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Original Article

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ABO blood group-incompatible living donor kidney transplantation: a prospective, single-centre analysis including serial protocol biopsies

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Abstract

Background. ABO incompatible kidney transplantation using antigen-specific immunoadsorption is increasingly performed but data on outcome, complications and protocol biopsies are still scarce. The present prospective single-centre study was aimed at these issues.

Methods. This was a prospective single-centre cohort study of 10 successive ABO incompatible living donor kidney transplantations at the University Hospital Basel from September 2005 to October 2007. The following parameters were closely monitored during the whole follow-up: graft function, albuminuria, blood group antibody titres, CD19+ cell count, total IgG and IgG subclasses, CMV antigenaemia, decoy cells in the urine, EBV and polyoma BK virus PCR in the blood. Protocol biopsies were performed on Days 0 and 7 after 3, 6, 12 and 18 months.

Results. Patient and graft survival is 100% after a median follow-up of 489 days (range 183–916 days). Median serum creatinine is 137 μmol/l (range 70–215 μmol/l), and median urine albumin–creatinine ratio (UACR) is 3.1 mg/mmol (range 0.6–7.8 mg/mmol) at the time of the last follow-up. All patients had sustained diminished CD19+ cell count and/or total IgG concentrations. Neither CMV antigenaemia nor EBV replication in the blood was observed. Seven patients had positive polyoma BK virus replication in the blood but none developed polyoma virus-associated nephropathy (PVAN). Protocol biopsies revealed rejection Banff IIa in three patients on Day 7, and in one patient after 3 and 6 months. Banff Ia rejection was found in five patients. All rejection episodes resolved. Mild signs of chronic antibody-mediated rejection were observed in five patients.

Conclusions. ABO-incompatible kidney transplantation seems to be successful and safe. Modifications of the current protocol may be possible and may further reduce potential side effects and costs.

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Introduction

The persistent shortage of deceased organ donors and the increasing number of patients waiting for a kidney transplant necessitate new solutions to expand the pool of potential kidney donors. Transplantation across blood group barriers has been performed since the pioneering work of G.P.J. Alexandre in the 1980s [1,2] and can increase the number of living donated kidney transplantations by at least 10% [3]. Recent studies using preconditioning regimens with anti-CD20 antibodies, immune globulins and removal of circulating blood group antibodies by plasmapheresis (PP) or selective immunoadsorption (IA) revealed similar short- and long-term patient and graft survival as observed in blood group-compatible transplantation [4–8]. Therefore, ABO-incompatible living donor kidney transplantation offers a promising strategy to reduce the number of patients waiting for a kidney transplant, yet the current experience in this field is still small. The present study summarizes a prospective single-centre experience of ABO blood group incompatible kidney transplantation at the University Hospital Basel.

Methods

The principles of ABO blood group incompatible kidney transplantation have been discussed in detail in two landmark publications [5,8].

Preparation and desensitization

In short, basic immunosuppressive therapy including tacrolimus [0.1 mg/kg body weight (BW) twice daily], mycophenolate mofetil (1000 mg twice daily, 500 mg twice

daily if BW was <50 kg) and prednisone (30 mg once daily) was started 2 weeks prior to transplantation. A single dose of rituximab (375 mg/m^2) was given 4 weeks prior to transplantation in an outpatient setting. Selective blood group antibody removal [selective immunoadsorption (IA)] was performed with a low-molecular carbohydrate column containing A or B blood group antigens linked to a sepharose matrix (Glycosorb®, Glycorex Transplantation, Lund, Sweden). Apheresis sessions were performed daily until the immunoglobulin (IgG) and isoagglutinine (IgM) antibody titres against donor erythrocytes were 1:8 or less, as determined by the indirect Coombs test for IgG and the saline method for IgM. The transplantation was then carried out the following day. If one of the two titres remained >1:8, additional IA was mandatory until the target titre was achieved. With each session, two plasma volumes, calculated with the formula of Kaplan [9], were processed. After the last IA, a single dose of intravenous (iv) immune globulins (IVIG, 0.5 g/kg BW) on Day-1 and an additional T-cell blockade with basiliximab 20 mg iv on Days 0 and 4 were administered.

