

# How to Grade Immunological Risk Using Sensitive HLA Donor-Specific Antibodies Detection Techniques

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## Abstract

*Since the pioneering work of Patel and Terazaki, the presence of an antidonor antibody of the IgG isotype, as demonstrated by a lymphocytotoxic assay on T-cells, has been a contraindication to transplantation due to the very high rate of graft loss reported. The advent of more sensitive and specific techniques of detection of anti-HLA antibodies (ELISA or Luminex) has questioned this dogma, with a number of reports showing that transplantation, despite the presence of a donor-specific antibody, could be done without excessive graft losses, despite higher rates of rejection.*

*We analyzed the results of a prospective observational study on the occurrence of acute antibody-mediated rejection and survival of patients and grafts, in kidney transplant patients with preexisting donor-specific HLA antibodies detected by Luminex. This study assess, for the first time, kidney graft survival and the gradation of the risk of acute antibody-mediated rejection according to the levels of donor-specific antibodies detected before transplantation. We have shown a dramatic increase in the risk of antibody-mediated rejection with increasing levels of preexisting donor-specific antibodies above 465 as detected by Luminex. We have also shown that patients transplanted with donor-specific antibody mean fluorescence intensity > 3,000 have a 3.8 increased risk of graft loss as compared to patients transplanted with donor-specific antibody mean fluorescence intensity < 3,000 (95% CI: 3.5-18.4; p < 0.0001). This stratification of immunological risk should be used to define acceptable graft and therapeutic strategies in sensitized patients on the waiting list. Thus, the matching of graft donors and recipients should take into account this precise analysis of the immunological profile of patients and the evaluation of the risk/benefit balance. (Trends in Transplant. 2010;4:3-10)*

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## Key words

**HLA antibodies. Antibody-mediated rejection. Graft survival. Kidney transplantation.**

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## Introduction

Anti-HLA immunization constitutes an immunogenetic hurdle to transplantation, leading to protracted waiting times that continue to increase for sensitized kidney transplant recipients<sup>1-3</sup>. In France, 25% of patients on the waiting list have a panel-reactive antibody level > 5%<sup>4</sup>, and in the USA, 32% of patients awaiting transplant are sensitized<sup>1</sup>. Despite efforts to diminish the risk of sensitization, namely recombinant erythropoietin, leukocyte-depleted transfusions, and the cessation of pre-graft transfusion protocols, the number of sensitized patients on transplant lists remains substantial. Moreover, loss of a prior graft has become the primary cause of anti-HLA sensitization.

Patel and Terasaki<sup>5</sup>, in 1969, demonstrated the efficacy of complement-dependent lymphocytotoxic crossmatch in defining immunological risk in renal transplantation. This became the standard methodology, still used today, for graft allocation. It became clear with time that it did not identify all preexisting donor-specific HLA antibodies (DSA). In recent years, techniques for detection of HLA antibodies have become more sensitive with the introduction of solid-phase assays, including enzyme-linked immunosorbent assays (ELISA), and multiple bead-based technology, of which the Luminex-based assays are the most frequently used. The clinical impact of the antibodies detected by these more sensitive techniques has yet to be fully evaluated in terms of graft survival and definition of acceptable grafts<sup>3</sup>. Studies of the clinical relevance of DSA in patients transplanted with negative crossmatches have been contradictory<sup>6-8</sup>.

The ability to quantify these antibodies<sup>9</sup> has added a further dimension of complexity to the equation. The Luminex technique has been used in recent studies to choose the type of desensitization according to DSA strength<sup>10</sup> and to determine acceptable DSA levels, allowing for successful kidney transplantation

following desensitization<sup>11</sup>. Forty years after the initial definition of immunological risk by Terasaki and Patel, the introduction of these more sensitive techniques revives and carries to a new level the basic question of the clinical relevance of donor-specific anti-HLA antibodies and their integration into current strategies of transplantation. Indeed, no single study has compared the sensitivity, specificity, and positive predictive value of classic or flow crossmatch, ELISA, and Luminex techniques in the prediction of acute antibody-mediated rejection and graft survival.

