The efficacy of antigen-specific immunoabsorption and rebound of anti-A/B antibodies in ABO-incompatible kidney transplantation

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Abstract

Background. As antigen-specific immunoabsorption (IA) using the Glycosorb®-ABO columns is becoming increasingly popular in ABO-incompatible (ABOi) transplantation, in this study, we retrospectively investigated the efficacy of Glycosorb®-ABO IA in vivo and ex vivo. We also assessed the risk of anti-A/B antibody (ABab) rebound before and after ABOi kidney transplantation.

Methods. A protocol for ABOi living donor kidney transplantation was used, combining four preoperative and three preemptive postoperative Glycosorb®-ABO IAs with rituximab and maintenance immunosuppression. ABabs were determined by a haemagglutination titration technique.

Results. ABOi kidney transplantation was attempted 45 times and 43 transplantsations were performed. Overall patient survival was 93% and graft survival was 91%. Mean follow-up was 4.5 years. Glycosorb®-ABO IA significantly reduced the ABabs in the majority of patients (P < 0.0001). However, in three patients (6.8%), the antibody elimination was incomplete. Inadequate adsorption of core-chain-dependent ABabs may explain this finding, but further studies are needed. In five patients, the preconditioning was interrupted before transplantation, resulting in ABab rebound. Yet, when preconditioning was re-started, the antibodies could be removed as planned. After ABOi transplantation, rebound of ABabs was seen in two patients (5%).

Conclusions. Glycosorb®-ABO IA in combination with rituximab effectively depletes ABabs in most patients, but owing to core-chain-dependent ABabs, Glycosorb®-ABO IA may be less effective than nonspecific techniques for antibody removal in some patients. Rebound before transplantation subsequent to interrupted preconditioning does not hamper a successful ABOi transplantation. Post-operatively, when this protocol for ABOi transplantation is followed, the risk of ABab rebound is small.

Keywords: ABO-incompatible; antibody rebound; antigen-specific immunoabsorption; Glycosorb®-ABO; rituximab

Introduction

With advances in immunosuppression and apheresis techniques over the past few years, ABO-incompatible (ABOi) kidney transplantation has grown in importance, extending beyond Japan [1–5]. The results in living donor ABOi kidney transplantation have also improved and several centres now report results comparable with ABO-compatible living donor transplantation [6–8]. Yet, in spite of improved results, common concerns are still the risk of anti-A/B antibody (ABab) rebound following ABOi transplantation and the increased risk of antibody-mediated rejection (AMR) in the early postoperative period [5, 6, 9].

Moreover, although preconditioning for the majority of patients is straightforward, for some patients, the preconditioning is complicated or even unsuccessful. By necessity, the treatment is sometimes interrupted. As suggested by studies on autoimmune diseases, antibody removal through therapeutic apheresis (TA) can stimulate and accelerate antibody production (herein referred to as rebound) once the therapy is stopped [10]. Whether an interruption in the preconditioning for ABOi transplantation will result in a rebound of ABabs and thereby preclude a successful ABOi transplantation has thus far not been studied. Other concerns, not extensively studied, include the risk of rebound following graft loss and the possibility of ABOi retransplantation in patients with a history of ABOi graft loss.

Finally, antigen-specific immunoabsorption (IA) as an alternative to nonspecific TA methods for the removal of ABabs is becoming increasingly popular [11]. At present, the Glycosorb®-ABO column (Glycorex Transplantation AB, Lund, Sweden) is the only product commercially available for this purpose. These columns contain the synthetic trisaccharide terminals of the A and B antigens, bound to a Sepharose matrix. An early in vitro study by Rydberg et al. [12] showed a nearly complete elimination of ABabs after just one passage. However, the same group also detected core-chain-dependent ABabs in some patients and argue that these will not be absorbed by the Glycosorb®-ABO column [13, 14]. The prevalence of
such core-chain-dependent ABabs and the risk of therapeu-
tic failure when using the Glycosorb®-ABO column is
unknown.

