Diagnosis and Treatment of Acute Rejection

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Outline-Acute Rejection

• Definitions-Histopathology

• Prevalence

• Significance

• Diagnosis (beyond the biopsy)

• Treatment
### Acute Rejection: Definitions
(Adapted from Banff ‘07 Update)

<table>
<thead>
<tr>
<th>Antibody-Mediated:</th>
<th>T cell-Mediated:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>“Rule of Three”</strong></td>
<td><strong>“Is it in the tubules/interstitum, or in the vessels?”</strong></td>
</tr>
<tr>
<td>PMNs &gt;&gt; monocytes</td>
<td>Monocytes &gt;&gt; PMNs</td>
</tr>
<tr>
<td>1. C4d+</td>
<td>IA: &gt;25% interstitial infiltration, 4-10 mononuclear cells/tubular cross-section</td>
</tr>
<tr>
<td>2. Presence of antidonor antibodies (DSA)</td>
<td>IB: &gt;25% interstitial infiltration, &gt;10 mononuclear cells/tubular cross-section</td>
</tr>
<tr>
<td>3. Acute tissue injury:</td>
<td>IIA: Intimal arteritis -mild-to-moderate (0-25% of lumenal area)</td>
</tr>
<tr>
<td>I. ATN-like (minimal inflammation)</td>
<td>IIB. Intimal arteritis -severe (&gt;25% of lumenal area)</td>
</tr>
<tr>
<td>II. Capillary and/or glomerular inflammation and/or thromboses</td>
<td>III. Transmural arteritis and/or fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocyte inflammation</td>
</tr>
<tr>
<td>III. Arterial inflammation</td>
<td><strong>Borderline</strong></td>
</tr>
<tr>
<td><strong>“Suspicious”</strong></td>
<td>10-25% interstitial infiltration, &lt;4 mononuclear cells/tubular cross-section</td>
</tr>
<tr>
<td>2 of 3 above (C4d, DSA or injury)</td>
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</table>

- **PMNs >> monocytes**
- **Monocytes >> PMNs**
Problems with current histopathologic diagnosis of Acute Rejection

• Antibody Mediated:
  – Lack of standardization of C4d staining
  – Inflammation *in absence of C4d* correlates with graft loss
  – C4d *in absence of DSA* correlates with graft loss

• T cell mediated:
  – Inflammation in areas of atrophy correlates with graft loss
Evidence that antibody-mediated injury occurs in the absence of C4d staining:

- 54 patients with +DSA but negative crossmatch underwent deceased donor KTX:
- 3 mo and 1y protocol biopsies were performed:

<table>
<thead>
<tr>
<th>3 mo biopsy result:</th>
<th>At 1 year post-transplant:</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min)</td>
<td>% with IF/TA</td>
</tr>
<tr>
<td>&quot;Subclinical AMR&quot; (N=14)</td>
<td>39</td>
</tr>
<tr>
<td>C4d+/DSA+/capillaritis+</td>
<td></td>
</tr>
<tr>
<td>&quot;Borderline&quot; (N=22)</td>
<td>46</td>
</tr>
<tr>
<td>C4d- /DSA+/capillaritis+</td>
<td></td>
</tr>
<tr>
<td>No AMR (N=9)</td>
<td>62</td>
</tr>
<tr>
<td>DSA+ only</td>
<td></td>
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</table>

Evidence that C4d predicts graft loss even in the absence of DSA:

- 173 subjects underwent biopsy for new onset late graft dysfunction (mean time after transplant 7.3 y)
- Subjects were divided into four groups based on C4d and DSA:
- After 2 years, both (C4d+) groups were at significantly greater risk for graft loss. Adjustment for inflammation (Banff i, t, g, and ptc scores) did not change the outcome.

