Lessons Learned from ABO-Incompatible Living Donor Kidney Transplantation : 20 Years Later

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From June 1982 to November 1989, 39 ABO-incompatible living kidney transplants were performed in 38 recipients. Pretransplant therapies included platelets donor transfusion (21/39), 2 to 5 plasmapheresis sessions (39/39), cyclosporin A with or without azathioprine (33/39) along with polyclonal Abs (36/39) and splenectomy at the time of transplantation (37/39). The last patient who received 2 ABO-incompatible transplants was previously splenectomized (end-stage renal failure due to a cortical necrosis following a traumatic spleen rupture). Three other patients who did not undergo a splenectomy at the time of transplantation were not included in that series but hyperacutely rejected their transplants during the first postoperative week. The 31 ABO-incompatible living related donor graft recipients are alive. Graft loss occurred from acute and/or hyperacute rejection in 5 cases (none below 15 years of age) and from chronic rejection in 8 cases. By contrast, among the 8 ABO-incompatible living unrelated donor graft recipients, only one renal graft is still functioning 20 years later. Graft survival rates are better in the group of patients < 15 years (100%, 89%, 78%, and 78% at 2, 5, 10, and 15 years respectively) compared with the group > 15 years (77%, 77%, 64%, and 59% respectively; NS).

Today, 20 years later, prospective randomized studies testing different steps in the preparation protocol are still lacking. Plasmaphereses were replaced by double filtration plasmapheresis and immunoadsorption. Splenectomy seems to be a prerequisite for successful ABO-incompatible living kidney transplantation but IV Ig globulins and rituximab are currently being successfully used without splenectomy along with the new immunosuppressive drugs. As the procedure remains unchanged, it might be reserved to patients where cadaver graft could not be a valuable alternative, especially for recipients < 15 years of age with a living related ABO-incompatible donor.

Keywords: *Living donor transplantation, Kidney transplantation, ABO-incompatible transplantation*

ABO-incompatible kidney transplantation (Tx) was first reported by Hume et al, Murray et al, and Starzl et al in 1955, 1960, and 1964, respectively [1,2,3]. Although long-term survival of the grafts was observed in some initial cases, overall experience indicated that hyperacute rejection could occur; therefore, crossing the ABO barrier was generally excluded in the kidney transplantation field. The concept of depleting anti-AB Abs was probably first introduced by Slapak et al in 1981 [4] when a renal transplant patient with major donor-recipient blood group incompatibility (A_1-incompatible kidney Tx) was successfully treated with the use of modified plasmapheresis for rejection. Eventually in 1990, the same group reported on pre-Tx immunoadsorption and plasmapheresis for ABO-incompatible Tx [5], followed by a high survival rate of 87%.

In March 1981, an ABO-incompatible cadaver kidney Tx was inadvertently performed at our institution because of an error in donor ABO typing. Despite the A_1 to O major ABO-incompatibility and a basic immunusuppressive therapy

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regimen including a short course of polyclonal Ab with azathioprine and steroids without calcineurin inhibitor and/or additional treatments, the kidney graft functioned well and is still functioning 22 years later. Noteworthy was the good histocompatibility matching between donor and recipient with 2 DR-matched antigens, the graft being allocated through Eurotransplant.

During the early 80s, bone marrow (BM) Tx was performed under certain circumstances, for example, when the donor was completely matched to the recipient for major Tx antigens i.e., HLAs. In that setting, the risk of transplanting ABO-incompatible BM was sometimes considered unavoidable: bidirectional ABO-incompatibility could be present when both iso-agglutinins and iso-antigens were incompatible between donor and recipient (eg, A donor to B recipient; B donor to A recipient) [6]. The immediate risk was hemolysis of RBCs that are contained in the graft: therefore, BM grafts were usually depleted of donor RBCs by various cell separation techniques. The delayed complications were also hemolysis and RBC aplasia because of persistent host iso-agglutinins but also hemolysis of recipient RBCs mediated by Abs produced by donor lymphocytes contained in the graft. Therefore, depletion of recipient circulating Abs were also performed [7]. The outcome after ABO-incompatible BM Tx varied from series to series but was not different from ABO-compatible BM Tx, demonstrating that it could be safely performed with adequate recipient preparation [8].

