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# Immunoadsorption in ABO-incompatible kidney transplantation in adult and pediatric patients with follow-up on graft and patient survival: First series from India

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**Abstract:**

**BACKGROUND:** There are no published reports on desensitization protocol for ABO-incompatible kidney transplants using Immuno-Adsorption (IA) plasmapheresis from India. IA offers certain advantages including processing of larger plasma volumes, quicker reduction of isoagglutinin titers and no requirement of replacement fluids.

**AIMS AND OBJECTIVES:** Authors' center evaluated success of desensitization protocol, and graft/patient outcomes when IA procedures were performed for desensitization in adult and pediatric ABO-incompatible kidney transplant patients.

**METHODS:** Patients undergoing ABO-incompatible kidney transplant with use of IA were evaluated at tertiary care center in north India. Patient records for 2-years were collated from hospital information system (HIS) and procedure forms.

**RESULTS:** Sixteen IA procedures were performed in five patients who underwent successful ABO-incompatible kidney transplant. Initial isoagglutinin IgG titer ranged from 32-512. Mean number of IA procedures performed to achieve the desired pre-transplant IgG titer ≤8 was 3.2. New IA column was used for each patient (and re-used for the same patient, if needed, after sterilization with Low temperature steam of formaldehyde). Mean plasma volume processed during each IA procedure was 4.5 times. No adverse events were observed during any IA procedure. All patients achieved successful desensitization. All patients continue to do well clinically with mean follow-up period of 8.8 months. Although IA was expensive, it offered advantages like specificity, larger plasma volume processing with desired reduction in titer, no 'replacement fluid' requirements and no adverse events in present case series.

**CONCLUSION:** IA plasmapheresis was universally successful in decreasing the ABO-isoagglutinin titers to desired level in all prospective ABO incompatible kidney transplant patients.

**Keywords:**

ABO incompatible, desensitization, immunoadsorption, kidney transplant, titer

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## Introduction

Unlike western countries, where deceased donor transplant is a norm and a national network manages the transplant waiting list-matching donors to recipients, India has predominantly living-related kidney donors from immediate family.[1] These "willing" living donors in the family are sometimes rendered unsuitable due to



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ABO blood group mismatch. ABO incompatibility is used to pose a considerable obstacle to expansion of donor pool around three decades back. Over a period of time, ABO-incompatible (ABOi) transplants have emerged as an alternative with numerous studies worldwide proving that long-term grafts and patient survival after ABOi transplant is comparable to ABO-compatible (ABOc) transplant.[2,3]

These comparable results have been achieved through desensitization that is usually achieved by B-cell depleting therapies and therapeutic apheresis besides conventional triple immunosuppression.[3] Rituximab, commonly used B-cell depleting therapy, suppresses new antibody production, while therapeutic apheresis removes preexisting blood group antibodies. Therapeutic apheresis technique for the removal of antibody already present in the body has evolved from non-selective conventional therapeutic plasma exchange (cTPE) to semi-selective cascade plasmapheresis (CP)/double-filtration plasmapheresis (DFPP) to highly selective immunoadsorption plasmapheresis (IA).[4] We would like to present our initial data on outcome results of ABOi kidney transplants using IA.

## Materials and Methods

### Settings

The study was performed in a tertiary health care center from January 2017 to December 2018. The study population comprised ABOi-kidney transplants during the study period that required IA as part of desensitization protocol.

### Transplantation of Human Organs and Tissues Act, 2014, India

In India, most of the solid-organ transplants are living-donor related. Transplantation of Human Organs and Tissues Rules, 2014[5] restricts organ donations to near-relatives living donors (including spouse, children, siblings, parents and grandparents) to curb the organ commercialization. Deceased donor program is still in budding stages and limited to very few institutions.[6] All prospective transplants have to be pre-approved by a local Organ Transplant Authorization Committee.