Since 2001, ABOi living donor kidney transplantation has
been attempted 45 times and 43 transplantations performed
at our center, using a protocol based on Glycosorb®-ABO
IA and rituximab [3]. The effects of this protocol on the
ABab levels have not been investigated so far.

We hypothesized that ABab rebound following ABOi
transplantation using Glycosorb®-ABO IA and rituximab
does not occur if the immunosuppression is maintained.
We also hypothesized that discontinuation of the immuno-
suppressive therapy before and after the ABOi transplan-
tation would result in rebound of the ABabs. We did not
expect any therapeutic failure using the Glycosorb®-
ABO system and we believed that ABOi retransplantation
would be feasible.

**Patients and methods**

Patient characteristics are shown in Table 1. All patients with a negative
complement-dependent cytotoxic crossmatch, a negative flow cytometric
crossmatch and a negative XM One™ crossmatch (AbSorber, Stockhol-
m, Sweden), undergoing preconditioning for living donor ABOi kidney
transplantation according to the protocol between September 2001 and
May 2010, were included in the study, in total, 36 adults and 8 children
(≤16 years of age at transplantation) [15, 16]. One adult patient under-
grew preconditioning twice. These two events were analysed separately.
The study therefore comprises 45 attempts at ABOi transplantation.
One patient was denied ABOi transplantation already at referral because
of a high ABab titre (1:1024). This patient was not included in the study.

Another eight patients received nonspecific TA to allow for removal of
ABabs together with other donor-specific antibodies. These patients were
excluded.

Data on the ABab titres and the number and type of IAs before and
after transplantation were collected, together with data on patient and graft
survival. Rejection episodes were recorded.

**The protocol**

The protocol for ABOi kidney transplantation has already been described
detail elsewhere [3, 17]. In summary, removal of the ABabs was
achieved by repeated Glycosorb®-ABO IA preoperatively on Days −6,
−5, −2 and -1 to attain an ABab titre of ≤1:8 at the day of transplantation.
Rituximab (anti-CD20) (MabThera®, Roche, Basel, Switz-
erland), 375 mg/m² body surface area, was given on Day −30 and oral im-
munosuppression (tacrolimus, mycophenolate mofetil and prednisolone)
instituted on Day −10. Day −1 intravenous immunoglobulin (Gammagard
S/D®; Baxter, Lesssines, Belgium) 0.5 g/kg was administered. Postopera-
tively, IA was performed on Days 2, 5 and 8. Additional IAs were per-
formed postoperatively if there was a rise in ABabs with a concomitant
impairment of kidney function.

For each patient, three Glycosorb®-ABO columns were purchased.
The columns were usually regenerated two to three times. However, in
patients with incomplete antibody elimination, as determined by ex vivo
ABab titrations of plasma after passage through the columns (see below),
the columns were discarded.