Based on that successful cadaver kidney transplant case and the ABO-incompatible allogenic bone marrow transplantation experience, a living donor ABO-incompatible kidney transplantation program was initiated at our institution in June 1982. Pretransplant removal of antibodies with plasmapheresis was chosen to prevent the occurrence of antibody-mediated hyperacute rejection, while the immunosuppressive regimen was started 3 days prior to transplantation [9,10]. The surgical procedure included a splenectomy [11]. However, despite chronic immunosuppression, anti donor blood-group antibody returned and persisted after successful Tx [12, 13]. In most patients, the graft continued to function well despite the continued presence of these antibodies and the persistence of the target antigen in the kidney—a situation termed *accommodation* by Bach et al [14].

Today, an ever-growing gap between the number of patients waiting for kidney transplantation and the number of donor organs available has become a major challenge throughout the world. To overcome this, kidney transplantation across ABO blood-group barriers, especially those involving living donors, are being performed with increasing frequency [15]. Recent refinements in immunosuppression and patient selection have increased both short- and long-term graft survival of ABO-incompatible kidney Tx with success approaching that of cadaveric and living donor transplants at 5 years [15-17]. But the mechanism by which the kidney graft can protect itself from the antibodies remains unclear.

The aim of the current study was to present the outcome and long-term results of a series of 39 ABO-incompatible living donor kidney Tx performed in the 80s at our institution and discuss the impact of the current modifications in preparation techniques along with the use of new immunosuppressive (IS) regimen.

Population

From June 1982 to November 1989, 39 ABO-incompatible living kidney transplants were performed in 38 recipients: 25 males, 13 females, including 1 boy who received a first ABO-incompatible transplant from his mother and a second from his uncle. Recipient mean age was 23 ± 10 year old. It included 35 first, 3 second, and 1 third transplants. Donor mean age was 42 ± 9 year old. All kinds of ABO-incompatibilities were encountered (A to O: 4; A_1 to O: 11; A_2 to O: 4; B to O: 11; B to A_1: 1; A_1 to B: 3; A_1B to A_1: 1; A_1B to B: 3; and A_1B to O: 1). There were 31 related living donors (mother to child: 20; father to child: 7; sister to sibling: 3; uncle: 1) and 8 unrelated living donors (wife to husband: 7; and husband to wife: 1).

Pretransplant therapies included platelets donor transfusion (21/39), 2 to 5 plasmapheresis sessions (39/39), cyclosporin A with or without azathioprine (33/39) along with polyclonal Abs (36/39): antilymphocytic (n = 27) or antithymocytic (n = 9) globulins and splenectomy at the time of transplantation (37/39). After the last plasmapheresis session, when the level of 1/4 (ABO antibodies) was reached, all recipients received 5 mL of substance A or B (depending on the incompatible blood group; blood group –specific substance extracted from porcine stomach, Behasil Corporation, Miami) diluted in 50 to 100 mL of saturated solution of plasma proteins,

administrated during a period of 30 to 60 minutes. Postoperative immunosuppressive regimen included aza-prednisolone (n = 6), CsA-prednisolone (n = 7), and CsA-aza-prednisolone (n = 26). To these 39 recipients, a small subgroup of 3 nonsplenectomized recipients must be added: the total of 42 recipients can be divided into 3 subgroups according to 3 successive eras and the type of IS therapy:

Era 1: (n = 6) Pre or per transplant splenectomy – no CsA
- Platelet transfusions (4/6)
- Plasmapheresis (6/6)
- Polyclonal AB from day 3 (6/6)
- Aza-prednisolone (6/6)
- Unrelated (0/6)