Gaston R et al, Transplantation 2010; 90: 68
Inflammation is meaningful even if it is not tubulitis

- DeKAF study of 337 patients with “new onset late graft survival” undergoing biopsies:
  - Inflammation in areas of tubular atrophy (“iatr”) was associated with decreased graft survival
  - NOT accounted for in current Banff grading schema

Mannon R et al, Am J Transplant 2010; 10: 2066
Prevalence of acute rejection - 2 eras

- **SRTR 1995-2000:**
  - Rates of AR >50% falling to <20%
  - After 1y, AR rates <3%

- **SRTR 2000-2008:**
  - 28,686 patients, 1st kidney transplant, on TAC/MMF/Pred +/- IL2ra
  - 1y Acute rejection:
    - Overall: 12.3%
    - +IL2ra: 11.6%
    - No induction: 13.0%

Gralla and Wiseman, Transplantation 2010; 90: 639
The Symphony Trial: Acute rejection with various immunosuppressive regimens and the impact on outcomes

- 12-month randomized open-label multicenter trial of 1645 KTX
- 4 Treatment arms (IL2ra induction in “low” arms, MMF/Prednisone for all)
  - Standard: CSA 150-300 ng/ml x 3 months, then 100-200 ng/ml

At 12 months:

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Acute Rejection (%)</th>
<th>Graft Survival (%)</th>
<th>GFR (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA “standard”</td>
<td>25.8</td>
<td>89.3</td>
<td>57.1</td>
</tr>
<tr>
<td>CSA (50-100 ng/ml)</td>
<td>24.0</td>
<td>93.1</td>
<td>59.4</td>
</tr>
<tr>
<td>TAC (3-7 ng/ml)</td>
<td>12.3*</td>
<td>94.2*</td>
<td>65.4*</td>
</tr>
<tr>
<td>SRL (4-8 ng/ml)</td>
<td>37.2</td>
<td>89.3</td>
<td>56.7</td>
</tr>
</tbody>
</table>

NEJM 2007; 357: 2562-75
Impact of Early Steroid Withdrawal on Incidence of Acute Rejection

- 5 year, randomized double blind trial
- Steroid withdrawal after 7 days vs steroid taper to 5 mg at 6 months
- 274 pts, TAC/MMF, induction agent determined by center practice

Steroid Withdrawal: Higher acute rejection rate, particularly with IL-2ra induction (24.2% vs 14.4%)

Control group: similar acute rejection rates (10.3% vs 11.9%) with either induction agent

For “high risk” patients:

IL2ra or Thymoglobulin to prevent acute rejection?

• 278 patients at elevated risk (PRA>20%, retransplant, high risk for DGF)
  • rATG x 4 vs. basiliximab x 2 and triple immunosuppression (CSA)

<table>
<thead>
<tr>
<th>Status @1year:</th>
<th>rATG (n=141)</th>
<th>Basiliximab (n=137)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute rejection</td>
<td>15.6%</td>
<td>25.5% (p=0.02)</td>
</tr>
<tr>
<td>AR requiring Ab therapy</td>
<td>1.4%</td>
<td>8.0% (P=0.005)</td>
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• 227 patients at elevated risk (PRA>30%, retransplant)
  • rATG x 8 vs. daclizumab x 5 and triple immunosuppression (TAC)

<table>
<thead>
<tr>
<th>Status @1year:</th>
<th>rATG (n=113)</th>
<th>Daclizumab (n=114)</th>
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<tr>
<td>Acute rejection</td>
<td>15.0%</td>
<td>27.2% (p=0.016)</td>
</tr>
<tr>
<td>AR requiring Ab therapy</td>
<td>2.7%</td>
<td>14.9% (P=0.002)</td>
</tr>
</tbody>
</table>

• Thymoglobulin more effective in preventing acute rejection in “high risk” patients

Brennan DC et al, NEJM 2006; 355: 1967
Noel C et al, JASN 2009; 20: 1385
Incidence of acute antibody-mediated rejection: pretransplant DSA vs no DSA

- 334 patients (CDC negative crossmatch)
- 67 were retrospectively determined to have preformed donor-specific antibody by flow cytometry
- No desensitization or depleting Ab induction

Amico P et al, Transplantation 2009; 7: 1681

- AMR in 55% of those with pre-tx DSA (but 45% did not!)
- Incidence of AMR in absence of DSA: 6%
Defining risk of acute rejection by ethnicity: still valid?

