

# ABO-incompatible kidney transplantation using immunoadsorption columns: First experiences in South Africa

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**Background.** ABO-incompatible kidney transplantation gives patients with chronic kidney disease requiring dialysis and without a blood group-compatible donor an alternative option for a kidney transplant.

**Objectives.** To describe our first experiences and outcomes with 3 patients using Glycosorb ABO immunoadsorption (IA) columns in performing ABO-incompatible living-donor kidney transplants. This is the first time this technique has been used in Africa.

**Methods.** As per the protocol, patients needed between 1 and 4 sessions of IA and received rituximab ~ one month before transplantation.

**Results.** All the patients achieved the target isohaemagglutinin antibody titre of 1:4 pretransplant. Only 1 patient with the highest initial screening titre (1:256) needed IA post-transplant. None of the patients experienced clinical rejection, and all had good graft kidney function at discharge and at the time of writing.

**Conclusion.** Glycosorb ABO IA is an effective technique in enabling ABO-incompatible living-donor kidney transplants to be performed successfully in a South African setting.

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Kidney transplantation provides the best long-term outcomes for patients with end-stage chronic kidney disease<sup>[1]</sup> who are well enough to receive a kidney transplant. The sources of donor kidneys include deceased donors and living donors. There is a shortage of deceased-donor organs worldwide, and especially in South Africa (SA), where deceased-donor donation rates are among the lowest in the world and where patients with chronic kidney disease on dialysis typically wait >5 years for a deceased-donor kidney transplant.

This situation means that living kidney donation options need to be more actively explored in those who cannot or do not want to wait many years for a deceased donor kidney transplant. Unfortunately, many of these patients do not have a living-donor option either. There are many possible reasons for this, including no relatives or friends offering to donate, while many who offer are not suitable to donate or are blood group or tissue incompatible.

Blood group incompatibility was long considered an absolute barrier to organ transplantation, because blood group antigens are not only expressed on red blood cells but on almost all cells in the body, including endothelial cells. Preformed anti-A or anti-B (or both) isohaemagglutinin antibodies develop soon after birth in all humans who do not have the cognate blood group, even without prior blood transfusions. These isohaemagglutinin antibodies are thought to result from contact with A- and B-like antigens in the intestines from exposure to various nutrients and bacteria,<sup>[2]</sup> and almost always induce hyperacute antibody-mediated rejection (ABMR) if present in a recipient transplanted with an ABO-incompatible organ, resulting in very poor graft outcomes.<sup>[3]</sup> As far back as the late 1980s, techniques were developed to overcome this phenomenon.<sup>[4]</sup> Initially these involved splenectomy and plasma exchange to reduce the titres of these isohaemagglutinin antibodies to a level that would allow safe transplantation. Subsequently, it was found that rituximab, an anti-CD20 monoclonal antibody that triggers destruction of

B cells (which produce antibodies), could be safely substituted for splenectomy.<sup>[5]</sup>

Plasma exchange was still needed to reduce isohaemagglutinin antibody titres, but this technique is non-selective and reduces all antibodies and many other proteins, including clotting factors, and is broadly immunosuppressive, with an increased risk of coagulation problems and infection. Outcomes of ABO-incompatible kidney transplants using plasma exchange show increased rates of rejection and infection, with a meta-analysis in 2018 by De Weerd and Betjes<sup>[6]</sup> showing 1-year uncensored graft survival of patients who were ABO-incompatible of 96% v. 98% in ABO-compatible controls (relative risk 0.97; 95% confidence interval 0.96 - 0.98;  $p < 0.001$ ),