Era 2: (n = 3) No splenectomy – CsA
- Platelet transfusion (3/3)
- Plasmapheresis (3/3)
- Polyclonal AB from day 3 (2/3)
- Aza-prednisolone (3/3)
- Unrelated (1/3)

Era 3: (n = 33) Splenectomy and CsA
- Platelet transfusion (17/33)
- Plasmapheresis (33 /33)
- Polyclonal AB from day 3 (30/33)
- CsA Aza-prednisolone (26/33)
- CsA – prednisolone (7/33)
- Unrelated (8/33)

Results

During era 2, all 3 not splenectomized recipients, hyperacutely rejected their transplants during the first postoperative week. Outcome of recipients from era 1 (no CsA) and 3 (all 33 with CsA) is summarized in Table 1 and in the following analysis. The 31 ABO-incompatible living related donor graft recipients are alive. Graft loss occurred from acute and/or hyperacute rejection in 5 cases (time to graft loss: 11 ± 3 days; no recipient below 15 years of age) and from chronic rejection in 8 cases (time to graft loss: 9.5 ± 4.8 years). By contrast, among the 8 ABO-incompatible living unrelated donor graft recipients, 2 died early from sepsis after being treated for rejection (2 and 3 months later), 2 lost their grafts from hyperacute rejection, and 3 from chronic rejection. Today, 20 years later, only 1 recipient (husband to wife with 2 DR matches) has a functioning graft (creatinine 1.0 mg/dL). Noteworthy is the evolution of a 9-year-old boy who received a first ABO-incompatible kidney transplant from his mother after being 20 months on dialysis. The cause of renal failure was a cortical necrosis following a traumatic spleen rupture. He lost that first transplant due to chronic rejection without any acute rejection episode. After 2 months on dialysis, he was transplanted with a second ABO-incompatible kidney transplant from his uncle. At the time of the second transplant, a laparotomy was performed and accessory hypertrophic spleen tissue was removed: 17 years later, the renal function remains excellent, with a creatinine level at 1.3 mg/dL. No cancers were encountered in that small series of patients.

Among the 39 recipients, 21 received donor platelet transfusions while the remaining 18 had random blood transfusions, but no difference was seen in the postoperative outcome or long-term survival. The ABO isoagglutinin titer was reduced below 1/4 with the 3 to 5 plasmapheresis in all recipients and almost to 0 by the administration of substance A or B. During the postoperative period, it increased more or less rapidly over the preoperative value but it could not be correlated to the grade, incidence of rejection and long-term outcome due to the small subgroups of patients.

Table 1. Outcome of 39 ABO-incompatible living related and unrelated donor kidney transplantation

	n	Mean age	No RRT before TP	Death	Graft losses		Functioning graft		
		M ± SD	%		HA rejection	Chronic rejection	n	FU (year) m + SD	Creatinine (mg/dL) m + SD (median)
Related Time to graft loss	**31**	**20 ± 7**	**35**	**0**	**5** 11 ± 3 days	**8** 9.5 ± 4.8 yrs	**18**	**17 ± 3**	**1.9 ± 1.3 (1.5)**
< 15 years Time to graft loss	9	11 ± 2	55	0	0	2 5.5 ± 4.8 yrs	7	16 ± 2	1.8 ± 0.6 (1.7)
> 15 years Time to graft loss	22	23 ± 6	28	0	5 11 ± 3 days	6 11 ± 4 yrs	11	18 ± 3	2.0 ± 1.6 (1.4)
Unrelated	**8**	**37 ± 9**	**12**	**2**	**4**	**3**	**1**	**20**	**1.0**

RRT: Renal replacement therapy TP: Transplantation HA: Hyperacute FU: Follow-Up

Graft survival rates were also studied in subgroups of patients: graft survival rates at 2, 5, 10, and 15 years in ABO-incompatible related living recipients are respectively 100%, 89%, 78%, and 78% for the subgroup < 15 years old and 77%, 77%, 64%, and 59% for the subgroup > 15 years old (NS) (Figure 1).

