Implementation of a Protocol for ABO-Incompatible Kidney Transplantation – A Three-Center Experience With 60 Consecutive Transplantations

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Background. A new protocol for ABO-incompatible kidney transplantation has recently been introduced. We report here on the joint experience of the implementation in Stockholm and Uppsala, Sweden and Freiburg, Germany.
Methods. The new protocol utilizes antigen-specific immunoadsorption to remove existing ABO-antibodies, rituximab, and intravenous immunoglobulin to prevent the rebound of antibodies, and conventional tacrolimus, mycophenolate-mofetil, and prednisolone immunosuppression. Sixty consecutive ABO-incompatible kidney transplantations were included in the study. The outcome is compared with the results of 274 ABO-compatible live donor transplantations performed during the same period.
Results. Two of the ABO-incompatible grafts have been lost (non-compliance and death with functioning graft). All the remaining 58 grafts had good renal function at a follow-up of up to 61 months. We did not observe any late rebound of antibodies and there were no humoral rejections. Graft survival was 97% for the ABO-incompatible compared with 95% for the ABO-compatible. Patient survival was 98% in both groups. There was a significant variation in preoperative A/B-antibody titer between the centers, with a median 1:8 in Uppsala, median 1:32 in Stockholm and median 1:128 in Freiburg. More preoperative antibody adsorptions were therefore needed in Freiburg than in Stockholm and Uppsala.
Conclusions. The new protocol was easily implemented and there were no graft losses that could be related to ABO-incompatibility. A significant inter-institutional variation in the measurement of anti-AB-antibodies was found, having a substantial impact on the number of immunoadsorptions and consequently on the total cost for the procedure. A standardized fluorescence-activated cell sorting technique for antibody quantification is much needed.

Keywords: ABO-incompatible, Antigen-specific, Immunoadsorption, Rituximab.

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A new protocol for ABO-incompatible kidney transplantation was introduced in 2001 (*1*). The new protocol utilizes antigen-specific immunoadsorption (GlycoSorb; Glycorex Transplantation AB, Lund, Sweden) rather than plasma exchange to remove existing anti-A or anti-B antibodies, and rituximab rather than splenectomy to prevent the rebound of antibodies in combination with conventional tacrolimus, mycophenolate mofetil, and prednisolone immunosuppression. This protocol has been successively implemented in some 20 European centers, notably in Sweden and Germany, but also in the United Kingdom, the Netherlands, Switzerland, Greece, France, and Spain. We report here on the joint experience of 60 consecutive ABO-incompatible live-donor (LD) kidney transplantations performed in Stockholm and Uppsala, Sweden and Freiburg, Germany. The outcome is compared with the results of 274 ABO-compatible LD transplantations performed during the same period.

MATERIALS AND METHODS

The first ABO-incompatible LD kidney transplantation utilizing the new protocol was performed in Stockholm in 2001 and the protocol has been implemented as a routine procedure there since 2002 (*2*). In Uppsala and Freiburg (*3*) it was implemented in 2004. Sixty consecutive ABO-incompatible LD kidney transplantations have so far been performed. All combinations of ABO-incompatibilities have been accepted except for a two-bloodgroup antigen mismatch, that is, with donor AB and recipient 0. There were 27 A1, 24 B, and 9 A2 major mismatches. The anti-A or anti-B titer against donor erythrocytes was determined using the saline method for immunoglobulin (Ig)M and the indirect Coomb′s test for IgG. The immunosuppressive protocol consisted of one dose of rituximab (375 mg/m^2) given 4 weeks before immunoad-