2000-2008: Acute Rejection in 23,240 1st tx recips, 0% PRA, on TAC/MMF/Pred

<table>
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<tr>
<th>Induction type</th>
<th>AA N=5704 (25%)</th>
<th>Non-AA N=17,540 (75%)</th>
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<tbody>
<tr>
<td>No induction</td>
<td></td>
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<tr>
<td>IL2ra rATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011 (35%) 1754 (31%)</td>
<td>16.1%* 12.7%*</td>
<td>5980 (34%) 6680 (38%)</td>
</tr>
<tr>
<td>1939 (34%)</td>
<td>13.5%*</td>
<td>4880 (28%) 9.8% 8.4%</td>
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*p<0.001

- In modern era, AA recipients still have 37% increased risk of acute rejection (adjusted analysis)

Gralla and Wiseman, ATC 2011
Summary: Risk of rejection

- Acute rejection is 10-12% in the first year in “low risk” patients
- Acute rejection is 15-30% with steroid withdrawal, CNI avoidance
- Acute rejection is 15-30% in “high risk” patients
- Antibody-mediated rejection: 5% in de novo setting, 35-50% in setting of known DSA without pretreatment

- **6y Graft Survival**
  - No AR: 74.4%
  - AR and return to baseline: 72.7%
  - AR and 85-95% of baseline: 67.0%
  - AR and 75-85% of baseline: 50.2%
  - AR and <75% of baseline: 38.0%

Recovery from acute rejection is associated with better outcomes.

Over time a smaller percentage of cases of rejection recovered to baseline.

Impact of Acute Rejection on Graft Survival: AUST/NZ 1997-2004

- Vascular rejection portends worse outcomes than cellular rejection, even with “good” response to therapy!

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<th>Response to therapy</th>
<th>Cellular rejection</th>
<th>Vascular rejection</th>
</tr>
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<tbody>
<tr>
<td>“Good” (return to baseline)</td>
<td>1.25 (ns)</td>
<td>2.35</td>
</tr>
<tr>
<td>“Poor”</td>
<td>1.74</td>
<td>2.23</td>
</tr>
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McDonald S et al, Am J Transplant 2007; 7: 1201
Why do kidneys fail?

Mayo Clinic: 1317 consecutive kidney transplants 1996-2006, biopsies performed in patients with failing grafts at a mean 4.7 mo prior to graft loss

- Of “IF/TA”
- 1/4 history of acute rejection
- Of “glomerular disease”
- 40% “transplant glomerulopathy” (~HLA Ab?)
- ~1/3 of graft loss is linked to alloimmune/rejection response

Summary:
Significance of rejection

• Cellular Rejection: if responsive to therapy, may not be of clinical consequence

• Vascular rejection: worse outcomes regardless of response to therapy

• Antibody-mediated rejection: the presence/development of anti-HLA DSA with other features of AMR likely has a cumulative effect on graft loss
Diagnosis of Acute Rejection - within and beyond the biopsy

• Additional features of the biopsy:
  – Genetic analysis
  – Cellular phenotyping (NK cells, macrophage/monocytes)
  – Non-HLA antibodies

• Urine/blood:
  – mRNA (perforin, granzyme B, FOXP3, TIM-3)

• Blood:
  – cell function assays (ELISPOT for IFN-γ; CD4 T cell ATP release)
Urinary mRNA transcripts: Can they diagnose rejection?

- Comparison of urinary cell levels of mRNAs (rtPCR) in 21 recipients with graft dysfunction and BPAR, and 25 recipients with stable graft function and normal biopsy results.

[Graph showing box plots for OX40, OX40L, and PD-1 mRNA levels in acute rejection and normal biopsy samples.]

- ROC curve analysis:
  - acute rejection sensitivity 95% specificity 92% (area under the curve=0.98, P<0.0001) using a combination of levels of mRNA for OX40, OX40L, PD-1, and levels of mRNA Foxp3.

Afaneh C et al, Transplantation 2010; 90: 1381
In the setting of delayed graft function (DGF): is it ATN or acute rejection?