Moreover graft survival rate of 8 ABO-incompatible living related donor recipients less than 15 years was compared to a cohort of 38 ABO-compatible living related donor recipients of the same age and transplanted during the same period (June 82 - November 89) under CsA based immunosuppressive therapy : no difference is demonstrated in Figure 2.

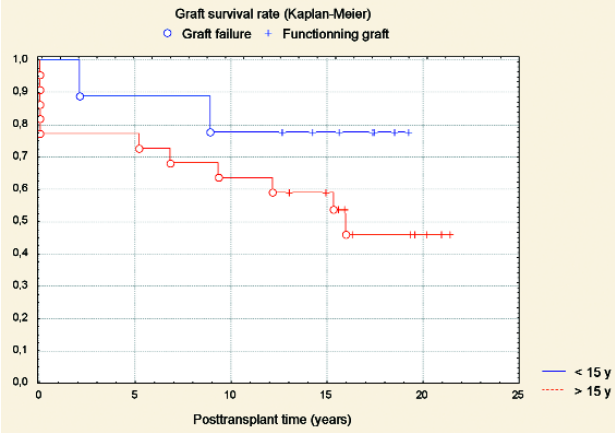

Figure 1. Kidney graft survival in recipients < (n = 9) and > (n = 22) 15 years of ABO-incompatible living related donor grafts.

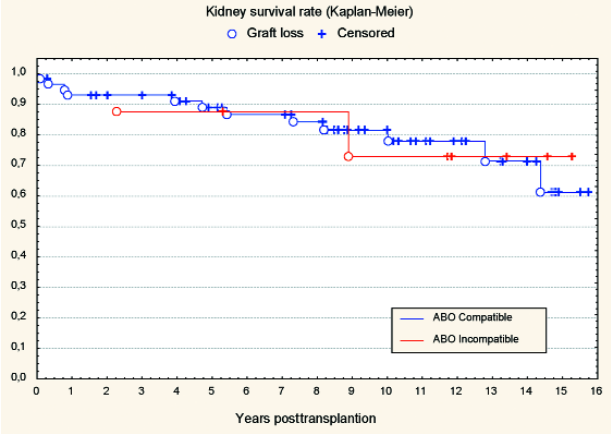

Figure 2. Kidney graft survival in recipients < 15 years (n = 8) of ABO-Inc-L-related D Kid Tx compared to a group of recipients < 15 years (n = 38) of ABO-Comp L-related Kid Tx, under CsA based immunosuppressive therapy.

Discussion

That series of 39 ABO-incompatible living donor kidney Tx represents the first attempt to overcome ABO-incompatibilities in preparing the living donor recipient by plasmapheresis, pretransplant immunosuppression, and per transplant splenectomy to get rid of the Abs. Based on the poor outcome of the 3 nonsplenectomized recipients from additive era 2, that study demonstrated that per or pretransplant splenectomy could play a role [11]. By contrast, the posttransplant recovery of the isoagglutinin titers could not predict posttransplantation outcome, especially with regard to the incidence of early acute or hyperacute rejection [12,13]. After a critic period of 3 weeks, isoagglutinin titers could increase over the original level without jeopardizing the kidney tissue even in the presence of antigens [13]. That phenomenon was eventually called *accommodation* by others [14].

