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ORIGINAL ARTICLE

Effect of donor-specific transfusions on the outcome of renal allografts in the cyclosporine era

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Summary

Despite the introduction of new immunosuppressive agents, a steady decline of functioning renal allografts after living donation is observed. Thus nonpharmacological strategies to prevent graft loss have to be reconsidered, including donor-specific transfusions (DST). We introduced a cyclosporine-based DST protocol for renal allograft recipients from living-related/unrelated donation. From 1993 to 2003, 200 ml of whole blood, or the respective mononuclear cells from the potential living donor were administered twice to all of our 61 recipient candidates. The transplanted subjects were compared with three groups of patients without DST from the Collaborative Transplant Study (Heidelberg, Germany) during a 6-year period. Six patients were sensitized without delay for a subsequent cadaveric kidney. DST patients had less often treatment for rejection and graft survival was superior compared with subjects from the other Swiss transplant centers ($n = 513$) or from Western Europe ($n = 7024$). To diminish the probability that superior results reflect patient selection rather than effects of DST, a 'matched-pair' analysis controlling for relevant factors of transplant outcome was performed. Again, this analysis indicated that recipients with DST had better outcome. Thus, our observation suggests that DST improve the outcome of living kidney transplants even when modern immunosuppressive drugs are prescribed.

Introduction

Pretransplant donor-specific transfusions (DST) have been shown to achieve a beneficial effect in living-related kidney transplantation in the azathioprine–prednisone era [1,2]. DST were introduced with the aim to induce unresponsiveness to allografts by modulation of the immune system [1,2]. After the introduction of cyclosporine, the beneficial effects of DST became controversial and were often not detectable [3–7]. Therefore, most transplant centers have abandoned such protocols. Logistical reasons, the fear of sensitization or transmission of infections like hepatitis and HIV in the early 1980s, have contributed to the disappearance of DST strategies. Nevertheless, several investigations from the last decade suggested a beneficial effect of DST [8–13]. In Bern, we introduced a cyclosporine-based DST protocol for renal allograft recipients from living-related/unrelated donation (LRD/LURD) in 1993. In the following, we report the outcome of these recipients transplanted between 1993 and 2003.

Materials and methods

Recipient and donor profiles of DST-positive study group from Bern

In Bern, we introduced a cyclosporine-based DST protocol for all renal allograft recipients from LRD/LURD in

January 1993. Until the end of December 2003, a total of 55 transplantations were performed in adults (>16 years) either from LRD ($n = 40$) or from LURD ($n = 15$) donors. Demographic characteristics are provided in Table 1 ('Bern'). All patients have initially received cyclosporine and prednisone. In addition, azathioprine ($n = 13$) or mycophenolate mofetil ($n = 18$) were added at the time of transplantation. Induction therapy with interleukin-2 receptor antibodies (IL-2 RA) was performed in 16 recipients.

Prescription of DST

Depending upon ABO/rhesus factor (Rh) phenotypes, either fresh (not stored whole blood) or a corresponding amount of mononuclear blood cells from the respective potential living donor were administered twice 2 months apart to all of our 61 recipient candidates. Whole blood (200 ml) was given in cases of ABO-blood group identity with Rh identity or compatibility (donor Rh–, recipient Rh+) ($n = 48$). In cases with ABO-blood group nonidentity but compatibility (e.g. donor blood group O and recipient blood group A), with Rh incompatibility or in the presence of allo-antibodies against donor erythrocytes, 20 ml of mononuclear blood cells (buffy coat) obtained from 200 ml whole blood was given ($n = 13$) to minimize unwanted immune reactions and side effects. The administration of whole blood or mononuclear cells occurred within 10 h following their harvesting. The second DST were not given in three recipients because sensitization occurred after the first transfusion. Prior and 14 days after each DST the following investigations were performed: T-lymphocyte cross-match, recipient panel reactive antibodies (PRA), allo-antibodies against erythrocytes (recipient) and direct Coombs test (recipient). None of the potential recipients received immunosuppressive coverage during the course of DST.

Table 1. Demographic and clinical features of the four groups of patients analyzed.

