ABO-incompatible kidney transplantation

Karoline Schousboe[1], Kjell Titlestad[2], Francois Baudier[3], Lars Ulrich Hansen[4] & Claus Bistrup[1]

ABSTRACT

INTRODUCTION: Kidney transplantation is the optimal treatment for many patients with end-stage renal disease (ESRD). Due to shortage of donor kidneys in Denmark, there is a need to expand the possibilities for donation. At the Odense University Hospital (OUH), we have introduced ABO-incompatible kidney transplantation. We used antigenspecific immunoadsorptions to remove blood group antibodies and anti-CD20 antibody (rituximab) to inhibit the antibody production. The aim of introducing the ABO-incompatible kidney transplantation at the OUH was to increase the rate of living donor kidney transplantation without increasing rejection or mortality rates.

MATERIAL AND METHODS: Retrospective evaluation. Eleven patients received ABO-incompatible kidney transplantation. The patients were followed for 3-26 months.

RESULTS: One patient had an antibody-mediated rejection, one patient suffered T-cell-mediated rejection, and one patient died of myocardial infarction with a functioning graft on the third post-operative day. Both rejections were treated effectively. Among the patients, the average serum creatinine level was 128 micromol/l.

CONCLUSION: The rejection and mortality rates for ABO-incompatible kidney transplantation at the OUH are similar to the results from ABO-compatible kidney transplantations performed at the OUH and at other hospitals.

Approximately 700 new cases of end-stage renal disease (ESRD) are diagnosed in Denmark per year [1]. Treatment consists of either dialysis or kidney transplantation, the latter resulting in better patient survival [2]. A major limitation on kidney transplantation in Denmark is the limited number of available donor kidneys. The waiting list currently counts 450 patients and the list is growing [3]. There is a need for expanding and optimizing the possibilities for kidney donation from deceased as well as living donors. In the past decade, the number of kidneys procured from diseased donors was approx. 125 per year [4]. To increase this number, The Danish Center for Organ Donation was established [5]. Furthermore, it is necessary to focus on optimized utilization of kidneys transplanted from living donors which have shown better patient and graft survival than those transplanted from deceased donors [6]. In recent years, an annual average of approx 75 (14 per million) living donor kidney transplantations have been performed in Denmark [3]. This donation rate is low among others due to exclusion of donors based on ABO-incompatibility between kidney donor and recipient (up to 40% in foreign studies [7]). ABO antigens are expressed on the surface of almost all cells in the body. Humans express antibodies against antigens that they do not express themselves. ABO-incompatibility causes hyper acute rejection in kidney transplantation if pretreatment is not given. Blood type A is separated into the subtypes A1 (80%) and non-A1 (20%). A1 is more immunogenic than B which, in turn, is more immunogenic than non-A1 [8].

Since 1989, ABO-incompatible kidney transplantations have been performed in Japan using extensive immunosuppressive regimes, including plasmapheresis and either splenectomy or spleen radiation and radiation of the graft during and after transplantation. In contrast, in 2003 Tydén et al from Sweden reported on ABO-incompatible kidney transplantation without the use of plasmapheresis, splenectomy and radiation in patients with a low concentration of blood type antibody measured by hemagglutinin titres [9]. Instead of plasmaphereses, removal of only anti-A or anti-B antibodies was performed by antigen-specific immunoadsorption. Induction with intravenous immunoglobulins (IVIG) was used to moderate the effect of the remaining antibodies. Contrary to splenectomy, anti-CD20 antibody (rituximab) was used to prevent the production of new antibodies. Prior to transplantation, triple immunosuppressive therapy was initiated. This Swedish protocol was introduced at the Odense University Hospital (OUH) in 2007 with the aim of increasing the donation rate in living donor kidney transplantation.

MATERIAL AND METHODS

We identified patients who had previously had a living donor rejected because of ABO-incompatibilty. Immunoglobulin (Ig)-G and IgM isohaemagglutinin titres were

! ABBREVIATIONS

CMV = cytomegalovirus
EBV = Epstein-Barr virus
ESRD = end-stage renal disease
Ig = immunoglobulin
IVIG = intravenous immunoglobulins
OUH = Odense University Hospital

ORIGINAL ARTICLE

1) Odense University Hospital, Department of Nephrology,
2) Department of Clinical Immunology,
3) Department of Vascular Surgery, and
4) Department of Urology

Dan Med Bul 2010;57(10):A4197

measured at screening, and before and after any transplantation. Isohaemagglutinin titres were defined as the inverted value of the highest plasma dilution of 0.9% saline that resulted in a weak positive agglutination reaction in recipient serum incubated with donor red blood cells [10].