## Clinical relevance of preformed HLA donor-specific antibodies in kidney transplantation

To investigate the clinical relevance of pre-graft DSA identified by strictly HLA-specific assays, we undertook a first study in which we retrospectively screened a series of 237 consecutive renal transplants performed in our unit by high-definition ELISA for their presence<sup>12</sup>. Our study showed that kidney graft survival at eight years was significantly worse in patients with DSA. The incidence of antibody-mediated rejection in patients with DSA was nine-fold higher than in patients without DSA and led to a significantly worse graft survival. The prevalence of antibody-mediated rejection in patients with DSA detected on historical serum was 32.3% and was significantly more elevated in patients with strongly positive DSA (score 6-8) and in patients with historical positive crossmatches. Thus, the presence of preformed DSA was strongly associated with increased graft loss in kidney transplants, related to an increased risk of antibody-mediated rejection.

To appraise the full clinical potential of DSA detected prior to transplantation, we conducted a prospective observational study in which we employed the capacity of the Luminex technique to identify with precision

and to quantify HLA-specific antibodies in order to grade increasing immunological risk. This study examined the impact of the strength of preformed DSA on the risk of acute antibody-mediated rejection occurrence and graft survival in deceased-donor kidney graft patients. The study included 402 consecutive deceased-donor single-organ kidney transplant patients performed in our unit between January 1998 and June 2006. Our graft strategy was the current worldwide strategy based on the negative National Institutes of Health lymphocytotoxic crossmatch test using complement-dependant cytotoxicity on the day of transplant.

Among the 402 consecutive deceased-donor single-organ kidney transplant patients performed in our unit between January 1998 and June 2006, 118 patients (29.4%) were sensitized, having antibodies against class I or class II HLA on any pretransplant sera. In 83 patients (20.6%), the antidonor specificity was determined by Luminex single antigen bead technique: 60 patients had DSA Luminex class I, 58 patients DSA Luminex class II, and 35 patients DSA Luminex class I and class II. Patients with DSA had a mean of 2.4 DSA<sup>1-6</sup> detected by Luminex on the peak serum. The mean fluorescence intensity (MFI) of the highest ranked donor-specific bead ( $MFI_{max}$ ) was 6,189 (301-18,069) and the mean of total MFI of the DSA detected by Luminex single antigen bead technique was 10,724 (301-48,204).

***Long-term outcomes of kidney graft in patients with preexisting donor-specific antibodies are significantly worse as compared to patients transplanted without donor-specific antibodies***

*Patients:* The mean follow-up time was 51.4 ± 30.6 months (range 1-132). Patient eight-year survival was similar in non-sensitized

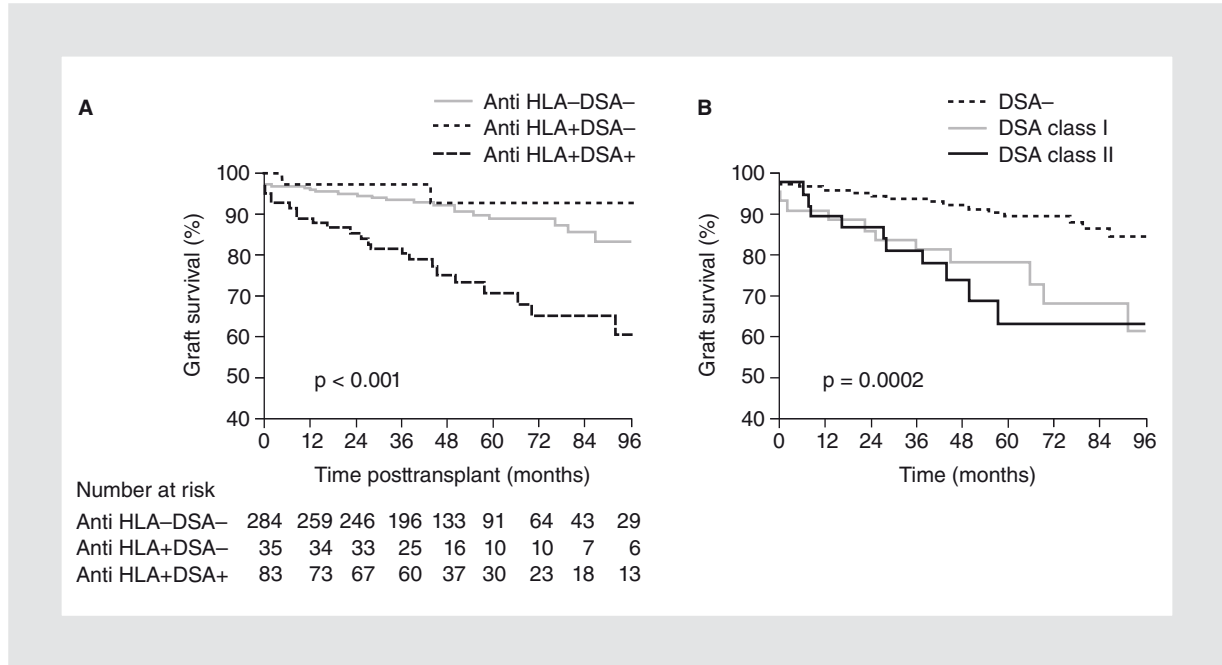
patients, sensitized patients without DSA, and patients with preformed DSA as assessed by Luminex technique: 90.2 vs. 91.2 and 90.9%, respectively ( $p = 0.98$ ).

