DELAYED IMMUNOSUPPRESSION WITHDRAWAL PRESERVES NONSENSITIZATION STATUS AFTER KIDNEY TRANSPLANT FAILURE

By

MICHAEL JIN CASEY

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2012

To my beloved wife Hana who’s support, love, and zeal has kept me going through numerous late nights

ACKNOWLEDGMENTS

I would like to thank my mentor Dr. Herwig-Ulf Meier-Kriesche who fully supported my decision to enroll in the Advanced Postgraduate Program in Clinical Investigation. I would like to thank Dr. Juan Scornik and Dr. Mark Segal for their invaluable advice and Dr. David Nelson for chairing my thesis committee. I would like to express my gratitude to Xuerong Wen not only for her exceptional statistical support, but also for her countless hours of study collaboration. I would like to express my appreciation to Dr. Marian Limacher who granted me the opportunity to enroll in the Advanced Postgraduate Program in Clinical Investigation and Eve Johnson for always keeping me on task throughout my graduate studies. Finally, I would like to give special thanks my lovely wife Hana and my dear children Nayeon and Rowan for tolerating the numerous hours I have spent cloistered away in my office working towards my degree.

This work supported in part by the NIH/NCATS Clinical and Translational Science Award to the University of Florida UL1 TR000064.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

DELAYED IMMUNOSUPPRESSION WITHDRAWAL PRESERVES NONSENSITIZATION STATUS AFTER KIDNEY TRANSPLANT FAILURE

By

Michael Jin Casey

December 2012

Chair: David R. Nelson
Major: Master of Science – Clinical and Translational Science

When kidney transplants fail, transplant medications are stopped to reduce risks associated with immunosuppression. However, retransplantation candidates are at high risk for sensitization which delayed immunosuppression withdrawal may minimize. We hypothesize that for patients with graft failure referred for retransplantation, delayed immunosuppression withdrawal preserves nonsensitization (PRA 0%) better than early immunosuppression withdrawal.

We retrospectively examined subjects transplanted at a single center between Aug. 1999 and Jan. 2011 with non-death related graft loss. Subjects were stratified by time to immunosuppression withdrawal after graft loss: >3 months, 1-3 months, and ≤1 month. Retransplant candidates were eligible for the main study where the primary outcome was nonsensitization at retransplantation evaluation. Subjects not referred for retransplantation were included in the safety analysis.

We found 102 patients, with 49 eligible for the main study. Nonsensitization rates at retransplantation evaluation were 66%, 38%, and 25% for immunosuppression withdrawal >3 months, 1-3 months, and ≤1 month respectively (p = 0.041). After

adjusting for cofactors such as blood transfusion and allograft nephrectomy, immunosuppression withdrawal >3 months remained significant for nonsensitization (adjusted odds ratio=5.09, 95% C.I. [1.09-23.72]). Mortality and infection-related death were not associated with delayed immunosuppression withdrawal in the main study group or total study population.

In this study, delayed immunosuppression withdrawal appears safe as a strategy to minimize sensitization in low risk retransplantation candidates. No adverse safety signals were seen, but further investigation with larger sample size is needed.

CHAPTER 1
INTRODUCTION

It is well known that kidney transplantation is life-sparing for patients with end stage kidney disease (ESRD) who are on the waiting list.[1-3] Projected lifetime almost doubles after deceased donor kidney transplantation compared with remaining on the waitlist.[4] However, when a kidney transplant recipient's graft fails, then the risk for death rises again.[5-6] In addition, an increased risk of sepsis and infection-related deaths are seen after transplant failure compared to patients who retain transplant function.[5,7] Yet, retransplantation with a subsequent kidney transplant is associated with a significant reduction in mortality when compared with their wait-listed counterparts with prior transplant failure.[8]

A growing number of patients with a failed kidney transplant are relisted for a subsequent kidney transplant. United States registry data show that approximately 20% of all ESRD patients on the kidney transplant waiting list have a prior failed transplant.[9] However, transplanting these patients in a timely manner is challenging because prior solid organ transplantation is a risk factor for human leukocyte antigen (HLA) sensitization which may limit the availability of compatible organs and prolong transplant waitlist time.[10] Prior studies have shown that transplant nephrectomy is a risk factor for sensitization.[11-13] In fact, kidney transplant failure itself carries a high risk of sensitization and is probably related to several factors such as sudden immunosuppression cessation, transplant nephrectomies, and blood transfusions.[14]