Parameter	1. Bern*	2. Matched Cases*	3. Switzerland	4. Western Europe
Total number of patients†	55	55	513	7024
Recipient sex				
Male (%)	62	62	64	64
Female (%)	38	38	36	36
Donor sex				
Male (%)	45	38	32	40
Female (%)	55	62	68	60
Recipient age (years, mean ± SE)	41 ± 2.0	41 ± 2.0	44 ± 0.59	39 ± 0.15
Donor age (years, mean ± SE)	48 ± 1.5	49 ± 1.6	50 ± 0.5	48 ± 0.1
Cold ischemia time (h, mean ± SE)	2.4 ± 0.1	2.7 ± 0.8	2.9 ± 0.3	2.8 ± 0.1
Panel reactivity				
0%	95	83	95	86
>0%	5	17	5	14
HLA-A+B+DR mismatch (mean ± SE)	3.1 ± 0.2	2.6 ± 0.2	3.1 ± 0.1	2.6 ± 0.0
Year of transpl.				
1993–1995 (%)	15	15	20	17
1996–1999 (%)	31	31	33	39
2000–2003 (%)	54	54	47	44
Original disease				
Diabetes (%)	6	4	9	5
ADPKD (%)	19	13	12	9
Pre-emptive transpl. (%)	36	9	29	13
Time on dialysis (months, mean ± SE)	20 ± 4	20 ± 3	33 ± 3	35 ± 1
First transplant (%)	96	96	92	90
Re-transplants (%)	4	4	8	10
Living related donation (%)	73	73	64	63
Living unrelated donation (%)	27	27	36	37
Race (White people) (%)	89	96	98	98

*$P > 0.10$ for all comparisons of 'Bern' versus 'Matched Cases', except $P = 0.02$ for pre-emptive transplantation and $P = 0.049$ for race.

†Not all patients with complete follow-up data.

HLA, human leukocyte antigen; ADPKD, autosomal dominant polycystic kidney disease.

Recipient and donor profiles of the three DST-negative control groups

From the international Collaborative Transplant Study (CTS) based in Heidelberg, Germany, we selected a DST-negative 'matched-case' control group of patients from Western Europe. For each of our 55 DST-positive patients, B. Döhler and G. Laux, members of the CTS study group, selected the best fitting DST-negative control person using an established internal algorithm. Specifically, allograft recipients and donors were matched for age, sex, LRD/LURD, original disease, cold ischemia time, first or re-transplantation, year of transplantation, time on dialysis, human leukocyte antigen (HLA) mismatches (with respect to HLA-identical siblings, there were only 4% in 'Bern', but approximately 10% in all other patient groups) and PRA. Demographic features of these subjects are summarized in Table 1 ('Matched Cases'). There were more pre-emptive transplantations in 'Bern' compared with 'Matched Cases', but the time on dialysis (months between the first dialysis and transplantation) was identical. In addition, there was a small difference with respect to race.

In addition to the 'Matched Cases', two DST-negative control groups were considered for comparison, as shown in Table 1 ('Switzerland', 'Western Europe'). They comprised the remaining DST-negative recipients from LRD or LURD from the other transplant centers in Switzerland and Western Europe.

Immunosuppressive protocol

More than 95% of the patients in all groups analyzed were started on calcineurin inhibitor therapy – with a clear preference of cyclosporine over tacrolimus – and on prednisone (data not shown). In addition, the majority of patients in all four groups received azathioprine or slightly more frequently mycophenolate mofetil (data not shown). The major differences with respect to immunosuppression between the four groups was the use of prophylactic antithymocyte globulin (ATG) at the time of transplantation. Whereas none of the patients from 'Bern' received ATG, a variable number of subjects in the other groups were given ATG: 'Matched Cases' (4%; $P = 0.15$ versus 'Bern'), 'Switzerland' (21%; $P = 0.0001$ versus 'Bern') and 'Western Europe' (14%; $P = 0.003$ versus 'Bern'). The prescription of IL-2 RA was comparable between 'Bern' (29%) and 'Matched Cases' (22%), $P = 0.38$. When compared with 'Bern', the groups of 'Switzerland' (18%) and 'Western Europe' (12%) often received less IL-2 RA, $P < 0.05$. However, these results need to be interpreted with some care, as the CTS survey on IL-2 RA was only based on positive answers with respect to their application; thus, no answer was regarded as no exposure to IL-2 RA. Prophylactic OKT-3 was not given to patients of the group 'Bern', and only prescribed to a small number of patients in the other groups (data not shown). Taken together, our patients from 'Bern' appeared to have received a slightly weaker induction therapy than other patients.

