Infectious Complications in Living-Donor Kidney Transplant Recipients Undergoing Multi-Modal Desensitization

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Abstract

Background: Pre-existing humoral barriers challenge the transplantation of living donor kidneys (LDK) into highly sensitized ABO- and human leukocyte antigen (HLA)-incompatible recipients. Conditioning these LDK recipients' immune systems is required before they undergo transplantation. We hypothesized that medical desensitization would yield higher post-transplantation rates of infection.

Methods: We conducted a study in which matched controls consisting of non-desensitized (NDS) LDK recipients were compared with desensitized (DS) recipients. Pre-transplantation desensitization included treatment with rituximab and mycophenolate mofetil followed by intravenous immunoglobulin (IVIg) and plasmapheresis. All participants in the study underwent induction therapy and maintenance immunosuppression. Primary outcomes included infection (opportunistic, local, systemic) within 12 mo after transplantation.

Results: Twenty-five patients underwent desensitization and LDK transplantation. Graft survival in the DS and NDS groups of patients was 96% and 98%, respectively. The mean 3- and 12-mo serum creatinine concentrations in the DS and NDS groups were 1.1 ± 0.2 mg/dL and 1.2 ± 0.3 mg/dL and 0.95 ± 0.4 mg/dL and 0.73 ± 0.8 mg/dL ($p=0.3$ and $p=0.01$), respectively. Thirty-six percent of the patients in the DS group had one or more infections, vs. 28% of those in the NDS group ($p=0.1$). No difference was observed in the frequency of opportunistic or systemic infections in the two groups. Local infections were statistically significantly more frequent in the DS group (60% vs. 30%, respectively; $p=0.02$).

Conclusion: Pre-operative desensitization in highly sensitized LDK recipients is followed by a similar incidence of opportunistic and systemic infections as in NDS patients. Local infections were significantly more frequent in the DS than in the NDS patients in the study. With careful monitoring of infectious complications, pre-transplant desensitization permits LDK transplantation into highly sensitized patients.

IN THE UNITED STATES, more than 115,000 individuals await kidney transplantation on the United Network for Organ Sharing (UNOS) cadaveric kidney waiting list [1]. Median wait times for kidney transplantation range from 855 d for patients with blood type AB to 1,935 d for those whose blood type is B-positive [2]. Patients who are either ABO-incompatible with a living donor or have antibodies to donors' human leukocyte antigen (HLA) wait even longer. Anti-HLA antibodies can develop as a response to previous transplants, pregnancy, or blood transfusions. The presence of circulating anti-HLA antibodies can produce a positive cross match result, which is predictive of hyperacute rejection of a transplanted organ or tissue, and has traditionally

been considered a contraindication to transplantation [3,4]. Lengthy UNOS wait times for organ transplants increase mortality and morbidity substantially, and foregoing transplantation because of an incompatible donor can have dire consequences [5].

Success in ABO- or HLA-incompatible living donor kidney (LDK) transplantation affords many patients previously deemed unsuited for transplantation an opportunity to receive a transplant [5–7]. Although previous work has shown the general safety and efficacy of desensitization of highly sensitized LDK recipients, a critical in-depth analysis of the infectious complications associated with this pre-transplant conditioning has yet to be done. Desensitization

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protocols generally include a combination of plasmapheresis, immunosuppression, and intravenous immunoglobulin (IVIg) [6–8]. A recent protocol that utilized plasmapheresis and low-dose IVIg before kidney transplantation produced significant survival benefit with desensitization in HLA-sensitive patients as compared with waiting for a compatible organ [8].

Whereas desensitization shows promise for moving HLA- and ABO-incompatible kidney transplant patients nearer to successful transplantation, an in-depth analysis of infectious complications associated with such desensitization remains to be completed. We evaluated the medical desensitization regimen (rituximab and IVIg) used at our institution for highly sensitized ABO- and HLA-incompatible LDK transplant recipients, with a critical focus on infectious complications. We hypothesized that all infectious complications would be more common in patients treated with the desensitization regimen than in controls who underwent LDK transplantation without desensitization, and that opportunistic and viral infections would be particularly common among these infectious complications.

