I have no financial relationships with commercial interests to disclose.
I would like to thank Drs. Heather Ross and Anne Dipchand for their slides and information on the Canadian Heart Transplant Allocation System.
I am not Canadian (yet)

AND

My presentation does include discussion of off-label or investigational use of bortezomib for desensitization.

Learning Objectives

• Describe the adverse consequences of sensitization in cardiac transplant candidates.
• Explain the Canadian approach to prioritizing sensitized patients.
• Identify pros and cons of prioritizing sensitized cardiac transplant candidates.
Background: Sensitization Risks

• Circulating antibodies against human leukocyte antigens (HLA) can occur in patients awaiting heart transplant due to:
  – Blood transfusions
  – Pregnancy
  – Previous organ transplant
  – Placement of ventricular assist device (VAD)

• Sensitized patients are at risk to develop hyperacute rejection at the time of heart transplant surgery

• Sensitized patients are also at higher risk for poor outcome after transplant:
  – Decreased survival
  – Increased rejection
  – Increased hemodynamic compromise rejection
  – Increased development of allograft vasculopathy


Registry Outcomes of Patients with Pre-transplant PRAs

• Retrospective study in 8,160 heart transplant recipients
• Used data from UNOS/OPTN registry, 2000-2004
• Demonstrated relationship between higher PRA levels at time of transplant and post transplant
  – Lower patient survival
  – Higher first-year rejection rates


Multivariate Cox Proportion Hazard Regression of Patient Survival

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA (continuous variable)</td>
<td>1.005</td>
<td>1.002 – 1.009</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PRA 1% - 10%</td>
<td>1.17</td>
<td>0.96 – 1.42</td>
<td>0.12</td>
</tr>
<tr>
<td>PRA 11% - 25%</td>
<td>0.94</td>
<td>0.99 – 1.00</td>
<td>0.73</td>
</tr>
<tr>
<td>PRA &gt; 25%</td>
<td>1.4</td>
<td>1.09 – 1.77</td>
<td>0.007</td>
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</tbody>
</table>
Consensus Conference on the Sensitized Patient Awaiting Heart Transplantation

- A consensus conference was held on April 8, 2008 to:
  - Assess the current status of sensitization in the pre-transplant patient
  - The use and efficacy of desensitization therapies
  - The outcome of desensitized patients after heart transplantation

- There were 71 participants across the field of heart transplantation who represented 51 heart transplant centers throughout the world.
- Survey data was submitted by 23 of the 51 participating centers prior to the conference.

Summary of the Pre-Conference Survey Results

- 8% of patients referred for heart transplant are sensitized and require treatment.
  - 12% of patients represent 39% of the total number of sensitized patients.
- 65% of centers use the virtual crossmatch.
- 48% of centers treat pre-transplant patients with elevated anti-B cell circulating antibodies (without elevated anti-T cell antibodies).
- 48% of centers will transplant across a donor specific antibody.
- The average threshold PRA level was 35% for initiation of desensitization treatment.
  - IVIG and plasmapheresis followed by rituximab and cyclophosphamide are the more commonly methods to reduce circulating antibodies.
  - On average, 60% of sensitized patients receive treatment that results in a significant reduction of circulating antibodies.
- 73% of transplant centers used patients with successful heart transplantation.
- 45% of centers use a special protocol for immunosuppression and/or post-operative therapies for transplanted patients who were treated for sensitization prior to transplant.

Clinical Challenges

- If circulating antibody levels in the pre-transplant patient are found to be significant, when should one intervene?
- What does the ability of an antibody to bind complement mean?
- What then can be done to lower these antibody levels?
- Does lowering of antibody levels make a difference in clinical outcome?
- How do you treat desensitized patients after heart transplantation?
Bortezomib

- Reversible inhibitor of 26S proteosome (degrades ubiquinated proteins - IKB)
- Inhibits NFkB critical in transducing nuclear signals of most cytokines
- Inhibits binding of myeloma cells to bone marrow stromal cells
- Causes plasma cell apoptosis
- Bortezomib is approved by the US Food and Drug Administration for treating multiple myeloma only

