Kabul Edilmeyen HLA Antijen Uyumsuzlukları

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Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation


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**TTS Consensus Guidelines 2013**

Recommendations of the Pre-Tx Group

a) Determination of ‘unacceptable HLA antigen mismatches’ should be a part of the kidney allocation algorithms. [2]

b) Accurate XM prediction depends on complete HLA typing. To minimize the incidence of unexpected positive XM in paired exchange registries, the donor should be typed at HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3, HLADRB4, HLA-DRB5, HLA-DQA, HLA-DQB, HLA-DPA, and HLA-DPB loci. [2]

c) A renal Tx can be performed without a prospective pre-Tx CDC or flow XM if SAB testing indicates the consistent absence of DSA

d) Risk assessment should include HLA antibody specificities identified in historic sera. [3]

e) If DSA is present but the CDC XM against donor T and B cells is negative, this should be regarded as an increased immunologic risk but not necessarily a contraindication to Tx, especially after elimination of DSA by desensitization. [2]

f) To optimize access to transplantation of highly sensitized recipients, inclusion of patients in special programs, such as kidney paired donation, AM, or desensitization, should be considered. [1]

g) HLA matching should be part of the allocation procedures to reduce the probability of developing HLA antibody, rejection, and graft loss. [2]

h) ABO incompatibility is no longer an absolute contraindication in kidney Tx and ABO-incompatible transplants can be successfully performed in recipients in whom isoagglutinin titers have been lowered to acceptable levels. [1]

i) Based on current evidence, no recommendation can be made for routine pre-Tx testing for non-HLA antibodies other than ABO. [2]
Determination of ‘Unacceptables’

Reasons

1. Calculation of cPRA/vPRA: more points for sensitized patients
ET Kidney Allocation System

Point score system

- HLA-A, B, DR Matching: 400
- Mismatch Probability: 100
- Waiting time (per day): 0.09
- Distance Factor: 300
- National Balance: no max
Determination of ‘Unacceptables’

Reasons

1. Calculation of cPRA/vPRA: more points for sensitized patients
2. Virtual Crossmatch
With increasing numbers of “unacceptables”, the patient’s chance to receive an organ offer diminishes.

In HI patients, identification of “many unacceptables” often results in extremely prolonged waiting times.

Conversely, unrecognized “unacceptables” lead to futile organ shipments or, if the crossmatch result is borderline positive, to impaired graft outcome or failure.
Currently Used Algorithms for the Determination of Unacceptable HLA Antigen Mismatches in Kidney Transplant Recipients


Tissue Antigens 2013

1. Leiden
2. Vienna
3. São Paulo
4. Basel
5. Berlin
6. Barcelona
Reasons for the Variation

1. Variation in antibody detection
2. Technical issues (SA testing)
3. Center-specific variations
   - definition of risk
   - apheresis
   - induction
   - immunosuppression
Pretransplant Immunologic Risk Assessment of Kidney Transplant Recipients With Donor-Specific Anti–Human Leukocyte Antigen Antibodies

Kwaku Marfo,1,2 Maria Ajaimy,1,3 Adriana Colovai,1,4 Liise Kayler,1,5 Stuart Greenstein,1,5 Michelle Lubetzky,1,3 Anjali Gupta,1,3 Layla Kamal,1,3 Graciela de Boccardo,1,3 Peter Masiakos,1,4 Milan Kinkhabwala,1,5 and Enver Akalin1,3,6

