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Pretransplant blood transfusions with cyclosporine in pediatric renal transplantation

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Abstract Pretransplant transfusions were repeatedly shown to be associated with improved graft survival in the "pre-cyclosporine era," and have recently been shown to be beneficial in patients on modern immunosuppressive regimes. In an attempt to improve this transfusion effect and minimize the potential development of cytotoxic antibodies, we have given these transfusions, with concomitant cyclosporine cover, prior to transplantation. Ninety-two renal transplantations were performed in 91 children in the study group (group 1) and all received pretransplant transfusions with cyclosporine cover. Results were compared with a preceding group of 102 children (104 transplantations) who had received pretransplant transfusions without cyclosporine cover (group 2). There were 70 cadaver and 22 living-related donor (LRD) transplants in group 1, and 88 cadaver and 16 LRD transplants in group 2. Graft survival rates (1- and 5-year) for cadaver transplantation were 96% and 90% in group 1 compared with 78% and 64% in group 2 (P=0.001). For LRD transplantation, these figures were 95% and 87% in group 1 and 81% and 69% in group 2. There was no difference between the two groups in terms of age at transplantation, sex, donor age, HLA-A, -B, -DR mismatches, or cold and warm ischemia times. All cadaver graft recipients received quadruple, sequential immunosuppression post transplant. However, 9 patients in group 1 were changed to tacrolimus for recurrent rejection episodes. No patient developed persistent lymphocytotoxic antibodies post transfusion or side effects of cyclosporine. Cyclosporine can be safely given with whole blood prior to transplantation with no adverse effect and no sensitization. Graft survival was significantly improved in this group of patients and graft loss due to rejection was exceptional. This effect should be further evaluated in prospective studies.

Key words Blood transfusion · Cyclosporine · Renal transplantation · Cytotoxic antibodies

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Introduction

A beneficial effect of pretransplant blood transfusions on graft outcome in renal transplantation was repeatedly demonstrated in the 1970s and early 1980s in both human and animal models [1, 2, 3, 4, 5]. The effect was more pronounced with increasing numbers of transfusions [6], although the greatest difference in outcome occurred with the first transfusion. Since the introduction of cyclosporine to the post-transplant immunosuppression protocol, such transfusions were widely abandoned amid reports that graft survival rates were equivalent in those patients who had and had not received pretransplant transfusions [7, 8]. In addition, since the early 1990s, the widespread use of recombinant human erythropoietin in patients with renal failure has obviated the need for transfusion in the majority of cases. In a recent, large, prospective, multicenter study, a significantly improved graft survival rate was again reported in recipients transfused pre transplant compared with those not transfused [9] The major drawback of these transfusions is the potential development of cytotoxic antibodies to donor lymphocytes. Immunosuppressive drugs such as azathioprine and cyclosporine have been administered at the time of transfusion in an attempt to minimize sensitization, without impairing the "transfusion effect" [10, 11, 12]. In one such study, cyclosporine given at the time of transfusion was shown, not only to prevent persistent sensitization, but also to increase anti-idiotypic antibody activity [13], a phenomenon reported to be associated with improved allograft outcome [14]. We describe our experience with pretransplant transfusions with concomitant cyclosporine.

Patients and methods

From July 1990 to November 1996, 91 children awaiting renal transplantation, whose cytotoxic antibody status was known to be negative, were included in the study protocol (group 1). The causes of end-stage renal failure are shown in Table 1. Two whole-blood transfusions were administered at an interval of 1 month. In cases of anticipated living-related donor (LRD) transplantation, two donor-specific transfusions were given.

Cyclosporine (Sandimmun) 10 mg/kg per day was commenced 4 days before the first transfusion and continued until 1 month after the second transfusion. In 14 patients with residual renal function (glomerular filtration rate>5 ml/min per 1.73 m^2), the dose of cyclosporine was reduced to 6 mg/kg per day in order to minimize the nephrotoxic effect of cyclosporine on residual renal function. In those patients who had previously received one or more blood transfusions, only one further transfusion was given, along with cyclosporine for 1 month.

