Transplant* 2009: **9**: 2459–65.
10. Al Lamki RS, Bradley JR, Pober JS. Endothelial cells in allograft rejection. *Transplantation* 2008: **86**: 1340–8.
11. Muczynski KA, Ekle DM, Coder DM, Anderson SK. Normal human kidney HLA-DR-expressing renal microvascular endothelial cells: characterization, isolation, and regulation of MHC class II expression. *J Am Soc Nephrol* 2003: **14**: 1336–48.
12. Villard J. Immunity after organ transplantation. *Swiss Med Wkly* 2006: **136**: 71–7.
13. Racusen LC, Colvin RB, Solez K et al. Antibody-mediated rejection criteria - an addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 2003: **3**: 708–14.
14. Solez K, Colvin RB, Racusen LC et al. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 2008: **8**: 753–60.
15. Tait BD, Hudson F, Brewin G, Cantwell L, Holdsworth R. Solid phase HLA antibody detection technology–challenges in interpretation. *Tissue Antigens* 2010: **76**: 87–95.
16. Worthington JE, Robson AJ, Sheldon S, Langton A, Martin S. A comparison of enzyme-linked immunoabsorbent assays and flow cytometry techniques for the detection of HLA specific antibodies. *Hum Immunol* 2001: **62**: 1178–84.
17. Gebel HM, Harris SB, Zibari G, Bray RA. Conundrums with FlowPRA beads. *Clin Transplant* 2002: **16** (Suppl 7): 24–9.
18. Tambur AR, Bray RA, Takemoto SK et al. Flow cytometric detection of HLA-specific antibodies as a predictor of heart allograft rejection. *Transplantation* 2000: **70**: 1055–9.
19. Taylor CJ, Kosmoliaptsis V, Summers DM, Bradley JA. Back to the future: application of contemporary technology to long-standing questions about the clinical relevance of human leukocyte antigen-specific alloantibodies in renal transplantation. *Hum Immunol* 2009: **70**: 563–8.
20. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969: **280**: 735–9.
21. Bray RA, Lebeck LK, Gebel HM. The flow cytometric crossmatch. Dual-color analysis of T cell and B cell reactivities. *Transplantation* 1989: **48**: 834–40.
22. Gebel HM, Bray RA. Sensitization and sensitivity: defining the unsensitized patient. *Transplantation* 2000: **69**: 1370–4.
23. Pei R, Lee JH, Shih NJ, Chen M, Terasaki PI. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. *Transplantation* 2003: **75**: 43–9.
24. Billen EV, Voorter CE, Christiaans MH, van den Berg-Loonen EM. Luminex donor-specific crossmatches. *Tissue Antigens* 2008: **71**: 507–13.
25. Billen EV, Christiaans MH, van den Berg-Loonen EM. Clinical relevance of Luminex donor-specific crossmatches: data from 165 renal transplants. *Tissue Antigens* 2009: **74**: 205–12.
26. Riethmuller S, Ferrari-Lacraz S, Muller MK et al. Donor-specific antibody levels and three generations of crossmatches to predict antibody-mediated rejection in kidney transplantation. *Transplantation* 2010: **90**: 160–7.
27. Claas FH. Clinical relevance of circulating donor-specific HLA antibodies. *Curr Opin Organ Transplant* 2010: **15**: 462–6.
28. Leffell MS, Zachary AA. Antiallograft antibodies: relevance, detection, and monitoring. *Curr Opin Organ Transplant* 2010: **15**: 2–7.
29. Cai J, Terasaki PI. Humoral theory of transplantation: mechanism, prevention, and treatment. *Hum Immunol* 2005: **66**: 334–42.
30. Duquesnoy RJ. Antibody-reactive epitope determination with HLAMatchmaker and its clinical applications. *Tissue Antigens* 2011: **6**: 525–34.
31. Duquesnoy RJ, Marrari M. Correlations between Terasaki's HLA class I epitopes and HLAMatchmaker-defined eplets on HLA-A, -B and -C antigens. *Tissue Antigens* 2009: **74**: 117–33.
32. Marrari M, Duquesnoy RJ. Correlations between Terasaki's HLA class II epitopes and HLAMatchmaker-defined eplets on HLA-DR and -DQ antigens. *Tissue Antigens* 2009: **74**: 134–46.