### Patient and donor selection with consent

Patients with end-stage renal disease who did not have ABOc donor in the family were informed about the ABOi transplant program. They were explained about the process of ABOi living donor transplant including the immunosuppressant regime and IA protocol with its potential benefit in reducing antibody titer and possible adverse effects such as citrate effect and changes in blood pressure. Informed consent was obtained from all patient–donor pairs who agreed to enroll in ABOi transplant program. All the prospective organ donors underwent extensive medical and psychological assessment in accordance with the institutional protocol. The donors who qualified these assessments were briefed about the transplant surgery, its duration, risks, length of stay in the hospital, etc. The donors then provided written consent for organ donation.

### Histocompatibility testing

In accordance with the THOTA, 2014, all patient–donor pairs underwent human leukocyte antigen typing to prove relationship. They also underwent antihuman globulin complement-dependent cytotoxicity (AHG-CDC) crossmatch and flow cytometry crossmatch (FCXM) for T-cell and B-cell to establish organ compatibility. All patient–donor pairs that had both AHG-CDC and FCXM negative were cleared for transplant. Posttransplant biopsy and Luminex single antigen bead assay were performed in patients with clinical signs suggesting of graft dysfunction or rejection.

### Desensitization protocol

The desensitization protocol consisted of immunosuppression and IA plasmapheresis.

### Rituximab and triple immunosuppression (immunosuppressant regime)

The immunosuppression regime was started with rituximab (anti-CD20 drug) that was administered as a single dose about 7–14 days before planned date of IA plasmapheresis to inhibit formation of new antibodies. Thereafter, IA was initiated to remove the existing blood group antibodies till the titer of 8 or lower was achieved. The other three immunosuppressive drugs (mycophenolate mofetil [MMF], tacrolimus, and glucocorticoids) were initiated before the surgery as per the standard hospital protocol. These three drugs were also used in ABOc kidney transplants.

Case 1 (pediatric patient): A single dose of rituximab (100 mg) was administered 2 weeks before transplantation. The induction regimen included two doses of basiliximab (10 mg on day 0 and day 4) and methylprednisolone (10 mg/kg) followed by oral prednisolone. The combination of tacrolimus (0.05 mg/kg) and MMF (600 mg/m$^2$/dose) was started 2 weeks before the transplant. A target tacrolimus blood level of 9–12 ng/mL (first 3 months) and 6–8 ng/mL (next 3 months) was maintained postoperatively.

Case 2–5 (adult patients): Oral MMF/MMF-S (500/360 mg/twice a day) and tacrolimus (0.05 mg/kg/day in two divided doses) were started 7 days before transplant day. One day before transplant, dose for both drugs is

doubled-MMF/MMF-S (1000/720 mg/twice a day) and tacrolimus (0.1 mg/kg/day in two divided doses). Methylprednisolone was administered perioperatively as single pulse dose of 500 mg. Posttransplant, tacrolimus trough level was maintained between 8 and 12 ng/ml till 3 month, 6–8 ng/ml till 6 months, and 5–7 ng/ml after 6 months. Prednisolone was started at 40 mg/day on day 1 and tapered to 20 mg/day at discharge. Thereafter, prednisolone is tapered from 20 mg/day to 5–7.5 mg/day over 3 months. Desensitization protocol in these patients additionally included IVIg and basiliximab/antithymocyte globulin (ATG) induction. After the last plasmapheresis, IVIg (0.4 mg/kg) was given one night before transplant. Basiliximab was given as (20 mg) first dose on day 0 (in operating room) and second dose on day 4. ATG was given as (3 mg/kg body weight) first dose on day 0 (in operating room) and second dose on day 2.

## Immunoadsorption

IA was initiated after median of 10 days (range, 7–14 days) after rituximab administration. IA consisted of centrifuge separating patient's plasma as the first step and passing it through a biospecific affinity IA column as the second step. The first step was performed using plastic disposable kit (P1R, Fresenius Kabi, Germany) on the apheresis equipment COM.TEC (Fresenius Kabi, Germany). The IA column used was Glycosorb®-ABO (Glycorex Transplantation AB, Sölvegatan 41, SE-223 70 Lund, Sweden). This column comes with tubing that is compatible and was used in conjunction with P1R kit. The attachments were made under sterile conditions (laminar airflow) in a manner that the separated plasma would be the "inflow" to the filter and the adsorbed plasma would be "reinfusion" in the P1R kit and thus, completing the circuit. The IA column was placed in a manner that flow of plasma through IA column was against gravity.