Follow-up

After transplantation, blood group IgM and IgG antibodies against the donor blood group were measured daily for 2 weeks, weekly until Day 31, and then 3, 6 and 12 months thereafter. Another three prophylactic IA were performed on Days 2, 5 and 8, with one plasma volume processed each time. From patient 8 on, regular prophylactic IA after kidney transplantation was discontinued if the clinical course was uneventful and the protocol biopsy on Day 7 revealed no signs of antibody-mediated rejection. Target tacrolimus trough levels were 10–12 ng/ml from Day 14 to 31, 8–10 ng/ml from Day 32 to 90, 6–8 ng/ml from Day 91 to 365 and 4–6 ng/ml thereafter. Target mycophenolate mofetil trough level was >2 mg/ml. Steroids [methylprednisolone iv and prednisone perorally (po)] were tapered: 500 mg iv on Day 0, 250 mg iv on Day 1, 100 mg iv on Day 2, 50 mg po from Day 3 to 6, 0.5 mg/kg BW po from Day 7 with a reduction by 5 mg every 2 weeks until 15 mg/day, then by 2.5 mg every 2 weeks until a maintenance dose of 0.1 mg/kg BW po was achieved. Primary prophylaxis with valganciclovir in cases with a high risk of CMV infection (donor CMV positive and/or recipient CMV positive) was performed during 3 months. Trimethoprim/sulfamethoxazol was given three times weekly for 6 months as a primary prophylaxis against *Pneumocystis jiroveci* pneumonia. Regular, at least monthly viral screening was done for Epstein–Barr virus (EBV; PCR), polyoma BK virus (BKV; PCR, decoy cells in the urine) and cytomegalovirus (CMV; estimation of the pp65 antigenaemia). The urine albumin to creatinine ratio (UACR; enzymatic quantification, normal value <2.26 mg/mmol), a marker of glomerular injury, was measured before transplantation, on Days 7 and 14, in months 1, 2, 3, 4, 5, 6, 9, 12, and annually thereafter. CD19+ cell count, total IgG concentration and IgG subclasses 1–4 were measured in the blood before rituximab therapy, the day before transplantation, in months 1, 6, 12 and annually thereafter. Protocol biopsies were taken on the day of transplantation ('zero biopsy', taken 45–60 min after the start of reperfusion), after 1 week, and after 3, 6 and 12 months. In general, two needle biopsy cores were obtained for morphologic work-up and processed as previously described elsewhere [10,11]. Electron microscopy was routinely performed in all specimens. Biopsies were judged according to the revised Banff criteria 2003 and 2005 [12,13]. All complications were prospectively recorded at each clinical visit.

Results

Patient characteristics

Between September 2005 and October 2007, 11 patients were enrolled and prepared for transplantation according to the protocol. One patient discontinued the preparation because of an acute stress disorder. The remaining 10 patients were successfully transplanted. As of 1 April 2008, patient and graft survival was 100% after a median follow-up of 489 days (range 183–916 days). Donor and recipient characteristics are shown in Table 1. All recipients' EBV serology was positive for IgG prior to transplantation. Two patients had a CMV high-risk constellation (donor CMV seropositive, recipient CMV seronegative), the recipient was CMV positive in three cases and both donor and recipient were CMV negative in five cases. The median number of HLA A, HLA B and HLA DR mismatches was 5 (range 4–6). No recipient had donor-specific HLA antibodies before transplantation as measured by single antigen beads on a luminex platform. The recipients' anti-A- or anti-B-IgM and IgG antibody titres against the donor blood group (donor erythrocytes) before start of the preparation and the total number of IA needed to achieve titres ≤1:8 are shown in Table 1. One patient (case 2) experienced symptomatic hypotension and bronchial obstruction a few minutes after the start of the first IA, probably due to continued therapy with an ACE inhibitor. IA was therefore replaced by total plasma exchange treatments.

Posttransplant follow-up

The follow-up data are shown in Table 2. All 10 recipients had immediate graft function after transplantation. Early posttransplant follow-up was uneventful in 8 out of 10 patients. Our first patient experienced biopsy-proven humoral rejection 13 days after transplantation. He was successfully treated with anti-thymocyte globulins (ATG Fresenius®, 4 mg/kg BW) and IVIG (0.4 g/kg BW) for 7 days, three additional IA and five PP sessions. One patient (case 4) has persistent, markedly elevated creatinine levels as a consequence of multiple operations due to recurrent ureter leakage and obstruction as well as recurrent bacterial pyelonephritis with graft injury. All 10 patients showed a rapid decline of UACR over time. Median UACR after 7 days was 29 mg/mmol ($n = 9$, range 9–658 mg/mmol), 6 mg/mmol after 3 months ($n = 10$, range 1–39 mg/mmol), 5 mg/mmol after 6 months ($n = 8$, range 1–27 mg/mmol) and 4 mg/mmol after 12 months ($n = 6$, range 1–25 mg/mmol). Table 2 shows the most recently measured UACR of each patient.