- T-cell immunoglobulin domain, mucin domain (TiM-3) is selectively expressed on the surface of T-helper (Th)1 cells
- Tim-3 mRNA expression in biopsies, peripheral blood leukocytes (PBL) and urinary cells (UC) were studied in 160 biopsies from 115 patients:

Manfro RC et al, Transplantation 2008; 86: 1869
Can one distinguish BKVAN from rejection using urine cytokines?

- CXCL9 and CXCL10 are induced by IFNγ: can a urinary chemokine assay determine AR from BKV or other causes of graft dysfunction?
- 156 subjects categorized as: healthy volunteer, stable KTX, AR, BKV, with CNI toxicity, or IFTA)

Jackson JA et al, Am J Transplant 2011; 11: 2228

- ROC curve for CXCL9: c-stat 0.87 (sensitivity 86%, specificity 80%) for AR or BKV vs other causes)
- Difficult to segregate the inflammation of BKV vs the inflammation of AR!
Acute rejection - Treatment

Steroids vs depleting Ab for acute rejection?

- Meta-analysis of 14 trials (965 patients) compared therapies for first AR episodes

- Ab was better than steroid in reversing rejection (RR 0.57) and preventing graft loss (death-censored RR 0.74)

- No difference in preventing subsequent rejection or death at 1 year

“Most trials were small, incompletely reported, especially for potential harms, and did not define outcome measures adequately.”


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<td>IVIg</td>
<td>High dose corticosteroids</td>
</tr>
<tr>
<td>Plasmapheresis</td>
<td>Depleting T cell therapy (rATG, ATGAM)</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Novel therapies</td>
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TPE/IVIg/Rituximab vs. IVIg for antibody-mediated rejection

- 24 patients with Ab-mediated rejection: all received solumedrol 500 mg x 3
- N=12: IVIg 2g/kg q 3 weeks x 4
- N=12: TPE x 4d with IVIg 100 mg/kg following TPE; then IVIg 2g/kg x 4 and RTX 375 mg/m^2 weekly x 2

% reduction in DSA at 3 mo

% Graft Survival at 36 mo

Treatment of Rejection
“standard practice”/ “in one’s experience”

- Patient with AKI:
  - US, UA/cx not revealing
  - Send blood HLA for DSA, BKV PCR, empiric treatment with corticosteroids while performing/awaiting biopsy (stain for SV40 and C4d)

- Treatment guided by biopsy:
  - TCMR Ia, Ib,IIa:
    - assess response to pulse IV corticosteroids, if improvement then continue for total 3-5 d; if no improvement after 1-2 (?) doses then advance to rATG 1.5 mg/kg x 4-14 doses
  - TCMR IIb:
    - rATG 4-14 doses
  - AHR:
    - Corticosteroids (rATG?), plasmapheresis, IVIg; consider rituxan or bortezomib
Bortezomib in the treatment of acute antibody-mediated rejection

- 6 patients with cellular and humoral rejection (C4d+, donor-specific antibodies) refractory to TPE/Rituxan/Thymoglobulin

- Bortezomib 1.3 mg/m² x 4 doses reversed rejection and reduced DSA levels

Donor-specific Ab levels (MESF)

Everly M et al, Transplantation 2008; 86: 1754
Bortezomib in combination with TPE/IVIg for antibody-mediated rejection

- 16 kidney-only and 4 kidney-combined organ recipients with de novo donor-specific antibody (DSA) and PTC C4d+ on biopsy
- IV corticosteroids followed by a 2-week cycle on days 1-4-8-11 of plasmapheresis and 1.3 mg/m² bortezomib; then IVIg 0.5 mg/kg x4.

Only two patients (10%) had undetectable DSA after treatment
Only 25% returned to their baseline renal function before AMR,
Urine protein/cr > 0.5 in 41% and > 1.0 in 18%.

Flechner SM et al, Transplantation 2010; 90:1486
Conclusions/Future Directions

• Acute rejection remains an important cause of early morbidity and later graft dysfunction/loss

• Refinements in the diagnosis of rejection (beyond Banff) are key to developing better treatment alternatives

• Many questions remain:
  – Do we treat inflammation in areas of IFTA? If so, how?
  – Do we treat +DSA? If so, when and how?
  – How to treat the patient with “subacute” antibody-mediated rejection?