## Box 2. Duration of immunoadsorption (IA) based on isohaemagglutinin antibody titre

Initial titre	Hours of IA (or number of hours if plasma volume (PV) is significantly different to 3 L)
≤1:4	No need for IA
1:8 (2 <sup>3</sup> )	3 (or 3 × PV/3 hours)
1:16 (2 <sup>4</sup> )	4 (or 4 × PV/3 hours)
1:32 (2 <sup>5</sup> )	5 (or 5 × PV/3 hours)
1:64 (2 <sup>6</sup> )	6 (or 6 × PV/3 hours)
1:128 (2 <sup>7</sup> )	7 (or 7 × PV/3 hours)
1:256 (2 <sup>8</sup> )	8 (or 8 × PV/3 hours)
<i>It is possible to treat higher titres, but the likelihood of treatment failure and rejection is increased</i>	
1:512 (2 <sup>9</sup> )	7 (or 9 × PV/3 hours)
≥1:1 024 (2 <sup>10</sup> )	8 (or 10 × PV/3 hours)

with 49% of reported causes of death of infectious origin in the ABO-incompatible patients v. 13% in the ABO-compatible controls ( $p=0.02$ ) and a higher rate of ABMR (10% v. 2%;  $p<0.001$ ) in the patients v. the controls. An earlier review showed that long-term patient survival was similar.<sup>[7]</sup>

In the early 2000s, Glycorex Transplantation AB, a Swedish health technology company, developed an immunoadsorption (IA) column (IAC) with the brand name Glycosorb ABO. The three different specificity columns remove either anti-A or anti-B isohaemagglutinin antibodies, or both simultaneously if needed. The columns contain synthetic terminal trisaccharide A or B (or both) blood group antigen covalently bound to a Sepharose matrix. During the IA procedure, plasma is separated from the other blood constituents in the same way as with plasma exchange (via filtration or centrifugation), but then instead of the plasma being discarded, as would be the case with plasma exchange, it is passed through the Glycosorb IAC, where the specific anti-A or anti-B (or both) isohaemagglutinin antibodies are bound. After passing through the IAC, the plasma is returned to the patient. Insignificant amounts of other antibodies or proteins are removed, and no replacement solution such as albumin or fresh-frozen plasma (FFP) is required.

Protocols using this method in combination with rituximab were developed by Tyden *et al.*<sup>[5]</sup> at the Karolinska Institute in Sweden. Outcomes with this procedure have been shown to be as good as ABO-compatible transplants in some studies.<sup>[8]</sup> A large meta-analysis in 2016 by Lo *et al.*<sup>[9]</sup> showed better outcomes for IA compared with plasma exchange, and also better outcomes for rituximab-treated patients compared with those who had splenectomy to prepare them for ABO-incompatible transplantation.

To date these IACs have been used successfully in >6 000 patients in many countries throughout the world, but not in Africa. The first use in an ABO-incompatible kidney transplant recipient took place at Groote Schuur Hospital in Cape Town early in 2023. Subsequently two more have been performed at the neighbouring UCT Private Hospital. More ABO-incompatible living-donor transplants are planned at both hospitals in the coming months.

Since our first case at Groote Schuur Hospital was publicised in the media, there has been strong interest from both patients on dialysis and their treating nephrologists, as well as from healthcare funders throughout SA, regarding this technique. Questions raised included which patients would be eligible for this procedure, how it is done, and the outcomes and costs incurred. An Indian study<sup>[10]</sup> has shown IA to have similar overall in-hospital costs to plasma exchange, despite the columns being relatively expensive. The reason for this could be related to the fact that most patients can be admitted on the day before the transplant for the first and possibly only IA procedure, unlike plasma exchange, which often requires admission a week before transplantation and multiple plasma exchange procedures before it is safe to proceed with transplantation.

In this case series, which includes our experiences with the first three patients using Glycosorb ABO IACs to allow safe kidney transplantation from ABO-incompatible living donors, we describe the protocol we used, the short-term patient outcomes, and what we have learned so far.

## Methods

The protocol we are currently using, and refining as we get more practical experience, is described below. The key elements are: (i) anti-A/B isohaemagglutinin titre measurement; (ii) B-cell depletion using rituximab; and (iii) antibody depletion – ideally done with an IAC.

