Article for Human Immunology

HLA antibody detection and kidney allocation within Eurotransplant

Auteurs:
Frans H.J. Claas & Ilias I.N. Doxiadis

Eurotransplant Reference Laboratory
Leiden University Medical Center. Department Immunohaematology and Blood Transfusion, Leiden, the Netherlands

Correspondence:
Prof.dr. F.H.J. Claas
Dept.of Immunohaematology and Blood Transfusion
Leiden University Medical Center
Albinusdreef 2, building 1
L1-105, portocabins
P.O.Box 9600
2300 RC/ Leiden
t. + 31.71.5263802
f. + 31.71.5265267
Summary

The HLA antibody status of a patient on the waiting list is one of the parameters for the allocation of donor kidneys within Eurotransplant. The introduction of more sensitive solid phase assays in addition to the current “golden standard”, complement dependent cytotoxicity (CDC), will have obvious impact on the allocation provided that their results will get the same value. However, in contrast to a positive CDC crossmatch due to donor HLA specific antibodies, which is considered a contraindication for transplantation, antibodies detected in the solid phase are rather a risk factor than a contraindication. Furthermore, the higher sensitivity of especially Luminex based screening assays will lead to a significantly higher number of highly sensitized patients and may affect the exclusivity of the acceptable mismatch program, which was initiated to give priority to highly sensitized patients. More data on the clinical relevance of antibodies detected by solid phase assays are necessary before their relevance for organ allocation can be established.
Introduction

In 1967 Eurotransplant started as an international kidney allocation organization aiming at optimal donor selection based on HLA matching (1). In the early days both HLA typing and antibody screening were performed by leukocyte agglutination but soon this assay was replaced by complement dependent cytotoxicity (2). Since it became clear that the presence of donor specific HLA antibodies before transplantation was associated with a high incidence of hyperacute rejection (3), such antibodies were considered a contraindication for transplantation. The policy became that the sera from the patients on the wait list were regularly screened for presence and specificity of the HLA antibodies. Foreign HLA antigens towards which the patients had formed antibodies were registered as non-acceptable HLA antigens in order to prevent the allocation of kidneys with these HLA antigens were allocated to a patient with specific HLA antibodies to the donor. For this reason the potential donor pool for sensitized patients is lower then for non-sensitized patients. Furthermore, it became clear that kidney allocation only on basis of HLA matching was not fair for patients with rare HLA phenotypes. These patients were waiting for a very long time without receiving any proper donor offer. For this reason the new Eurotransplant kidney allocation program was introduced in 1996 (4). For every donor kidney which becomes available within the Eurotransplant area, all patients on the waiting list receive points on basis of several parameters such as the degree of HLA match with the donor, waiting time, distance to the donor center and match prognostic index. The latter implies extra points for patients with rare HLA phenotypes and for sensitized patients. The higher the degree of sensitization the more points a patient will receive. In the past the degree of sensitization was based on the percentage panel reactive antibodies (% PRA), which was determined by screening the serum of the patient against a panel of leukocytes donors in complement dependent cytotoxicity. The percentage PRA reflects the percentage of panel donors, which showed positive reactions with the patients’ serum. However, during external proficiency testing it became clear that the percentage PRA is a very unreliable marker (5). Due to the
variable composition of the panels used, the percentage PRA of the same serum may vary between 5 and 80%. In contrast, the antibody specificities reported by the different laboratories participating in the external proficiency showed less variation. Therefore, a virtual PRA was introduced which is based on the specificities of the antibodies detected in the serum of a patient in relation to the frequency of the target antigens in the organ donor population. In order to obtain a uniform and reliable PRA value within Eurotransplant, a tool to calculate the virtual PRA is made available on the Eurotransplant website (www.eurotransplant.eu). After introducing the antibody specificities detected in the serum of the patient, the program calculate the chance that a patient will have a positive crossmatch with the kidney donor population of Eurotransplant (figure 1).