Apoptosis of Plasma Cells in vitro by Bortezomib

Bortezomib Experience in Renal Transplantation

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Indication</th>
<th>Adjunctive Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idica (2008)</td>
<td>11</td>
<td>Devese post tx</td>
<td>AMR+ACE</td>
<td>Reversal of Rejection</td>
</tr>
<tr>
<td>Trivedi (2008)</td>
<td>11</td>
<td>Devese post tx</td>
<td>Cell specific indo, thio, bios, ON i anti-prolif</td>
<td>No</td>
</tr>
<tr>
<td>Sheer (2010)</td>
<td>4</td>
<td>AMR</td>
<td>No</td>
<td>No Effect</td>
</tr>
<tr>
<td>Wahner (2010)</td>
<td>7</td>
<td>AMR</td>
<td>No</td>
<td>Reversal of AMR</td>
</tr>
<tr>
<td>Sberro-Soussan (2010)</td>
<td>4</td>
<td>AMR</td>
<td>No</td>
<td>No Effect</td>
</tr>
<tr>
<td>Gaines (2010)</td>
<td>2</td>
<td>Devese pre tx</td>
<td>No</td>
<td>Mild</td>
</tr>
</tbody>
</table>

(p < 0.0001)
Current Desensitization Therapies

- High dose intravenous gammaglobulin (IVIG) has been reported to effectively lower circulating antibodies.
- Kidney transplant literature suggests plasmapheresis is helpful in the short term, but circulating antibodies return over time.
- Rituximab, a monoclonal antibody selective against CD20 on B-cells, has also been used with variable response.
- An optimal protocol has yet to be established.

The Effectiveness Of A Standardized Desensitization Protocol In Reducing Calculated Panel Reactive Antibodies (cPRA) In Sensitized Heart Transplant Candidates

- Between 2007 and 2009, 9 patients awaiting heart transplantation with cPRA > 50% were identified, utilizing a mean fluorescence intensity (MFI) threshold of 7500.
- All 9 patients were treated with:
  - Intravenous Immunglobulin (IVIG) 2gm/kg on treatment days 1 and 30 and intravenous rituximab 3g on days 7 and 21.
  - 3 patients received treatments of plasmapheresis for 5 days.
  - 2 patients received a course of bortezomib (1.3 mg/m^2), as they did not initially respond to the IVIG/rituximab treatment.


Results

- Mean baseline cPRA was found to be 73%, which following treatment, reduced to a mean level of 22% (p=0.021).
- 8/9 patients exhibited a significant reduction in cPRA.
  - The remaining patient demonstrated no reduction in cPRA following treatment, but nonetheless continued to receive therapy.

Results

Desensitization Therapy Conclusion

- The use of desensitization therapy decreases the cPRA, thus increasing the chances that an acceptable donor heart will be available for the sensitized patient awaiting heart transplantation.
- The optimal regimen for desensitization needs to be determined in controlled randomized studies.
Wouldn’t it be great to have a pilot study to see if prioritizing sensitized patients improves post-transplant outcomes?

• Rhetorical question (not AR)
Canadian Sesquicentennial

- Canada founded July 1, 1867
- Four colonies of British North America (New Brunswick, Nova Scotia, Lower Canada (Quebec) and Upper Canada (Ontario)) joined to form the Dominion or Confederation of Canada at the Charlottetown Conference in Charlottetown, Prince Edward Island.

Canadian Cardiac Transplant Network

PURPOSE: to advance the practice of cardiac transplantation and mechanical circulatory assist device therapy in Canada, including:

- Attention to national issues in cardiac transplantation (e.g. cardiac donor management/organ allocation/listing criteria/organ sharing) including the setting of guidelines, standard protocols, and the promotion of formal policies.
- Promotion of cardiac transplant and mechanical circulatory assist-related research (in both de novo and long term patients).
- Development and support of a national cardiac transplant and mechanical circulatory assist device database.
- Advocacy for patients who require or have undergone cardiac transplantation.
- Education (promoting professional development including fellowship programs/guest speakers/educational programs).

Current Listing Algorithm

* v. Dec 2012

- Status 4
- Status 4S
- Status 3.5
- Status 3
- Status 2
- Status 1
- Status 0 (On hold)
Status 4

Current
1) Mechanically ventilated patient on high-dose single or multiple inotropes±temporary mechanical circulatory support (e.g., Intra-aortic balloon pump, extracorporeal membrane oxygenation (ECMO), or Abiomed BVS 5000, or Biomedicus), excluding long-term ventricular assist devices (VAD).
2) Patient with VAD malfunction or complication, such as thrombosis, systemic device-related infection, mechanical failure, or life-threatening arrhythmia.
3) Patient should be recertified every 7 days as a Status 4 by a qualified physician, if still medically appropriate.

Historical
• Only change since 2006 is addition of the word “temporary”

Challenge
• No mechanism for “recertification” every 7 days left in algorithm

Status 4S
1) High PRA (>80% using the Canadian cPRA calculator)

Status 3.5

Current
1) High-dose or multiple inotropes in hospital, and patients not candidates for VAD therapy or no VAD available.
2) Acute refractory ventricular arrhythmias.