### Box 1. Preparation for ABO-incompatible transplant

Before the transplant team decide to proceed with the transplant, the following must occur:

#### 1. 4 weeks before planned transplant date:

- Final complement-dependent cytotoxic crossmatch, i.e. before rituximab given (as false-positive results are common if done after rituximab), and measure anti-A or anti-B antibody titres in recipient as relevant.
- Rituximab 375 mg/m<sup>2</sup> given as a single-dose intravenous infusion (usually 600 - 900 mg, rounded off to the nearest 100 mg) given with premedication (promethazine 25 mg and hydrocortisone 100 mg) 2 - 4 weeks (preferably the latter) before the planned transplant date.

#### 2. 2 weeks before planned transplant date:

- Tacrolimus 0.15 mg/kg/d and mycophenolate mofetil 750 mg bd. Measure tacrolimus and mycophenolic acid (MPA) level after 5 - 7 days and adjust doses appropriately. Aim for tacrolimus trough level 8 - 12 ng/mL and MPA trough level 2 - 4 mg/L.

#### 3. 1 week before planned transplant date:

- Stop angiotensin-converting enzyme inhibitors, e.g. enalapril, or angiotensin II receptor blockers, e.g. losartan, as reactions, e.g. skin flushing, and rarely anaphylactoid reactions to the Glycosorb IACs may occur in patients on these drugs.

#### 4. Day before planned transplant date:

- Admit patient for final dialysis session (if on haemodialysis) and Glycosorb IA; these can be done concurrently. Measure anti-A or anti-B antibody titres in recipient as relevant.

## Preparing a patient for ABO-incompatible kidney transplant

Confirm the donor and recipient blood groups and that human leucocyte antigen (HLA) crossmatches, i.e. complement-dependent cytotoxic (CDC) and if indicated flow cytometry crossmatches, are negative.

If the donor is blood group A and the recipient blood group O or B, check the A subtype. Any non-A1 donor blood group A (A2, Ax, etc.) with a recipient anti-A isohaemagglutinin titre  $\leq 1:16$  can be considered as an O blood group and will only need rituximab and no further procedures to prepare the patient for transplant (from an ABO perspective). Higher anti-A isohaemagglutinin titres may need IA.

For blood group A1 and B donors to ABO-incompatible recipients (Box 1), the target anti-A and anti-B titre pretransplant is  $\leq 1:4$ . From the day before transplant to 2 weeks post-transplant, daily samples are sent to the on-site Western Cape Blood Services blood bank using a dedicated blood bank form designed for requesting and reporting on these tests.

It is possible to achieve large reductions in isohaemagglutinin titres with even a single prolonged session using Glycosorb IACs, as they are not easily saturated. In an average-sized patient, for every 1 hour of running the plasma through the IAC at 50 mL/min (3 L/h), halving of the titre should be anticipated if their plasma volume is close to 3 L. Repeat titres should be done during the session at the planned stoppage time to assess effectiveness and how much longer to continue, if needed, as well as after the first session to assess the overall response for the session. This means continuing the IA session at least until the first result is available, and if the target was not achieved, to continue until it is estimated that the target would be achieved.

As shown in Box 2, the number of planned hours is the exponent value of the isohaemagglutinin titre.

If using a Glycosorb anti-A/B column, e.g. for an AB donor to an O recipient or if no anti-A or anti-B column is available, the maximum plasma flow recommended is 40 mL/min, so correspondingly 25% longer sessions will be needed to achieve the target titre.

Rebound or increase in titre by at least one dilutional factor, e.g. 1:4 to 1:8, overnight is not uncommon. A second IA session using a Glycosorb IAC can be used if the target titre of  $\leq 1:4$  is not achieved. The timing of this can be challenging, and if the titre is  $\geq 1:8$  and the surgery cannot be postponed by one day, an IA session will need to be completed early on the morning of surgery. If the target titre is not achieved after this second session, a third session of IA will be needed, or converting to conventional plasma exchange, preferably with a centrifugal plasma exchange machine, may be required.