The immunosuppressive protocol for cadaver kidney transplant recipients comprised prophylactic antibody (antithymocyte globulin, OKT3, or anti-LFA1) (Table 2) with the introduction of cyclosporine (Sandimmun) on day 6 post transplant, initially intravenously at a dose of 1 mg/kg and subsequently orally at a dose sufficient to maintain trough levels between 150 and 250 ng/ml. The switch from Sandimmun to Neoral was started for all patients in both groups in 1996. Prednisone, 60 mg/m^2 per day with dose reduction over a period of 6 months to 7.5 mg/m^2 per day, and azathioprine, 1.5 mg/kg per day, were started on day 1. In those children receiving LRD kidneys, no prophylactic antibody was given, except for 1 patient, and cyclosporine was started on day 1. Acute rejection episodes were confirmed on renal biopsy and treated with three doses of intravenous methylprednisolone, 1 g/1.73 m^2, given on alternate days.

In the previous cohort of patients transplanted between 1987 and 1990 (group 2), 171 kidney transplantations were performed, including 20 LRD transplants. These patients had been transfused without cyclosporine cover prior to transplantation. As patients in goup 1 did not develop cytotoxic antibodies following transfusion under cyclosporine cover or only very low IgM titers, we selected from group 2 only those patients who had not developed cytotoxic antibodies post transfusion. This was done as it has often been found that graft survival is not as good in responders compared with non-responders. We therefore selected 104 transplants performed in 102 patients (88 cadaveric, 16 LRD). The immunosuppressive protocol for cadaveric kidney transplant recipients was as described above. LRD recipients in this group generally received quadruple sequential immunosuppressive therapy, as described for the patients receiving cadaveric grafts in group 1.

Five of the cadaveric graft recipients in group 1 and 7 in group 2 had previously been transplanted. For LRD recipients, 3 patients in group 2 had previously been transplanted with a cadaveric transplant. Patient data for both groups are presented in Table 1 and Table 3.

In addition, those patients at risk of graft thrombosis (recipients weighing less than 15 kg, donor age less than 5 years, more than one renal artery, previous history of arteriovenous fistula or graft thrombosis) received a prophylactic low molecular weight heparin treatment during the first 3 weeks. This protocol started in our institution in December 1988.

Statistical analysis

The significance of differences was evaluated using Student's *t*-test. The log-rank test was used to estimate statistical differences of graft survival rates.

Table 1 Causes of end-stage renal failure (*CAD* cadaveric, *LRD* living-related donor)

	Group 1		Group 2	
	CAD	LRD	CAD	LRD
Glomerular diseases	24	7	28	3
Hereditary nephropathies	14	4	11	7
Hypoplasia/dysplasia	15	3	16	2
Obstructive uropathies	15	4	17	4
Vasculitis	1	1	6	–
Tubulointerstitial nephritis	–	–	3	–
Others	1	3	7	–
Totals	70	22	88	16

Table 2 Induction therapy in patients receiving CAD transplants

	Group 1	Group 2
Antithymocyte globulins	50	39
OKT3	1	47
Anti-LFA1	10	–
No induction	9	2
Totals	70	88

Results

Ninety-two pediatric renal transplantations (70 cadaveric, 22 LRD) were performed in 91 patients between July 1990 and November 1996. Three patients became sensitized with 2.5%–5% with IgM reactive antibodies. These antibodies were transient and had disappeared at the time of transplantation. No other patient receiving donor-

Table 3 Patient data

	Group 1		Group 2	
	CAD	LRD	CAD	LRD
No.	70	22	88	16
Sex M/F	42/28	13/9	43/45	11/5
Mean age at transplantation (years)	11	11	11.1	9.7
Primary/retransplant	65/5	22/0	81/7	13/3
Mean donor age (years)	15.1	38.3	18.7	36.2
Mean no. ABDR incompatibilities	3	3	3	2
Mean cold ischemia time (h)	30.9	6.6	35.3	2.8
No. of hemodialysis sessions after transplant	1.2±1.9		2.9±6.4	
Mean warm ischemia time (min)	47	42	56	52
Mean creatinine clearance at 1 year (ml/min per 1.73 m^2)	60	62	57.6	67

Table 4 Causes of graft loss

	CAD		LRD	
	Group 1 (*n*=70)	Group 2 (*n*=88)	Group 1 (*n*=22)	Group 2 (*n*=16)
Acute or chronic rejection (non-compliance)		16 (1)	1 (1)	4
Vascular thrombosis	3	8	1	1
Recurrence of primary disease	1	5		1
Deaths	2	5		
Totals	6	34	2	6

specific transfusions with cyclosporine coverage developed cytotoxic antibodies. Eleven patients developed side effects during the course of cyclosporine. An increase in blood pressure occurred in 4 patients, 1 of whom developed seizures. A temporary increase in creatinine was observed in 2 patients with residual renal function. Hypertrichosis was observed in 5 patients. In group 2, we selected 102 patients (88 cadaveric, 16 LRD) who had not developed cytotoxic antibodies post transfusion.