### Table 1. Patient characteristics and results*

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Adult patients</th>
<th>Paediatric patients</th>
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| No. of patients undergo-
 ing preconditioning | 36 (37 attempts) | 8 |
| No. of transplanted pa-
 tients (%) | 35 (95) | 8 (100) |
| ABOi (donor → recipi-
 ent) (%) | | |
| A1 → 0 | 14 (37.8) | 4 (50.0) |
| B → 0 | 6 (16.2) | 1 (12.5) |
| A2 → 0 | 6 (16.2) | 1 (12.5) |
| B → A | 5 (13.5) | 0 (0) |
| A1B → B | 2 (5.4) | 0 (0) |
| AB → A | 2 (5.4) | 1 (12.5) |
| A1 → B | 1 (2.7) | 1 (12.5) |
| B → AB | 1 (2.7) | 0 (0) |
| Median age at transplantation in years (IQR) | 35.1 (27.1–58.5) | 12.3 (3.7–13.1) |
| Female: male (%) | 11:26 (30.70) | 4:4 (50:50) |
| Median follow-up time (IQR) in months | 50.0 (28.5–75.4) | 60.1 (44.1–76.7) |
| No. of pretransplant IA, median (IQR) | 4 (4.0–5.0) | 0 (0.75–4.75) |
| No. of posttransplant IA, median (IQR) | 3 (2.0–3.0) | 4 (2.25–4.75) |
| Anti-A/B IgM titre at referral, median (IQR) | 1:32 (1:16–1:64) | 1:16–1:32 (1:2–1:64/1:128) |
| Anti-A/B IgG titre at referral, median (IQR) | 1:16 (1:8–1:16) | 1:8 (1:2–1:16/1:32) |
| Results | | |
| Canceled transplantation (%) | 2 (5.4) | 0 (0) |
| Patient survival among transplanted patients (%) | 33 (94.3) | 7 (100) |
| Graft survival (%) | 31 (88.6) | 7 (100) |
| Acute cellular rejection (% of patients)† | 4 (11.4) | 0 (0) |
| Acute AMR (% of patients)† | 2 (5.7) | 0 (0) |
| Anti-A/B IgM titre at transplantation, median (IQR) | 1:4 (1:2–1:8) | 1:4 (1:2–1:4) |
| Anti-A/B IgG titre at transplantation, median (IQR) | 1:1 (1:1–1:2) | 1:2 (1:1–1:2) |
| GFR§ | 70.3 (52.5–82.7) | 53.5 (43.0–97.0) |
| Median GFR (IQR) at 3 months | 76.7 (53.1–87.5) | 58.5 (46.0–78.0) |

*Data for the adult and pediatric patients were analyzed separately.
†Preconditioning for ABOi kidney transplantation was attempted twice in one patient.
‡Including two patients with late acute rejections and graft failure as a result of nonadherence.
§In the adult patients, GFR was calculated using the Cockroft–Gault formula (milliliters per minute). In the paedi-
atriac patients, GFR was measured by the clearance of iohexol (mL/min/1.73m²).
Since the introduction of the protocol, some changes have been made. These are previously described elsewhere [17].

**ABab measurements**

A haemagglutination titration technique was used to measure the ABabs. The titration was initially done using a tube technique and from February 2006 by a gel technique. This method has already been described elsewhere [18]. Forty-six percent of the samples were analysed by gel technique. Titrations were done at referral, after treatment with rituximab but before the start of IA and before as well as after each IA and on a daily basis during the in-hospital stay. Titration was also done regularly after discharge and whenever there was an impairment of kidney function. In addition, ABab titrations were done on plasma samples obtained ex vivo, after passage through the Glycosorb®-ABO columns, in order to assess the efficacy of the columns. In total, 1640 plasma samples were analysed, i.e. 35 samples per patient on average. However, a full data set could not be obtained for all patients.

**Rebound of ABabs**

Clinically significant rebound of ABabs in ABOi transplantation has not been uniformly defined [19–22]. In studies on autoimmune diseases, rebound of antibodies commonly refers to a rise in antibodies equal to the level at baseline or higher [10]. In ABOi transplantation, Japanese studies have correlated an antibody titre ≥1:32 postoperatively to an increased risk of AMR [19]. As we intended to study rebound both before and after ABOi transplantation, an increased risk of AMR could not be used as the single indicator of critically high ABabs levels. Therefore, rebound herein refers to a return of the ABabs to the baseline level and/or a rise in the ABab titre to 1:32 or higher. However, changes in ABab titres below 1:8 were deemed to be of little clinical significance and were therefore disregarded.

**Patient and graft survival**

Data on patient and graft survival as well as other relevant clinical information were obtained from patient records.

**Rejection**

Biopsies were taken when there was a clinical suspicion of rejection and were assessed by a transplant pathologist. Rejections were graded according to the Banff criteria [23, 24]. C4d-staining was done by either immunofluorescence or immunohistochemistry.

**Graft function**

Graft function was assessed at 3 and 12 months after transplantation using the glomerular filtration rate (GFR). In adult kidney recipients, GFR was calculated by the Cockroft–Gault formula, whereas in the pediatric group, GFR was measured by clearance of iohexol [25].

