HLA Antibody Detection
And Donor Specific Antibody Significance

Dr. Paolo MALVEZZI
Clinique de Néphrologie
CHU Grenoble - France

Tehran, August 2016
The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.

The New England Journal of Medicine

SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION*

Ramon Patel, M.R.C.P., and Paul I. Terasaki, Ph.D.

Abstract Crossmatch tests of the prospective kidney-transplant donor’s lymphocytes with the serum of the prospective recipient in 225 transplants showed that eight of 195 with negative crossmatch failed to function immediately, in contrast to 24 of 30 with positive crossmatch (p less than 0.001). Immediate failure occurred in significantly higher numbers among patients with a higher risk of having antibodies, such as multiparous females and patients receiving secondary transplants. The effect was not a nonspecific one, for more immediate failures occurred among transplants from unrelated than among those from related donors. The corresponding frequency of positive crossmatch was also lower among related donors. The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.
Crossmatch, PRA and HLA antibodies

Complement dependent cytotoxicity  FACS  Luminex/ELISA

CELL BASED CYTOTOXICITY  CELL BASED ASSAY  BEAD ASSAY

Tinckam KJ and Chandraker A. CJASN 1: 404, 2006
Luminex - Single Antigen Technology

- Plastic bead
- HLA ag
- Patient’s Antibodies
Luminex - Single Antigen Technology

Plastic bead

HLA ag

Ab with fluorochrome

LASER

BEAD IDENTIFICATION

FLUORESCENCE INTENSITY
Limits and confusion around Luminex technology

- Luminex is an *in vitro* technology: it *does not* give information on the pathogenic nature of the antibody.
- It confirms the presence, even at low concentrations, of specific HLA ab in the patients serum.
- It may give false positive and false negative results that may condition access to transplantation if virtual crossmatch is performed.
Luminex: what is MFI?
How do we interpret MFI?

• Mean Fluorescence Intensity is the result of many factors:

> Ag density on the bead
> Ab concentration in the serum
> Ab affinity

MFI does not mean Antibody strength!!!
Prozone effect

- Antibody Excess (Prozone)
- Equivalence (Optimum Proportions of Antigen and Antibody)
- Antigen Excess
Prozone effect: how to get rid of it

- Too much antibody is present in the serum and interferes with the detection system.
- Too much antibody is present and complement and/or IgM present in the serum interferes with the detection system.

To prevent this phenomenon a few methods are useful:
- Heat inactivation (56° for 30’)
- Dilution of patient serum
- Use of EDTA or DTT in order to destroy complement bonds
Prozone effect

BS Carey et al. Transplant Immunology 37 (2016) 23–27
Prozone effect: recommendations

- When using Luminex technology:
  - Pretreat samples with EDTA
  - Dilute samples, especially when MFI is >15000 in order to abrogate the prozone effect.

HLAab detection: considerations

- Excellent instruments to detect HLA ab
  - LCT assays to determine *in vivo* cytotoxicity of the antibodies
    - *Low sensibility, but inexpensive and will allow to avoid hyperacute rejection*
  - Luminex Single Antigen assay: to detect and identify all circulating HLA antibodies
    - *Extremely sensible, expensive, needs a good lab with experience*
    - *Will allow to determine whether patient is at risk for antibody mediated rejection*
Clinical significance of DSA

- Antibodies directed against HLA antigens of the donor are known to be deleterious both when they are found BEFORE and when they appear AFTER transplantation:
  - They lead to early kidney dysfunction
  - Due to acute and chronic antibody mediated rejection
Chronic antibody-mediated rejection (CAMR)
DSA and the kidney graft: time lapse

**Function**
- Acute clinical ABMR
- Graft dysfunction
- Indolent ABMR

**Histopathology**
- Chronic ABMR
- IFTA
- Graft arteriosclerosis
- Transplant glomerulopathy

- Endothelial injury
- Peritubular capillaritis
- Glomerulitis
- Persisting microvascular inflammation

**DSAs**
- Complement activation
- C4d
- ENDATs
- Persisting or de novo DSAs

**ENDATs:** endothelial-associated transcripts
**IF/TA:** interstitial fibrosis/tubular atrophy

Loupy et al. Nat Rev Nephrol 2012
Clinical significance of DSA: they are bad!

Death-censored allograft survival stratified by the presence of antibody within 1 year. Moreover, patients had HLA-specific antibodies within 1 year had worse survival rate than in patients had antibodies beyond 1 year (log-rank P<0.001). HLA, human leukocyte antigen.

P-C Lee et al. Transplantation 2009;88: 568–574
Clinical significance of DSA: DQ

De novo DQ DSA, death-censored: allograft survival by group

Graft survival (%)

No DSA, n=149
DQ-only DSA, n=34
DQ+other DSA, n=20

1yr(%) 3yrs(%) 5yrs(%)
99 99 98
100 85 78
90 75 70

P<0.0001

Years Post-transplant

MC Freitas et al. Transplantation 2013;95: 1113-1119
Clinical significance of DSA: IgM

Everly M et al. Transplantation 2014;97: 494-501
Clinical significance of DSA: IgG subtype and IgM

Everly M et al. Transplantation 2014; 97: 494-501
Complement binding and MFI:
de novo DQ

MC Freitas et al. Transplantation 2013;95: 1113-1119
Our experience in Grenoble…

- Are complement binding assays really necessary?
Study design

- Were included all kidney transplant patients in our center that were grafted between 2005 and 2015
- Were excluded previously immunized patients.
- All patients underwent at least once yearly Luminex ab screening test.
- If screening test was positive for class 2 ab → DSA identification was performed by Immucor® SA Luminex and complement binding was evaluated by Immucor® C3d kit.
- SA Luminex positivity: MFI > 1500
- C3d Assay positivity: MFI > 750
Study population

Grenoble University Hospital

Between 2005-2015

924 kidney transplant patients

Non sensitized
759

Developed dn class II DSA
41

HLA sensitized
165
Results

- Mean follow-up before *de novo* DSA identification: 39.8 ± 23 months
- 65% developed DQ ab
- 27% developed DR ab
- 7% developed DP ab
- 44% had positive C3d assays
Comparison of standard and C3d assays
(loess smoothing)
Only DSA beads considered

R = 0.9 p<0.001
Kidney graft biopsy at DSA detection

<table>
<thead>
<tr>
<th>Histology lesion</th>
<th>C3d +</th>
<th>C3d -</th>
<th>Total biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute AMR</td>
<td>5 (55%)</td>
<td>4 (45%)</td>
<td>9</td>
</tr>
<tr>
<td>Chronic AMR</td>
<td>7 (100%)</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>No AMR</td>
<td>3 (27%)</td>
<td>8 (73%)</td>
<td>11</td>
</tr>
</tbody>
</table>

27
Kidney function one year later: 50% GFR decrease and/or graft loss

Progression (50% clearance decrease)
- No progression
- Progression

Chi^2 p-value = 0.04
How do we proceed? Before transplantation

• Check for preformed ABs in Luminex regularly

• If living donor is available but with DSAs and negative CM:
  – Luminex + LCT assays to determine in vitro (MFI) and in vivo (cytotoxicity) characteristics
  – Desensitization

• If deceased donor is available:
  – No transplant with preformed (MFI >3000??) DSAs
  – No transplant with + LCT crossmatch
How do we proceed after transplantation

• Verify for HLA ab at M3 , M12 then every year

• If DSA appear:
  – Biopsy
    • If no lesions or active lesions of AMR appear:
      – Treatment with rituximab and PE
    • If chronic lesions with no activity:
      – No treatment a part from nephroprotective measures
Thank You