In that population, the recipient preparation for transplantation included several plasmaphereses (3 to 5) along with IV injection of substance A and/or B to get rid of the isoagglutinins prior to transplantation. The immunosuppressive regimen consisting in a quadruple drug induction therapy (in the last 26 patients) was also initiated 3 days before transplantation, along with splenectomy at the time of transplantation. Despite that heavy treatment and irrespectively of the isoagglutinin titers, and its recovery, hyperacute rejection was commonly seen during the first postoperative days [12,13]. Some of them did respond to OKT3 therapy while others failed. Over that period, isoagglutinin titers either returned to normal or continued to increase to very high levels despite the presence of antigens at the endothelial surface [12,13]. That observation was eventually reported and the mechanism explained by others [18,19]. By contrast, for Galili et al [20], the prevention of the anti-gal response may decrease the immune rejection of ABO-incompatible allografts. So far, no cancers were observed during the follow-up period in the survivors.

Today, almost 20 years later, each part of the global protocol was further assessed by a different group, but prospective randomized studies are still lacking. Plasmaphereses were replaced by immunoadsorption column by several Japanese centers [15] and others [21]: a major drawback was the cost-benefit ratio of that procedure, which could not demonstrate any advantage on the incidence of hyperacute and/or acute rejection rate [15]. The efficacy of substance A and/or B to delete the last circulating isoagglutinins could no longer be assessed, as that substance is no longer used for

human therapeutic purposes due to its animal origin. To get rid of the Abs, IV Ig globulins are currently being successfully used by Park et al [22] along with the new, more-potent IS drugs, such as tacrolimus and mycophenolate mofetil.

Furthermore, the need for per transplant splenectomy remains controversial [21,23]. Indeed, 20 years earlier, we demonstrated the prerequesite of that procedure with the IS regimen used during that era of transplantation [11]. At that time, splenectomy was also proposed by others [24] for cadaver kidney transplantation. The rationale for performing splenectomy is that spleen is an (Ig M - Ig G producing) B-cells reservoir. Indeed, 3 of our patients without splenectomy hyperacutely rejected their kidney graft (see era 2). The hypothesis was eventually confirmed by the outcome of the second related live donor kidney graft, which was performed along with the ablation of the accessory spleens. Today splenectomy is often avoided, especially in children with the risk for pneumococcal infections. Indeed several authors did avoid the procedure for A_2 to O donor – recipient pairs [21]. Others are proposing the use of new monoclonal Abs such as rituximab (anti-CD 20 monoclonal Ab infusions) [21,25].

Despite a high incidence of hyperacute and/or acute rejection, the best clinical results were seen (Figure 1) in well-matched live donor kidneys, especially in small children where the survival curves seem to plateau after the first critical period (Figure 1). That observation was eventually done by Yanza et al in liver Tx [26] and also by West et al [27] in heart-lung transplantation. That could be explained by the fact that preformed natural Abs (isohemagglutinins) against AB antigens are present at birth as IgG as a result of transport of maternal Abs through the placenta, but not as a result of self-production. Maternal Abs disappears from the neonate after 2 weeks, but at approximately 8-12 weeks, the newborn infant starts producing IgM and IgG of its own. Adult levels are reached by the age of 5-10 years. Although the stimulus for the production of Ab to A and/or B determinants remains uncertain, one commonly held hypothesis is that it is a response to the presence of A and/or B saccharides on bacteria or other microorganisms that colonize the infant gastrointestinal tract [28].

By contrast, the worst results were encountered in the living unrelated donor subgroup in which 2 patients died: one from pneumopathy and the other from multiple organ failure. Two other recipients presented a hyperacute rejection of their ABO-incompatible kidneys, which were not reversible despite OKT3 therapy. Three others had a chronic rejection and were back on dialysis. The remaining patient is still alive with a well-functioning kidney 20 years later, she is a woman (current creatinine of 1.0 mg/dL) who was sharing 2 DR antigens with her husband who donated a kidney.

Since that period, no randomized prospective studies have been made in that field [28]. Except for the introduction of new, more-potent IS drugs, which have decreased the incidence of rejection, the whole procedure remains more or less unchanged and might be reserved for patients where cadaver graft could not be a valuable alternative. That first ABO-incompatible LD Kd Tx trial, its success, emphasized that the procedure could help small children.

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