TABLE 2. Clinical outcomes of DSA+ versus DSA− kidney transplant recipients

<table>
<thead>
<tr>
<th></th>
<th>DSA+ (n=66)</th>
<th>DSA− (n=307)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient survival</td>
<td>99%</td>
<td>95%</td>
<td>0.32</td>
</tr>
<tr>
<td>Graft survival</td>
<td>88%</td>
<td>90%</td>
<td>0.66</td>
</tr>
<tr>
<td>1-year patient survival</td>
<td>98%</td>
<td>96%</td>
<td>0.70</td>
</tr>
<tr>
<td>1-year graft survival</td>
<td>93%</td>
<td>96%</td>
<td>1.0</td>
</tr>
<tr>
<td>6-month graft survival</td>
<td>94%</td>
<td>96%</td>
<td>0.57</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>11%</td>
<td>12%</td>
<td>0.84</td>
</tr>
<tr>
<td>Cellular rejection</td>
<td>6%</td>
<td>9%</td>
<td>0.63</td>
</tr>
<tr>
<td>Antibody-mediated rejection</td>
<td>5%</td>
<td>1%</td>
<td>0.07</td>
</tr>
<tr>
<td>Mixed rejection</td>
<td>—</td>
<td>2%</td>
<td>0.60</td>
</tr>
<tr>
<td>Acute rejection within 6 months</td>
<td>8%</td>
<td>6%</td>
<td>0.49</td>
</tr>
<tr>
<td>Acute rejection within first year</td>
<td>9%</td>
<td>8%</td>
<td>0.80</td>
</tr>
<tr>
<td>Chronic antibody-mediated rejection</td>
<td>3%</td>
<td>2%</td>
<td>0.35</td>
</tr>
<tr>
<td>Delayed graft function</td>
<td>44%</td>
<td>38%</td>
<td>0.49</td>
</tr>
<tr>
<td>Last mean creatinine, mg/dL</td>
<td>1.4±0.6</td>
<td>1.4±0.6</td>
<td>1.00</td>
</tr>
<tr>
<td>Mean spot urine protein/creatinine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.5 g/day</td>
<td>86%</td>
<td>89%</td>
<td>1.0</td>
</tr>
<tr>
<td>0.5–1.0 g/day</td>
<td>5%</td>
<td>5%</td>
<td>1.0</td>
</tr>
<tr>
<td>&gt;1.0 g/day</td>
<td>9%</td>
<td>6%</td>
<td>0.4</td>
</tr>
<tr>
<td>BKV-viremia</td>
<td>14%</td>
<td>15%</td>
<td>0.85</td>
</tr>
<tr>
<td>CMV-viremia</td>
<td>2%</td>
<td>9%</td>
<td>0.06</td>
</tr>
<tr>
<td>Median follow-up, mo</td>
<td>20 (6–50)</td>
<td>26 (6–50)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

DSA, donor-specific anti–human leukocyte antigen antibodies; BKV, BK virus; CMV, cytomegalovirus.

No difference in outcome between patients with and without pre-Tx DSA

- Tx, if CDC and flow T- and B-cell XM were negative
- HLA Ab specificities with >5,000 MFI for HLA-A, -B, -DR and >10,000 MFI for HLA-DQB were reported as “unacceptables”
- If DSA, induction with ATG and Ivlg
Heidelberg Algorithm for Transplantation of High-Risk Sensitized Patients

1. Pre-Tx identification of high-risk patients
2. Good HLA-match
3. ET Acceptable Mismatch Program
4. Pretreatment with PPh/IA + Rituximab + ATG
5. Infection prophylaxis
6. Apheresis after Tx
7. Post-Tx antibody monitoring
8. Protocol biopsies on post-Tx days 7 and 90
9. Desensitization in positive-XM living-Tx
Presensitized Kidney Transplant Recipients (ELISA)

Heidelberg - Before 2007

Heidelberg - After 2007

Graft survival (%)

Time post-transplant (years)

Ab- n=1,001
Ab+ n=231

P < 0.001

P = 0.97

Ab+ n=90
Ab- n=735

CTS Serum Study (www.ctstransplant.org)
Variation in Ab Detection
Heidelberg Kidney Waiting List
n=569

CDC (T cell) positive: 7% n = 37
ELISA positive: 15% n = 86
Lum-SA positive (≥1,000 MFI): 82% n = 467
Luminex Single Antigen Bead Assay