When kidney transplants fail, transplant medications are commonly stopped to reduce the risks associated with immunosuppression, namely infection and premature death.[15-16] However, when to withdraw immunosuppression is still an unanswered

question. Potential benefits of delayed immunosuppression withdrawal include rejection risk reduction, allograft nephrectomy risk reduction, and preservation of residual renal function[17-18], but another more intriguing benefit may be minimizing the risk of sensitization that may occur soon after kidney transplant failure.[14] By minimizing the risk of sensitization, patients with a failed kidney transplant may have a greater chance at receiving a subsequent life-sparing kidney transplant. We hypothesize that in patients with kidney transplant failure who were referred for retransplantation, delaying the withdrawal of immunosuppression medications reduces the risk of sensitization compared to early immunosuppression withdrawal. In addition, we evaluate whether delayed immunosuppression withdrawal is associated with significant additional risk compared to early immunosuppression withdrawal, especially in potential retransplant candidates.

CHAPTER 2
METHODS

The study utilized data from the Organ Transplant Tracking Record (OTTR) and the electronic medical record system at the Shands Transplant Center at the University of Florida. The OTTR database was developed to document and store patient demographic and clinical characteristics, laboratory test results, status of referral, listing, transplant, complications, graft failure, and death. The study protocol was approved by the University of Florida Institutional Review Board.

Study Participants

A retrospective cohort study was conducted. The study participants consisted of adult kidney transplant recipients transplanted between August 1999 and January 2011 who experienced non-death related graft failure. Subjects were analyzed by time to immunosuppression withdrawal after kidney transplant failure and they were divided into three categories: ≤ 1 month, 1-3 months, and >3 months. Immunosuppression withdrawal was defined as the point when all noncorticosteroid immunosuppressant drugs were stopped. Subjects who were subsequently evaluated for kidney retransplantation were eligible for the main study group. Subjects not evaluated for subsequent kidney retransplantation were analyzed only for safety endpoints.

Study Outcomes

The primary outcome was rate of nonsensitization among subjects evaluated for retransplantation (main study group). Nonsensitization was defined as a panel reactive antibody (PRA) level of 0%. We chose to study nonsensitization (PRA = 0%) because it was an unambiguous endpoint where there was a clear distinction between subjects with and without HLA alloantibodies. This simplified endpoint removed any speculation

associated with trying to assign relative importance to different degrees of PRA change. Also, the outcome of nonsensitization was probably less biased by the different lab techniques used to determine PRA over time. Prior to primary transplant, the peak PRA was used to determine whether a patient was nonsensitized (PRA=0%) or sensitized (PRA>0%). After graft failure, the PRA at the time of retransplant evaluation was used.

Secondary outcomes from the main study group were PRA level at the time of evaluation for retransplantation, 3-year relisting rate, and 3-year retransplantation rate. All subjects—whether evaluated or not evaluated for retransplantation—were studied for the safety outcomes 3-year mortality, 3-year infection related mortality, and 3-year infection free survival (time to first infection). Date of graft failure served as the starting point for all reported 3-year outcomes.

Potential covariates for nonsensitization include donor type (deceased v. living donor), recipient age at the time of graft failure, black race, gender, diabetes as cause of end stage renal disease, nonsensitization status prior to primary kidney transplant, HLA mismatch, induction with a lymphocyte depleting agent, acute rejection, primary graft survival length, duration from graft failure to re-evaluation PRA, transplant nephrectomy, and blood transfusion. Acute rejection was defined as a biopsy confirmed cellular rejection Banff 1A or higher or biopsy confirmed antibody mediated rejection.

Statistical Analysis

Continuous variables were compared using F test for more than two comparison groups. Categorical variables were analyzed using chi-square test or Fisher Exact Test. Multivariate logistic regression analysis was employed to identify the significant risk factors for nonsensitization at the time of evaluation for retransplantation. Multivariate time dependent Cox model was utilized to assess time to events adjusting for

immunosuppression withdrawal time. In the multivariate models, rates of event occurrence in the patients with immunosuppression withdrawal >3 months and 1-3 months were compared with those of <1 month (baseline), respectively. All statistical analyses were conducted with SAS 9.2 (Cary, NC).

HLA Antibody Detection

Throughout the entire study, HLA antibodies were screened (antibody positive or negative) by solid phase assay (ELISA, Luminex screen and/or Flow PRA). PRA was determined by cytotoxicity (years 1999-2006) or by calculated PRA using single antigen beads (years 2006-2012). All antibody tests were performed by solid-phase assays using commercial reagents for enzyme immunoassay and flow cytometry. The tests used to measure HLA antibodies were enzyme immunoassay (GTI, Waukesha, WI), Luminex screen, Flow PRA and single antigen beads (One Lambda, Canoga Park, CA). For the latter test, raw median fluorescence intensity values > 1,000 were considered positive and values of 1,000 to 3,000 were considered positive but acceptable for potential donors carrying the target antigen. Donor specificity was assigned for antibodies to HLA-A, -B, -C, -DR, and -DQ.