Table 1. Donor and recipient characteristics

	Donor			Recipient							
	Sex	age	BG	BG	Sex	Age	Relationship	Original kidney disease	Time on waiting list (years)	Initial BG antibody titres IgM IgG	Number of IA needed before and after transplantation
1	F	59	B →	A_1	M	47	Partner	DN	Pre-emptive	1:64 1:8	4 6 + 5 PP
2	F	43	AB→	B	M	48	Partner	IgA-N	3.3	1:16 –	4 (PP) 2 (PP)
3	M	28	A_1 →	0	M	51	Brother-in-law	PKD	3.9	1:128 1:256	11 3
4	F	66	A_1 →	0	M	67	Partner	IgA-N	Pre-emptive	1:64 1:64	5 1
5	M	40	A_1 →	0	M	40	Friend	IgA-N	4.0	1:16 1:64	5 2
6	M	36	A_1 →	0	F	34	Partner	PKD	Pre-emptive	1:32 1:32	4 2
7	F	42	A_1 →	0	M	54	Sister	PKD	1.7	1:256 1:256	16 1
8	F	61	A_1 →	0	M	70	Friend	GS	0.7	1:2 1:8	4 0
9	F	55	A_1 →	0	M	55	Partner	CIN	4.9	1:128 1:1024	13 0
10	M	73	A_1 →	B	F	69	Partner	DN	0.8	– 1:32	4 0

BG: blood group; DN: diabetic nephropathy; IgA-N: IgA-nephropathy; GS: glomerulosclerosis; PKD: polycystic kidney disease; CIN: chronic interstitial nephritis; PP: plasmapheresis.

Table 2. Follow-up data

Patient	Follow-up (day)	Last creatinine (μmol/l)	Last UACR (mg/mmol)	Last aMDRD (ml/min/ 1.73 m^2)	BKV	Complications
1	916	125	4.1	53	+	
2	747	98	1.6	70	+	
3	729	153	5.4	42	+	Deep venous thrombosis after travelling
4	526	207	4.1	30	–	6 × surgical revision due to ureter leakage, 2 × transplant pyelonephritis cataract operation
5	505	143	0.6	47	+	
6	489	84	1.2	67	+	
7	376	131	1.1	52	–	2× transplant pyelonephritis
8	273	148	7.8	41	+	
9	229	215	2.0	28	+	Lymphocele operation; nephrectomy (own kidney: infected kidney stones with recurrent urinary tract infection); herpes zoster
10	183	70	5.1	72	–	
Median	489	137	3.1	50		
Range	183–916	70–215	0.6–7.8	28–72		

UACR: urine albumin creatinine ratio (normal value: <2.26 mg/mmol); aMDRD: calculation of glomerular filtration rate by the abbreviated MDRD formula [14]; BKV: polyoma BK PCR in the blood.

Complications during follow-up

The complications of each patient are shown in Table 2. Thus far, no CMV reactivation (positive p-65 antigenaemia), CMV disease or EBV replication in the blood has been observed. BKV replication in the blood was found in seven patients (see Table 2): first positive PCR results were seen after 7, 8, 11, 37, 46, 49 and 108 weeks (median 37 weeks), respectively. None of these patients had signs of a polyoma virus-associated nephropathy (PVAN) in the protocol biopsies following the positive BKV replication in the blood.

Table 3. Acute and chronic histological findings in protocol biopsies according to the Banff classification [12,13]; relevant acute lesions are highlighted

Patient	Histological findings	Zero-biopsy	Day 7	Month 3	Month 6	Month 12	Month 18
1	Acute lesions	N	IIA	N	N	N	IA
	Chronic lesions	N	N	N	N	N	Mild IF/TA
2	Acute lesions	N	N	IA	N	N	Not done
	Chronic lesions	N	N	N	N	N	Not done
3	Acute lesions	N	N	IIA	IIA	N	N
	Chronic lesions	N	N	N	N	N	Mild IF/TA
4	Acute lesions	Ins. mat.	Ins. mat.	PN	PN; IIA	N	–
	Chronic lesions	Ins. mat.	Ins. mat.	N	Mild IF/TA	Mild IF/TA	–
5	Acute lesions	N	IIA	N	N	N	–
	Chronic lesions	N	N	N	N	N	–
6	Acute lesions	N	N	N	N	N	–
	Chronic lesions	N	N	N	N	N	–
7	Acute lesions	N	N	IA	IA	–	–
	Chronic lesions	N	N	Mild IF/TA	Mild IF/TA	–	–
8	Acute lesions	Not done	N	CNI	IB	–	–
	Chronic lesions	Not done	N	N	N	–	–
9	Acute lesions	N	IIA	IA	IA	–	–
	Chronic lesions	N	N	N	Mild IF/TA	–	–
10	Acute lesions	N	N	N	–	–	–
	Chronic lesions	N	N	N	–	–	–