**Antibody detection.**
For a long period of time, all antibody screening and crossmatching in the tissue typing laboratories affiliated to Eurotransplant was performed with complement dependent cytotoxicity only. Actually, even nowadays complement dependent cytotoxicity is the standard crossmatch assay within Eurotransplant as CDC is considered a reliable tool to avoid hyperacute rejection. However, alternative solid phase based antibody screening assays like ELISA, Flow-PRA and Luminex (6) have been introduced in many tissue typing laboratories affiliated to Eurotransplant and several of these centers use these assays, in addition to CDC, to determine antibody specificities in the sera of their patients on the waiting list. The reason for this policy is that these new assays, which also detect non-complement fixing antibodies, are more sensitive than CDC. However, it remains to be established whether the antibodies detected in these assays are clinically relevant, which is the reason why no recommendations have been established yet with regard to the use of the results of these alternative antibody screening assays. However, proficiency testing in Eurotransplant clearly showed that the determination of HLA antibodies specificities in these new assays, and especially in Luminex, is not very reliable as shown by the results reported by the different laboratories. Many antibody specificities are reported by individual centers which cannot be confirmed by other centers. One of the main problems with Luminex-based screening assays is the lack of a clear discrimination between positive and negative reactions. Often a gradual decrease in antibody reactivity (either reflected in mean fluorescence intensity, MFI, or in numbers of channel shift) is observed. As long as there is not an evidence based consensus on
the assignment of a positive and negative reaction, the results of Luminex screening assays should not be interpreted in the same way as the results of a CDC screening but, at the moment, it is impossible to force the different tissue typing centers to have a uniform policy on the use and interpretation of these assays.

The acceptable mismatch program.

Although the clinical relevance of these new antibody screening assays is not clear, the transplant centers within Eurotransplant are free to introduce the antigens recognized as non-acceptable mismatches for their sensitized patients in the Eurotransplant computer system. This will have an impact on the match prognostic index, one of the parameters in the kidney allocation algorithm, and, as a consequence also a minor effect on the kidney allocation within Eurotransplant.

However, antibody specificities only detected by Luminex are not taken into consideration for the special program which Eurotransplant has introduced for highly sensitized patients, the so called acceptable mismatch program (7). This program was initiated to increase the chance of finding a crossmatch negative donor kidney for long waiting highly sensitized patients with HLA specific antibodies detectable in CDC and reactive with more than 85% percent of the panel. In contrast to the non-cytotoxic antibodies, detected in ELISA, Flow and Luminex, which should be considered risk factors for transplantation, antibodies detectable in CDC are considered a clear contraindication for transplantation (8). The aim of the acceptable mismatch program is to define those HLA antigens toward which the patient did not make these detrimental antibodies and use this knowledge for the allocation of donor organs to highly sensitized patients (9). Patients included in this program have the highest priority in the Eurotransplant allocation program. Any donor kidney, which is compatible with the combination of the patients’ HLA antigens and one or more acceptable mismatches, is mandatory allocated to this patient. So far only patients who are highly sensitized on basis of the results of the CDC antibody screening are considered eligible for the acceptable mismatch program. Patients with Luminex antibodies only are excluded. One of the reasons for this policy is that antibodies detectable in Luminex only are not considered to be a contraindication for transplantation (10). This was confirmed by a study of Van den Berg-Loonen et al. (11), who showed in a retrospective study that in patients transplanted in the acceptable mismatch program on basis of a negative CDC crossmatch the presence of donor specific antibodies detectable in Luminex was not
associated with a poorer graft survival compared to patients without donor specific antibodies in Luminex. The presence of donor specific antibodies in Luminex was associated with a higher incidence of acute rejection in the first six months but this did not affect graft survival at the long term. This observation was made in a small patient cohort and certainly needs confirmation in a larger study group. However, also a pilot study performed by our center showed that the clinical relevance of these Luminex antibodies is not clear (table 1). Furthermore, the recent observation that Luminex assays can detect HLA antibodies in sera of non-sensitized males is another reason to be careful with the interpretation of the results of these antibody screening assays (12).

