Is cross-match relevant in liver transplantation?

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Cross-matching in organ transplantation

- Crossmatching: the process of testing recipient’s serum against donor cells to ensure there are no donor-specific HLA Abs (DSA) in the patient’s serum that can cause rejection of the transplanted organ.

Evolution of cross-match technology


- A simple and practical test for donor-specific Abs (DSA)- the complement-dependent lymphocytotoxicity (CDC) cross-match test.

Subsequently:
- Flow cytometry (donor or surrogate cells)
- Ab specificity analysis: solid-phase immunoassays (SPI), in particular single Ag bead assay
- Precision and accuracy

New methods are highly sensitive and we do not fully understand the clinical significance of Abs detected by these methods.
Cross-match (XM) target cells

- Isolated T and B lymphocytes
- T cells express HLA class I (A, B, C) only
- B cells express both HLA class I & II (DR, DQ, DP)

Methods for Ab screening and XM in organ transplantation

<table>
<thead>
<tr>
<th>Method</th>
<th>Pretreatment screening</th>
<th>Pretreatment XM</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC/CDC</td>
<td>+++</td>
<td>+++</td>
<td>Prevention of HAV or early AMR</td>
</tr>
<tr>
<td>T/C, modified</td>
<td>+++</td>
<td>+++</td>
<td>Prevention of HAV or early AMR</td>
</tr>
<tr>
<td>ELISA genetic</td>
<td>+++</td>
<td>–</td>
<td>Detection of HLA antibodies</td>
</tr>
<tr>
<td>ELISA specific</td>
<td>+++</td>
<td>–</td>
<td>Detection of HLA antibodies</td>
</tr>
<tr>
<td>LUM genetic</td>
<td>+++</td>
<td>–</td>
<td>Detection of HLA antibodies</td>
</tr>
<tr>
<td>LUM phenotype</td>
<td>+++</td>
<td>–</td>
<td>Specification of HLA antibodies</td>
</tr>
<tr>
<td>LUM SAB</td>
<td>+++</td>
<td>–</td>
<td>Comprehensive specification HLA antibodies</td>
</tr>
</tbody>
</table>

HLA matching in heart, lung and liver transplantation

Not routinely applied to heart, lung or liver transplants for the following reasons:

- Patient pool is too small for matching
- Cold ischaemia time is too short
- Most heart, lung and liver transplants are performed for reasons of clinical urgency. This overrides matching considerations.

This does not mean HLA matching does not have an effect

Can permit an acceptable mismatch (avoid HLA Ags to which the recipient is sensitized)
Methods for Ab screening and XM for each type of organ transplant

<table>
<thead>
<tr>
<th>Organ(s) / tissue(s)</th>
<th>Posttransplant screening</th>
<th>Generic methods</th>
<th>XM</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>+++</td>
<td>RFT/CDC/PC</td>
<td>CDC/ROSAM*</td>
<td>Prevention of HLA or early AMR</td>
</tr>
<tr>
<td>Heart</td>
<td>+++</td>
<td>RFT/CDC/PC</td>
<td>CDC/ROSAM*</td>
<td>Prevention of HLA or early AMR</td>
</tr>
<tr>
<td>Liver</td>
<td>!</td>
<td>RFT/CDC/PC</td>
<td>CDC/ROSAM*</td>
<td>Prevention of HLA or early AMR</td>
</tr>
<tr>
<td>Pancreas/Islets</td>
<td>+++</td>
<td>RFT/CDC/PC</td>
<td>CDC/ROSAM*</td>
<td>Prevention of HLA or early AMR</td>
</tr>
<tr>
<td>Intestinal</td>
<td>!</td>
<td>RFT/CDC/PC</td>
<td>CDC/ROSAM*</td>
<td>Prevention of HLA or early AMR</td>
</tr>
</tbody>
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 XM and liver transplant/patient survival

Historically (technology limited to CDC):

Positive cross-match considered a contraindication to all solid organ transplants except liver (Gordon, Surgery 1986)

However, since early 1990s:

Increased graft loss rates in liver transplant patients with a positive XM (Ogura/Busuttil, Cite Transplant 1994; Castillo-Rama, Liver Transplant 2008; Goh, retransplants (Liver Transplant 2010)

The role of DSAs in liver graft failure remains unclear

The role of DSAs in liver graft failure remains unclear
Results suggested that positive XM grafts should not be considered a contraindication for LDLT. XM positive grafts had no influence on patient survival at 3yr.

Equal overall rejection rate in pre-transplant flow-cytometric cross-match negative and positive adult recipients in liver transplantation.

Findings were supportive of little risk associated with preformed DSA in deceased donor liver transplantation.