Patients and Methods

Donor–recipient matching

This retrospective study was approved by the University of Virginia institutional review board (IRB), and the need for informed consent was waived. A matched case–control study design was used to compare outcomes in desensitized (DS) and non-desensitized (NDS) recipients of LDK transplants. Each desensitized patient was hand-matched with two non-desensitized patients who served as controls. The patients and controls were matched closely for the cause of their end-stage renal disease (ESRD) according to categories including congenital, immune-related, and hyper-tensive disease; diabetes mellitus (DM) type 1 or DM type 2; and age (± 3 y), prior dialysis requirement, and prior transplantation.

Desensitization protocol

All patients treated with the desensitization protocol had either a positive flow-cytometric crossmatch result for identified anti-donor HLA antibodies (some also had anti-ABO antibodies with titers $>1:8$). Medical desensitization included rituximab (375 mg/m^2) given intravenously (IV) and mycophenolate mofetil (500 mg bid beginning 6–8 wks pre-operatively, followed by IVIg (0.5 g/kg) and plasmapheresis beginning 2 wks pre-operatively. Patients undergoing desensitization had a negative flow-cytometric crossmatch result before transplantation and anti-ABO antibody titers $<1:8$ before proceeding to transplantation. Subsequent LDK transplantation was done at our institution.

Immunosuppression and antibody testing

Both DS and NDS patients underwent induction immunosuppressive therapy post-operatively (with rabbit anti-human thymocyte globulin in a total dose of 6 mg/kg) followed by maintenance immunosuppression (with tacrolimus, with a target 12-h trough plasma concentration of

$8\text{--}10\text{ mg/mL}$), mycophenolate mofetil [typically at 2 g qd], and prednisone [tapered to $5\text{--}10\text{ mg qd}$].

Prophylaxis

Patients scheduled for LDK donation who were considered to be high-risk for cytomegalovirus (CMV) infection (donor +/recipient–) received valganciclovir 900 mg for 6 mo. Those considered to be at moderate-risk for CMV infection (recipient+) received valganciclovir 450 mg for 3 mo. Acyclovir was given for prophylaxis against viral infection to all patients at low-risk for CMV infection. Prophylaxis against *Pneumocystis pneumonia* (PCP) consisted of trimethoprim–sulfamethoxazole given twice weekly for life. Kidney recipients did not undergo surveillance for either CMV or BK virus other than when indicated clinically.

Post-operative care, outcomes, and statistics

All patient follow-up was done at our transplant center by a team of surgeons, transplant nephrologists, and nurse coordinators. Close contact was maintained with all transplant recipients both before and after transplantation. All laboratory work, including bacterial, viral, and fungal cultures, as well as assays for immunosuppressant drug concentrations, were evaluated by our transplantation team. Infections were captured thoroughly with this approach and were entered into our IRB-approved data base by study personnel.

Primary outcomes included opportunistic, local, and systemic infections occurring within 12 mo of transplantation; secondary outcomes included patient and graft survival and acute rejection. An infection was categorized as opportunistic if it was of a kind that would affect an immunocompromised but generally not an immune-competent host. Viral infections were considered opportunistic for purposes of this study. Local infections were defined as those confined to a single organ system and not associated with bacteremia, viremia, or fungemia, and included symptomatic urinary tract, sinus, pulmonary, renal, dermal, and throat infections. Asymptomatic bacteriuria was not considered an infection for purposes of the study. A cytomegalovirus (CMV)-positive serostatus without evidence of symptoms of infection was not included as an infection. Systemic infections were considered to be any that were disseminated, including bacteremia, viremia, and fungemia. Pathogen-based categories were also evaluated. The primary focus among infections was on those occurring within 1 yr after transplantation because these were thought to potentially relate to the desensitization regimen. Patient and graft survival was evaluated for 1 yr after transplantation. Graft function was monitored through each patient's serum creatinine concentration. Acute rejection was diagnosed by kidney biopsy and treated with additional immunosuppressive medications tailored to the individual patient.