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sorption. This was followed by a conventional triple-drug immunosuppressive protocol consisting of tacrolimus, mycophenolate mofetil, and prednisolone/prednisone, starting 7 to 10 days before immunoadsorption. Postoperatively the desired tacrolimus trough level was 12–15 ng/ml. Mycophenolate mofetil was given at a daily dose of 2 g. The prednisolone/prednisone was tapered according to local practice. All patients received antiviral prophylaxis with valganciclovir/valaciclovir for 3 months and cotrimoxaxol for 6 months. Preoperatively the anti-A or anti-B antibodies were removed using antigen-specific immunoadsorption (GlycoSorb ABO; Glycorex Transplantation AB, Lund, Sweden). The GlycoSorb ABO column is a low-molecular carbohydrate column with A or B blood-group antigen linked to a Sepharose matrix. A detailed description of the apheresis procedure has recently been published (*4*). The protocol calls for four preoperative apheresis sessions and we aim for a preoperative antibody titer of IgG <1:8. If this is not achieved after four sessions, the transplantation is postponed for a week and four more sessions are performed. Before transplantation 0.5 g/kg of intravenous immunoglobulin (IVIG) was administered. Postoperatively three more apheresis sessions were given every third day over a total period of 9 days in Stockholm and Uppsala, while in Freiburg postoperative adsorptions were only performed if there were signs of rebound of antibodies (a twofold increase in antibody titer).

RESULTS

Sixty ABO-incompatible live-donor kidney transplantations were performed during the period of analysis (2002–2006). There was no difference in graft- or patient survival and in graft function between the ABO-incompatible and the ABO-compatible transplantations (Table 1). One of the ABO-incompatible grafts was lost as a consequence of noncompliance 22 months after transplantation (the patient stopped immunosuppressive therapy). One patient died with a functioning graft 4 months posttransplantation because of severe Clostridium colitis. All the remaining 58 grafts had good renal function at a follow-up of up to 61 months. We did not observe any late rebound of antibodies and there were no ABO antibody-mediated humoral rejections. The preoperative A/B antibody titer varied substantially between the centers with a low median titer 1:8 in Uppsala, a median titer of 1:32 in Stockholm, and a high median titer of 1:128 in Freiburg (Table 2). As a consequence, more preoperative antibody adsorptions were needed in Freiburg (median 5) than in Stockholm and Uppsala (median 4). Actually in three and five cases in Stockholm and Freiburg, respectively, the transplantation was cancelled because of persistent high antibody titers after up to nine GlycoSorb-adsorptions. In Stockholm and Uppsala postoperative preemptive adsorptions were performed according to the protocol, while in Freiburg the postoperative preemptive adsorptions were omitted. However, even so, no humoral rejections were observed.

DISCUSSION

This three-center pooled analysis reveals some very interesting findings. First of all, the study demonstrates that good results can be achieved in ABO-incompatible kidney transplantation with the present protocol and that the initial successful experience in Stockholm was not due to a single-center effect. On the contrary, the protocol was easily implemented in the other two centers and the results were replicated. Consequently, there were no graft losses that could be related to the ABO-incompatibility and when compared with the ABO-compatible transplantations no difference in graft- or patient

TABLE 1. Comparison of graft and patient survival and graft function in ABO-incompatible and ABO-compatible living-donor (LD) transplantations

	N	Graft losses	Actual graft survival	Actual patient survival	Actual serum creatinine (μmol/L) mean and range	Follow-up mean and range
ABO-incompatible LD tx	60	1 non-compliance 1 DWFG	97%	98%	127 (42–203)	17.5 (2–61) months
ABO-compatible LD tx	274	7 AHR+2 technical 6 DWFG	95%	98%	133 (53–360)	21.1 (2–63) months

AHR, acute humoral rejection; DWFG, death with functioning graft.

TABLE 2. Demographics of ABO-incompatible kidney recipients, antibody titre before adsorption, and number of pre- and postoperative adsorptions

		Age		IgG antibody titre		Preoperative adsorptions		Postoperative adsorptions	
Center	N	Mean	Range	Median	Range	Median	Range	Median	Range
Stockholm	26	30.8	1–63	32	1–128	4	0–9	3	0–16
Freiburg	21	45.3	21–63	128	8–1024	5	1–12	0	0–6
Uppsala	13	46.3	19–69	8	1–32	4	1–5	4	1–5

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survival or in graft function was found. Furthermore, no rebound of antibodies with or without humoral rejection was observed in any of the 60 consecutive patients. Since a rebound of antibodies has been reported when other protocols have been used (*5*, *6*), an explanation for this difference must be sought in differences between the protocols. One such difference is the use of rituximab instead of splenectomy. Indeed, this has been suggested by others (*7*) who found no antibody-mediated rejections and sustained low postoperative antibody titers when rituximab was used rather than splenectomy. Another important factor may be the preapheresis induction period with full-dose tacrolimus and mycophenolate mofetil, which may well decrease antibody production (*8*, *9*).