Liver recipients harbouring anti-donor preformed lymphocytotoxic antibodies exhibit a poor allograft survival at the first year after transplantation: Experience of one centre

Findings show a direct correlation between the presence of anti-donor preformed Abs and poor allograft survival in liver transplantation.
Pre-formed anti-donor HLA-Ab and reduced graft survival

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Number of Tx</th>
<th>HLA-Ab Assay</th>
<th>Clinical Correlation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Njoku 2008</td>
<td>206 adult</td>
<td>CDC-T cell CMI, Flow-PRA Pred.</td>
<td>No correlation with early ACR or LCM-UDX, FM positive</td>
<td>Transplant Immunol 14:131</td>
</tr>
</tbody>
</table>

Pre-formed Antibodies Detected by Cytotoxic Assay or Multibead Array Decrease Liver Allograft Survival: Role of Human Leukocyte Antigen Compatibility

Although HLA typing is not a prerequisite for liver transplantation, screening of HLA Abs with Luminex techniques and CDC CM may be useful in the detection of at-risk patients that could benefit from increased surveillance and tailored therapy following transplantation.

Pre-formed anti-donor Abs decrease liver graft survival

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<tr>
<td>Castillo-Rama 2008</td>
<td>156 adult</td>
<td>CDC I and II CMI, Pre-Tx Luminex Screen for HLA-Ab</td>
<td>Both CDC and Luminex detected Abs associated with shorter graft survival 2 year post-Tx. Luminex class I Abs Abs associated with ACR</td>
<td>Liver Transplantation 14:154</td>
</tr>
</tbody>
</table>
CDC XM positive LDLT recipients show significantly poorer outcomes than XM negative recipients.

Pre-formed donor-specific anti-HLA (MFI) and chronic rejection in liver transplantation

High MFI DSA are associated with chronic rejection

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<tbody>
<tr>
<td>O’Leary 2011</td>
<td>30 adult with CR and 30 control adult without CR</td>
<td>Lumines DSA</td>
<td>In the CR group the frequency of DSA and high MFI DSA was higher than in the control group</td>
<td>Am J Transplant 11:10888</td>
</tr>
</tbody>
</table>
The impact of positive CM on liver allografts

- Shortly after tx recipients may develop:
  - Thrombocytopenia
  - Hepatocyte swelling
  - Hypocomplementemia
  - Hepatocanalicular cholestasis
- AMR may be accompanied by ACR
- Correlated with preformed DSA causing high titer XM

CM positive patients show more C4d staining

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</thead>
<tbody>
<tr>
<td>Lunz 2012</td>
<td>89 adult</td>
<td>CDC, XM</td>
<td>C4d staining more often in biopsies from XM-positive recipients within 3 weeks post-Tx. Biopsies from DSA- recipients showed more often ACR compared to DSA- patients.</td>
<td>Am J Transplant 12:171</td>
</tr>
</tbody>
</table>

Liver allograft from a XM+ recipient obtained 10 days after transplantation for primary biliary cirrhosis. The patient developed otherwise unexplained hyperbilirubinemia and developed a significantly fall in platelet counts. Comparison of the H-E-stained slide (top) and C4d-stained slide (bottom). The portal tracts (PT) from each slide are shown at higher magnification in the left inset. Note the positive C4d staining in the portal vein, central veins and capillaries (CV). The arrows show vessels. Liver allograft from a XM+ recipient obtained 10 days after transplantation for primary biliary cirrhosis. The patient developed otherwise unexplained hyperbilirubinemia and developed a significantly fall in platelet counts. Comparison of the H-E-stained slide (top) and C4d-stained slide (bottom). The portal tracts (PT) from each slide are shown at higher magnification in the left inset. Note the positive C4d staining in the portal vein, central veins and capillaries (CV). The arrows show vessels.
C4d staining in liver biopsies within 3 weeks of transplant

<table>
<thead>
<tr>
<th>Staining</th>
<th>CM+</th>
<th>CM-</th>
</tr>
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<tbody>
<tr>
<td>Acute</td>
<td>26%</td>
<td>33%</td>
</tr>
<tr>
<td>Chronic</td>
<td>29%</td>
<td>31%</td>
</tr>
<tr>
<td>Portal</td>
<td>30%</td>
<td>31%</td>
</tr>
<tr>
<td>Sinusoidal</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Values obtained using regression models adjusted for gender, donor, recipient, HLA class I/II, previous transplant, and number of pre-existing MHC class I Abs.

Lunz et al., Am J Transplant 2012

Human Leukocyte Antigen Crossmatch Testing Is Important for Liver Retransplantation

Because MHC class I Abs have a deleterious effect on liver re-graft survival, CM testing should be performed before liver retransplantation.

