Moving from Manual to Automated Standardization of Anti –A & Anti-B Titration in ABOi Renal Transplantation

Dr. Ravindra Pratap Singh
Consultant Transfusion Medicine
MD- Transfusion Medicine (PGIMER, Chandigarh)
Fellow in Transfusion Science, UK

ADITYA BIRLA MEMORIAL HOSPITAL (ABMH), PUNE

19TH NOVEMBER 2016 : TRANSMEDCON2016BHOPAL
Introduction

- Transplantation has established itself as the superior mode of renal replacement therapy, with respect to both –outcomes as well as cost-effectiveness.

- The first successful kidney transplantation was performed by Murray in 1954 between identical twins after confirming their genetic identity with skin grafts.

- Several advancements have been made since then in the field of renal transplantation, making it the preferred form of renal replacement therapy.
BARRIERS TO RENAL TRANSPLANTATION

- HLA sensitization

- ABO incompatibility (ABOi)

  **ABO Barrier:**

  1. Nearly 30% of patients on the kidney waiting list are sensitized due to previous transplant, blood transfusion, or pregnancy.

  2. There is a 35% chance that any 2 individuals will be ABO incompatible:

     - 1/3 of potential live donors are excluded immediately due to ABO incompatibility.
# ABOi Renal Transplantation Chart

<table>
<thead>
<tr>
<th>BLOOD GROUP</th>
<th>PATIENT</th>
<th>DONOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>O</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>A</td>
<td>◯</td>
<td>✓</td>
</tr>
<tr>
<td>B</td>
<td>◯</td>
<td>◯</td>
</tr>
<tr>
<td>AB</td>
<td>◯</td>
<td>◯</td>
</tr>
</tbody>
</table>
Rejection of the transplanted organ remains the major limitation to transplantation success.

Rejection refers to the recognition of, and immune response to, the allograft by the recipient’s immune system, resulting in allograft destruction.

**Transplantation antigens:**

- Major histocompatibility complex (MHC)
- **ABO blood group antigens**
- Monocyte and endothelial cell antigens
- Minor transplantation antigens
Cellular events leading to allograft rejection
Cellular events leading to allograft rejection

- **Hyperacute rejection:**
  - It is caused by pre-formed recipient antibodies reacting with antigens on the endothelial surface of the allograft, activating the complement and the coagulation cascades.
  - These antibodies are directed against antigens of **ABO blood group** systems or against **HLA class I antigens** (Positive T cell Crossmatch).

Proposed Mechanism of Hyper acute rejection in ABOi organ transplantation
Cellular events leading to allograft rejection

• **Accelerated acute rejection:**
  - It refers to rejection episodes occurring 2 - 5 days post transplant and is mediated by the **humoral immune system**.
  - There is pre-transplant sensitisation of recipient to donor antigens; however, antibody titres are too low to give a positive lymphocytotoxicity crossmatch, but ‘second set’ antibody production occurs rapidly after transplant.
  - Patients who have **negative lymphocytotoxicity crossmatch**, but **a positive flow cytometry crossmatch** are at a greater risk of developing this form of rejection.

• **Acute rejection:**
  - Acute rejection is defined as an immunological process resulting in a serum creatinine increase of ≥ 0.4 mg/dl over baseline and histological confirmation.
Current practice

Accommodation

- ABOi renal graft functioned well without rejection in recipients having high titers of anti – A/B antibodies – “Accommodation”
  - It is regarded as an acquired resistance of an organ to immune mediated damage.

- The mechanism of accommodation is yet to be elucidated. It was postulated that accommodation might be involved in (Lynch & Platt, 2008):
  - Change in antigen & antibodies.
  - Modified control of complement.
Current practice

A. Induction and Antibody reduction Therapy

B. Determine isoagglutinin Titer
A. Induction and Antibody reduction Therapy

Figure. Protocol for preoperative and postoperative immunomodulation and immunosuppression for ABO-incompatible transplant recipients. IVlg indicates intravenous immunoglobulin; PP, plasmapheresis.
B. Determination of isoagglutinin titer

- Accurate measurement of isoagglutinin titer is an important aspect for successful ABO-I kidney transplantation. If the isoagglutinin titer is underestimated compared to the actual titer of patient, we could consider a patient as safe for transplantation and it could lead to rejection or short duration of allograft survival (Crew & Ratner, 2010).