CHAPTER 3
RESULTS

Out of 1651 kidney transplants performed between August 1999 and January 2011, 102 subjects were identified as having non-death related graft failure. Forty-nine subjects were subsequently evaluated for kidney retransplantation and eligible for the main study group. Of the main study participants, 12, 8, and 29 subjects had their immunosuppression withdrawn after graft failure at ≤ 1 month, 1-3 months, and > 3 months respectively (Figure 3-1). Delayed immunosuppression withdrawal after graft failure was seen in subjects with residual renal function and those with an anticipated short waitlist time. In the >3 months immunosuppression withdrawal group, the median duration of prolonged immunosuppression was 11.9 months [range 3.8-49.7] after graft failure. Five patients (17%) in the > 3 months immunosuppression withdrawal group maintained their immunosuppression due to a functioning non-renal solid organ transplant. Baseline characteristics—including blood transfusion, allograft nephrectomy, and acute rejection—were statistically similar among all three immunosuppression withdrawal groups (Table 3-1). Early graft losses made up a substantial portion of the main study group such that the median duration of primary graft survival was 19 months with no significant differences between the immunosuppression withdrawal groups (Table 3-1).

Efficacy Outcomes for Delayed Immunosuppression Withdrawal

Nonsensitization after Graft Failure

Prior to initial kidney transplantation, similar rates of nonsensitized subjects were observed in the three immunosuppression withdrawal groups. However after transplant failure, notable reductions in nonsensitized subjects (increased sensitization rates) were

seen in the ≤ 1 month and 1-3 months immunosuppression withdrawal groups, while only a mild reduction was seen in the > 3 month immunosuppression withdrawal group. Consequently, a statistically significant separation in nonsensitization rates after transplant failure developed between these groups, p =0.041 (Figure 3-2). Of subjects who converted from nonsensitized prior to primary graft placement to sensitized, 20% had testing for HLA antibodies at the time of graft failure and none had detectable antibodies at that time.

After adjusting for covariates including nonsensitization prior to kidney transplantation, the multivariate logistic regression model showed that the > 3 months immunosuppression withdrawal group continued to have better preservation of nonsensitization status compared to the ≤ 1 month immunosuppression withdrawal group (adjusted odds ratio [aOR]=5.09 [95% C.I. 1.09-23.72]). No benefit was seen in the 1-3 month immunosuppression withdrawal group compared to the ≤ 1 month immunosuppression withdrawal group (aOR=0.58 [95% C.I. 0.07-4.99]). None of the other model covariates such as blood transfusion or graft nephrectomy were significantly associated with nonsensitization after transplant failure (Figure 3-3, Table 3-2).

PRA after Graft Failure

PRA values (mean±standard deviation) at the time of retransplant evaluation in the ≤ 1 month, 1-3 months, and > 3 months immunosuppression withdrawal groups were 39±10, 45±17, and 17±5 respectively (p =0.045), and the change in mean PRA (ΔPRA) from prior to primary transplant were +35, +21, and +10 PRA percentage points respectively (Figure 3-4). A multivariate linear regression model showed that the >3 month immunosuppression withdrawal group had a smaller rise in ΔPRA compared to

the ≤ 1 month group (p = 0.056). No significant difference in ΔPRA was seen between the ≤ 1 month and 1-3 months immunosuppression withdrawal groups (p = 0.36). Other model covariates—recipient gender, induction with a lymphocyte depleting agent, acute rejection, graft nephrectomy, and blood transfusion—were not significantly associated with ΔPRA (Tables 3-3).

Relisting and Retransplantation

Relisting rates after transplantation failure in the ≤ 1 month, 1-3 months, and > 3 months immunosuppression withdrawal groups were 75%, 25%, and 76% respectively. Retransplantation rates in the ≤ 1 month, 1-3 months, and > 3 months immunosuppression withdrawal groups were 25%, 0%, and 48% respectively. The 1-3 months immunosuppression withdrawal group had a significantly smaller rate of relisted subjects (p =0.031) and no subjects were retransplanted. After excluding the 1-3 month immunosuppression group, a trend toward more retransplantations was seen with the > 3 months immunosuppression withdrawal group compared to the ≤ 1 month immunosuppression withdrawal group (p =0.17). Similar rates of retransplantation with living donor kidneys were seen—33% versus 29%—between the ≤ 1 month and > 3 months immunosuppression withdrawal groups, respectively.