Transplantation should proceed within 24 hours after the anti-A/B isohaemagglutinin titre drops to  $\leq 1:4$ .

Induction of immunosuppression is with basiliximab (20 mg on day 0 and day 4), unless there are concomitant HLA donor-specific antibodies (DSAs) present, in which case antithymocyte globulin should be used. High-dose methylprednisolone is used as per local protocol.

Anti-A/B isohaemagglutinin titre measurement continues daily post-transplant for at least the first 10 days and then on alternate days if titres are not increasing, until 2 weeks post-transplant. If titres rise to 1:8 without any signs of rejection, the situation can be watched, but once they reach  $\geq 1:16$  or there is evidence of deteriorating graft function at lower titres, then Glycosorb IA (preferably) or plasma exchange should be continued daily until titres drop to  $\leq 1:8$  or until 2 weeks post-transplant.

After 2 weeks, titres can be watched and allowed to rise if there are no other signs of rejection – this phenomenon is called accommodation.

Biopsies should be performed at any sign of rejection. The signs of ABMR due to ABO incompatibility are the same as for HLA incompatibility. The latter should be tested for by screening HLA antibodies to look for HLA donor-specific antibodies. C4d-positive staining on biopsy without any other signs of ABMR is not unexpected (it may be a sign of accommodation) and does not require treatment for rejection.

ABMR that occurs later can be treated with Glycosorb IA/plasma exchange daily as above. If rejection is thought to be HLA mediated, plasma exchange should be performed and not IA. Severe acute ABMR that is resistant to this treatment may require splenectomy.

If recipients require FFP for any reason during the first 2 weeks, they should receive AB blood group FFP, which should contain no anti-A or anti-B isohaemagglutinin antibodies, which may induce rejection by acting as DSAs. If they require a blood transfusion, this must be recipient blood group compatible.

### Clinical cases

Patient 1 was 35 years old and had been on haemodialysis and on a deceased-donor waiting list for 9 years, as she had no blood group-compatible living-donor options. Waiting times had increased during the COVID-19 pandemic, as transplantation had been put on hold. She was blood group B. When she heard about ABO-incompatible transplants becoming an option, her brother, who was blood group A, was tested to be a potential donor. They were tissue compatible, being haploidentical with only a 3/10 HLA-mismatch and no DSAs, and he was therefore worked up as a donor nephrectomy.

Patient 2 was a 68-year-old, blood group O dialysis patient who had been on haemodialysis for 1 year. He had no willing living-donor options except for his ABO-incompatible wife, and did not want to

wait for years on a deceased-donor waiting list for a deceased-donor kidney transplant, by which stage he might not be transplantable. His wife was blood group A, and they were tissue compatible with an 8/10 HLA-mismatch and no DSAs.

Patient 3 was 40 years old and had developed ABMR 2 years after her first kidney transplant and soon after giving birth to her only child. Despite plasma exchange, her graft failed. She had been back on haemodialysis for almost 3 years. She was tested against her ABO-compatible aunt to whom she was found to be highly sensitised. We identified that she had a sister who had never been tissue typed or crossmatched against her, as her sister was blood group incompatible. Her sister was therefore tested in the hope that she was HLA identical. While not HLA identical, she was haploidentical and with only a 4/12 mismatch. The patient only had one strong DSA against the 4 mismatched alleles and had negative CDC and flow cytometry T- and B-cell crossmatches to her sister.

As per our protocol, blood group A subtyping was done to look for non-A1 blood group, as this can be treated practically as a blood group O donor, making desensitisation unnecessary, unless the recipient has very high anti-A antibody titres. All three donors were blood group A1.

### Results

Fig. 1 shows the anti-A antibody titres for the 3 patients at screening, before rituximab, on admission before Glycosorb IA, after the first IA (and the second IA as needed on the day of transplant surgery in patients 2 and 3), and on the days following surgery.

Patient 1 had a significant drop in titre after rituximab and achieved the target level of 1:4 ( $2^2$ ) after the first IA session pretransplant, and did not require another session thereafter.