Statistical analyses

Differences between groups 'Bern' and 'Matched Cases' were analyzed by the *t*-test for equal means (donor age, cold ischemia time, HLA-mismatches, recipient age, and time on dialysis) and the chi-squared test (original disease, donor sex, PRA, donor relationship, recipient race, recipient sex, transplant year, graft number, and pre-emptive transplantation) with Bonferroni correction, as appropriate. Graft survival rates were calculated according to Kaplan and Meier [14]. Statistical significance for graft survival was estimated using the log-rank test. Analysis of rejection treatment was carried out in patients who had a functioning graft at 1 year and for whom it was known whether or not rejection treatment was carried out in the preceding year. All statistical analyses were performed with SAS (version 8.02; SAS Institute, Cary, NC, USA) and *P*-values <0.05 were regarded as significant.

Results

Sensitization rate and outcome following DST

A total of 61 potential allograft recipients received DST from the potential donor. Note, we did not observe serious side effects of transfusions, such as viral infections, severe transfusion reactions or hemolysis.

Following one ($n = 3$) or two ($n = 3$) DST, a total of six subjects (10%) developed a positive T-cell crossmatch (LRD: $n = 5$ and LURD: $n = 1$; three females and three males) precluding transplantation from living donation. Sensitized patients were immediately put on the waiting list for cadaveric renal transplantation. Subsequently, five of these six sensitized subjects received a cadaveric transplant with a mean waiting time of 12.2 months (range of 2–18 months) for the four patients with blood group A, and 2.7 months with blood group O. These waiting times were even shorter than the average waiting time for a cadaveric kidney in Switzerland (Dagmar Vernet, National Coordinator, Swisstransplant, Geneva; pers. comm.). However, we admit that this may not be the case in other regions of the world. Currently, four of these five patients have a well-functioning renal allograft, whereas one patient lost his kidney because of a severe acute rejection episode 1 week after engraftment. The sixth patient, who was

sensitized following a DST, came from a foreign country and was lost to follow-up. The remaining 55 nonsensitized subjects went on for transplantation and formed our study group 'Bern'.

Overall graft survival

Allograft survival at 6 years for our DST study group 'Bern' and the three DST-negative control groups 'Matched Cases', 'Switzerland' or 'Western Europe' were 98%, 82%, 84% and 81% (Fig. 1), indicating a trend toward a better outcome in the DST study group. At 6 years, the following number of patients were under analysis: 'Bern' ($n = 18$), 'Matched Cases' ($n = 10$), 'Switzerland' ($n = 121$) and 'Western Europe' ($n = 1515$).

The difference between 'Bern' and 'Western Europe' was statistically significant ($P = 0.03$), and the divergence between 'Bern' and 'Switzerland' just failed to reach significance ($P = 0.055$). Importantly, there was no increasing graft loss over time in the study group 'Bern', whereas late graft losses were observed in all other patient groups. In one out of 55 allografts of our subjects, a perioperative compression of the main of two renal arteries due to a hematoma occurred that lead to ischemia of the two upper thirds of the allograft. Subsequently, this graft was lost after several months because of declining kidney function of the remaining lower pole of the allograft.

Graft survival on an intention-to-treat base

On an intention-to-treat base, namely including the six sensitized patients as 'failures', the 6-year graft survival of our patients from the study group 'Bern' was 88.5%. For obvious reasons, such an analysis could not be performed with the patients from the other groups.

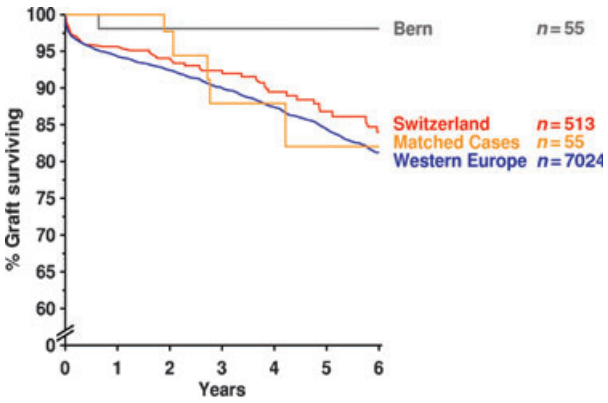

Figure 1 Graft survival after living renal allograft transplantation in patients with donor-specific transfusions (DST) ('Bern') and without DST ('Switzerland', 'Western Europe', 'Matched Cases').