N: normal; CNI: calcineurin inhibitor toxicity; PN: pyelonephritis; IF/TA: interstitial fibrosis and tubular atrophy; Ins. mat.: insufficient material; IA: immunoadsorption.

Immunological follow-up data

IgM and IgG antibody titres against the donor blood group remained below 1:8 during the entire follow-up period, with only one exception (patient 8, IgG titre 1:16 after 1 month). A strong and sustained suppression of CD19+ cells, which represent the B-lymphocyte subpopulation, by a single dose of rituximab and mycophenolate mofetil was found. The mean CD19+ cell count in 9 out of 10 patients before application of rituximab was 96/μl ($n = 9$, median 63/μl, range 40–233/μl). One day before transplantation, median CD19+ cell count was as low as 5/μl ($n = 9$, range 0–9/μl), which was still found almost unchanged 1 month after transplantation ($n = 7$, median 2/μl, range 0–16/μl). Over time, the CD19+ cell count rose slightly to a median 12/μl after 6 months ($n = 7$, range 5–17/μl) and 25/μl after 12 months ($n = 5$, range 2–32/μl), respectively. Still, this number is far below the initial cell count and below the lower limit in normal controls (200–400/μl). Patient 8 suffers from chronic lymphatic leukaemia: in this case, we found an extraordinarily high CD19+ cell count before transplantation (7219/μl); during the follow-up, the number of CD19+ cells declined stepwise, with the last measured value of 966/μl (13% of pre-treatment value) after 7 months. IgG levels in the blood before rituximab treatment were within or above the normal range ($n = 10$, median 11.3 g/l, range 8.2–20.4 g/l, normal range 6.5–15 g/l) in all patients. One month after transplantation, four out of eight IgG levels were below the lower limit ($n = 8$, median 6.0 g/l, range 4.6–11.7 g/l). After 6 and 12 months, total IgG levels were below the lower limit in five and two patients, respectively (6 months: $n = 9$, median 6.3 g/l, range 5.3–11.4 g/l, 12 months: $n = 6$, median 6.9 g/l, range 4.9–9.6 g/l). Concerning IgG subclasses, no uniform pattern could be found, with most patients having low quantities of IgG 1–4 throughout, in consistence with the reduced total amount of IgG (data not shown).

Graft biopsies

The results of the protocol biopsies are shown in Table 3 ($n = 46$ including 2 with insufficient material). All episodes of acute interstitial or humoral rejection found in protocol biopsies were treated with steroid pulse therapy. Patient 3, who presented with a slight deterioration of graft function at the time of his 3-month protocol biopsy, was additionally treated with IVIG (0.4 g/kg BW) for 5 days. Six diagnostic biopsies due to unexplained deterioration of graft function were performed in three patients (patients 1, 4 and 9; two biopsies each). The first diagnostic biopsy from patient 1 was performed within the first 2 weeks after transplantation and revealed histological changes classified as Banff IB. Another diagnostic biopsy after 14 months was performed during steroid withdrawal: it revealed acute interstitial rejection Banff IA that was treated with steroid pulse therapy and reintroduction of low dose prednisone. Patient 4 had two diagnostic biopsies during the first month, one showing signs of an acute humoral rejection (Banff IIA) that was treated with IVIG (0.4 g/kg BW) for 5 days. A second diagnostic biopsy showed no specific pathology. In patient 9, the two diagnostic biopsies (Days 18 and 41 posttransplant) showed acute tubular necrosis in both samples. Zero biopsies showed normal renal tissue with a negative C4d immunostaining in all cases ($n = 8$). C4d immunostaining became diffusely positive in all but one of the 36 follow-up biopsies (one biopsy revealed only focal C4d positivity).