Univariate statistical analysis of categorical data included the Fisher exact test or χ^2 test. An unpaired *t*-test or the Mann–Whitney U test was used for continuous variables. Data are presented as means $\pm$ standard deviation (SD). A value of $p < 0.05$ was considered significant. Statistics were completed with SPSS software version 19 (SPSS, IBM, Armonk, NY).

Results

Baseline demographics

Twenty-five consecutive highly immunologically sensitized patients, all of whom were initially crossmatch-positive, were identified as having undergone desensitization and subsequent LDK transplantation at our institution between January 2003 and July 2009. Six of the patients were ABO-incompatible with their donors and 17 were HLA-incompatible. Two patients were both ABO- and HLA-incompatible. Fifty patients who underwent LDK transplantation between January 2003 and July 2009 were used as controls and matched two-to-one with the DS patients based on cause of ESRD and age. Forty-eight percent (12/25) of the DS patients were suited for matching with two controls having the same cause of ESRD as the patient (DM type 1, DM type 2, hypertension, Alport syndrome, calcineurin toxicity, focal segmental glomerulonephritis [FSGS], IgA nephropathy, or polycystic kidney disease [PKD]). The remaining DS patients were matched with controls whose cause of ESRD was in the same category as that of the patient, including congenital and immune-related processes. Fifty-two percent (13/25) of the desensitized patients were matched with two controls that were within 3 yrs of the patient's age.

Of the NDS group, were 36% (18/50) female, as compared with 48% (12/25) of the DS group ($p=0.3$). The mean age of the DS group was 42.1 ± 18.3 yrs, as compared with 42.1 ± 18.4 yrs for the control group ($p=0.9$). The mean body mass index (BMI) of the DS patients was 28 ± 5.3 kg/m², as compared with 28.2 ± 6.1 kg/m² for the NDS patients ($p=0.7$). A positive CMV serostatus was seen in 60% (15/25) of the DS patients as compared with 52% (26/50) of the NDS patients ($p=0.6$). A significantly greater number of patients in the DS group (44%, 11/25) than in the NDS group (6%, 3/50; $p=0.0002$) had previously had one or more attempted kidney transplantations.

Following medical desensitization, all of the DS patients ultimately achieved a negative crossmatch status before transplantation. Immediate complications related to desensitization included a transfusion reaction (fevers, rigors) requiring hospitalization in one patient undergoing treatment with IVIG. The first round of desensitization was unsuccessful in a second patient in whom it did not produce a negative crossmatch. The patient underwent subsequent nephrectomy of a previously transplanted kidney, with a further regiment of desensitization repeated, which produced a negative crossmatch.

Patient and graft survival

At one year after LDK transplantation, patient survival in both the DS and NDS cohorts was 100%, with graft survival of 96% in the DS group as compared with 98% in the NDS group. One patient in the DS group underwent nephrectomy of a transplanted kidney, on post-operative day 42. This patient experienced acute antibody-mediated rejection (BANFF grade II) and severe acute tubular damage on post-operative day 8, which was refractory to medical management. A transfusion reaction following a splenectomy performed to mitigate the rejection necessitated extracorporeal membrane oxygenation (ECMO). In the NDS group, one patient experienced graft lost on post-operative day 4 because of acute graft thrombosis and widespread cortical and medullary necrosis. A second patient had graft loss at 23 mo post-operatively.

The mean 3-mo serum creatinine concentration of patients with surviving grafts was 1.1 ± 0.2 mg/dL in the DS group vs. 0.95 ± 0.4 mg/dL in the NDS group ($p=0.3$). The average serum creatinine concentration at 1 y after transplantation was 1.2 ± 0.3 mg/dL in the DS group vs. 0.73 ± 0.8 mg/dL in the NDS group ($p=0.01$). Rejection occurred more frequently in the DS than in the NDS group. Seven patients (28%) in the DS group and four (8%) in the NDS group experienced rejection ($p=0.03$) (Table 1).