Non-Alloimmunized Patients

Heidelberg Kidney Waiting List

79%

MFI ≥1,000

0%

0%

Percentage of Patients

Luminex SA  ELISA Screening  CDC
<table>
<thead>
<tr>
<th>HLA Specificities</th>
<th>Median MFI (Range)</th>
<th>Frequency in Patients (%)</th>
<th>Frequency in general population (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*24:02</td>
<td>4193 (1336-12197)</td>
<td>17.8</td>
<td>8.7</td>
</tr>
<tr>
<td>A*24:03</td>
<td>4043 (1010-11025)</td>
<td>15.6</td>
<td>0.1</td>
</tr>
<tr>
<td>A*23:01</td>
<td>6730 (3595-7108)</td>
<td>11.1</td>
<td>1.7</td>
</tr>
<tr>
<td>A*31:01</td>
<td>1967 (1036-3802)</td>
<td>11.1</td>
<td>2.4</td>
</tr>
<tr>
<td>A*25:01</td>
<td>2411 (1554-5004)</td>
<td>8.9</td>
<td>1.9</td>
</tr>
<tr>
<td>A*30:01</td>
<td>1725 (1253-3561)</td>
<td>8.9</td>
<td>1.3</td>
</tr>
<tr>
<td>A*30:02</td>
<td>1827 (1183-2783)</td>
<td>6.7</td>
<td>0.9</td>
</tr>
<tr>
<td>A*32:01</td>
<td>3887 (3312-7329)</td>
<td>6.7</td>
<td>3.1</td>
</tr>
<tr>
<td>A*34:01</td>
<td>1477 (1398-1509)</td>
<td>6.7</td>
<td>0.0</td>
</tr>
<tr>
<td>B*15:12 (B76)</td>
<td>1991 (1001-5505)</td>
<td>20.0</td>
<td>0.0</td>
</tr>
<tr>
<td>B*37:01</td>
<td>1599 (1026-5130)</td>
<td>17.8</td>
<td>1.3</td>
</tr>
<tr>
<td>B*08:01</td>
<td>2466 (1003-9862)</td>
<td>11.1</td>
<td>12.5</td>
</tr>
<tr>
<td>B*15:11 (B75)</td>
<td>5836 (1503-9723)</td>
<td>8.9</td>
<td>0.0</td>
</tr>
<tr>
<td>B*44:02</td>
<td>3100 (1654-8542)</td>
<td>6.7</td>
<td>9.0</td>
</tr>
<tr>
<td>B*45:01</td>
<td>1723 (1012-5452)</td>
<td>6.7</td>
<td>0.4</td>
</tr>
<tr>
<td>B*46:01</td>
<td>2225 (1102-12232)</td>
<td>6.7</td>
<td>0.0</td>
</tr>
<tr>
<td>B*49:01</td>
<td>7354 (1091-7569)</td>
<td>6.7</td>
<td>1.3</td>
</tr>
<tr>
<td>B*51:01</td>
<td>3606 (1643-4137)</td>
<td>6.7</td>
<td>4.5</td>
</tr>
<tr>
<td>B*59:01</td>
<td>4613 (2364-5882)</td>
<td>6.7</td>
<td>0.0</td>
</tr>
<tr>
<td>B*15:16 (B63)</td>
<td>2586 (1067-4852)</td>
<td>6.7</td>
<td>0.0</td>
</tr>
<tr>
<td>C*05:01</td>
<td>1340 (1005-1690)</td>
<td>11.1</td>
<td>9.1</td>
</tr>
<tr>
<td>C*17:01</td>
<td>2065 (1573-8586)</td>
<td>11.1</td>
<td>0.7</td>
</tr>
<tr>
<td>C*01:02</td>
<td>1482 (1217-1975)</td>
<td>6.7</td>
<td>2.9</td>
</tr>
<tr>
<td>C*03:03 (Cw9)</td>
<td>1679 (1268-2516)</td>
<td>6.7</td>
<td>5.5</td>
</tr>
<tr>
<td>DRB1*12:01</td>
<td>1532 (1183-2159)</td>
<td>6.7</td>
<td>1.5</td>
</tr>
<tr>
<td>DRB1*16:02</td>
<td>1169 (1114-1240)</td>
<td>6.7</td>
<td>0.1</td>
</tr>
<tr>
<td>DQA1<em>01:03/DQB1</em>06:03</td>
<td>1224 (1025-3175)</td>
<td>8.9</td>
<td>6.5</td>
</tr>
<tr>
<td>DQA1<em>03:02/DQB1</em>03:03</td>
<td>3548 (1117-10124)</td>
<td>8.9</td>
<td>4.5</td>
</tr>
<tr>
<td>DQA1<em>01:02/DQB1</em>06:04</td>
<td>1450 (1363-2742)</td>
<td>6.7</td>
<td>3.2</td>
</tr>
<tr>
<td>DQA1<em>02:01/DQB1</em>03:01</td>
<td>2201 (1295-3641)</td>
<td>6.7</td>
<td>18.5</td>
</tr>
<tr>
<td>DQA1<em>03:02/DQB1</em>03:02</td>
<td>2810 (2126-10081)</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>DQA1<em>02:01/DQB1</em>03:03</td>
<td>3957 (1486-8004)</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>DPA1<em>01:04/DPB1</em>11:01</td>
<td>1811 (1679-2607)</td>
<td>8.9</td>
<td></td>
</tr>
</tbody>
</table>

Gombos, …, Süsal
AJT 2012
## TABLE 4. Technological advantages and limitations of Luminex HLA SAB