Discussion

The goal of ABO incompatible living donor kidney transplantation is to increase the number of kidney transplantations and to avoid or shorten the time on dialysis. The rate of success and potential risks must be equal to those after blood group-compatible transplantations. The present

analysis shows a proof of concept. The number of living donor kidney transplantations at the University Hospital Basel has been increased by 20% (61 instead of 51) since the start of the ABO incompatible program in September 2005. Four patients waiting >3 years for a graft could be transplanted, and pre-emptive transplantation was possible in three cases. Seven patients with blood group O, the blood group with the longest waiting time for a graft from a deceased donor, were given the opportunity for transplantation. In general, blood group O recipients (patients 3–9) had higher blood group antibody titres, particularly IgG titres, than patients with blood group A or B (patients 1, 2 and 10), and therefore needed a more intensive preconditioning. Of note, all recipients with blood group O were transplanted with an organ from a donor with blood group A_1. Patient and graft survival is excellent and does not differ from blood group-compatible kidney transplantation. Even patients with very high initial blood group antibody titres (e.g. patient 9, IgG titre 1:1024) have been successfully transplanted. Therefore, high initial anti-donor blood group antibodies should not be an exclusion criterion for ABO incompatible transplantation as also shown by Donauer [7] and Shimmura [15]. Regular IA posttransplant was performed in our first seven patients as originally suggested by Tydén [5]. Motivated by the results of Donauer [7], routine posttransplant IA was stopped in our last three patients despite very high titres in one case (patient 9) without negative impact on A/B-antibody levels or renal function. These results suggest that regular IA posttransplant in the absence of clinical or histological evidence of graft dysfunction is not needed. Markers of chronic graft injury, e.g. deterioration of kidney function, albumin excretion in the urine or chronic changes in the serial protocol biopsies, did not increase over time despite persisting C4d positivity in all graft biopsies. Hence, the relevance of C4d-positive graft biopsies as a marker of acute or chronic humoral rejection differs between ABO-incompatible and donor-specific anti-HLA antibody-positive grafts. We share the observation of Haas and colleagues [16] that C4d positivity in ABO-incompatible grafts does not necessarily mean harmful antibody-mediated rejection but has to be interpreted in the context of additional clinical, histological and laboratory findings.

The spectrum of adverse events in the present analysis is comparable with ABO-compatible kidney transplantation. Major complications that required re- or prolonged hospitalization were due to surgical reasons and were unrelated to ABO incompatibility or its treatment. None of the patients experienced CMV disease, CMV antigenaemia or EBV replication. These findings suggest that the current protocol does not carry an enhanced risk of over-immunosuppression. Nevertheless, 7 out of 10 patients experienced repeatedly positive BKV replication in the blood that did not proceed to overt BKV nephropathy probably because of rapid lowering of the maintenance immunosuppression and subsequent disappearance of the virus in the blood. The onset of BKV replication in the blood and the occurrence of BKV nephropathy can correlate with the total amount of applied immunosuppression [17]. Patients getting an ABO-incompatible graft are additionally treated with rituximab. Therefore, it can be speculated that the high number of patients showing BKV replication in this study might be caused by this additional immunosuppressant. Still, the balancing act of adequate immunosuppression remains a challenge. Of note is the highly significant and sustained suppression of CD19 + B cells after a single dose of rituximab and mycophenolate mofetil as already shown by Genberg and colleagues [18]. B-cell depletion, likely supported by therapy with mycophenolate mofetil, led to a decrease of total IgG levels and its subclasses in the blood. Although not clinically relevant until now, these findings deserve long-term evaluation and raise the question of whether rituximab is really necessary. Recently, successful ABO incompatible transplantation without use of rituximab or splenectomy has been published [19]. Therefore, this issue needs further investigation as well as the usefulness of single dose IVIG therapy immediately before transplantation. The last patient to receive an ABO-incompatible kidney transplant at our centre (data not shown) experienced an anaphylactic reaction to IVIG immediately after the infusion began. IVIG was discontinued, nevertheless the patient received a kidney transplant the following day with an uneventful follow-up. Hence, IVIG may be safely withdrawn from the current protocol.

In conclusion, ABO-incompatible kidney transplantation has become a standard procedure at our centre with an equal success rate and adverse event profile as a blood group-compatible transplantation. The number of living donor kidney transplants could be increased by 20%. The rapidly rising experience in this field, preferably gained by randomized controlled studies, may lead to further simplifications of the currently used protocols. This will help reduce potential side effects, lower costs and propagate this concept.

Conflict of interest statement. None declared.

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