Graft function 1 year after transplantation

Graft function is reported to the CTS registry according to the serum creatinine level in four different categories i.e. <130, 130–259, 260–400, and >400 μmol/l. From the DST study group 'Bern', 59% of patients were in the category with creatinine levels <130 μmol/l. The values for the 'Matched Cases', 'Switzerland' or 'Western Europe' were 48%, 52%, and 45%, respectively. Although none of these differences reached statistical significance, these results suggest that renal graft function may be better preserved in DST patients over a more prolonged follow-up period.

Treatment of rejections in the first year after transplantation

The percentages of patients having been treated for acute renal allograft rejection during the first year were analyzed (Fig. 2). Only three patients (6%) from the study group 'Bern' received rejection therapy, significantly less frequently than subjects in 'Switzerland' (21%) and in 'Western Europe' (20%); $P = 0.01$ for both comparisons. In the 'Matched Cases' group, a total of 14% of subjects were treated for rejections.

Patient survival

The 6-year patient survival of our DST-treated patients was 100% and the corresponding numbers of the other three groups ranged between 92% and 93% (Fig. 3). These values did not reach statistical significance. At 6 years, the following patient numbers were included: 'Bern' ($n = 18$), 'Matched Cases' ($n = 10$), 'Switzerland' ($n = 121$) and 'Western Europe' ($n = 1583$).

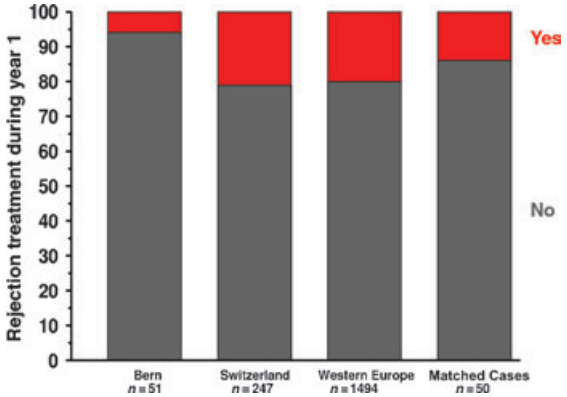

Figure 2 Percentage of patients treated for allograft rejection during the first year after living renal allograft transplantation. Patients were treated with donor-specific transfusions (DST) ('Bern') and without DST ('Switzerland', 'Western Europe', 'Matched Cases').

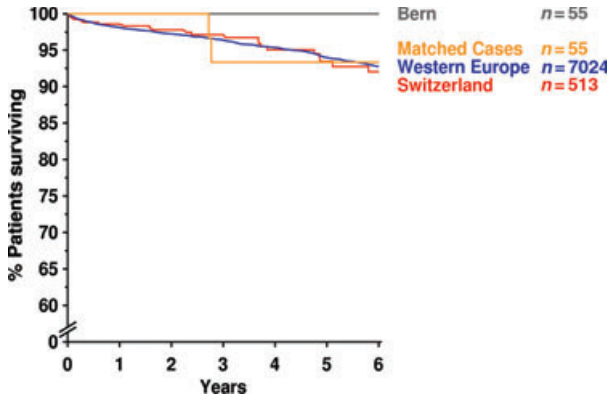

Figure 3 Patient survival after living renal allograft transplantation. Patients were treated with donor-specific transfusions (DST) ('Bern') and without DST ('Switzerland', 'Western Europe', 'Matched Cases').

Discussion

For the analysis of the effect of DST in the present investigation, we did not use combined endpoints as considered in the majority of drug trials in the field of renal transplantation to demonstrate superiority of novel therapeutic strategies. We rather preferred to show the individual endpoints. All these endpoints, such as graft survival, patient survival, renal function and number of treated rejections at 1 year, revealed a clear tendency or in some cases even statistical significance in favor of DST. The percentage of grafts functioning after 6 years was approximately 15% higher in our DST-treated patients than in all other DST-negative recipients from the remaining transplant centers in Switzerland or Western Europe. This effect was associated with a tendency for better kidney function and a more than three times lower incidence of treatment for acute rejection during the first year despite absence of prophylactic ATG induction therapy. To diminish the probability that the superior results in DST patients primarily reflect patient selection rather than an effect of DST, a 'matched-pair' analysis controlling for all the known and available relevant factors for transplant outcome was performed. Such an analysis was made possible by the extensive number of patients registered in the CTS. This analysis again indicates that the recipients with DST had a slightly better renal function with an approximately 16% higher 6-year graft survival rate. Importantly, the DST-negative control patients were more than twice as likely to receive rejection treatment during the first year. Thus, taken together, these results favor a beneficial effect of DST. Naturally, we cannot exclude a certain bias with respect to a center-effect regarding patient management and selection for transplantation. Furthermore, we do not have robust data on pregnancies and third-party, non-DST transfusions prior to transplantation that may also influence our results.

