New Insights into Antibody Mediated Graft Injury after Pediatric Liver Transplantation

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Antibody Mediated Graft Injury after Pediatric Liver Transplantation

- Isohemaglutinins – ABO incompatibility
- ‘Auto antibodies – de novo post tx autoimmune hepatitis
- Donor specific antibodies

ABO Incompatibility

- In USA/Europe < 5% of pediatric liver tx
- More common in countries with only LD option: upto 18% in Japan
- Indications: fulminant liver failure, size considerations
ABO Incompatibility

Mechanism of graft injury
- Preformed isohemagglutins bind to graft vasculature which express ABO antigens
  - Starzl et al. NEJM 1968;278:642
- Complement activation, vessel damage, inflammation, diffuse thrombosis
- Hemorrhagic necrosis

Complement activation, vessel damage, inflammation, diffuse thrombosis

ABO Incompatibility

Mechanism of graft injury
- After ABO incompatible liver tx tempo of graft injury slower
- Hyperacute hemorrhagic necrosis rare
- Hepatic necrosis within 3 weeks
- Intrahepatic biliary strictures most common within 3 months

ABO Incompatibility

Effect of age: < 1 year protective
- Isohemagglutins not produced in newborns
- Low titers up to about one year of age
- First evidence: heart recipients < 1 year of age tolerated ABO incompatible grafts
  - West et al. NEJM 2001:11:793
ABO Incompatibility

Single center results – Kyoto
Impact of recipient age on outcome of ABO
Incompatible living-donor liver transplantation.

Egawa et al. Transplantation 2004;77:403

- N = 523
- 18% (99) ABO liver tx
- Prior to era of Rituximab, infusion therapy, splenectomy

RESULTS: Patient survival

Figure 1. Actuarial survival of patients with ABO-I, ABO-compatible, and ABO-identical grafts.

Egawa, et al

RESULTS: Patient and Graft Survival by Age

Figure 2. Actuarial patient (A) and graft (B) survival of patients with ABO-I living-related liver grafts in four age groups (>1-4 years old, >4-16 years old).
ABO Incompatibility

Liver recipients < 1 year of age
- 1.5% children had antibody mediated rejection
- 26% > 1 year of age

RESULTS: Long term antibody levels

Fig 3. All long term survivors in all 4 age groups showed persistent low IgG and IgM antibody levels Tolerization of recipient B and plasma cells to donor antigens?

ABO Incompatibility

Complications: ABO-I recipients (Kyoto):
- Sig higher hepatic necrosis
  - 8% vs 0%
- Sig higher intrahepatic biliary complications
  - 15% vs 0.6%
- Sig higher incidence of hepatic a. thrombosis
  - 10% vs 1-3.5%
- No different in incidence of acute, chronic rejection
ABO Incompatibility

Present status of ABO incompatible living donor liver transplantation in Japan

Japan study group for ABO-I liver tx
- N = 291
- Follow-up: 8 mo – 15 year
- Local infusion therapy – 2000
- Rituximab - 2003

ABO Incompatibility

Japan – update: Treatment strategies

- Plasmapheresis before and after tx
- Splenectomy (N = 97)
- Portal infusion (N = 41, mostly adult)
- Hepatic artery infusion (N = 68, mostly adult)
- PVI + HA1 (N = 23)
- NB: up to 37% incidence of vascular complications
- Rituximab (N = 59)

Present status of ABO incompatible living donor liver transplantation in Japan

Egawa et al. Hepatology 2008
Present status of ABO incompatible living donor liver transplantation in Japan

Incidence of intrahepatic biliary complications and hepatic necrosis by age

Egawa et al. Hepatology 2008

Effect of Age and Era

Effect of Age

Effect of Era – adults only

Egawa et al. Hepatology 2008

ABO Incompatibility: Japanese experience

Multivariate analysis of risk factors for patient death
- ICU status
- Adult age
- High pre-op IgM titers
- High post-op IgM titers
- Severe bacterial/fungal infection
ABO Incompatibility: Japanese experience

Multivariate analysis of risk factors for antibody-mediated rejection
- Age > 1 year
- Increase in post-operative antibody titer
- Pre-op titer not significant
  - Even if reduced, still at risk for antibody-mediated rejection

ABO Incompatibility

Current recommendation for pediatrics
- < 1 year of age likely safe
- > 1 year of age, reserve for urgent cases
- Monitor pre and post tx antibody levels
- If LD, can pretreat: pheresis, IVIG, rituximab
- Post transplant
  - Titers > 1:16 consider pheresis + IVIG
  - Higher or climbing titers, treat with rituximab
  - Number of rituximab courses dependent on antibody response and graft function

ABO Incompatibility

The modern era – keep it simple for the children
- Standard immunosuppression and MMF
- Avoid increased risk of infection - splenectomy
- Avoid risk of vascular complications – infusion therapies
ABO Incompatibility

Successful ABO incompatible pediatric liver transplantation utilizing standard immunosuppression with selective postoperative plasmapheresis.