<table>
<thead>
<tr>
<th>Technological advantages</th>
<th>Technological limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative: enables precise identification of all antibody specificities in complex sera (DSA)</td>
<td>Some positive results can be caused by antibodies to denatured HLA.</td>
</tr>
<tr>
<td>Comprehensive: distinguishes antibodies to all common alleles for HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3/4/5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1</td>
<td>Occasional high background binding requiring repeat testing and absorption protocols.</td>
</tr>
<tr>
<td>Semiquantitative: enables determination of antibody levels (high, intermediate, and low)</td>
<td>Variable HLA protein density on beads. Blocking factors may cause false-negative or misleading low assessment of antibody levels (prozone?). IgM and C1 can block IgG binding.</td>
</tr>
<tr>
<td>Sensitive: enables detection of weak antibody levels</td>
<td>Lot-to-lot variation requiring validation. Vendor-specific variation.</td>
</tr>
<tr>
<td>Rapid: enables real-time antibody monitoring for DSA, HLAi transplantation. Pretransplantation and posttransplantation antibody monitoring (assist diagnosis of AMR). Virtual XM</td>
<td></td>
</tr>
<tr>
<td>Enables detection of non-HLA–specific antibodies (e.g., MICA)</td>
<td>Reagents not standardized</td>
</tr>
<tr>
<td>Detection and differentiation between immunoglobulin class and isotype (e.g., complement fixing and noncomplement fixing C4d and C1q)</td>
<td></td>
</tr>
</tbody>
</table>

AMR, antibody-mediated rejection; DSA, donor-specific HLA antibodies; HLAi, HLA incompatible; MICA, major histocompatibility complex class I–related chain A; SAB, single-antigen beads; XM, crossmatch.
Determination of “Unacceptable HLA Antigens”

- CDC-PRA (B-cell plates)
- ELISA-PRA
- Luminex Single Antigen
- Additional Abs are (dependent on immunological history) risk factors
Pre-Tx Luminex SA Testing
CDC- and ELISA-*negative* Kidney Tx Recipients
cut-off 1,000 MFI

Süsal et al. Transplantation 2011
Impact of SAB-Detected Pre-Tx DSA CDC- or ELISA-\textit{positive} Kidney Tx Recipients 
cut-off 1,000 MFI

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Graft survival over time post-transplant (years) for different DSA status groups.}
\end{figure}

\textbf{DSA I- II+} n= 37
\textbf{DSA I- II-} n=268
\textbf{DSA I+ II-} n = 73
\textbf{DSA I+ II+} n = 35

\textit{Süsal et al. unpublished}
Equity in Access to Transplantation
1. Apply only to sensitized patients with <85% PRA
2. All highly immunized patients who fulfill the criteria of the ET ‘Acceptable Mismatch’ (AM) Program should be reported to ET for acceptance to the AM Program
3. UAM must be determined at active listing of the patient on the waiting list and must be checked and actualized regularly, at least once a year and after every immunizing event
4. Sera of all patients should be tested using CDC and solid phase assays
5. Immunization history (questionnaire)
6. SA method should be used at least at the time of active listing
7. Technical issues associated with SA testing should be considered
8. If the quarterly performed screening tests indicate significant changes in the profile or strength of antibodies, SA test should be reapplied
9. Alternatively, yearly application of the SA test could be utilized in patients with an immunization history in their anamnesis if the center is practicing desensitization in patients with weaker DSA that were not defined as UAM
10. For the determination of UAM, B-cell specific cytotoxic techniques should also be utilized under the usage of DTT (B-CDC&B-CDC-DTT)

11. All HLA class I and class II antibody specificities that were identified in the CDC testing are a contraindication to transplantation and have to be registered in the ET Allocation-Software as UAM.

12. In high risk patients all antibody specificities that give positive reactions above 3,000 MFI should be defined as UAM
   a. retransplant recipients
   b. recipients with class I and class II antibodies in CDC or solid phase screening assays, such as bead-PRA, flow-PRA or ELISA

13. In immunized patients with a less pronounced risk for immunological graft loss, all antibody specificities that give positive reactions above 5,000 MFI should be defined as UAM
   a. first transplant recipients with only HLA class I or only class II antibodies in solid phase assays, such as bead-PRA, flow-PRA or ELISA
Expected Problems

1. Prolonged waiting time in sensitized patients (instead of a maximum of 100 points, linearly increasing score points are required)
Reduction of Donor Frequency with Different UAM Determination Techniques

Waiting List Patients with 50-85% current vPRA (n=10)

- Without UAM: 62%
- CDC: 56%
- CDC + ELISA PRA: 29%
- vPRA: 31%
- Luminex SA Cutoff 1000 MFI: 10%
- Luminex SA Cutoff 3000 MFI: 19%
- Luminex SA Cutoff 5000 MFI: 25%
Expected Problems

1. Prolonged waiting time in sensitized patients (instead of a maximum of 100 points, linearly increasing score points are required)

2. Scientific basis is not thoroughly explored
   1. No clinical test which detects all and only clinically relevant antibodies
   2. Many patients lose their pre-Tx DSA after Tx, even if they are C1q binding
Thank you very much for your attention!

 EFI 2017
 in Mannheim/Heidelberg
Pre-Tx Luminex SA Testing
CDC- and ELISA-Negative Kidney Tx Recipients
cut-off 1,000 MFI

Süsal et al. Transplantation 2011