Historical
• Sensitized patients until 2010

Revision (Oct 2012)
• Delete “no VAD available”
Status 3

Current
1) VAD not meeting Status 4 criteria.
2) Patients on inotropes in hospital, not meeting above criteria.
3) Heart/Lung recipient candidates.
4) Cyanotic congenital heart disease with resting saturation <60%.
6) Adult-sized complex congenital heart disease with increasing dysrhythmic or systemic ventricular decline.

Historical
• CHD criteria adapted from pediatrics listing algorithm in 2006

Status 2

Current
1) In-hospital patient, or patient on outpatient inotropic therapy not meeting the above criteria.
2) Patients listed for multiple organ transplantation (other than heart-lung).

Historical
• Included cyanotic congenital heart disease and Fontan (PLE or plastic bronchitis) until 2012

Revision (Oct 2013)
• Status of multi-organ transplants

Status 1

Current
1) Adult with cyanotic CHD: resting O₂ saturation 65–75% or prolonged desaturation to less than 60% with modest activity (i.e., walking).
2) Adult with Fontan palliation with protein-losing enteropathy
3) All other out-of-hospital patients.

Historical
• 1) and 2) moved from Status 2 in 2012
Mandatory Sharing for Status 4/4S Patients

The Canadian Cardiac Transplant Network has endorsed and formalized a system whereby hearts are allocated nation-wide to the patients most in need of transplantation. The principle of the organ-sharing agreement, as outlined by the Canadian Cardiac Transplant Network, is as follows:

• The OPO will offer the donor heart to the Canadian site with the highest status recipient in the geographic area. The OPO will also notify the Canadian program(s) with a potentially appropriate Status 4 or 4S recipient(s) nationwide of the potential donor heart. Mandatory discussion in a timely fashion, physician to physician, will ensue to allocate the organ, the principle being that the recipient with the longest current listing as Status 4 be given priority. If consensus is not reached, final allocation will be made by the center to which the heart was originally offered.

• If an organ becomes available in a province without a cardiac transplant program (adult or pediatric), it will be offered to the program with the longest listed patient from that province. That transplant program will then follow the established organ allocation algorithm.
Mandatory Sharing for Status 4/4S

Mechanical Circulatory Support

Contentious Issues/Challenges

- VAD not eligible for Status 4:
  - on inotropes (2005)
  - intubated for implantation (2005)
  - with GI bleeding (2010)
  - with superficial driveline infection (2010)

- Rehab period following VAD implantation:
  - minimum 3 weeks Status 0 (on hold)
  - some centres waiting 3-6 months in some patients

- VAD “not available”:
  - historical (no longer acceptable)

Mechanical Circulatory Support

Current Discussion

- Should stable outpatient VADs even be a Status 3? Are they disadvantaging Status 2 patients?
  - Ongoing discussion

- Can a VAD outpatient have a “complication” warranting a Status 4 listing?
  - Consensus is NO – algorithm being revised
Sensitized Patients

• 2006 – Status 3.5
  High PRA (>80%), or PRA >20% with 3 prior positive crossmatches (in the setting of negative virtual or actual donor/recipient specific crossmatch and appropriate size and blood type of the prospective donor).

• Became “4S” in 2010 after extensive discussions
  • Definitions
  • Impact on organ allocation to high status patients
  • Practical implementation
  • Access to national pool of donors

Sensitized Patients - II

• 2012 – revised to current wording
  High PRA (>80% using the Canadian cPRA calculator )

• Programs may elect to list a highly sensitized patient as a 4S but MAY NOT cross a prospective positive virtual (or real) crossmatch

Sensitized Patients - III

• Unacceptable HLA Antigens, are antigens to which the recipient has HLA antibodies that the HLA laboratory has determined are present and clinically significant.

• Indeterminate HLA antigens are antigens to which the recipient has reactivity in the HLA antibody assay, of undetermined, or unknown clinical significance.
Sensitized Patients - IV

- **cPRA** for the purpose of 4S status will be calculated based on **Unacceptable HLA Antigens** to HLA-A, B, C, DRB1, DRB3/4/5 and DQB 1 ONLY.
- Antibodies to DQA1, DPA1 and DPB1 will not be considered in the cPRA calculation.
- Indeterminate HLA Antigens will not be considered in the cPRA calculation.

Sensitized Patients - V

- **Current** unacceptable antigens are those detected in the recipients **most recent serum** and entered in the registry.
- **Cumulative** unacceptable antigens are those detected in **all sera tested** and entered in the registry.