Patient and graft survival following cadaveric transplantation

In group 1, 1 patient died 2 weeks post transplant due to overwhelming sepsis. A second death occurred 3 years post transplant with a functional graft. The cause of death is unclear as the patient died suddenly during a football game.

In the remaining 68 patients, graft loss was seen in 2 patients in the 1st month due to vascular thrombosis, and in 1 patient 39 months post transplant due to the recurrence of primary disease (Table 4). Graft loss was not due to rejection in any case. Graft survival rate was thus 96% at 1 and 2 years post transplantation, 93% at 3 years, and 90% at 4 and 5 years (Fig. 1).

In group 2, 5 deaths occurred in the 1st post-transplant year due to bleeding complications (1 patient), cytomegalovirus myocarditis (1 patient), post-transplant lymphoproliferative disease (1 patient), Kaposi sarcoma (1 patient), and after a return to dialysis (1 patient).

In the remaining 81 patients, 15 grafts were lost in the 1st year post transplant, 2 in the 2nd year, 6 in the 3rd year, 2 in the 4th year, and 1 in the 5th year post transplant. Rejection accounted for graft loss in 13 of these patients (Table 4). Graft survival rate was 77% at 1 year, 75% at 2 years, 68% at 3 years, 65% at 4 years, and 64% at 5 years.

In comparison, graft survival for the 151 patients who received cadaveric transplants between 1987 and 1990, including the responders, was 81%, 77%, 70%, 65%, and 64% at respectively 1, 2, 3, 4 and 5 years, which is not different from the graft survival in the subgroup of non-responders. As shown in Fig. 1, the difference in graft survival between group 1 and group 2 was significant (log rank, $P<0.0014$).

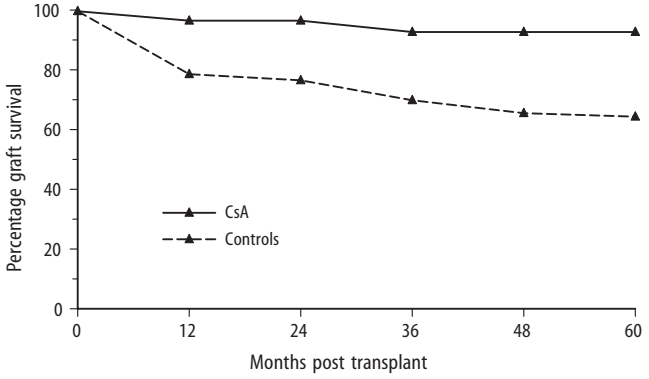

Fig. 1 Actuarial graft survival following cadaveric renal transplantation (*CsA* cyclosporine)

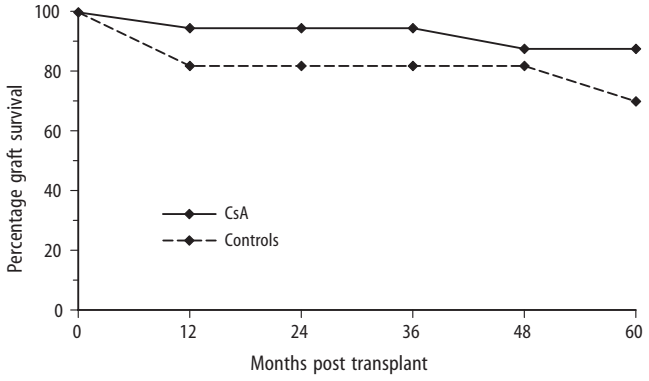

Fig. 2 Actuarial graft survival following living-related donor renal transplantation

Patient and graft survival following LRD transplantation

In group 1, 1 graft was lost within a month of transplantation due to vascular thrombosis; a second was lost 40 months post transplant due to chronic rejection related to non-compliance (Table 4). Graft survival was 95% at 1 year and 87% at 5 years (Fig. 2).