Four weeks prior to transplantation, rituximab was infused (375 mg/m^2). Triple immunosuppressive treatment with tacrolimus 0.25 mg/kg, mycophenolate mofetil 2 g and prednisolone 30 mg daily was started 14 days prior to the expected date of transplantation (day -14). The target B-tacrolimus concentration of 15 ng/ml was reduced to 10 and 5 ng/ml one and two months after transplantation, respectively. Three months after transplantation, mycophenolate mofitil was reduced to 1 g. On the day of transplantation, the dose of prednisolone was increased to 100 mg, followed by a daily reduction of 10 mg until reaching a dose of 20 mg/day which was continued for the rest of the first postoperative month. The prednisolone dose was reduced to 15 and 10 mg in the second and third months, respectively. After six months, withdrawal from prednisolone was considered. Anti-viral prophylaxis with aciclovir was given to all patients for three months except when kidneys from cytomegalovirus (CMV)-positive donors were transplanted into CMV-negative recipients. These patients were treated with valganciclovir. The patients received sulfamethoxazole/trimethoprim (400/80 mg) once daily for three months as prophylaxis against pneumocystis jirovecii pneumonia.

Preoperatively, anti-A or anti-B antibodies were removed from plasma by antigen-specific immunoadsorption (Glycosorb AB0; Glycorex Transplantation AB, Lund, Sweden). Three sessions of immunoadsorptions of double plasma volumes were performed on days -5, -4 and -1. If the IgG and/or IgM isohaemagglutinin titres for anti-A or anti-B antibodies were greater than 1:32, another three to four sessions were performed, and transplantation was postponed for one week. If the titres exceeded 1:(256-)512 at screening, five preoperative sessions were planned. After the last preoperative session, all patients were given IVIG 0,5 g/kg (Immunglobulin, CSL Behring). The first two patients received postoperative immunoadsorption of one plasma volume on days 1, 3, and 6, respectively. Based on experiences from other centres [11], this strategy was changed and the following recipients received postoperative immunoadsorptions only if their titres exceeded 1:32.

RESULTS

Eleven ABO-incompatible transplantations were performed with a follow-up of 3-26 months (median eight months). The first patient was transplanted in June 2007 and the remaining ten patients were transplanted from the beginning of 2008 to the spring of 2009. Nine of the eleven donor kidneys were removed by laparoscopy. Characteristics of donors and recipients, human leukocyte antigen mismatches, CMV- and Epstein-Barr virus (EBV)-status, and dialysis modality are shown in **Table 1**. Blood types of donor and recipient, effect of immunoadsorptions on IgG titres, serum creatinine and prednisolone dose at follow-up are shown in **Table 2**. Only IgG titres are shown as IgG is more specific for antibody-meditated rejection than IgM [12].

Patient number two developed a sterile peritonitis on day -41. Subsequently, the IgG titre rose to 1:1,000, and the patient received four extra immunoadsorptions prior to transplantation. On day 42, the patient again showed a rise in titre to 512 as well as graft dysfunction. A biopsy from the graft showed antibody-mediated rejection which was treated with a high dose of prednisolone, IVIG and nine antigen-specific immunoadsorp-

TABLE 1

Donor and recipient characteristics and number of human leukocyte antigen mismatches on loci AB and DR. Diagnosis, cytomegalovirus, Epstein-Barr virus, and dialysis status of the recipient.

Patient	Donor		Recipient			HLA AB-DR	CMV	EBV	Dia-
no.	gender	age (years)	sex	age (years)	Diagnosis	mismatches	don/rec	don/rec	lysis
1	M	59	M	32	IgA nephropathy	1:1	N/P	P/P	HD
2	F	45	M	54	Polycystic kidney disease	2:1	P/P	P/P	PD
3	F	62	M	66	Nephrosclerosis	3:2	P/NS	P/P	PD
4	F	44	M	48	Unknown	3:2	N/N	N/N	HD
5	M	51	M	54	Unknown	0:0	P/P	P/P	PD
6	M	48	M	35	Unknown	4:2	N/P	P/P	HD
7	F	35	F	26	Glomerulosclerosis	3:2	NA/N	P/P	PD
8	F	43	M	55	Type 1 diabetes	3:1	N/N	P/P	HD
9	M	53	M	55	IgA nephropathy	1:1	P/P	P/P	HD
10	M	55	M	62	Reflux nephropathy	2:1	P/P	P/N	HD
11	F	59	M	60	Chronic pyelonephritis	3:1	P/N	P/P	PRE