During the IA procedures, all patients were slowly infused 10% calcium gluconate (prophylactic; 10 ml diluted in 50 ml normal saline for every 1000–500 ml plasma processed) to counter the adverse effects of citrate.

## Glycosorb column

The Glycosorb ABO column is a biospecific low-molecular carbohydrate column with A or B blood group antigen linked to a sepharose matrix. The column specifically depletes anti-A or anti-B antibodies without any apparent side effects. Glycosorb-A column depletes anti-A antibody and Glycosorb-B column depletes anti-B antibody in the recipient. There is no loss of plasma in IA procedure and therefore there is no requirement of "replenishment fluid."

## Reuse of immunoadsorption column

The column was rinsed with normal saline before and after each procedure. The column was sterilized with low temperature steam of formaldehyde after each procedure for reuse. The column, if required, was reused for the same patient for maximum of four times. Patients were explained about reuse explicitly and their written consent was obtained before each procedure.

## ABO antibody titer

The blood group antibody titer was done by column agglutination technology (Ortho-Clinical Diagnostics). The cassettes used were anti-IgG, anti-C3d, and polyspecific (Ortho BioVue System, Ortho-Clinical Diagnostics, High Wycombe, UK), and the technique was low ionic salt solution indirect antiglobulin test (LISS-IAT). The procedure was the same as published previously,[7] and only IgG was considered to decide upon patient management. The titer was done before and after each IA procedure, daily for 7 days posttransplant, and at least twice weekly till 6 weeks posttransplant.

## Ethical clearance

ABOi kidney transplant has become "standard of care" for patients. The personal identifiers were removed before evaluation of data and complete confidentiality was maintained. The Institutional Review Board has approved the study protocol including reuse of column. The column features on the "list of single use devices that can be reused" issued and maintained by hospital infection control committee (HICC). HICC has laid down the provision that it can be used in the same patient (autologous) for a maximum of four times after proper sterilization, after each use.

## Statistical analysis

To summarize the data, mean and median were used for continuous variables, whereas counts and percentage were used for categorical variables.

## Follow-up

Patients were on regular follow-up for any clinical symptom or sign. Posttransplantation antibody titers were done daily for 1st week and then at least twice weekly for 6-weeks. Serum creatinine (S. Cr) and urine output were monitored in postrenal transplantation patients. Follow-up was measured as graft and patient survival.

# Results

During the study period, 16 IA procedures were performed in total five patients who underwent successful ABO-incompatible kidney transplantation. The demographic details of the patients and donors including their blood group and relationship are described in Table 1. Four of the 5 (80%) organ donors

**Table 1: Demographic Details**

| Patient | | | | | Donor | | Antibody | Baseline Titer |
|---|---|---|---|---|---|---|---|---|
| Case Number | Age | Gender | Diagnosis | Blood group | Relationship with patient | Blood Group | ABO antibody | Titer IgG |
| Case 1 | 3 | F | VUR induced CKD-5 | B Pos | Mother | A Pos | anti-A | 32 |
| Case 2 | 23 | M | CKD-5 on MHD | O Pos | Mother | A Pos | anti-A | 256 |
| Case 3 | 36 | M | CKD-5 on MHD | O Pos | Father | B Pos | anti-B | 512 |
| Case 4 | 39 | M | CKD-5 on MHD | O Pos | Mother | A Pos | anti-A | 256 |
| Case 5 | 59 | M | CKD-5 on MHD | O Pos | Sister | A Pos | Anti-A | 512 |