**Statistics**

Statistical analysis was performed using JMP 8.0 software (SAS Institute, Cary, NC, USA) including univariate analysis of variance, multivariate analysis of variance, chi-square test and Wilcoxon’s rank-sum test as applicable.

**Results**

ABOi living donor kidney transplantation was attempted 45 times, and 43 ABOi kidney transplantations were performed; two preconditioning attempts failed. One-year patient survival was 100% and graft survival was 96%. The overall patient and graft survival, the incidence of rejection and GFR at 3 and 12 months are displayed in Table 1 with the results of the adult and paediatric kidney transplantations analysed separately. Three patients without functioning grafts died during the study period. In patients with functioning grafts, the overall patient survival was 100%. Mean follow-up time was 4.5 years ± 26 months.

**ABab measurements**

Changes in the anti-A/B IgG and IgM showed the same pattern. For that reason, the demonstration of data was limited to changes in anti-A/B IgG. Moreover, there was no significant difference in anti-A/B IgG and IgM between the adults and children, and data regarding ABab titres were therefore analysed together. The overall changes in anti-A/B IgG are displayed in Figure 1a and b, including both adult and paediatric patients.

**Rituximab**

Rituximab was given at a median of 27 days (interquartile range (IQR) 15–32 days) before transplantation and 3 weeks before the start of IA. There was no change in ABab titres when comparing the ABab titres at referral with those after rituximab administration but before IA and intravenous immunoglobulin (median anti-A/B IgG 1:32 at both time points) (P = 0.79).

**Changes in ABab titers during Glycosorb®-ABO IA before transplantation**

Thirty-four of the 45 ABOi preconditioning attempts (76%) were uneventful and transplantation was performed according to plan. Overall (including all patients), the immediate effect in vivo of the preoperative IAs was a reduction in ABabs by 2 titre steps (median) (IQR 1–3) when comparing ABab titres before and immediately after IA. However, the antibodies rose between treatments and the median change from the start of one treatment to the next varied between 0 and 1 titre step. Yet, a significant decrease in ABab titres was achieved after four preoperative IAs (P = 0.0001) (Figure 1).

In the group of patients with an uneventful preconditioning, altogether four preoperative IAs reduced the ABabs in vivo by a median of 3 titre-steps (IQR 2–3 titre-steps) and when the efficacy of each Glycosorb®-ABO column was assessed, that is, by analysis of plasma samples obtained ex vivo, after passage through the columns, no ABabs could be detected (median ABab titre 1:<1 (IQR 1:<1–1:1)), indicating that the antibody elimination was complete.

Eleven preconditioning attempts (24%) were complicated. The complications were of two types:

- An unexpectedly poor response to the Glycosorb®-ABO immunoadsorption, leading to additional immunoadsorptions; and
- Medical conditions occurring in the recipient or donor, not directly related to the ABO incompatibility, leading to an interruption of the treatment.

One patient had complications of both types.

**Weak response to the Glycosorb®-ABO IA**

In 7 of the 44 patients (16%), four preoperative Glycosorb®-ABO IAs did not reduce the ABabs enough to allow for transplantation. All of these patients had an ABab titre at referral within the fourth quartile range (1:64–1:512). In these patients, four preoperative IAs reduced the ABabs by 1.5 titre steps (median) compared with a 3 titre step reduction (median) in the other 10 patients with similar starting titres (P = 0.04). Yet, in four of these seven patients, the plasma samples obtained ex vivo, after passage through the Glycosorb®-ABO columns, indicated that the antibody
elimination during Glycosorb®-ABO IA was complete and that saturation of the columns did not occur. These four patients received an additional four to six preoperative IAs, and three could then undergo the ABOi transplantation, two immediately and one patient after a 3-year interruption (see below). For one patient, the transplantation was canceled.