CMV = cytomegalovirus; don = donor; EBV = Epstein-Barr virus; F = female; HD = haemodialysis; HLA AB-DR = human leukocyte antigen on loci AB and DR; IgA = immunoglobulin A; M = male; N = negative; NA = not analyzed; NS = not significantly positive; P = positive; PD = peritoneal dialysis; PRE = preemptive; rec = recipient.

TABLE 2

Effect of antigen-specific immunoadsorption on immunoglobulin G-titres, serum creatinine and present dose of prednisolone.

Patient no.	Don/rec blood types	Anti-A or -B IgG-titres at screening	Anti-A or -B IgG-titres prior to adsorption	Number of preoperative adsorptions	Anti-A or -B IgG-titres at transplantation	Number of postoperative adsorptions	Anti-A or -B IgG-titres postoperatively (min/max)[a]	Follow-up (months)	Serum creatinine at follow-up (micromol/l)	Prednisolone daily dose at follow-up (mg)
1	Non-A1/O	32	512	3	16	3	4/62	26	142	0
2	B/O	128	1000	7	16	9	4/512	19	163	7,5
3	B/O	16	16	3	4	0	0/16	14	120	0
4	A1/B	8	4	3	4	0	0/1	10	132	5
5	B/O	64	32	3	1	0	1/1	10	99	5
6	A1/O	256	256	5	8	0	4/8	8	104	2,5
7	A1/O	512	512	5	16	0	8/16	6	71	2,5
8	A1/B	16	64	3	4	0	1/4	3 days[b]	180	70
9	B/O	128	256	3	32	0	1/4	5	130	10
10	B/A	16	8	2	8	0	0/1	4	159	10
11	B/A	32	16	3	1	0	0/1	3	156	Anti-rejection treatment

don = donor; IgG = immunoglobulin G; rec = recipient.
a) Lowest and highest measured value. b) Death due to coronary infarction.

tions. Seventeen days later, the titre was reduced to 1:8 and serum creatinine was 110 micromol/l [10].

Patient number three, who was not significantly positive for CMV, received a CMV-positive kidney and was initially treated with aciclovir. The patient had a CMV viraemia one and six months after the operation, respectively, and was twice treated with valganciclovir for three months. At follow-up, the patient was CMV-free. Patient 11, who was CMV-naïve, had a CMV viraemia on day 22. Mycophenolate mofetil was reduced and aciclovir was substituted with valganciclovir. Due to a further increase in CMV copies, mycophenolate mofetil was further reduced and tacrolimus and prednisolone were also reduced. Thereafter, the patient had a mild cellular rejection, Banff grade 1A, and was treated with prednisolone.

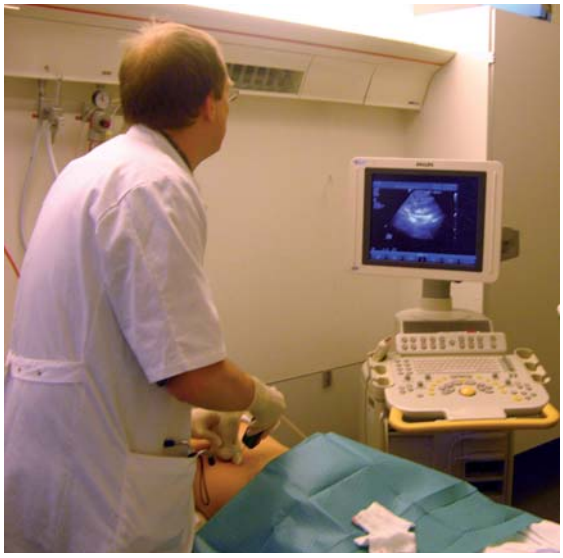
Patient eight, a 43-year-old patient with type 1 diabetes, who had received dialysis therapy for a period of ten years, died on day three of acute myocardial infarction with a functioning graft and an serum creatinine level of 180 micromol/l. At follow-up, the other ten patients had a well-functioning graft with a mean serum creatinine level of 128 micromol/l. None of the eleven patients had bacteraemia, BK-virus or EBV infection.