Heffner et al. Liver Transpl 2006;12:972

- N = 16
- Quadruple immunosuppression with dacluzimab
- No pre tx plasmapheresis
- No splenectomy
- One post tx pheresis
- No difference in patient-graft survival compared to ABO-matched graft

De novo Autoimmune Hepatitis

Clinical Definition

- Post transplant de novo 'immune' hepatitis
- Graft dysfunction after excluding other causes
- Biopsy evidence of hepatitis similar to autoimmune hepatitis
- Autoimmune markers positive
- Hypergammaglobulinemia

Definition of d-AIH: pathology

(A) Chronic hepatitis with moderate portal and interface mononuclear cell infiltrate, including plasma cells (haematoxylin-eosin staining);

(B) reticulin staining showing bridging collapse

N. Kerkar et al., De-novo autoimmune hepatitis after liver transplantation, Lancet 351 (1998) 409-413

Positive autoimmune markers

- Presence of autoantibodies in d-AIH patients (listed in decreasing order of frequency):
  - Anti-smooth muscle antibody
  - Anti-nuclear antibody
  - Anti-LKM
  - Anti-mitochondrial


- p-ANCA, anti- SLA, anti-human asialoglycoprotein receptor

Presence of AutoAbs alone ≠ d-AIH

- The presence of autoAbs has consistently been reported following LT, in 15-41% of peds recipients.

- However, the incidence of d-AIH on the other hand is only noted in 2.1-5.2% of peds pts.

De novo –AIH: UCLA Experience

- Rejection and steroid dependence: unique risk factors in the development of pediatric posttransplant de novo autoimmune hepatitis
- Retrospective case controlled study
- 41 of 619 children transplanted at UCLA 1984-2003

Aims:
- Identify clinical characteristics & risk factors for development d-AIH
- Evaluate response to treatment.

Results

- Overall incidence d-AIH: 6.6%
- Mean time LT → d-AIH: 7.0 +/- 1.2 years

Predictors of developing d-AIH

- The following variables were analyzed and found NOT to be significant in the development of d-AIH:
  - gender
  - race
  - primary immunosuppression
Significant Predictors of d-AIH development

Prednisone Requirements 2 Years Post Liver Transplant

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of Patients off of Prednisone by 2 Years Post-LT</td>
<td>8%</td>
<td>31%</td>
<td>0.0084**</td>
</tr>
</tbody>
</table>

**Chi-square = 6.94, df=1.**

Rejection Post-LT in the Case and Control Groups

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>CASE</th>
<th>CONTROL</th>
<th>P-VALUE *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Number of Total Biopsy Proven Rejection Episodes</td>
<td>2.8</td>
<td>1.0</td>
<td>0.0008</td>
</tr>
<tr>
<td>Mean Number of Acute Steroid Sensitive Rejection Episodes</td>
<td>2.0</td>
<td>0.7</td>
<td>0.0016</td>
</tr>
<tr>
<td>Mean Number of Chronic Rejection Episodes</td>
<td>0.6</td>
<td>0.3</td>
<td>0.10</td>
</tr>
<tr>
<td>Mean Number of Steroid Resistant Rejection Episodes</td>
<td>0.2</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean Time from LT to Acute Rejection (Years +/- 95% CI)</td>
<td>2.0 +/- 0.7</td>
<td>1.1 +/- 0.8</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* P-values are based on two-tailed student’s t-test
**Unanswered Questions**

- What is the target in the liver graft of the 'auto' antibody?
- Is de novo AIH hepatitis a marker of immune deregulation?
- Any relationship to donor specific antibodies?