Safety Outcomes for Delayed Immunosuppression Withdrawal

Subjects Referred for Retransplantation (Main Study Group)

In subjects referred for retransplantation, the 3-year mortality rate in the main study group was 14% (7/49 subjects). The number of deaths for the ≤ 1 month, 1-3 months, and > 3 months immunosuppression withdrawal groups, were 1 (8.3%), 4 (50%), and 2 (6.9%) respectively (Table 3-4). Only one subject died from an infection related cause and another subject died from malignancy (chronic myelogenous

leukemia). Both subjects were from the 1-3 months immunosuppression withdrawal group. Cardiovascular events were the most common cause of death occurring in 3 out of 7 subjects. Due to the low number of death events, no meaningful statistical conclusion was derived from mortality and delayed immunosuppression withdrawal. However, a multivariate time dependent Cox model found no difference in infection-free patient survival (time to first infection) between all three immunosuppression withdrawal groups, $p = 0.61$ (Figure 3-5). Excluding nonmelanoma skin cancers, only one malignancy was diagnosed and was mentioned previously.

Subjects Not Referred for Retransplantation (Safety Analysis Only Group)

The 53 subjects who were not referred for retransplantation (safety analysis only) were an average of nine years older than the main study group (Table 3-5). The 3-year mortality rate was 51% (27/53 subjects) with a majority of deaths occurring within a year after graft failure. Half of these deaths (13/27 subjects) were infection-related. In this group, both patient mortality and infection related mortality were significantly higher compared to the main study group (Figure 3-6). In subjects not referred for retransplantation, there were 16, 16, and 21 subjects in the ≤ 1 month, 1-3 months, and > 3 months immunosuppression withdrawal groups respectively. Multivariate time dependent Cox models showed that the 1-3 months and > 3 months immunosuppression withdrawal groups were not associated with increased death ($p = 0.60$ and $p = 0.84$ respectively) or infection related death ($p = 0.57$ and $p = 0.31$ respectively) compared to the < 1 month immunosuppression withdrawal group (Tables 3-6 and 3-7). Also, delayed immunosuppression withdrawal was not associated with shorter time to first infection. Excluding subjects with nonmelanoma skin cancers, three subjects were diagnosed with malignancies (renal cell carcinoma, melanoma, and

pancreatic cancer) and they were alive at the study's conclusion. None of these subjects with malignancy were in the > 3 months immunosuppression withdrawal group.

All Subjects (Main Study Group and Safety Analysis Only Group)

Similarly, a combined safety analysis was performed for all 102 subjects who were evaluated for retransplantation (main study group) and not evaluated for retransplantation (safety analysis only group). For the combined safety analysis, multivariate time dependent Cox models showed that the 1-3 months and >3 months delayed immunosuppression withdrawal groups were not associated with increased death (p = 0.27 and p = 0.58 respectively) or infection related death (p = 0.53 and p = 0.26 respectively) when compared to the ≤1 month immunosuppression withdrawal group (Tables 3-8 and 3-9). Not surprisingly, subjects not referred for retransplantation were at greater risk for death (aOR=2.9, 95% C.I. [1.16-7.48]) and infection related death (aOR=10.3, 95% C.I. [1.23-85.7]). Comparable to earlier results, delayed immunosuppression withdrawal was not associated with a shorter time to first infection in the combined safety analysis.

Table 3-1. Baseline characteristics for subjects referred for retransplantation evaluation

Baseline characteristics	All patients *n* = 49	IS withdrawal ≤ 1 month *n* = 12	IS withdrawal 1~3 months *n* = 8	IS withdrawal > 3 months *n* = 29	*p* value
Age at graft failure, y.o., mean ± SD	43 ± 14	43 ± 13	42 ± 10	43 ± 15	0.9931
Female gender, *n* (%)	20 (41)	8 (67)	3 (38)	9 (31)	0.1203
Black race, *n* (%)	15 (31)	3 (25)	3 (38)	9 (31)	0.9419
ESRD - diabetes, *n* (%)	6 (16)	3 (27)	1 (20)	2 (10)	0.3688
Deceased donor, *n* (%)	41 (84)	9 (75)	8 (100)	24 (83)	0.4118
Nonsensitized prior to 1^{o} graft, *n* (%)	35 (71)	7 (58)	5 (63)	23 (79)	0.3305
≥ 3 HLA mismatches, *n* (%)	33 (77)	9 (82)	6 (75)	18 (75)	>0.999
Induction medicine, lymphocyte depleting agent, *n* (%)	12 (24)	5 (42)	1 (13)	6 (21)	0.3342
Acute rejection, *n* (%)	14 (29)	3 (25)	2 (25)	9 (31)	>0.999
1^{o} Graft survival, months, median [25%, 75% quartile]	19 [5, 49]	19 [7, 51]	26 [9, 46]	14 [3, 49]	0.3546
Blood transfusion recipient, *n* (%)	17 (35)	4 (33)	5 (63)	8 (28)	0.1789
Nephrectomy after graft failure, *n* (%)	18 (49)	4 (36)	3 (60)	11 (52)	0.6465