In an attempt to define the relevant factors accounting for the good result of our approach, we analyzed all the available studies about DST (Tables 2 and 3). Unfortunately, many of the relevant information are missing and not stated in Tables 2 and 3, such as the important waiting time on dialysis [15]. Thus the conclusions derived reflect tendencies rather than firm statements. First, the 1- and 5-year graft survival was better in virtually all DST patients compared with the controls when the time period before the introduction of cyclosporine A was considered (Table 2). After the introduction of cyclosporine A, the 1- and 5-year graft survival increased in both DST and non-DST-treated subjects and the differences in favor of DST became smaller (Table 3). Secondly, the overall percentage of patients sensitized ranged between 5% and 29% (Tables 2 and 3). Although there is some evidence that the administration of immunosuppressive drugs during the application of DST reduces the sensitization rate, several studies revealed a low sensitization rate comparable with our studies (≅10%) without immunosuppression [8,16–18]. From the other studies, it is unknown whether or not sensitization due to DST precluded or delayed subsequent cadaveric transplantation. In our study no substantial delay until cadaveric transplantation after sensitization was observed. This observation is not surprising given the restricted number of HLA antigens infused by DST. Although of concern with respect to the waiting time for an allograft, the problem of sensitization was probably overstated prior to the availability of erythropoietin in the past because many of these patients received multiple blood transfusions from a large number of donors to treat anemia in addition to DST. Thirdly, whether fresh or stored blood constituents were used for DST appeared not to influence the sensitization rate or the impact on graft survival (Tables 2 and 3). Similarly, the number of DST applied and the interval between DST and transplantation were not found to be critical factors. However, we are convinced that it might be wise to wait at least 3 weeks after the last transfusion to detect a possible sensitization.

Traditionally, DST protocols have been highly variable with respect to the use of whole blood [1,2,5,8, 10,12,13,16–22] versus buffy coat [3,7,9,10,12,18,21], stored [5,8,16,17,20,21] versus fresh [1,2,7,13,21,22] transfusions, actual number of administered DST, presence or absence of various types of immunosuppressive coverage, time interval between DST and transplantation and duration of follow-up (Tables 2 and 3). A major deficiency of these investigations was the controls considered. Some of the studies had no controls at all [3,9,20,21], others

Table 2. Reported strategies and results of donor-specific transfusions (DST) in patients with calcineurine inhibitor-free maintenance immunosuppression.

References	Number of patients (DST/without DST, if applicable)	Number and type of DST (BC/WB/F/S)	Interval: DST to transpl.	Immunosuppressive coverage yes/no	% of patients sensitized	1 year graft survival (%) DST/without DST	5 year graft survival (%) DST/without DST
Salvatierra *et al*. [2]	23/108 (historical)	3; WB/F;	2 weeks to 6 months	No	29	94/79	n.a.
Whelchel *et al*. [16]	40/38 (historical)	4; WB/S;	14 days	No	8	90/68 (at 6 months)	n.a.
Light *et al*. [17]	20/25 (historical)	3; WB/S;	15–149 days	No	10	78/68 (at 9 months)	n.a.
Norman *et al*. [18]	121/42 (historical)	1–3; WB or BC;	n.a.	No	13	96/69	83/59
Salvatierra *et al*. [1]	302/191 (concurrent HLA identical group)	3; WB/F;	2 weeks to 6 months	No/yes: some patients AZA	12 (AZA) and 21 (no coverage)	93/90	84/85 (at 3 years)
Flye *et al*. [12]	163/57 (HLA identical siblings; concurrent)	3–6; WB or BC;	n.a.	Yes: AZA	7	94/91	79/80
Anderson *et al*. [10]	163/54 (HLA identical siblings; concurrent)	3–6; WB or BC;	n.a.	Yes: AZA	7	95/93	79/82

BC, buffy coat; WB, whole blood; F, fresh; S, stored; AZA, azathioprine; n.a., no information available.

Table 3. Reported strategies and results of donor-specific transfusions (DST) in patients with calcineurine inhibitor-based maintenance immunosuppression.