All infections

One or more infections occurred within the first year after transplantation in 36% (9/25) of the DS group, as compared with 28% (14/50) of the NDS group ($p=0.1$). The average number of infections per infected patient was 1.6 ± 0.7 in the DS group, vs. 1.4 ± 0.7 in the NDS group. Cumulatively, 56% (14/25) of the patients in the DS group, vs. 36% (18/50) of those in the NDS group ($p=0.6$), had at least one infection within the respective follow-up periods. The mean time to infection was 6.1 ± 10.1 mos in the DS group as compared with 3.0 ± 5.9 mos in the NDS group ($p=0.07$).

Opportunistic infections

No difference was observed in the frequency of opportunistic infections in the DS (20%) and NDS groups (8%) ($p=0.1$). One patient in the DS group experienced *Nocardia* pneumonia at 3 mo after transplantation. Viral infections in desensitized patients included CMV pneumonia (11 mos after transplantation), picornavirus pneumonia (9 mos after transplantation), adenovirus nephritis (6 mos after transplantation), and herpes zoster (shingles, 12 mos after transplantation). Viral infections in the NDS group included BK nephritis in two patients, adenovirus gastroenteritis, and a digital adenoviral paronychia in one patient. There was no difference in the incidence of viral infection in the DS and NDS groups (16% vs. 8%, respectively, $p=0.4$).

TABLE 1. REJECTION EPISODES IN DESENSITIZED AND NON-DESENSITIZED PATIENTS

Type of rejection	BANFF grade	Months after transplantation
Desensitized		
ACR	IIA	26
ACR		4
ACR	IB	0.36
ACR	IA	0.46
Acute AMR	II	6
T-cell-mediated acute rejection	IB	8
ACR	IIA	9
Acute AMR	II	0.26
Mixed ACR and AMR		4
Non-desensitized		
ACR		0.26
Mixed acute B cell mediated	II	11
T cell-mediated acute rejection	IB	11
Acute vascular humoral rejection		0.26
ACR		1

ACR = acute cellular rejection; AMR = antibody-mediated rejection; BANFF = Banff classification of renal allograft pathology.

TABLE 2. LOCAL INFECTIONS IN DESENSITIZED AND NON-DESENSITIZED PATIENTS

Cause of end-stage renal disease	Infection	Mo post-operative
Desensitized		
Chronic GN	<i>Streptococcus pneumoniae</i> sinusitis	1
FSGS	Staphylococcal extremity dialysis graft infection	2
Hypertension	<i>Escherichia coli</i> UTI	4
Dysplastic kidneys	<i>S. pneumoniae</i> bronchitis	6
Dysplastic kidneys	<i>E. coli</i> UTI	6
Congenital horseshoe kidney, hypertension	<i>E. coli</i> UTI	8
GN	<i>Nocardia</i> pneumonia	3
GN	Picornaviral pneumonia	9
PCKD	<i>Klebsiella oxytoca</i> UTI	1
PCKD	CMV pneumonia	11
FSGS	Adenoviral tubulointerstitial nephritis	6
Reflux uropathy	Streptococcal pharyngitis	6
FSGS	Herpes zoster shingles	12
Non-desensitized		
Calcineurin toxicity	<i>Proteus</i> UTI	3
Calcineurin toxicity	<i>S. pneumoniae</i> pneumonia	4
GN	Staphylococcal thrombophlebitis of extremity soft tissue	1
Diabetes mellitus type 2	Coagulase-negative staphylococcal UTI	0.43
MPGN	<i>E. coli</i> UTI	1
Hypertension	BK nephritis	11
Hypertension	<i>E. coli</i> UTI	10
Hypertension	<i>E. coli</i> UTI	8
Hypertension	Adenoviral gastroenteritis	9
Posterior urethral valves	<i>E. coli</i> UTI	5
Posterior urethral valves	<i>Klebsiella</i> UTI	12
Dysplastic kidney	Non-hemolytic streptococcal UTI	7
Alport syndrome	<i>S. pneumoniae</i> sinusitis	4
Alport syndrome	Adenoviral paronychia	8
Alport syndrome	Coagulase-negative staphylococcal UTI	11

CMV = cytomegalovirus; FSGS = focal segmental glomerulosclerosis; GN = glomerulonephritis; MPGN = membranoproliferative glomerulonephritis; PCKD = polycystic kidney disease; UTI = urinary tract infection.