DISCUSSION

Acceptable graft function was achieved in all patients undergoing ABO-incompatible kidney transplantation from a living donor at OUH until September 2009. Patient two had an antibody-mediated rejection which was treated efficiently with antigen-specific immunoadsorption, IVIG and prednisolone, and patient 11 had a cellular rejection which was treated conventionally with glucocorticoids. The follow-up period of 3-26 months was too short to assess the long-term prognosis. However, to evaluate the safety of continuing this regimen, an assessment of the short-term prognosis was considered important. Other studies with short follow-up periods of 1-48 and 3-17 months, respectively, have reported no antibody-mediated rejections [11, 13]. In patient two, high anti-B titres of 1:1,024 preceded by peritonitis prior to transplantation could have caused the antibody-mediated rejection.

In previous studies including plasmaferesis and splenectomy prior to transplantation, isohaemagglutinin titres exceeding 1:128 have been associated with more frequent rejections and reduced graft survival [12]. Therefore, according to the Tydén-protocol, 1:128 was the maximum permissible level of IgG anti-A or anti-B titre prior to ABO-incompatible transplantation. However, comparison of titres in different studies is difficult due to variations in the laboratory methods employed at different centres [14]. Consequently, blood samples from patients were were sent to the laboratory used by Týden, revealing titres at 2-3 times lower level than those of the analyses performed at the OUH [10], which indicates that the titre level may be of limited importance. Furthermore, initial isohaemagglutinin titres may not be predictive of immunoadsorption success nor of outcome of the ABO-incompatible kidney transplantation [11].

Patient 11 developed a cellular rejection after reduction of immunosuppressive therapy due to CMV infection. Patient eight died with a functioning graft due to myocardial infarction three days after the transplantation. The patient suffered from diabetes, which is the



Ultrasound-guided graft biopsy.

greatest risk factor for cardiovascular disease after kidney transplantation, and the greatest risk is seen during the first three months [15].

Taken together, of the eleven ABO-incompatible transplanted patients, one case showed antibody-mediated rejection, another cellular rejection, and a third died with a functioning graft. The short and varying follow-up period (3-26 months) of the ABO-incompatible kidney transplantations performed at the OUH does not allow for comparison with results of ABO-compatible living person kidney transplantations at the OUH, which in 2007 showed a one-year patient survival of 94.9%. The one-year graft survival of these patients was 97.2%. A study comparing 15 ABO-incompatible kidney transplantations with 30 ABO-compatible kidney transplantations with a longer follow-up period averaging three years showed no significant differences in acute rejections, patient or graft survival between the two groups [16].

In previous studies, postoperative immunoadsorptions were planned regardless of the titre level. The assumption was that a low isohaemagglutinin titre was not necessarily a marker of lack of antibody production, but more likely showed that antibodies were adsorbted by the graft [13]. In a recent publication from *Freiburg*, 15 of 22 patients had low titres and were not given postoperative immunoadsorptions [17]. None of the patients developed antibody-mediated rejection. This resulted in major economic savings as the cost of a single-use immunoadsorption column was 5,200 €. Based on these findings, our strategy was changed and postoperative immunoadsorptions were not performed in the final nine patients. None of these patients developed titre increase or antibody-mediated rejection.

Since 1983, a prednisolone-free protocol for ABO-compatible kidney transplantations has been used at the OUH [18]. In ABO-incompatible kidney transplantations with an elevated risk of antibody-mediated rejection, we have administered prednisolone according to the Týden protocol. Unfortunately, prednisolone is associated with various side effects, i.e. infections, increased risk of cardiovascular disease, transplantation-associated diabetes, hypertension, hyper-lipidemia, osteoporosis and weight gain. Therefore, according to a steroid sparing protocol published in 2008 by *Galliford et al* [19], we started reducing prednisolone. In two patients, prednisolone was withdrawn one year after transplantation without any known impact on graft function. However, the risk of rejection associated with late steroid withdrawal remains uncertain [20].