33. El Awar N, Terasaki PI, Nguyen A et al. Epitopes of human leukocyte antigen class I antibodies found in sera of normal healthy males and cord blood. *Hum Immunol* 2009: **70**: 844–53.
34. 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**: 2625–9.
35. 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**: 2561–70.
36. Amico P, Honger 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**: 1681–8.
37. Lefaucheur C, Loupy A, Hill GS et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J Am Soc Nephrol* 2010: **21**: 1398–406.
38. Aubert V, Venetz JP, Pantaleo G, Pascual M. Low levels of human leukocyte antigen donor-specific antibodies detected by solid phase assay before transplantation are frequently clinically irrelevant. *Hum Immunol* 2009: **70**: 580–3.
39. Zachary AA, Sholander JT, Houp JA, Leffell MS. Using real data for a virtual crossmatch. *Hum Immunol* 2009: **70**: 574–9.
40. Gloor JM, Winters JL, Cornell LD et al. Baseline donor-specific antibody levels and outcomes in positive crossmatch kidney transplantation. *Am J Transplant* 2010: **10**: 582–9.
41. Akalin E, Pascual M. Sensitization after kidney transplantation. *Clin J Am Soc Nephrol* 2006: **1**: 433–40.
42. Hourmant M, Cesbron-Gautier A, Terasaki PI et al. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. *J Am Soc Nephrol* 2005: **16**: 2804–12.
43. Terasaki PI, Ozawa M. Predictive value of HLA antibodies and serum creatinine in chronic rejection: results of a 2-year prospective trial. *Transplantation* 2005: **80**: 1194–7.
44. Mihaylova A, Baltadjieva D, Boneva P et al. Clinical relevance of anti-HLA antibodies detected by flow-cytometry bead-based assays–single-center experience. *Hum Immunol* 2006: **67**: 787–94.

45. Lachmann N, Terasaki PI, Budde K et al. Anti-human leukocyte antigen and donor-specific antibodies detected by luminex posttransplant serve as biomarkers for chronic rejection of renal allografts. *Transplantation* 2009: **87**: 1505–13.
46. Mao Q, Terasaki PI, Cai J et al. Extremely high association between appearance of HLA antibodies and failure of kidney grafts in a five-year longitudinal study. *Am J Transplant* 2007: **7**: 864–71.
47. Cecka JM. Calculated PRA (CPRA): the new measure of sensitization for transplant candidates. *Am J Transplant* 2010: **10**: 26–9.
48. Bray RA, Nolen JD, Larsen C et al. Transplanting the highly sensitized patient: the Emory algorithm. *Am J Transplant* 2006: **6**: 2307–15.
49. Claas FH, Doxiadis II. Human leukocyte antigen antibody detection and kidney allocation within Eurotransplant. *Hum Immunol* 2009: **70**: 636–9.
50. Jordan SC, Pescovitz MD. Presensitization: the problem and its management. *Clin J Am Soc Nephrol* 2006: **1**: 421–32.
51. Vo AA, Lukovsky M, Toyoda M et al. Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med* 2008: **359**: 242–51.
52. Stegall MD, Gloor J, Winters JL, Moore SB, DeGoey S. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. *Am J Transplant* 2006: **6**: 346–51.
53. Mulley WR, Hudson FJ, Tait BD et al. A single low-fixed dose of rituximab to salvage renal transplants from refractory antibody-mediated rejection. *Transplantation* 2009: **87**: 286–9.
54. Colovai AI, Vasilescu ER, Foca-Rodi A et al. Acute and hyperacute humoral rejection in kidney allograft recipients treated with anti-human thymocyte antibodies. *Hum Immunol* 2005: **66**: 501–12.
55. Gloor J, Stegall MD. Sensitized renal transplant recipients: current protocols and future directions. *Nat Rev Nephrol* 2010: **6**: 297–306.
56. Loupy A, Suberbielle-Boissel C, Zuber J et al. Combined posttransplant prophylactic IVIg/anti-CD 20/plasmapheresis in kidney recipients with preformed donor-specific antibodies: a pilot study. *Transplantation* 2010: **89**: 1403–10.
57. Gloor J, Cosio F, Lager DJ, Stegall MD. The spectrum of antibody-mediated renal allograft injury: implications for treatment. *Am J Transplant* 2008: **8**: 1367–73.