Another observation is the finding that postoperative preemptive antibody adsorptions may not be needed. Thus in one center postoperative immunoadsorptions were only performed in 7 out of 21 patients showing a slight rebound of antibodies. However they still did not observe any humoral rejections. Whether this will hold true in a larger series remains to be shown. The reason for performing preemptive adsorptions postoperatively rather than waiting for increasing antibody titers initially was that interpretation of postoperative antibody titers is obscured by the fact that a low antibody titer does not preclude antibody production since the antibodies may actually be absorbed by the graft. In the event of an increase in antibody titers the kidney may already be saturated and it may be too late to intervene. But again, the impact of preemptive immunoadsorptions in the absence of detectable antibody titers can be questioned.

A third important observation of this study was the substantial difference in antibody titers in the patients included in Stockholm, Uppsala, and Freiburg. Since there is no reason to believe that there is a difference in the degree of ABO-immunization between the three centers, the only possible explanation is differences in titration techniques. Such inter-institutional variation in the measurement of anti-A/B antibodies has previously been reported (*10*). Since the number of preoperative antibody adsorptions is based entirely on the measured antibody titer, variations in technique have a substantial impact on the number of procedures and, consequently, on the total cost for the procedure. Assuming that the three populations have the same degree of immunization, it seems that the techniques used in Freiburg and Stockholm for titration were too sensitive when compared with that in Uppsala; however, even with the lower sensitivity there was no humoral rejections or antibody rebound. Obviously, a standardized fluorescence-activated cell sorting technique for antibody quantification is much needed.

REFERENCES

1. Tydén G, Kumlien G, Fehrman I. Successful ABO-incompatible kidney transplantations without splenectomy using antigen-specific immunoadsorption and rituximab. *Transplantation* 2003; 76: 730.
2. Tydén G, Kumlien G, Genberg H, et al. The Stockholm experience with ABO-incompatible kidney transplantations without splenectomy. *Xenotransplantation* 2006; 13: 105.
3. Donauer J, Wilpert J, Geyer M, et al. ABO-incompatible kidney transplantation using antigen-specific immunoadsorption and rituximab: A single center experience. *Xenotransplantation* 2006; 13: 108.
4. Kumlien G, Ullström L, Losvall A, Persson LG, Tydén G. Clinical experience with a new apheresis filter that specifically depletes ABO blood group antibodies. *Transfusion* 2006; 46: 1568.
5. Ishida H, Tanabe K, Ishizuka T, et al. Differences in humoral immunity between a non-rejection group and a rejection group after ABO-incompatible renal transplantation. *Transplantation* 2006; 81: 665.
6. Gloor JM, Lager DJ, Moore SB, et al. ABO-incompatible kidney transplantation using both A2 and non-A2 living donors. *Transplantation* 2003; 75: 971.
7. Sonnenday CJ, Warren DS, Cooper M, et al. Plasmapheresis, CMV hyperimmune globulin, and anti-CD20 allow ABO-incompatible renal transplantation without splenectomy. *Am J Transplant* 2004; 4: 1315.
8. Tanabe K, Tokumoto T, Ishida H, et al. Excellent outcome of ABO-incompatible living kidney transplantation under pretransplantation immunosuppression with tacrolimus, mycophenolate mofetil, and steroids. *Transplant Proc* 2004; 36: 2175.
9. Mannami M, Mitsuhata N. Improved outcomes after ABO-incompatible living-donor kidney transplantation after 4 weeks of treatment with mycophenolate mofetil. *Transplantation* 2005; 79: 1756.
10. Kobayashi T, Saito K. A series of surveys on assay for anti-A/B antibody by Japanese ABO-incompatible Transplantation Committee. *Xenotransplantation* 2006; 13: 136.

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