Localized and disseminated infections

Localized, non-disseminated infections occurred in 56% (13/25) of the patients in the DS group and 30% (15/50) of the patients in the NDS group ($p=0.08$) (Table 2). No difference was observed in the incidence of systemic infections in the DS and NDS groups (4% vs. 4%, respectively, $p=1.0$). One patient in the DS group experienced symptomatic propionibacteremia, whereas one patient in the NDS group had *Escherichia coli* "urosepsis" and another had symptomatic streptococcal bacteremia.

Discussion

Contrary to our hypothesis, medical desensitization with rituximab and mycophenolate mofetil followed by IVIg and plasmapheresis did not result in a greater frequency of infectious complications in highly-sensitized LDK transplant recipients than in NDS controls. Although a greater number of local infections were observed in the DS patients, the difference did not reach statistical significance. No statistically significant difference in the overall incidence of infection (opportunistic, local, or disseminated [bacteremia, viremia, fungemia]) was identified in the first year after transplantation.

In addition, the regimen used for desensitization and described above resulted in excellent patient and graft sur-

vival, which were comparable to previously reported results [8–10]. Episodes of organ rejection episodes were significantly more frequent in the DS patients in the first year after transplantation, which was an expected finding in light of the DS patients' allosensitization before transplantation. Other investigators have reported rates of rejection of 24.1% [9] to 28.6% [10] for LDK transplantation. Although rejection was more common in the DS patients in our study, cumulative graft loss was not. Additionally, the mean creatinine concentration of DS patients were also significantly higher than that of NDS patients at 1 y after transplantation ($p=0.01$), but this did not translate into acute graft loss. The long-term implications of this for renal allografting are unclear.

Although work has been done on the efficacy of medical desensitization before organ transplantation, few specific data exist about the infectious complications related to it. One study of 15 ABO-incompatible LDK recipients who underwent pre-transplant desensitization with immunoadsorption and rituximab found no difference in their frequency of infection and that of ABO-compatible donors who did not receive the same pre-operative immunosuppression [11]. In another study of ABO-incompatible patients, who underwent pre-operative plasmapheresis and splenectomy and were treated with tacrolimus, mycophenolate mofetil, and methylprednisolone, 16% experienced acute pyelonephritis and

33% had acute rejection of LDK transplants [12]. A third study found that 4.8% of kidney transplant patients receiving a preoperative immunosuppressive regimen developed opportunistic infections [13]. By comparison, opportunistic infections were seen in 12% of the patients in our DS group. Moreover, our findings resemble those in a recent study by Vo et al., in which rituximab in conjunction with plasmapheresis and IVIg did not appear to increase the risk of infectious complications in LDK recipients [14]. The unique feature of the desensitization protocol in our study was administration of mycophenolate mofetil before transplantation.

Perhaps the most surprising outcome of our study was the similar incidence of viral infections in the DS and NDS groups, with a higher rate of localized bacterial infections (mainly consisting of UTIs) in the DS group. We had hypothesized initially that because desensitization targets the B-cell and antibody-based immunity associated with antibody-mediated rejection, infections associated with adaptive immunity would be more common. This was not the case. It is possible that the IVIg administered with the desensitization protocol in our study provided temporary passive humoral immunity to infectious agents [15]. On the other hand, the desensitization protocol may have affected the innate immune response to bacterial infections to a greater degree than expected. Whether it would be possible to devise an intervention that would augment the innate immune response in DS patients to a degree that would reduce the incidence of localized bacterial infection to its baseline value remains to be seen.