Five-year regraft survival in (— —) adult patients with human HLA class I Abs (n = 80, 67.8%) and (— —) adult patients without HLA class I Abs (n = 38, 32.3%). Class I Abs were associated with significantly poorer 1-, 3-, and 5-year graft survival (second liver transplant survival rates of 76.0% versus 54.7% at 1 year (P = 0.046), 67.2% versus 45.1% at 3 years (P = 0.042), and 67.2% versus 43.5% at 5 years (P = 0.03)) after retransplantation in patients with and without class I Abs, respectively.

Goh et al., Liver Transplantation 2010

Preformed HLA Cl I Abs have a deleterious effect of liver re-graft survival

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<tr>
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<th>HLA-Ab Abs</th>
<th>Clinical Correlation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goh 2010</td>
<td>1288 re-Tx; 129 adult; 23 pediatric</td>
<td>Pre-Tx Hepatitis</td>
<td>Pre-Tx HLA-Ab was significantly associated with poorer second graft survival in adult recipients</td>
<td>Liver Transplantation 18:328</td>
</tr>
</tbody>
</table>
Acute Humoral Rejection in an ABO Compatible Combined Liver-Kidney Transplant—The Kidney Is Not Always Protected

Heightened suspicion for acute humoral rejection of the renal allograft is necessary when performing combined liver-kidney transplants to highly sensitized patients due to previous organ transplants

Donor-Directed MHC Class I Antibody Is Preferentially Cleared from Sensitized Recipients of Combined Liver/Kidney Transplants

All patients with humoral rejection of their kidney had preformed anti-class II Abs. Liver allografts may not be fully protective of the renal allograft, especially with pre-existing MHC class II DSA. Long-term and careful follow-up will be critical to determine the impact of DSA on both allografts

Impact of pre-tx HLA-Ab in combined kidney-liver transplantation

<table>
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<th>HLA-Ab assay</th>
<th>Clinical Correlation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Der 2011</td>
<td>Combined Liver Kidney 6 patients</td>
<td>CDC-C6M, legacy DSA</td>
<td>AMLR of the kidney allograft was associated with pre-formed Class II DSA and not Class I DSA.</td>
<td>Am J Transplant 11:941</td>
</tr>
</tbody>
</table>

2011
Combined liver-kidney transplantation - relevance of the XM

- presensitization has a negative impact on patient survival and kidney graft survival
  
  Reichman, Am J Transplant, 2009
  Dar, Am J Transplant, 2011
  Askar, Transplantation, 2011

- preformed DSA can promote AMR in the kidney
  
  Reichman, Am J Transplant, 2009

- in sensitized patients with pre-formed class II DSA, the liver may not be fully protective
  
  Dar, Am J Transplant, 2011

Non-HLA alloAbs

In liver transplantation, data on a possible effect of pre-transplant non-HLA alloAbs (e.g. MICA, AECA) (other than ABO isoagglutinins) are not available

MICA = MHCI-related chain A
AECA = anti-endothelial/epithelial cell Ab

Conclusions (i)

- the impact of allosensitization (DSA) in liver transplant outcome remains controversial
- the liver can absorb HLA-Ab without exhibiting AMR
- several groups have reported correlation between pre-transplant DSA with positive CDC-XM, and lower 1-year graft survival (Castillo-Rama)
Conclusions (ii)

- positive XM correlates with early C4d (Lunz)
- in chronic rejection, the frequency of preformed DSA with higher MFI is higher than in controls (O’Leary)
- in liver re-transplantation, preformed alloAbs for the 2nd graft are associated with poor graft survival (Goh)
- Liver grafts may not be fully protective of kidney grafts (Dar) and preformed DSA may promote AMR in the kidney in combined kidney-liver transplantation (Reichman)

Liver allografts – pre-transplant considerations/recommendations

- liver may be (partially) resistant to Ab-mediated damage, but high levels of DSA may be associated with poorer outcomes and should be considered a risk factor for graft dysfunction
- a cross-match should be performed in sensitized recipients
- pre-transplant screening for HLA Abs is recommended for risk stratification and HLA typing of recipient and donor should be available
- in sensitized recipients of combined LK transplants, the liver may not fully protect against AMR in the kidney and should be included in risk assessment

Tait et al, Am J Transplant 2013

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Assistant Director: John Lunz PhD

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- ROTRF
- American Society of Transplantation
- American Liver Foundation
Impact of allo sensitization remains controversial

• pre-transplant HLA testing and XM are currently not routine procedures

But:

• a pre-transplant positive T cell XM and pre-transplant DSA are associated with poor graft survival
  
  Opelz, Hum Immunol, 2009
  Castillo-Rama, Liver Transplantation, 2008
  O’Leary, Am J Transplant, 2011

• pre-formed HLA Abs are associated with poorer survival of re-transplants
  
  Lunz, Hum Immunol, 2009

• ACR is more common in patients who were DSA and XM positive and demonstrated C4d in biopsies as early as 3wk post transplant
  
  Lunz, Hum Immunol, 2009