Current Approach to 4S

- If a program wishes to list a patient as 4S to have access to the national pool of donors, then:
  - The patient must have a cumulative Class I and II cPRA of >=80%. AND
  - The patient will not be permitted to have a positive VXM to ABC or DRDQb.

- If a program wishes to cross a VXM to any or all of the ABC, DRDQb and DRDQp (either class I or Class II or both):
  - This is permitted, BUT the patient **cannot** be listed as a 4S.
  - This is regardless of whether a patient would have "met" the 4S criteria.
Sensitization
Controversies/Challenges

• Desensitization
  – Virtually no support for sharing if ANY antigen is crossed (historical or present)
  – The recalculated cPRA needs to remain >80% to remain a status 4S (for the purposes of mandatory sharing)

Conflict Resolution and QA

• In the event of a dispute in organ allocation between centres, then a member of the current CCTN Executive is to serve as a mediator and can be contacted 24/7 by the physicians and surgeons participating in the organ allocation discussion.

• All Status 4 and 4S patients, whether adult or pediatric, are reviewed annually at a meeting of representatives from all of the transplant centres (i.e. Canadian Cardiac Transplant Network meetings) as a means of quality assurance.

2015 4S Update

• 96 patients were listed status 4S from January 2010 to September 2015.
  – 52 were transplanted as status 4S,
  – 7 were transplanted as a different status,
  – 5 de-listed,
  – 4 died waiting and 28 remain active.

• Of 52 transplants,
  – Mean age was 47 years; 46% male
  – 44% had dilated cardiomyopathy
  – Blood group O -42%
  – 53% had a VAD as BTT
  – All patients received induction
  – Maintenance immunosuppression was standard and included tacrolimus and MMF, in addition to prednisone.

Clarke et al, ISHLT 2016 on behalf of CCTN
2015 4S Update

- Mean follow up - 28 months (1 week – 5.3 years),
  - 9 patients died (17%)
  - Primary graft failure/AMR accounted for 1/3 of deaths.
- Kaplan-Meier 1-year survival - 88%
- AMR occurrence
  - Pathologic AMR - 12 patients (23%)
  - Clinical AMR - 3 patients in the first year post-transplant (5.8%)
  - Only clinical AMR was treated
- 17% of patients developed de novo DSA and demonstrated no correlation to AMR (clinical or pathologic).
- 32% of patients had at least 1 - 2R cellular rejection in the first year and 15.4% of patients had CAV 1 at follow up

Limitations of Current Antibody Evaluation

- "Biologically speaking, antibody strength refers to the intensity of affinity and avidity for a particular antigen–antibody complex."
  - kinetics of antigen–antibody binding, or more accurately, antigen–antibody dissociation
- MFI challenges
  - Not transferable between centres
  - Not strictly quantitative
  - neat MFI values do not always accurately depict antibody strength
  - 'prozone' effect – interference with binding of the secondary detection reagent, giving false-negative results
  - EDTA treatment (6%) does not always remove all inhibitory factors
- C1q assay limited by low sensitivity and inability to detect the presence of weak antibodies
- Titration studies - costly

Potential challenges to high priority for the highly sensitized.....

- Role of mean fluorescent intensity (MFI) antibody titer
  - PRA methods – 2008-2011 standardized across various Canadian laboratories – align practices for calling ‘antibody’
  - Natural alignment with proficiency testing in 2012, 2015
- What do pre-formed antibodies actually mean?
  - Not all circulating anti-HLA antibodies are necessarily bad
  - MFI, C1q binding
- Should all eligible highly sensitized patients be tried on desensitization therapy to lower antibody levels before being listed 4S?
  - There are clearly pts where desensitization may be only option
  - Why cross an antibody if you can avoid it?
  - We have no RCT on desensitization, uncertain timing of transplant, toxicity/cost of therapies
Can or should we do this in the United States?

Pros

• Allows patients who presently have prolonged waits for transplant with concomittant deterioration on waiting list.
• Seems to work equitably with decent outcomes in Canada

Cons

• Canada and the US are different
Cons

• Much larger US population with multiple competing centers in same city or region (i.e., Philadelphia Metropolitan area has four heart transplant centers and Toronto Metropolitan area has one though they are the same size in population.

Cons

• How do we define sensitized patients who should be prioritized?
• What assays do we use?
• Didn’t we just go through a revision of the UNOS heart allocation system?

Cons

• Could do this within UNOS regions
• Smaller geographically and with history of collaboration via Regional Review Boards
• Sensitization can be incorporated into a Heart Allocation Score as part of a future, ideal heart prioritization system.
• Can follow outcomes from the 4S Canadian “pilot” study.