References	Number of patients (DST/without DST, if applicable)	Number and type of DST (BC/WB/F/S)	Interval: DST to transpl.	Immunosuppressive coverage yes/no	% of patients sensitized	1 year graft survival (%) DST/without DST	5 year graft survival (%) DST/without DST
Cheigh *et al*. [20]	65	3; WB, S	≤2 months	CYA	5	96	92 (at 2 years)
Salvatierra *et al*. [13]	71/47 (historical)	3; WB/F	2 weeks to 6 months	Yes: AZA	8	100/87	98/66 (at 4 years)
Davies *et al*. [21]	86	1–3; WB or BC, F or S	1 day to 2 weeks	Yes: AZA, CYA	n.a.	90	n.a.
Velidedeoglu *et al*. [8]	344/93 (concurrent)	3; WB/S	n.a.	No	15	n.a.	61/43
Barber 1994 [5]	130/123 (concurrent)	4; WB/S	14 days	No	n.a.	n.a.	73/73 (at 4 years)
Okazaki *et al*. [9]	247	3; BC	n.a.	No/yes: DSG or AZA	14 (no IS) 8 (AZA or DSG)	99	89
Sharma *et al*. [11]	15/15 (prospective randomized trial)	1;WB/F	24 h	Yes: CYA	n.a.	86/75	n.a.
Otsuka *et al*. [4,7]	40/13 (concurrent)	3; BC/F	≤3 weeks	Yes: CYA	8	n.a.	88/76
Lezaic *et al*. [22]	19/40 (not stated)	3; WB/F	≥1 month	Yes: AZA	16	100/87	100/58
Amada *et al*. [3]	173	3; BC	4 weeks	No/yes: DSG or AZA	11	n.a.	90
Present publication	61/55 (matched cases)	2; WB or BC/F	>1 month	No	10	98/100	98/82

BC, buffy coat; WB, whole blood; F, fresh; S, stored; AZA, azathioprine; CYA, cyclosporine A; DSG, deoxyspergualine; IS, immunosuppression; n.a., no information available.

focused either on historical [13,17–19] or on a kind of parallel control group [1,2,4,5,7,8,11,12]. The present investigation appears to be the only one, which considered well-matched individuals. Only in one small study a prospective randomized study design was used [11]. In that study, 15 patients received 250 ml of DST 24 h preoperatively with cyclosporine A coverage and the remaining 15 subjects did not receive a DST [11]. The DST group demonstrated significantly fewer acute rejection episodes within 3 months of transplantation (seven versus two), a markedly better graft function and a clear trend toward a higher 1-year allograft survival (85.5% vs. 74.8%) [11]. Thus all these observations indicate that DST is of benefit. This observation is in line with a beneficial effect of DST observed in the only prospective randomized multi-center trial in the field of nonliving kidney transplantation, where as a significantly higher 5-year graft survival of 79% vs. 70% was encountered in 205 subjects that received three random pretransplant transfusions, when compared with the 218 patients without transfusions [23].

Although our sensitization rate of 10% precluded the respective transplant procedures, it may well also have prevented the waist of some of these living donor organs because of acute rejection in case of an actual transplantation.

The exact underlying immunological mechanism of the benefit of DST is unknown. It has been postulated that exposure to donor antigens may induce donor-specific hyporeactivity by elimination of the recipient's alloreactive lymphocyte clones against the donor [22,24,25].

A recent hypothesis stated that the induction of $CD4^+$ regulatory T cells by DST may be relevant [26,27]. Furthermore, DST can have a synergistic effect with T-cell co-stimulatory blockade in the induction of tolerance in different transplant models [28,29]. In this respect, the tolerance inducing effect of DST may involve the indirect antigen recognition pathway [28].

Finally, DST may be a way to test which potential recipients will reject their allografts after transfusion by the development of cytotoxic antibodies that keep these patients away from receiving these grafts [24]. In comparison with most other respective centers, we used a relatively highly 'immunogenic' DST protocol by the application of fresh blood products without any concomitant immunosuppressive treatment. We speculate that this strategy may be responsible for a particularly strong modulation of the immune system, as mentioned above, leading to a low number of rejection episodes with consequently a good outcome.

Although longer follow-up periods are required for a definite conclusion, our 6-year observation strongly suggests that DST improve the outcome of living kidney transplants even when modern immunosuppressive drugs are prescribed. A future large prospective and randomized trial with and without the application of DST (whole blood or buffy coat, as indicated) should be considered to confirm and extend our results.

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