Patient 2 had an increase in titres after screening, which did not decrease after the use of rituximab. He also had a rebound in antibody titre overnight after the first IA session, which necessitated a second IA session on the morning of surgery, but titres remained low thereafter.

Patient 3 had a very high titre at screening of 1:256 ( $2^8$ ), which spontaneously decreased before receiving rituximab, but did not decrease further following rituximab. She also did not show a good response to the first IA session and needed a second session on the morning of surgery. Her titre remained low until day 8, when it suddenly increased from 1:4 to 1:16 ( $2^4$ ) despite her creatinine being at its lowest level since the transplant at 98  $\mu\text{mol/L}$ . She therefore received her third session of IA with titres reducing to 1:8 ( $2^3$ ) for 2 days thereafter, and again needed a fourth session on day 11 when her titre increased to 1:32 ( $2^5$ ), but titres subsequently remained at acceptable levels.

Fig. 2 plots serum creatinine levels and demonstrates that all three patients had good responses in terms of graft kidney function. Patient 2 developed ureteric obstruction, diagnosed on day 8, which required percutaneous nephrostomy placement on day 9, and subsequently had a complicated urological course, but at the time of writing has good renal function with all the urological issues resolved. Patient 3 also developed a ureteric stricture, diagnosed on day 13, which required a nephrostomy the same day. A double-J stent was placed on day 15 and the patient was discharged on day 17 after the nephrostomy was removed. Neither had a stent placed at the time of transplant.

All the patients have latest estimated glomerular filtration rates of between 47 and 87 mL/min/1.73 m<sup>2</sup> using the CKD-EPI formula (patient 1 at 87 mL/min 6 months post-transplant, patient 2 at 47 mL/min at 5 months post-transplant, and patient 3 at 57 mL/min at 3 weeks post-transplant).