In three of the seven patients with an inadequate antibody removal after four preoperative IAs, the efficacy of the Glycosorb®-ABO IAs was inferior compared with the other patients. Plasma obtained ex vivo after passage through the Glycosorb®-ABO columns was repeatedly positive for ABabs at a titre ≥1:8 (range 1:8–1:32), indicating that the antibody elimination was only partial. One of these patients had already undergone an ABOi transplantation with early graft failure (A₁ → 0) and was accepted for a second transplantation with A-incompatibility (A₂ → 0). One patient was transplanted after 10 preoperative Glycosorb®-ABO IAs, with an ABab titre of 1:32 at the day of transplantation. (The clinical course was otherwise uncomplicated.) In one patient, core-chain-dependent ABabs were suspected after three Glycosorb®-ABO IAs. The therapy was therefore stopped and replaced with therapeutic plasma exchange (TPE). The ABabs were subsequently reduced as expected (Figure 2).
Interrupted preconditioning

In five patients, the preconditioning was interrupted (for 1–33 months) and the immunosuppressive therapy discontinued for medical reasons not directly related to the ABO-incompatibility, namely peritonitis in two patients, hypoxia in one patient, a donor contraindication in one patient and side effects of the oral immunosuppression in one patient who had already received nine preoperative IAs. In these patients, a rebound of ABabs was seen once the preconditioning was halted (Figure 3). Yet, whenever the preconditioning was restarted, including another series of four preoperative IAs, the ABabs could be removed as planned.

Changes in ABab titres after transplantation

In 35 of the 43 transplanted patients (81%), the postoperative course was essentially uneventful. These patients did not experience rebound of ABabs, acute rejection or graft failure.

Graft failure

Four patients experienced graft failure (9.3%). They each had an uneventful preoperative course and none needed more than four preoperative IAs to attain an A/B antibody titer of 1:8 at the day of transplantation. Two of these grafts were lost early due to vascular complications: one patient had a thrombosis of the main renal artery (POD 8 (C4d-)) and one other patient had a thrombosis of the main renal vein (POD 3) (C4d-). These two kidneys were explanted. After transplantectomy and discontinuation of immunosuppression, there was a rebound of ABabs. One of these patients was later accepted for a second ABOi transplantation (see above) (Figure 4). Two patients experienced late graft failure as a result of rejection due to nonadherence [POD 395 (C4d+) and POD 613 (C4d-) (both Banff IA)] (see below). None had any significant rebound of ABabs, despite discontinuation of the immunosuppression.

Rejection

A total of 4 of the 43 patients experienced acute rejection (9.3%): Of these, two experienced early acute cellular rejection (Banff IA and Banff 2A, both C4d+) (4.7%). In addition, the two patients with late graft failure as a result of nonadherence were diagnosed with a mixed cellular and AMR at the time of graft failure (4.7%) (see above). None of the patients experienced early acute AMR.

Rebound

Excluding the two patients with early graft failure, who experienced rebound of ABabs only after transplantectomy, rebound after transplantation was seen in 2 of the 43 patients. In both patients, a poor response to the Glycosorb®-ABO IA was observed before transplantation and one patient received additional TPE (see above). After transplantation, this patient...
received another two series of Glycosorb®-ABO IA with limited effect. Yet again, the ABabs were reduced by TPE (Figure 2). The other patient received a total of 14 postoperative Glycosorb®-ABO IAs. However, none of the two experienced AMR.

Discussion

In this study, a protocol for ABOi kidney transplantation using Glycosorb®-ABO IA and rituximab was used. The efficacy of Glycosorb®-ABO IA was assessed and the ABab levels and their impact on outcome were analysed.

First, there are several ongoing studies evaluating rituximab as a means to reduce donor-specific antibodies prior to transplantation. In the present study, all patients received rituximab 4 weeks before the transplantation. However, rituximab treatment alone did not have any effect on the ABab levels over a 3-week period before transplantation, i.e. from rituximab administration to start of IA. We therefore do not believe that rituximab itself, in the absence of a B-cell suppressive effect does not reduce the antibodies before transplantation either [26–28]. In contrary, rituximab (as part of an immunosuppressive protocol) seems effective in preventing rebound of ABAbs after transplantation, although the mechanism of action is not fully understood [8, 29].