In group 2, 3 grafts were lost within a month of transplantation, due to rejection in 2 patients and vascular thrombosis in 1. Two further grafts were lost in the 5th post-transplant year due to chronic rejection and recurrence of primary disease (Table 4). Graft survival was 81% at 1 year and 68% at 5 years (Fig. 2). The difference in graft survival between group 1 and 2 did not

reach statistical significance due to the small number of patients in the groups (P=0.2).

Acute rejection episodes following cadaveric transplantation

Of the 70 patients in group 1, 3 lost their graft early from a non-immunological cause. Among the remaining 67, 24 had 1 rejection episode and 17 had more than 1 rejection episode. This rejection crisis occurred during the 1st month in 25 patients, between the 1st and the 6th month in 18, and later in 8 patients. Twenty-six had no rejection episode.

Of the 88 children in group 2, 10 lost their graft early from a non-immunological cause. Among the remaining 78, 23 had 1 rejection episode, 18 had 2 rejection episodes, and 11 had more than 2 rejection episodes. This rejection crisis occurred during the 1st month in 31 patients, between the 1st and the 6th month in 22 patients, and later in 23 patients. Twenty-six had no rejection episode.

Acute rejection episodes following LRD transplantation

In group 1, 5 patients experienced 1 acute rejection episode and 1 patient experienced 2; 1 of these 2 children lost her graft at 40 months due to non-compliance. Fifteen patients had no episodes of rejection.

In group 2, 13 patients experienced 1 (9 children) or several (4 children) rejection episodes. Seven of these occurred within a month of transplantation. Three patients had no rejection episodes.

Modification of treatment

In group 1, 9 cadaver kidney recipients and 1 LRD recipient were changed from cyclosporine to tacrolimus at a mean of 6.6 months post transplant due to recurrent rejection episodes. In 1 further patient (LRD recipient), azathioprine was replaced by mycophenolate mofetil at 35 months post transplant. No patient in group 2 had their immunosuppressive therapy changed.

Effect of low molecular weight heparin

In the control group, 7 of the 9 patients who experienced graft thrombosis were transplanted before we had started the low molecular weight heparin protocol in 1988. This complication occurred in only 4 of the 92 patients of group 1 who were transplanted after the protocol was started. However, Table 5 shows that, overall, graft survival was not superior in patients who had received prophylactic heparin.

Table 5 Graft survival (%) in patients who have or have not received prophylactic low molecular weight heparin (LMWH) following CAD renal transplantation

	Group 1		Group 2	
	LMWH + (45 patients)	LMWH – (25 patients)	LMWH + (34 patients)	LMWH – (54 patients)
Month 12	95.5	92	70.6	81.5
Month 24	95.5	92	67.6	79.6
Month 36	95.5	87	61.7	72.2
Month 48	95.5	87	56	70.3
Month 60	95.5	87	53	70.3

Discussion

There are many factors to account for the improvement in graft survival rates over the past decade. Molecular biology techniques allow more-accurate determination of HLA groups, potentially improving the compatibility between donor and recipient. Post-operative care of transplant recipients, including the identification and treatment of infectious complications, has also improved. Newer immunosuppressive agents, such as tacrolimus and mycophenolate mofetil, along with monoclonal antibodies and antilymphocyte globulins as induction and rescue therapy, are also responsible in part for the advances in pediatric transplantation seen in the last few years. For these reasons, the use of historical controls in a study relating to outcome parameters is clearly subject to criticism. However, our policies with regard to donor specifications, timing of transplantation, and immunosuppression in the immediate post-transplant period did not change between 1986 and 1996. Since this protocol consisting of pretransplant blood transfusions under cyclosporine cover was introduced in 1990, graft survival has improved dramatically; in particular, graft loss due to rejection is now exceptional. A single kidney has been lost due to rejection, and this was due to non-compliance. In contrast, among the patients who had received pretransplant blood transfusion without cyclosporine cover, 19 patients have lost their grafts as a result of rejection. This improvement in outcome was present prior to the introduction of tacrolimus rescue therapy in 1995.