*Grafts:* Five- and eight-year death-censored graft survival was, respectively, 89.2 and 83.6% in non-sensitized patients, 92.5 and 92.5% in sensitized patients without DSA, and finally 71.2 and 60.8% in patients with preformed DSA detected by Luminex technique. Kaplan-Meier analysis revealed that patients with preformed DSA had a significantly lower graft survival as compared to sensitized patients without DSA and non-sensitized patients ( $p < 0.001$ ) (Fig. 1A). There was no difference in graft survival analyzed according to the class of the maximum DSA identified pre-graft ( $p = 0.8$ ). Patients with DSA had poorer graft survival regardless of whether the maximum DSA was class I or II ( $p < 0.0002$ ) (Fig. 1B).

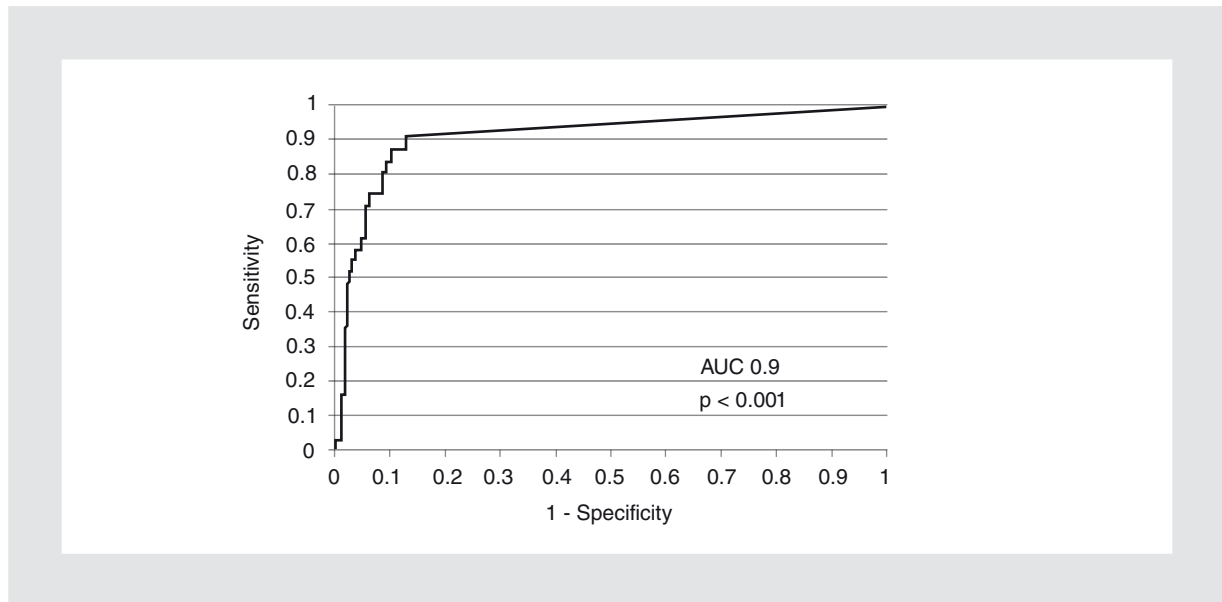
***Antibody-mediated rejection risk according to quantification of donor-specific anti-HLA antibodies by Luminex***