Moreover, in patients with interrupted preconditioning (n = 5), there was a rebound of ABAbs once the IA and oral immunosuppression was stopped, again indicating that single-dose rituximab as monotherapy despite a long-term B-cell suppressive effect does not reduce the antibodies before transplantation [30]. Yet, even with rebound of ABAbs, all of these patients were eventually able to undergo a successful ABOi transplantation (GFR 49–105 mL/min at 12 months, 0% rejection or graft loss).

Second, the epitope of most ABAbs is the terminal trisaccharide + core chain [13, 14, 32, 33]. Such core-chain-dependent ABAbs are, most probably, not adsorbed by the Glycosorb®-ABO system [12]. The prevalence of core-chain-dependent ABAbs is not known. When basing the protocol for ABOi transplantation on Glycosorb®-ABO IA, we believed that those would be rare. However, in the present study, in 3 of the 44 patients (6.8%), the ABAbs were not removed as efficiently as expected during Glycosorb®-ABO IA. TPE, tested on one patient, on the other hand, reduced the antibodies as anticipated. Regrettably, we were not able to analyse the specificities of the ABAbs in our group of patients but in a recent study, on healthy blood donors, the ABAbs were predominately core-chain dependent in 3 of the 23 (13%) individuals [33]. These findings suggest that a large proportion of the ABAbs, even before ABOi transplantation, in some 7–13% patients may be core-chain dependent. There is also a risk that such antibodies may develop after an ABOi transplantation [13].

Furthermore, there are four different core saccharides and consequently four A/B antigen subtypes. On the erythrocyte surface, core saccharide chain Type 2 predominate, while studies have shown that, although core chain Type 2 is most prevalent on the graft endothelium, core chain Type 4 may be predominant in kidney tissue overall [34]. As a consequence, the use of erythrocytes when measuring the immunologic activity against the ABOi allograft may be suboptimal [35, 36]. The potential diversity in the specificity of core-chain-dependent ABAbs may also explain why the graft function was stable, in patients transplanted, despite an incomplete antibody removal during Glycosorb®-ABO IA (n = 2, GFR at 12 months: 87 and 73 mL/min). A clinically established method to assess the specificity of the ABAbs would therefore be most beneficial in deciding what technique to use for antibody removal [33].

After transplantation, a correlation between postoperative rebound of ABAbs to titres ≥1:32 and AMR has been observed, occurring in approximately 15–17% of patients [19, 21]. Conversely, in this study, rebound was observed in only two patients (5%) (maximum postoperative IgG 1:32 and 1:64) and even in these patients graft function at 3 and 12 months was good (GFR 72–93 mL/min). The risk of ABAb rebound after transplantation according to our protocol therefore seems small and the impact of rebound uncertain.

Finally, among the patients who experienced graft failure (n = 4), those undergoing transplantectomy had a rapid rebound of ABAbs, once the graft was removed. In one of these patients, we aimed at a second transplantation, with desensitization against the same antigen. To our knowledge, there are no reports of successful repeated ABO-incompatibility retransplantation. This was our only attempt and it was abandoned after eight preoperative IAs with only minimal effect on the anti-A IgG titre. In the two patients with late graft failure but without transplantectomy, on the other hand, the ABAbs did not increase significantly. One possible explanation is that the grafts in these patients still exerted some immunomodulatory activity, possibly by suppressing the ABAb production or by absorbing the ABAbs. Whether transplantectomy in these patients would trigger a rebound of the ABAbs, we do not know.
To summarize, we conclude that ABOi kidney transplantation using Glycosor®-ABO IA in combination with rituximab effectively depletes ABAbs in the majority of patients but owing to core-chain-dependent ABAbs, non-specific TA may be more effective than Glycosor®-ABO IA in some patients. We also conclude that rebound before transplantation subsequent to interrupted preconditioning or after transplantation does not seem to have any negative impact on graft function.

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Conflict of interest statement. None declared.

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