**Donor Specific Antibodies**
Donor specific antibodies

Relevance of DSAs after liver tx
- Distinguished preformed from de novo DSA
- Role of preformed DSA in kidney tx proven 40 years ago
  Terasaki et al. NEJM 1969:280:735
- What about the liver?
- Conflicting evidence especially for role of de novo DSA as a cause for graft injury

DSAs

- Relevance of DSAs after pediatric liver tx
- Very little data

Positive DSA associated with forms of late graft dysfunction:
- Fibrosis
- De novo AIH
- Rejection

Negative DSA
- Associated with tolerance

DSA Pediatric Liver Tx

Increasing concern: progressive fibrosis in protocol biopsies of long-term pediatric liver tx survivors
- What is mechanism of graft injury
- ? role of antibody mediated rejection
- Association with positive DSA?
Long-term Complications
Chronic Hepatitis and Fibrosis

Progressive histologic damage in liver grafts following pediatric liver tx:
- Protocol biopsies in > 5 yr survivors
- Normal histology 68% at 1 yr, 45% at 5 yrs and 31% at 10 yrs
- Chronic hepatitis most common abnormality
- Incidence of fibrosis increased with time – in 91% at 10 yrs
- Only predictive finding was autoantibody positivity

Chronic hepatitis and fibrosis are confirmed by other studies:
- Chicago: fibrosis in 61 of 63 > 3 year survivors
  - Not correlated with other recognized complications
  - Liver functions tests could be normal
- The Netherlands
  - 1 and 5 year protocol biopsies fibrosis had increased from 31% to 65%, by 5 years fibrosis was severe in 25%
  - Related to transplant related factors, cold ischemia time, graft type, age of donor and recipient

positive DSA Pediatric Liver Tx

Progressive graft fibrosis and donor-specific human leukocyte antigen antibodies in pediatric late liver allografts
- Miyagawa Hayashino et al. (Kyoto) Liver Transpl 2012;18:1333
- 79 stable patients > 5 years after liver tx
- Normal graft function
- Protocol biopsies
Results

- Positive DSA (Luminex) in 32 (41%)
  - 30/32 Class 2 positive only
- Bridging fibrosis/cirrhosis: centrilobular based
  - Positive DSA = 88%
  - Negative DSA = 17% (p < 0.001)
- Mild/indeterminate rejection: 15 of 32
  - Positive DSA = 47%
  - Negative DSA = 14% (p = 0.004)

C4D staining
Focal vs diffuse

- Diffuse strong PV C4D stain >50% triads
- Focal PV C4D stain <20% triads
- Nonspecific portal stroma C4D stain

- DSA +: 78% C4D + 5/25 diffuse
- DSA -: 23% C4D + 0 diffuse

Progressive graft fibrosis over time after transplant

Liver Transplantation 2012;18:1333
Percent of patients with positive DSA increases with severity of fibrosis

No association between time post tx and positive DSA

Liver Transplantation 2012;18:1333

Relationship of DSA to Late Fibrosis

Conclusion:

Does graft injury – fibrosis – occur over a period of chronic inflammation induced by donor specific antibody mediated rejection?

The Flipside:
Tolerant patients and DSA
Tolerant patients and DSA

- DSA negative in all patients weaned off
- Liver Transplant recipients weaned off immunosuppression lack circulating donor specific antibodies.
  - Girnita et al Human Immun 2010;71:274

In a drug withdrawal protocol
- 19 tolerant patients: no DSA +
- 9 of 12 weaning patients: DSA+ (only 4 successfully weaned)
- 6 of 7 failed withdrawal had DSA +

Predicting operational Tolerance in Pediatric Living Donor Liver Transplantation by Absence of HLA Antibodies

- HLA antibodies are more common in non-tolerant patients and tend to have higher MFI

Highest MFI in tolerant vs non tolerant patients after tx

Waki, Transplantation, 2013

- pretransplant peak MFIs not sig different
Donor-specific HLA antibodies in pediatric liver transplant recipients: Culprits or innocent bystanders?

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Aim and Hypothesis

- **Aim:** To assess the impact of DSA on long-term outcomes in pediatric LTx recipients
- **Hypothesis:** Patients with d-AIH and/or episodes of late acute or chronic rejection are more likely to have post-transplant DSA

Methods: Study Design

- 46 LTx recipients who were ≥1 year post-LTx
- Serum was collected at routine clinic visits
  - Samples were blinded
- Antibody detection was performed using Luminex single antigen class I and II beads
  - HLA-A, HLA-B, HLA-DR, HLA-DQ
- Donor-recipient mismatched HLAs were compared
  - Positive DSA: >1000 mean fluorescence intensity (MFI)
Methods: Patient Phenotypes

- Non-tolerant patients (n=16)
  - Late graft dysfunction requiring dual or triple immunosuppression
  - 2 with biopsy-proven chronic rejection
- With d-AIH (n=11)
  - Based on biopsy and autoantibodies
- Without d-AIH (n=5)
  - Late acute cellular rejection ≥1 year post-LTx