*M-Male; F-Female; MHD- Maintenance Hemodialysis

**Table 2: Procedure Details**

| Case Number | Base-Line Titer | Total IA procedures | Pre-Surgery Titer | Weight of patient (kgs) | Blood volume of patient (mls) | Mean blood volume processed/ procedure (mls) | Mean Plasma Volume processed/ procedure (times) |
|---|---|---|---|---|---|---|---|
| Case 1 | 32 | 2 | 4 | 10.5 | 715 | 2860 | 4.0 |
| Case 2 | 256 | 4 | 8 | 52.9 | 4765 | 19536 | 4.1 |
| Case 3 | 512 | 4 | 8 | 50.3 | 4465 | 21208 | 4.75 |
| Case 4 | 256 | 3 | 4 | 76 | 5625 | 22129 | 5.0 |
| Case 5 | 512 | 3 | 8 | 55 | 5187 | 23806 | 4.6 |

**Table 3: Follow-up of patients**

| Case Number | Days from Sx to Dx | Creatinine at Dx | Titer at Dx (IgG) | Total Follow-up (in days) | Creatinine at last follow-up | Titer at last follow-up (IgG) |
|---|---|---|---|---|---|---|
| Case 1 | 8 | 0.5 | 8 | 560 | 0.6 | 8 |
| Case 2 | 8 | 1.9 | 8 | 395 | 1.6 | 8 |
| Case 3 | 9 | 1.2 | 4 | 210 | 2.0 | 8 |
| Case 4 | 9 | 1.5 | 16 | 120 | 1.6 | 8 |
| Case 5 | 8 | 1.2 | 8 | 30 | 1.1 | 4 |

* Sx- Surgery; Dx- Discharge

were female. Cases 2–5 were on dialysis at the time of transplantation.

The pre-IA isoagglutinin IgG titer ranged from 32 to 512. Median number of IA procedures performed to achieve the desired pretransplant IgG titer ≤8 was 3 (2–4). The mean plasma volume processed by each IA procedure was 4.3 (2–5) times. Table 2 describes the details of IA procedures performed in these four patients. No adverse events (citrate toxicity/blood pressure fluctuation) or complications (fever/infection) were observed during pretransplantation IA procedure.

A new IA column was used for each patient (and successfully reused for the same patient, if needed, after sterilization with ethylene oxide). Overall, the authors used one column per patient. Five columns were used for total 16 IA procedures (mean: 3.3 procedures per column).

In the posttransplantation phase, case 1 presented with symptoms of urosepsis and rising antibody titer. Investigations revealed *Escherichia coli* (sensitive to meropenem). Prompt antibiotic therapy was instituted. In view of a continuing uptrend in titers (8 → 64), two sessions of CP were instituted with a consequential decline to 16. All the laboratory parameters had normalized (S. Cr 0.4 mg/dL), and there was no evidence of graft dysfunction at the time of discharge. No posttransplantation plasmapheresis procedure was performed in any other case. No signs and symptoms of antibody-mediated rejection reported in any patient. All four ABOi kidney transplant patients continue to do well clinically with a mean follow-up period of 11 months (4–19). Table 3 provides the details of time period of patient follow-up including S. Cr and antibody titer at the last follow-up.

## Discussion

### Successful desensitization using immunoadsorption

ABOi kidney transplants have become a successful alternative "standard of care" for patients who do not have suitable ABOc donor. ABOi transplants have achieved long-term graft and patient survival results comparable to ABOc transplants.[3] This is, to the best of authors' knowledge, the first report on successful use of IA plasmapheresis for desensitization in ABOi

kidney transplants from India. In the present report, IA plasmapheresis successfully achieved the target ABO antibody titer in all four patients and thus allowing kidney transplant.

The desensitization protocol, required to achieve patient–donor ABO compatibility, is primarily based on reduction of antibody production and removal of already present antibody in the system. Rituximab (monoclonal antibody against a B-cell surface marker; anti-CD20) reduces new antibody production by inhibiting B-cell. On the other hand, plasmapheresis can remove already present antibody in the recipients' blood. Previous reports have successfully used cTPE/CP/DFPP to achieve the desired pretransplant ABO antibody titer. However, these procedures come with inherent drawbacks. These procedures are either nonselective or semi-selective and also result in loss of "desirable" proteins including albumin, coagulation factors, and protective antibodies. These drawbacks were overcome with recent availability and subsequent use of IA at authors' institute.