Acute antibody-mediated rejection occurred in 8% of kidney transplant patients. Among the 32 patients with antibody-mediated rejection, were DSA-positive by Luminex single antigen bead technique. The patients with antibody-mediated rejection had an average of three DSA detectable by Luminex single antigen bead technique on peak sera (range 0-6), with a mean  $MFI_{max}$  of 7,852 (466-17,574) and a mean total MFI of the DSA of 15,350 (823-42,472).

Receiver operating characteristic curve analysis determined that an MFI of 465 is associated with maximal specificity and sensitivity regarding the occurrence of antibody-mediated rejection (AUC 0.9;  $p < .0001$ ) (Fig. 2).



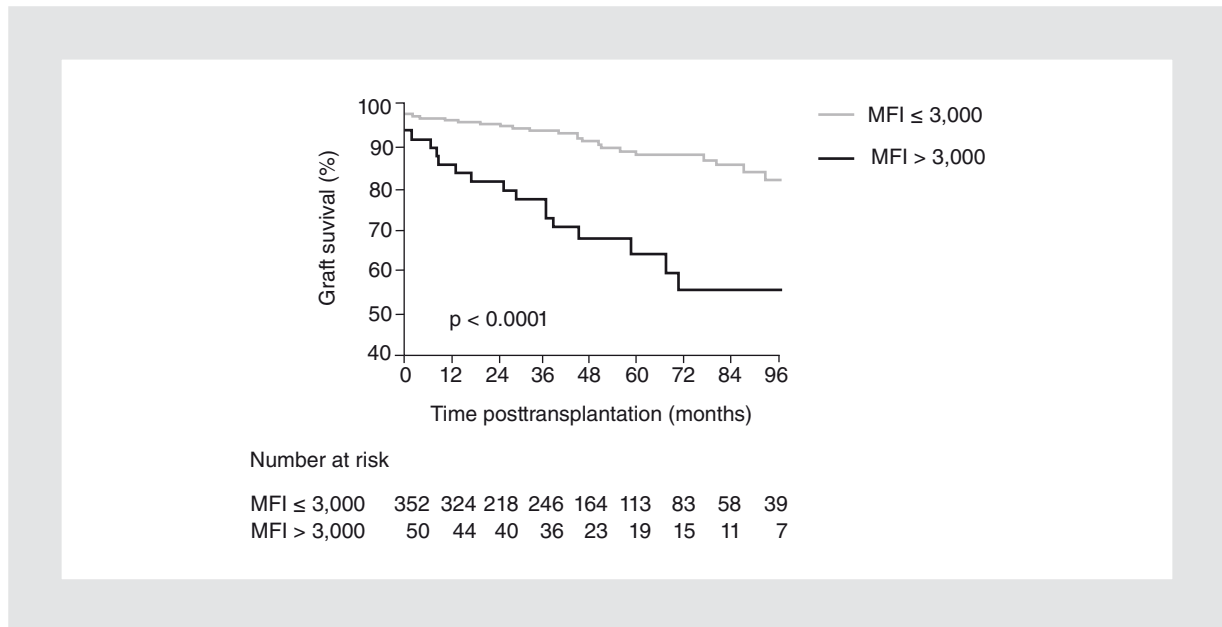
**Figure 1.** Kaplan-Meier estimates of graft survival. According to the presence of anti-HLA antibodies and donor-specific antibodies on the pre-graft serum in kidney transplant population (Panel A). According to the presence of Class I or Class II of the highest pre-graft donor-specific antibodies on the pre-graft serum (Panel B). Donor-specific anti-HLA antibodies were detected by Luminex single antigen technique. P values were calculated with the use of the log-rank test. DSA: donor-specific antibodies.