- Stable patients (n=23)
  - Normal liver function on tacrolimus monotherapy
  - No history of late acute cellular rejection
- Tolerant patients (n=7)
  - Normal liver function off chronic immunosuppression ≥2 years
  - Immunosuppression discontinued due to PTLD (n=3) and non-adherence (n=4)

Results: Overall Prevalence

- 26/46 patients (57%) with +DSA
  - Range of 1-4 DSA per patient
  - Mean MFI/DSA 10903 ± 8463
  - Mean cumulative MFI/patient 17718 ± 16791
Results: DSA Loci
Most DSA were directed at class 2 loci

- DQ 48%
- B 5%
- A 2%
- DR 45%

Results: Demographic Data
+DSA (n=26) -DSA (n=20) p value
Time since LTx (years) 11.6 ± 4.4 11.9 ± 6.2 0.84
Age at LTx (years) 2.3 ± 3 5.6 ± 4.9 0.01
Age at Sample (years) 13.9 ± 4.3 17.5 ± 5.2 0.02
Male 38% 50% 0.55
Biliary atresia 77% 65% 0.51
Acute liver failure 8% 20% 0.38
H/o PTLD 12% 15% 1.0

Results: Past Allograft Dysfunction
+DSA (n=26) -DSA (n=20) p value
Re-transplant recipient 19% 15% 1.0
H/o early rejection 58% 47% 1.0
H/o late rejection 31% 30% 1.0
Number of late rejection episodes 0.5 ± 1 0.4 ± 0.7 0.61
Results: Current Allograft Function

<table>
<thead>
<tr>
<th></th>
<th>+DSA (n=26)</th>
<th>-DSA (n=20)</th>
<th>p value</th>
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<tbody>
<tr>
<td>ALT (U/L)</td>
<td>28 ± 21</td>
<td>31 ± 34</td>
<td>0.80</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.7 ± 0.5</td>
<td>0.7 ± 0.4</td>
<td>0.95</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>63 ± 112</td>
<td>37 ± 36</td>
<td>0.39</td>
</tr>
<tr>
<td>De-novo AIH</td>
<td>9</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>Non-tolerant without d-AIH</td>
<td>1</td>
<td>4</td>
<td>0.15</td>
</tr>
<tr>
<td>Stable</td>
<td>14</td>
<td>9</td>
<td>0.77</td>
</tr>
<tr>
<td>Tolerant</td>
<td>2</td>
<td>5</td>
<td>0.21</td>
</tr>
<tr>
<td>Chronic rejection</td>
<td>2</td>
<td>0</td>
<td>0.50</td>
</tr>
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Results: DSA Association with Clinical Status

Results: DR DSA Association with Clinical Status
Results: DQ DSA Association with Clinical Status

<table>
<thead>
<tr>
<th>% of Patients with HLA-DQ DSA</th>
<th>De-novo DSA</th>
<th>Non-tolerant</th>
<th>Stable</th>
<th>Tolerant</th>
</tr>
</thead>
<tbody>
<tr>
<td>De-novo DSA</td>
<td></td>
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<tr>
<td>Non-tolerant</td>
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<tr>
<td>Stable</td>
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<tr>
<td>Tolerant</td>
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Results: Multivariate Predictors of DSA

<table>
<thead>
<tr>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time since LTx (years)</td>
<td>0.81</td>
<td>0.65-1.02</td>
</tr>
<tr>
<td>Age at LTx (years)</td>
<td>0.51</td>
<td>0.32-0.82</td>
</tr>
<tr>
<td>Re-transplant recipient</td>
<td>73.7</td>
<td>1.9-2874</td>
</tr>
<tr>
<td>De-novo AIH*</td>
<td>34.5</td>
<td>1.29-913</td>
</tr>
<tr>
<td>Non-tolerant without d-AIH*</td>
<td>47.9</td>
<td>0.5-4489</td>
</tr>
<tr>
<td>Stable*</td>
<td>17.2</td>
<td>0.89-333</td>
</tr>
</tbody>
</table>

Conclusions

- DSA are common post-LTx
  - Class II > Class I
- DSA were detected less often in tolerant patients
- DSA were detected more often in non-tolerant patients with d-AIH
- DSA did not always translate to allograft dysfunction
Conclusions

We haven’t yet answered the Question:
Donor-specific HLA antibodies in pediatric liver transplant recipients:

Guilty????

Innocent????

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