Strengths of the present study include a dedicated focus on the infectious complications associated with medical desensitization in the unique group of highly immunologically sensitized LDK recipients. Understanding the infection-related morbidity associated with this will help ensure that patients are not subjected to undue risk for major complications of medical desensitization. In addition, our study is the first to report a matched-control strategy to correct for renal disease and patient age as factors potentially associated with the risk of infection in LDK recipients.

Weaknesses of the present study include the inability to achieve a perfect donor-recipient match for all patients in terms of age and cause of ESRD, owing to the limited number of patients in the study. Other variables, not included in the study, could also increase or decrease a patient's likelihood of experiencing infectious complications. For example, the panel reactive antibody (PRA) score, a history of prior transplantation, and treatment for rejection might all be associated with infectious disease risk but were not included in the current analysis. Lastly, because of the unique immune status of the subset of patients in our study, it may not be possible to generalize the rates at which they experienced infection to other kidney transplant patients.

Collectively, LDK transplant recipients who are highly sensitized and undergo a pre-operative medical desensitization regimen have a high incidence of short-term infectious complications, but at a rate generally similar to that of NDS patients. Care must be taken to recognize and appropriately manage the higher incidence of local infections in DS patients. However, the regimen used for desensitization in our study enables an immunologically challenging group of patients to successfully undergo LDK transplantation. Future work is certainly required to further evaluate and understand the long-term infectious complication that can affect these pa-

tients and to attempt to modify them to improve the outcomes of LDK transplantation.

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References

1. UNOS Transplant Trends. Accessed December 3, 2012 from www.unos.org.
2. UNOS Organ Procurement and Transplantation Network. Kidney Kaplan-Meier median waiting times for registrations listed (based on OPTN data as of November 23, 2012). www.unos.org. Accessed December 3, 2012.
3. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969;280:735-739.
4. Williams GM, DePlanque B, Lower R, et al. Antibodies and human transplant rejection. *Ann Surg* 1969;170:603-616.
5. Stegall MD, Dean PG, Gloor JM. ABO-incompatible kidney transplantation. *Transplantation* 2004;78:635-640.
6. Magee C. Transplantation across previously incompatible immunologic barriers. *Transplant Int* 2006;19:87-97.
7. Jordan SC, Vo AA, Peng M. Intravenous gammaglobulin (IVIg): A novel approach to improve transplant rates and outcomes in highly HLA-sensitized patients. *Am J Transplantation* 2006;6:459-466.
8. Montgomery RA, Lonze BE, King KE, et al. Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med* 2011;365:318-326.
9. Kute VB, Vanikar AV, Trivedi HL, et al. Desensitization protocol for highly sensitized renal transplant patients: a single-center experience. *Saudi J Kidney Dis Transpl* 2011;22:662-669.
10. Zhang W, Chen D, Chen Z, et al. Successful kidney transplantation in highly sensitized patients. *Front Med* 2011;5: 80-85.
11. Genberg H, Kumlien G, Wennberg L, et al. Long-term results of ABO-incompatible transplantation with antigen-specific immunoadsorption and rituximab. *Transplantation* 2007; 84:S44-S47.
12. Ishikawa N, Yagisawa T, Sakuma Y, et al. Transplantation of ABO-incompatible and living unrelated donor-recipient combinations. *Transplant Proc* 2008;40:2292-2293.
13. Kenmochi T, Saigo K, Maruyama M, et al. Results of kidney transplantation from ABO-incompatible living donors in a single institution. *Transplant Proc* 2008;40:2289-2291.
14. Kahwaji J, Sinha A, Toyoda M, et al. Infectious complications in kidney-transplant recipients desensitized with rituximab and intravenous immunoglobulin. *Clin J Am Soc Nephrol* 2011;6: 2894-2900.
15. Gelfand EW. Intravenous immune globulin in autoimmune and inflammatory diseases. *New Engl J Med* 2012;367: 2015-2025.

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