The targets for these "natural" HLA antibodies are probably denatured HLA antigens attached to the Luminex beads. This finding questions the specificity and relevance of antibodies detected in solid phase assays, where isolated HLA molecules are used as targets for antibody reactivity. It remains to be established to what extent these isolated molecules reflect the antigenicity of the HLA molecules present on the cells in the transplanted organ. Actually, this was one of the reasons why we have developed a panel of single HLA antigen expressing cell lines for the detection of HLA antibody specificities (13). These cell-bound HLA antigens have the same conformation as the HLA antigens expressed on the surface of cells, which is probably not always the case for the isolated HLA molecules used in the newly developed HLA antibody detection assays. The results of antibody screening against these single HLA antigen expressing cell lines is probably more relevant than the results of antibody screening against a panel of single HLA antigen expressing beads. Another reason why Luminex antibodies are excluded from the acceptable mismatch program is the fact that inclusion of Luminex based antibody specificities would result in an enormous increase of the number of highly sensitized patients as external proficiency testing showed that many more HLA specificities are found in Luminex assays compared to CDC. The Eurotransplant community has accepted that highly sensitized patients included in the acceptable mismatch program get the highest priority in the Eurotransplant allocation program and implementation of all Luminex based HLA antibody specificities would significantly affect the exclusivity of the program (table 2).

Future developments.
It is clear that HLA antibody detection assays like ELISA, Flow and Luminex are more sensitive than the standard complement dependent cytotoxicity assay. However, the
clinical relevance of the antibodies detected is not clear. A positive CDC crossmatch must be considered a contra-indication for transplantation whereas donor specific antibodies detected exclusively in the other assays should rather be considered a risk factor (8) Further studies are necessary to clarify the actual relevance of donor specific antibodies detected in for instance Luminex only. First of all, a general agreement should be reached on the assignment of positive and negative antibody reactivity in Luminex. A next step should be a multicenter study to define whether antibody titer, immunoglobulin subclass (14) or the capacity to fix complement (15) are parameters on basis of which one may finally decide which antibodies detectable in Luminex are clinically relevant. Only if it becomes clear that a well defined subpopulation of Luminex antibodies have a similar clinical relevance as antibodies detected in CDC, these antibody specificities will get the same value and relevance in the acceptable mismatch program.

Acknowledgements
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References
2. Terasaki PI, McClelland JD Microdroplet assay of human serum cytotoxins Nature 1964; 204: 998


Legend to figure 1.

Web based tool to calculate virtual PRA on basis of HLA antibody specificities.
Table 1.

Variable clinical effect associated with the presence of donor specific HLA antibodies in pretransplant sera as detected by Luminex single antigen beads.

<table>
<thead>
<tr>
<th>Patient</th>
<th>specificity DSA</th>
<th>Graft survival</th>
<th>Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HLA-A2</td>
<td>7 days</td>
<td>yes</td>
</tr>
<tr>
<td>2</td>
<td>HLA-A2</td>
<td>&gt; 6 years</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>HLA-B8</td>
<td>&gt; 8 years</td>
<td>no</td>
</tr>
<tr>
<td>4</td>
<td>HLA-A24</td>
<td>3 years</td>
<td>yes</td>
</tr>
<tr>
<td>5</td>
<td>HLA-DR11</td>
<td>&gt; 4 years</td>
<td>no</td>
</tr>
<tr>
<td>6</td>
<td>HLA-DR4</td>
<td>12 years</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>HLA-DR4</td>
<td>3 years</td>
<td>yes</td>
</tr>
</tbody>
</table>

Note: all patients were highly sensitized and have been transplanted on basis of a negative CDC crossmatch.
Table 2

Impact of virtual PRA and Luminex based screening on sensitization grade.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Antibody specificity</th>
<th>% PRA</th>
<th>v-PRA</th>
<th>v-PRA (luminex)</th>
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<tbody>
<tr>
<td>1</td>
<td>A23</td>
<td>13</td>
<td>4</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>A11, A25, A33, A68</td>
<td>49</td>
<td>20</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>B18, B38, B39</td>
<td>37</td>
<td>18</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>B27</td>
<td>9</td>
<td>8</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>B27</td>
<td>25</td>
<td>8</td>
<td>96</td>
</tr>
<tr>
<td>6</td>
<td>A68</td>
<td>22</td>
<td>5</td>
<td>96</td>
</tr>
</tbody>
</table>

Results are data from the external proficiency testing program of Eurotransplant in 2008.
Web based tool to calculate virtual PRA based on HLA antibody specificities