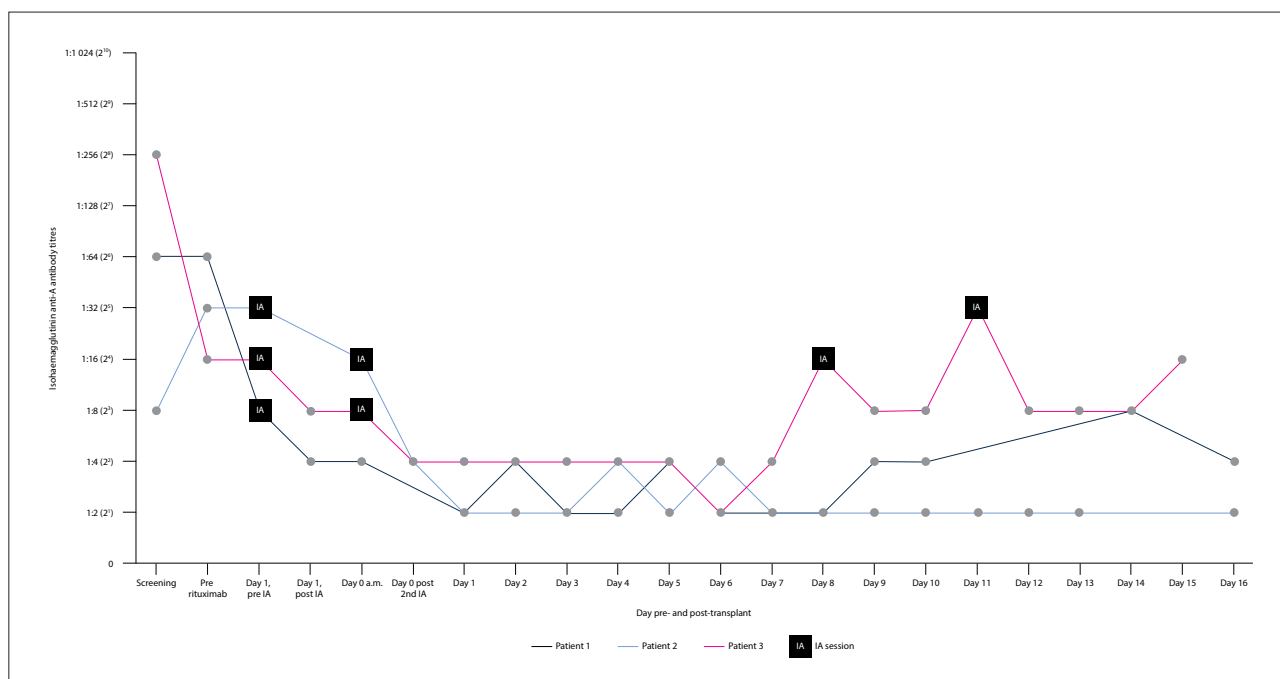


Fig. 1. Anti-A antibody titres for the 3 patients pre- and post-transplant. (IA = immunoadsorption.)