- IgM antibody mediates complement activation and endothelial damage in AMR, and it is more rapidly removed by plasmapheresis than IgG. However, IgG titers are more emphasized for patient eligibility, rejection risk, and plasmapheresis guidance.

- Reporting both IgM and IgG titers has been recommended by a working group from US centers (Montgomery et al., 2004).

- Importantly, measured titers are method-dependent and considerably variable according to assays.

- The goal of isoagglutinin titer to prevent hyperacute rejection is variable across transplantation centers, ranging from $\leq 1:8$ to $\leq 1:32$ before transplantation (Crew & Ratner, 2010).

- In our institution, the titer is lowered to $\leq 1:8$ before transplantation.
Background & Aims

- Various methods are available and mostly conventional, are used in transplant immunology centers worldwide.
  - ABO antibody titration variation are higher with conventional manual and semi automated available methodologies which will have direct impact on grafted renal survival.

- The study was conducted from August 2015 to September 2016 at Department of Transfusion Medicine, Aditya Birla Memorial Hospital, Pune.
  - The aim is to standardized automated ABO antibody titration by SPRCA method, NEO, Immucor Gamma, USA with traditional tube and CAT technology.
  - We performed IgM & IgG ABO antibody titration for renal transplant patients by conventional tube (saline & AHG) & CAT, Biorad, Switzerland (Saline & AHG) and same compared with ABO IgM & IgG antibody titration on automation by Solid Phase Red Cells Adherence (SPRCA) Technique on Neo, immucor Gamma, USA.
Material & methods

- Population in Study:
  - No. of patients - 20
    - Male – 19 (95%); Female – 1 (5%)
    - Age: 9 Years to 62 Years (Average – 37.4 Years)
    - Blood Type (N=20):
      - O – 10
      - A – 3
      - B – 7

<table>
<thead>
<tr>
<th>Blood Group - Patient</th>
<th>Blood Group - Donor</th>
<th>Total (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>A</td>
<td>7</td>
</tr>
<tr>
<td>O</td>
<td>B</td>
<td>2</td>
</tr>
<tr>
<td>O</td>
<td>AB</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>AB</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>AB</td>
<td>2</td>
</tr>
</tbody>
</table>
Material & methods

• A total of 98 times serial dilution and titration of anti-A &/or anti-B antibodies were performed by tube and CAT (Biorad, Switzerland) technique and same was performed by SPRCA, Neo, immucor Gamma, USA.

  – Starting isoagglutinin titer:

    • Mean: 1:128
    
    • Range 1:1 to 1:1024
Material & methods

- Principle of Conventional Tube, CAT & SPRCA Antibody Titration
Material & Methods

• Preferred Sample at our centre:
  – Serum (clotted) for manual methods.
    • For IgG antibody titration – In EDTA plasma sample, precipitation of proteins leads to false positive results.
  – Plasma (EDTA) good for automated system – Neo Immucor Gamma, USA.

• Removal of IgM antibodies:
  – Available methods – use of sulfahydrl reagents (DTT/2ME)
  – Heat Inactivation of IgM antibody¹:
    • Study recommended serum treatement at 63 °C for 10 minutes completely inactivate IgM abs and IgG testing results were comparable to DTT treated serum.
    • We use dry heating block incubator and on validation IgM inactivation achieved at 63 °C for 15 minutes.