**Figure 2.** Receiver operating characteristic (ROC) curve. For the mean intensity of fluorescence of highest pre-graft donor-specific antibody detected by Luminex single antigen technique, associated with acute antibody-mediated rejection. AUC: area under the ROC curve.

The prevalence of antibody-mediated rejection rises significantly with increasing MFI of highest pre-graft DSA detected by Luminex technique on peak pre-graft serum:

0.9% in patients with MFI < 465; 18.7% in those with MFI of 466-3,000; 36.4% for MFI of 3,001-6,000; and 51.3% for patients with MFI > 6,000 ( $\text{Chi}^2 = 138.1$ ;  $p < 0.0001$ ).



**Figure 3.** Kaplan-Meier estimates of graft survival according to the mean fluorescence intensity of highest pre-graft ranked donor-specific antibodies detected by Luminex single antigen technique in the entire cohort of kidney transplant patients. DSA: donor-specific antibodies; MFI: mean fluorescence intensity.

### **Graft survival according to quantification of donor-specific anti-HLA antibodies by Luminex**

The eight-year graft survival decreases progressively with rising Luminex MFI: 82.5% in patients with MFI < 465; 78.4% for patients with MFI of 466-3,000; 60.6% for those with MFI of 3,001-6,000; and 55.9 ± 9.6% for patients with MFI > 6,000 ( $p < 0.001$ ). As shown in figure 3, the graft survival in patients with MFI > 3,000 was significantly lower than that of patients with MFI ≤ 3,000 ( $p < 0.0001$ ). The relative risk of graft loss for patients transplanted with pre-graft DSA higher than 3,000 was 3.8 (95% CI: 3.5-18.4;  $p < 0.0001$ ) as compared to with MFI DSA lower than 3,000.

### **Estimation of the immunological risk prior to transplantation in clinical practice**

Our study focuses on the estimation of the immunological risk of a given patient based

on the antibody profile as defined prior to transplant by Luminex analysis. It assesses for the first time, using the recent immunological techniques, kidney graft survival and the gradation of the risk of acute antibody-mediated rejection according to the levels of DSA detected before transplantation. This analysis has the advantage of being performed much earlier than any crossmatch assay and helps define a transplant strategy for any patient on the waiting list. Based on this strategy, the clinician may then decide to go on with the crossmatch or to stop the process before any crossmatching. So, the utilization of the Luminex single antigen bead technique has expanded the role of histocompatibility testing beyond the traditional one, identifying the contraindications to transplantation, to a personalized appraisal of immunological risk. In the present environment of organ scarcity and financial and logistical constraints, the evaluation before transplantation of the immunological profiles of patients on the waiting lists helps to guide deceased-donor kidney allocation<sup>13</sup>. Stratification of immunological risk before transplantation should be used not only for deceased-kidney allocation,

but also to establish priority programs with the aim of increasing graft access by highly sensitized patients and also to guide the immunosuppressive therapy and monitoring of recipients. Thus, the transplantation strategy for each patient should weigh the risk/benefit ratio based on defined immunological risk prior to transplant.

In recent years, a major change in renal transplantation has come from the recognition of the importance of antibody-mediated rejection, recognized initially only as the cause of hyperacute rejection, but now known to be responsible for acute and chronic lesions<sup>14</sup>. Our study underlines the fact that acute antibody-mediated rejection is a major factor in the evolution of HLA-incompatible kidney transplants and is associated with higher rates of graft loss. For treatment of acute antibody-mediated rejection, we used a specific treatment based on intravenous immune globulin products known to have powerful immunomodulatory effects<sup>15</sup>. Treatment of acute antibody-mediated rejection has evolved from intravenous immune globulin-based regimens to combination therapies, with plasmapheresis, intravenous immune globulin, and rituximab, leading to an amelioration of the graft survival of patients with acute antibody-mediated rejection<sup>16-19</sup>.