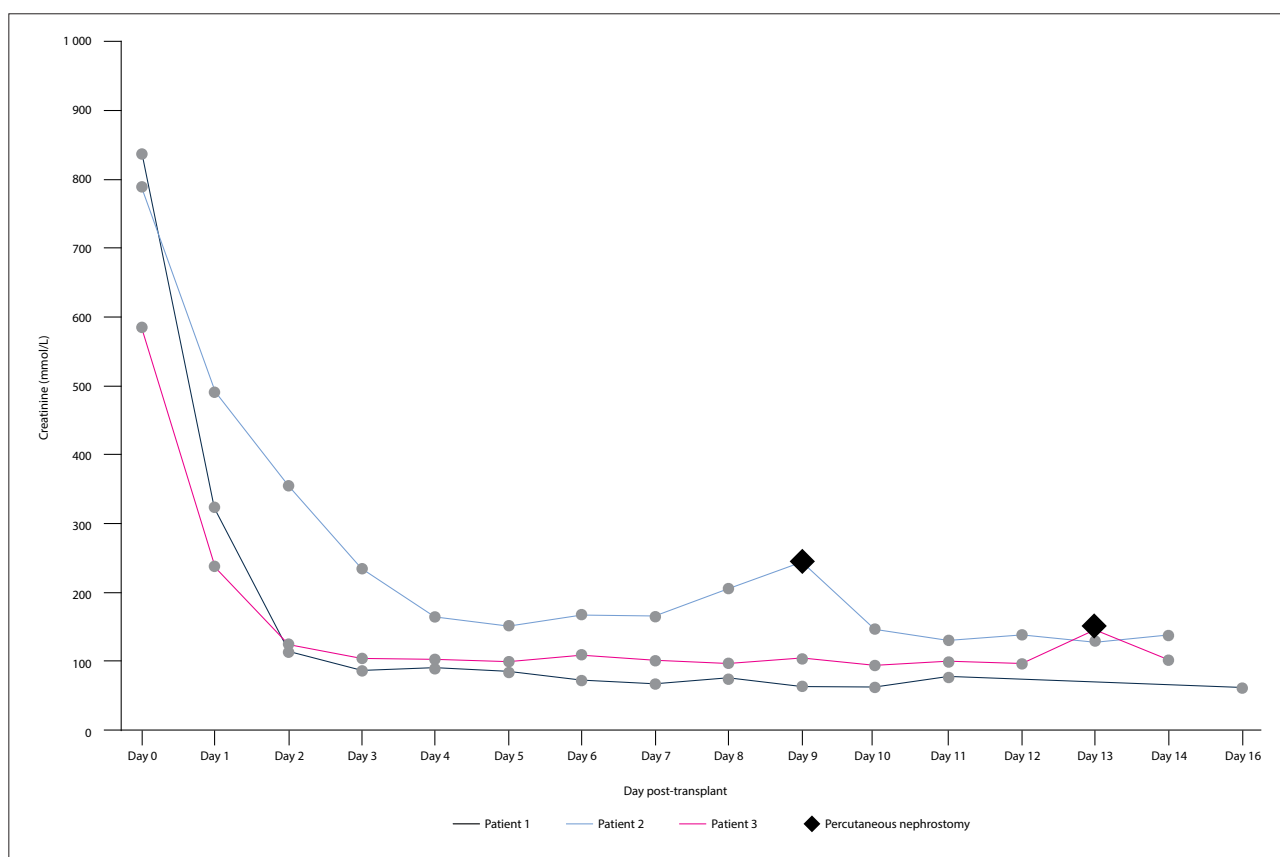


Fig. 2. Serum creatinine levels for the 3 patients post-transplant.

No patient required a biopsy to exclude rejection, and all the patients were discharged on prophylaxis against cytomegalovirus (valganciclovir), tuberculosis (isoniazid) and *Pneumocystis jirovecii* (co-trimoxazole).

## Discussion

Our first experiences with ABO-incompatible living-donor kidney

transplants using Glycosorb IACs have shown that this is an effective technique in allowing ABO-incompatible transplants to be performed successfully in an SA setting.

Between one and four sessions were needed in this small series of 3 patients, with the patient with the highest screening anti-A titre needing the most sessions.

Overnight rebound in titres was seen in 1 patient. This is a well-described phenomenon, as antibodies equilibrate from the extravascular into the intravascular space. It is also seen with conventional plasma exchange.

Antibody titres were not measured after day 14 as they do not correlate with the risk of rejection thereafter, even when they do increase. This phenomenon, called accommodation, has long been described in relation to ABO-incompatible transplantation. Although it is not well understood, at least three mechanisms seem to play a role, as described in detail by Platt and Cascalho<sup>[11]</sup> in a recent review. The first mechanism involves resistance to injury which, depending on the pathways that are activated, potentially limits susceptibility to ischaemia-reperfusion injury and hyperacute rejection. The second and third mechanisms involve changes in expression of the A and B glycoprotein antigen so that binding of antibodies to the graft decreases, and changes in the properties of graft-specific antibodies in ways that decrease the pathogenic impact of binding to their cognate antigen.

As stated in our protocol, C4d-positive staining on biopsy without any other signs of ABMR is not unexpected and may be a sign of accommodation, and does not require treatment for rejection without concomitant histological or immunological evidence of ABMR.<sup>[11]</sup> Our protocol does not include protocol biopsies to look for this phenomenon.

Despite this accommodation, occasional late ABMR has been described in ABO-incompatible kidney transplant recipients and is usually related to septic events. It has been theorised that this rejection is related to a rapid increase in IgM cross-reacting antibodies which are produced during these events.<sup>[12]</sup>

Who should be considered for this type of transplant? These first three patients demonstrate a good selection of the criteria that we would suggest. Firstly, patients need to be transplant candidates as per generally acceptable and local centre criteria. Secondly, they should have no other blood group and HLA-compatible donor options. If the alternative option is a highly HLA-sensitised ABO-compatible donor, then a less HLA-sensitised ABO-incompatible donor (for whom the recipient does not need plasma exchange to desensitise) may be a better long-term option, as rejection is less likely in the short and long term.<sup>[13]</sup> Finally, the anti-A or anti-B isohaemagglutinin titres should not be too high. We would suggest  $\leq 1:256$  ( $2^8$ ) at screening, but as can be seen from our patients, these titres can increase or decrease significantly, either spontaneously or after rituximab and after IA.

## Conclusion

This short case series demonstrates that ABO-incompatible living-donor kidney transplantation using Glycosorb IACs can be performed successfully in an SA setting. This procedure allows patients without any other donor options to be successfully transplanted. Although

an expensive procedure in terms of the cost of the IAC, it is relatively straightforward to perform with three simple tools – rituximab, isohaemagglutinin titres, and Glycosorb IACs. The long-term benefits in dialysis costs saved and in patient quality of life and life expectancy should not be underestimated.

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**Conflicts of interest.** None.

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