Anti – A/B Antibody Titration Process Manual Method

Diluted pt’s serum

Scheme for serial dilutions for titration of serum

Serum

LISS/NS

Scheme for serial dilutions for titration of serum
Anti – A/B Antibody Titration Process Manual Method
Anti – A/B Antibody Titration Process on Neo, Immucor Gamma

IgM Ab Titration

IgG Ab Titration
Anti – A/B Antibody Titration Process on Neo, Immucor Gamma
Anti – A/B Antibody Titration Process on Neo, Immucor Gamma
IgM Titer by SPRCA
IgG Titer by SPRCA
IgG Titer by SPRCA
# Result

<table>
<thead>
<tr>
<th>Titration Difference</th>
<th>Tube Vs CAT</th>
<th>Tube Vs SPRCA</th>
<th>CAT Vs SPRCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2</td>
<td>92</td>
</tr>
<tr>
<td>1</td>
<td>92</td>
<td>93</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>98</td>
<td>98</td>
</tr>
</tbody>
</table>

## IgM Titration Difference

- **Tube Vs SPRCA**
- **CAT Vs SPRCA**
- **Tube Vs CAT**

### IgM A/B Antibody Titration Comparison

![Graph showing IgM Titration Difference](image)
## Result

<table>
<thead>
<tr>
<th>Dilution Difference</th>
<th>Tube Vs CAT</th>
<th>Tube Vs SPRCA</th>
<th>CAT Vs SPRCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>91</td>
</tr>
<tr>
<td>1</td>
<td>93</td>
<td>92</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>98</td>
<td>98</td>
</tr>
</tbody>
</table>

IgG A/B Antibody Titration Comparison

![Graph showing the comparison of IgG A/B Antibody Titration](image)

- **Tube Vs CAT**
- **Tube Vs SPRCA**
- **CAT Vs SPRCA**
Discussion

• In our study, ABO antibodies titration was done on all three methods (tube, CAT & SPRCA) and results compared our automated titration method with tube and CAT methods.

• At our hospital, pre transplant critical titer is considered as 1:8.

• In IgM anti-A &/or anti-B titration, of 98 tests, in tube method recorded low as compared to CAT & SPRCA (difference of 1 dilution in 92 tests and 2 dilution in 6 tests), while in IgG anti-A &/or anti-B titration, of 98 tests, in tube method, titration difference with CAT & SPRCA (difference of 1 dilution in 93 tests and 2 dilutions in 3 tests and 3 dilutions in 2 tests) is significant.
# Discussion

## Comparison of Various Titration Methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tube method</th>
<th>Column agglutination</th>
<th>SPRCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A column ingredient</td>
<td>Not needed</td>
<td>Sephadex gel or glass bead</td>
<td>Immobilized RBC Membrane on polystyrene Microwells</td>
</tr>
<tr>
<td>Use of RBC</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Antihuman globulin</td>
<td>Yes</td>
<td>Yes</td>
<td>IgG coated RBCs (Indicator Cells)</td>
</tr>
<tr>
<td>Secondary antibody</td>
<td>No</td>
<td>No</td>
<td>Yes (IgG)</td>
</tr>
<tr>
<td>Deletion of IgM</td>
<td>DTT/2ME/Heat Inactivation</td>
<td>DTT/2ME/Heat Inactivation</td>
<td>Not Needed</td>
</tr>
</tbody>
</table>
| Interpretation              | **IgM & IgG: Agglutination** | **IgM & IgG: Agglutination** | **IgM**: Agglutination  
**IgG**: Uniform adherence of IgG coated RBCs on reaction surface |
| Result                      | Titer       | Titer                | Titer                                                                |
| Instrument                  | Not Needed (Manual) | Semi-automated        | Needed                                                               |
| Cost                        | Low         | Intermediate         | Intermediate                                                         |
| Assay time                  | IgM: 20 – 30 min  
* IgG: 90 – 120 min | IgM: 30 – 40 min  
* IgG: 60 – 90 min | IgM: 15 – 20 min  
IgG: 40 – 50 min |

*Include heat inactivation of IgM antibody & process time*
The accuracy and precision is best with automation as compared to conventional tube and CAT technique.

Both conventional and CAT techniques were cumbersome, time taking and carries higher chances of variation of results reporting due to interpersonal variation in techniques and interpretations.

Automated SPRCA is on the other side more convenient, free from variation as right from sample dilution to addition of reagent and interpretation is done by machine.

Due to constant and reproducible results were observed on SPRCA than conventional tube and CAT technique, we have successfully shifted on automation from traditional methods.
THANK YOU