Importantly, our study shows that even in the absence of clinical acute antibody-mediated rejection, the long-term graft course is worse in patients with preexisting DSA. The recently described entity of subclinical antibody-mediated rejection<sup>20,21</sup>, in which progressive morphologic lesions are found on biopsy in the absence of overt clinical rejection, may account for this different course. These progressive lesions lead to chronic humoral rejection, first described in 2001<sup>22</sup> and now recognized to be a distinct cause of late graft dysfunction and loss<sup>23,24</sup>.

Luminex analysis permits pre-graft characterization of the antibody profiles in sensitized

patients and gives improved definition of safe (antibody-negative) and at-risk (antibody-positive) HLA specificities. The first step in the transplant strategy for sensitized patients is to define whether a graft with minimal immunological risk is possible. Whenever possible, kidney transplantation should be performed in the absence of DSA. Virtual cross-matching, recently promoted by the United Network for Organ Sharing<sup>25</sup>, consists in selecting potential donors without HLA-specificities against receiver's antibodies, predicting a negative crossmatch. The use of the virtual crossmatch permits expanding the geographic regions from which kidneys can be drawn and reduces waiting time and deaths on the waiting list<sup>26-28</sup>. Furthermore, it is a good indicator to reduce the risk of antibody-mediated rejection<sup>29</sup>.

In practice, we must evaluate, for each sensitized patient on the waiting list, if the listing of forbidden antigens permits a donor pool sufficient to assure transplantation. For some highly sensitized patients, use of virtual crossmatching leads to an insufficient number of donors. Thus, alternatively, the access to transplantation of these patients can be augmented in three ways: priority programs, desensitization regimens, or by increased immunological risk of transplantation with preexisting DSA.

Priority programs, such as the Eurotransplant "Acceptable Mismatch" program<sup>30</sup>, gives priority to any hypersensitized patient when there is no incompatibility between the class I antigens of the donor and those of the recipient, including permitted antigens, and no more than one donor/recipient incompatibility. This program has shown that the probability for a hyperimmunized patient to be grafted with a kidney with a negative crossmatch rises from 17 to 58% over two years<sup>31</sup>. Class, et al.<sup>30</sup> have published graft survivals of such patients identical to that of non-immunized patients and superior to that of immunized patients grafted without the aid of the permitted antigen.

Desensitization protocols based on high-dose intravenous immunoglobulins (IVIg)<sup>32-34</sup> have been used with success. The desensitization protocol used by our group is based on high-dose IVIg, given as three monthly treatments of IVIg 2 g/kg given over two days<sup>32</sup>. Other possibilities, including plasmapheresis/low-dose IVIg/anti-CD20 antibody<sup>35</sup> or the combination IVIg/rituximab, can be efficacious in desensitizing patients awaiting transplantation<sup>36</sup>.

Transplantation in the presence of DSA requires a careful estimation of the immunological risk, as assessed by remote crossmatches and levels of antibodies detected by Luminex technique. It will then be up to the transplant teams to decide, well before transplantation, if these patients are to be grafted with historical DSA, and up to what level, or possibly with remote positive crossmatch. For such patients at high immunological risk, specific posttransplant protocols are used<sup>10,37-40</sup>. In our experience, patients transplanted with a remote positive crossmatch received, post-transplant, in addition to the standard induction with thymoglobulin, three monthly rounds of high-dose IVIg. Nonetheless, the data in the literature are, for the present, strictly observational. Little data is available on the latest advances in immunosuppressive drugs. The use of rituximab in association with IVIg as prophylaxis against acute rejection in positive-crossmatch transplants has been disappointing so far<sup>41</sup>. For such transplants at high immunologic risk, close follow-up is indispensable, notably with systematic quantification of anti-HLA antibodies posttransplantation and protocol biopsies including C4d staining.

Access to and results of transplantation can therefore be improved in sensitized patients. The data presented in this paper emphasize the importance of precisely characterizing the status of the anti-HLA pretransplant immunization using sensitive techniques. This should allow optimization of donor immunological selection.

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