The mechanism of improved graft survival in patients receiving pretransplant transfusions has never been clarified. Various explanations have been put forward. The first is that of a “selection effect,” which means the identification of patients who develop anti-HLA antibodies post transfusion, and hence those most at risk of rejection (responders) versus non-responders [15]. The more-recent reports demonstrating an even greater benefit of transfusions when immunosuppressive therapy (azathioprine/cyclosporine) is administered concurrently make this hypothesis less tenable.

Other authors have proposed a clonal deletion effect of the transfusion of HLA antigen-specific donor T lymphocytes [16]; the transfusion constitutes an initial allo-

immunization and the graft a rechallenge. The resulting activated T lymphocytes are particularly sensitive to immunosuppressive treatments, which preferentially destroy actively dividing cells. Takiff et al. [17] reported that this effect is more pronounced when immunosuppression is started prior to transplantation.

Another hypothesis is the induction by the transfusion of a suppressor T cell response. Many studies have shown that alloreactivity in mixed lymphocyte cultures may be reduced following blood transfusions [18, 19].

Finally, it has been reported that blood transfusions can induce the production of anti-idiotypic antibodies capable of inhibiting the action of HLA class 1 and 2 lymphocytotoxic antibodies, with subsequent immunological unresponsiveness [20]. It has been shown in an animal model that as class 1 alloreactivity declines in response to cyclosporine, anti-idiotypic activity increases [21]. Other authors have reported a reduced risk of rejection and subsequent graft loss in patients transfused under cyclosporine cover and have attributed this to the formation of anti-idiotypic antibodies [22, 23]. The same benefit on graft survival was also reported in rats by Cofer et al. [24].

Our experience with pretransplant transfusions with concomitant cyclosporine has been a positive one. The first benefit is that none of the patients who have received blood transfusion under cyclosporine coverage have developed anti-HLA antibodies that were of any significance. Prior to this protocol, the rate of immunization following blood transfusions in children awaiting renal transplantation was close to 30%. Moreover, in those patients who were transplanted after this protocol, graft loss due to rejection has been exceptional, occurring in only 1 patient following non-compliance. The rate of acute rejection crisis was comparable to that of the historical control group, but these rejection episodes were less severe, as graft loss due to rejection was exceptional. The improved graft survival may indeed be due in part to the switch from cyclosporine to tacrolimus in 10 patients from group 1 with recurrent rejection episodes. However, improved graft survival was present in our series before tacrolimus was introduced as rescue therapy. Another explanation for the better results in patients from group 1 with cadaver donors is a lower rate of acute tubular necrosis. Indeed, there was a trend to a lower incidence of acute tubular necrosis in patients from group 1, in whom the number of hemodialysis sessions after transplantation was lower compared with patients from group 2 (1.2±1.9 in group 1 vs. 2.9±6.4 in group 2, $P<0.05$) (Table 1).

Graft thrombosis is a main cause of graft loss in children. This was indeed the case in our experience before we started a prophylactic low molecular weight heparin therapy in patients at risk of graft thrombosis [25]. However, the graft survival rate was not better in those patients who had received prophylactic heparin.

The current risks of the main viral infections transmitted by transfusions are now very low [26]. This is due to improvements in laboratory testing, screening of donors, and recruitment of low-risk donors. The risk of transmission of the human immunodeficiency virus (HIV) has been estimated as 1 in 493,000, whereas the risk of transmission for the hepatitis C virus (HVC) was calculated to be 1 in 103,000. Although very few patients require blood transfusion since the introduction of recombinant erythropoietin, we believe that blood transfusions may still be justified in preparation for transplantation if graft survival is improved. Since we started our protocol in 1990, no case of major viral contamination, including HIV and HVC, was observed in our patients following blood transfusion. We consider that the improvement of graft survival also outweighs the risks and cost of a 2-month treatment with cyclosporine.

In conclusion, the benefit of pretransplant transfusions in adults has been confirmed in a recent prospective study [9]. Our results show that pretransplant blood transfusions with cyclosporine cover are associated with improved graft survival rates and lower incidence of graft loss due to rejection than those transfused without cyclosporine cover. We believe this should be further evaluated by controlled studies.

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