## Acceptable titer before surgery

Acceptable titer differs from one institute to another; most of the published reports have given ≤4 to ≤32 as acceptable titers.[7] The target titer of anti-ABO antibodies immediately before transplant was ≤4 in the Stockholm and Freiburg groups.[8,9] Guidelines for antibody-incompatible transplantation by the British Transplantation Society[10] recommend that pretransplant hemagglutination titer ≤8 as acceptable titer. The present study, therefore, used pretransplant titers as ≤8 as acceptable. This is further strengthened by reports from various centers across India including authors' center.[1,4,11-13]

## Advantages of immunoadsorption

### *Selectivity*

IA selectively removes the specific ABO antibody by adsorbing the antibody onto the antibody-specific antigen. This technique only removes the antibody, thus leaving other molecules and proteins (including albumin, coagulation factors, and protective antibodies) in recipients' blood. Selectivity allowed larger volumes to be processed that resulted in higher reduction in antibody titers. The authors processed large plasma volumes per IA procedure (mean 4.5 volumes) and reused the column (up to 4 times) for the same patient. This translates into shorter period for desensitization, early surgery, and shorter hospital stay.

### *No replacement fluid*

cTPE/CP/DFPP requires replacement fluid in the form of fresh frozen plasma (FFP), normal saline, or albumin. In IA, recipients' plasma is neither exchanged and replaced (as in conventional TPE) nor is lost (as in CP), therefore negating any need for replacement fluid. This translates into multiple advantages for the patient. First, larger plasma volumes can be processed without worrying about transfusion of excessive donor FFP as compared to cTPE and CP. Second, no 'replacement fluids' precludes FFP related adverse transfusion events, thus enhancing the safety of the IA procedures.

## Limitations of immunoadsorption: No "negative-balance"

The entire patients' plasma, after passing through the IA column, is returned back to the patient. This does not permit "negative balance" during the procedure, which is sometimes desirable considering fluid retention and edema. In addition, the patient is being given citrate for anticoagulation and calcium infusion to counter citrate toxicity during the procedure. This can make processing of large volumes (more than five plasma volume) difficult in patients, especially in "anuric" patients. Citrate anticoagulant and calcium infusions may result in volume overload, edema, and even congestive heart failure. The authors, therefore, limited processed volume per procedure to maximum five plasma volumes though the titer reduction through IA could have been more efficient, say at seven plasma-volumes processed.

## Reuse of column and monetary savings

The use IA is limited by its' relatively higher cost in comparison to cTPE and CP. In a country like India, where patients must spend "out-of-pocket" and there is no government supported insurance program, affordability is an issue and routine use of IA is very limited. The authors have tried to overcome this limitation by reusing the column. Although manufacturers recommend single use, the authors reused the column for the same patient and achieved good results. Reuse of IA column is reported from Switzerland[14] as well as India.[11] If cost was not an issue, it would have been prudent to use it according to the manufacturers' instructions and prefer use of IA in patients with high antibody titer (≥256), and cTPE/CP for patients with lower titer (≤128). However, since the use is presently guided by the only consideration of "affordability," the use is limited to patients who can afford the cost of IA column.

## Post-op therapeutic plasma exchange

While few earlier studies[15,16] recommend protocol driven postsurgery TPE, our experience suggest that it is not warranted and should only be considered in situations where titer rebounds to ≥32 along with clinical signs and symptoms and laboratory parameter deterioration.[2,17,18]

## Limitation of the study

Small sample size is a limitation of this study. Studies

with larger sample size are needed to corroborate successful use of IA plasmapheresis in ABOi kidney transplants.

## Conclusion

IA plasmapheresis was universally successful in decreasing the ABO isoagglutinin titers to desired level in all prospective ABOi kidney transplant patients.

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### Conflicts of interest
There are no conflicts of interest.

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