CONCLUSION

It has been possible to implement ABO-incompatible kidney transplantations at the OUH based on: rituximab instead of splenectomy and antigen-specific immunoadsorption instead of plasmapheresis, combined with IVIG induction and conventional triple immunosuppressive therapy. During the process, the protocol was modified: planned postoperative immunoadsorptions were omitted and prednisolone was either reduced or withdrawn. The single cases of antibody-mediated rejection, cellular rejection, and death are estimated to be at the same level as previous results from ABO-compatible and -incompatible kidney transplantation studies using living donor kidneys performed both at the OUH and as reported in other studies.

The implementation of ABO-incompatible kidney transplantation at the OUH increases the number of possible suitable living donors. Consequently, some patients may avoid a long waiting period while queing for donation from a deceased donor which may, in turn, have a positive impact on graft function and survival [2]. Furthermore, patients on the waiting list may see a reduction in the waiting time to receive a kidney from a deceased donor.

CORRESPONDENCE: *Karoline Schousboe,* Department of Nephrology, Odense University Hospital. E-mail: kschousboe@dadlnet.dk

ACCEPTED: 16 July 2010

CONFLICTS OF INTEREST: None

LITERATURE

1. The Danish Society of Nephrology. Danish Nephrology Registry, Annual Report. 2006.
2. Meier-Kriesche HU, Port FK, Ojo AO et al. Effect of waiting time on renal transplant outcome. Kidney Int 2000;58:1311-7.
3. www.scandiatransplant.org. (1 May 2010).
4. The Danish Society of Nephrology. Danish Nephrology Registry, Annual Report. 2008.
5. Linnet J and Brennum J. Dobbelt livreddende behandling. Ugeskr Læger 2009;13:1112-3.
6. Cecka JM. The OPTN/UNOS Renal Transplant Registry. Clin Transpl 2005;1-16.
7. Kumlien G, Ullstrom L, Losvall A et al. Clinical experience with a new apheresis filter that specifically depletes ABO blood group antibodies. Transfusion 2006;46:1568-75.

8. Rydberg L. ABO-incompatibility in solid organ transplantation. Transfus Med 2001;11:325-42.
9. Tyden G, Kumlien G, Fehrman I. Successful ABO-incompatible kidney transplantations without splenectomy using antigen-specific immunoadsorption and rituximab. Transplantation 2003;76:730-1.
10. Just SA, Marcussen N, Sprogoe U et al. Acute antibody-mediated rejection after ABO-incompatible kidney transplantation treated successfully with antigen-specific immunoadsorption. Nephrol Dial Transplant 2010;25:310-13.
11. Donauer J, Wilpert J, Geyer M et al. ABO-incompatible kidney transplantation using antigen-specific immunoadsorption and rituximab: a single center experience. Xenotransplantation 2006;13:108-10.
12. Shimmura H, Tanabe K, Ishikawa N et al. Role of anti-A/B antibody titers in results of ABO-incompatible kidney transplantation. Transplantation 2000;70:1331-5.
13. Tyden G, Kumlien G, Genberg H et al. The Stockholm experience with ABO-incompatible kidney transplantations without splenectomy. Xenotransplantation 2006;13:105-7.
14. Kumlien G, Wilpert J, Safwenberg J et al. Comparing the tube and gel techniques for ABO antibody titration, as performed in three European centers. Transplantation 2007;84:S17-S19.
15. Aalten J, Hoogeveen EK, Roodnat JI et al. Associations between pre-kidney-transplant risk factors and post-transplant cardiovascular events and death. Transpl Int 2008;21:985-91.
16. Genberg H, Kumlien G, Wennberg L et al. Long-term results of ABO-incompatible kidney transplantation with antigen-specific immunoadsorption and rituximab. Transplantation 2007;84:S44-S47.
17. Geyer M, Donauer J, Pisarski P et al. Preemptive postoperative antigen-specific immunoadsorption in ABO-incompatible kidney transplantation: necessary or not? Transplantation 2007;84:S40-S43.
18. Birkeland SA, Elbirk A, Rohr N et al. Optimization of cyclosporine monotherapy--long-term graft function. Transplant Proc 1994;26:2501-6.
19. Galliford J, Charif R, Chan KK et al. ABO incompatible living renal transplantation with a steroid sparing protocol. Transplantation 2008;86:901-6.
20. Oettl T, Zuliani E, Gaspert A et al. Late steroid withdrawal after ABO blood group-incompatible living donor kidney transplantation: high rate of mild cellular rejection. Transplantation 2010;89:702-6.