IS=immunosuppression. HLA=human leukocyte antigen

Table 3-2. Adjusted odds ratios for nonsensitization after transplant failure

Effect	Adjusted odds ratio	95% confidence limit		*p* value
		Lower limit	Upper limit	
IS withdrawal, 1-3 months v ≤ 1 month	0.58	0.067	4.989	0.618
IS withdrawal, >3 months v ≤ 1 month	5.09	1.090	23.719	0.038*
Induction, LDA v. other	0.19	0.028	1.336	0.095
Female recipient	0.29	0.064	1.307	0.107
PRA prior to 1^{o} graft, PRA=0 v PRA>0	1.37	0.331	5.666	0.665
Acute rejection	0.30	0.056	1.611	0.160
Allograft nephrectomy	0.57	0.124	2.631	0.473
Blood transfusion	2.83	0.499	16.058	0.240

IS=immunosuppression. LDA=lymphocyte depleting agent. PRA=panel reactive antibody.
**p* value < 0.05

Table 3-3. Linear regression model for ΔPRA

Parameter	Δ PRA estimate	Standard error	*p* value
IS withdrawal 1~3 months v ≤1 month	n/a	n/a	0.358
IS withdrawal >3 months v ≤1 month	n/a	n/a	0.056
Induction, LDA v. other	17.4	12.3	0.165
Female recipient	6.2	11.0	0.574
Acute rejection	13.2	10.9	0.235
Allograft nephrectomy	2.4	10.2	0.817
Blood transfusion	15.1	11.0	0.177

ΔPRA=change in mean PRA from prior to 1^{o} graft to after graft failure
IS=immunosuppression.
LDA=lymphocyte depleting agent.
n/a=not applicable

Table 3-4. Three year mortality stratified by immunosuppression withdrawal duration in the main study group

IS withdrawal group	Number of deaths	Cause of death
≤1 month (*n* =1 2)	1	CVD
1~3 months (*n* = 8)	4	CVD (2 subjects) CML sepsis
>3 months (*n* = 29)	2	Abdominal bleed renal failure

IS=immunosuppression. CVD= cardiovascular disease. CML=chronic myelogenous leukemia

Table 3-5. Baseline characteristics for all patients with kidney transplant failure

Characteristics	All patients (*n* = 102)	Referred for RTX (*n* = 49)	Not referred for RTX (*n* = 53)	*p* value
Age at graft failure, mean Yrs ± SD	47 ± 14	43 ± 14	52 ± 14	0.0012
Female gender, *n* (%)	39 (39)	20 (41)	19 (38)	0.7743
Black race, *n* (%)	35 (35)	15 (31)	20 (40)	0.5449
Diabetes as 1^{o} cause of ESRD, *n* (%)	8 (10)	6 (16)	2 (5)	0.1376
Nonsensitized prior to 1^{o} graft, *n* (%)	73 (72)	35 (71)	38 (72)	0.9759
Deceased donor, *n* (%)	83 (84)	41 (84)	42 (84)	0.9648
Induction therapy lymphocyte depleting agent, *n* (%)	26 (26)	12 (24)	14 (27)	0.7358
Acute rejection, *n* (%)	34 (33)	14 (29)	20 (38)	0.3266
CMV infection, *n* (%)	10 (10)	6 (12)	4 (8)	0.4254
BKV infection, *n* (%)	4 (4)	1 (2)	3 (6)	0.6187
1^{o} Graft nephrectomy, *n* (%)	39 (49)	18 (49)	21 (50)	0.9046

RTX=retransplantation

Table 3-6. Time dependent Cox model for 3-year mortality in subjects not referred for retransplantation

Parameter	Adjusted odds ratio	95% confidence interval		*p* value
		Lower limit	Upper limit	
IS withdrawal, 1-3 months v ≤ 1 month	1.342	0.448	4.016	0.5991
IS withdrawal, >3 months v ≤ 1 month	1.138	0.319	4.060	0.8419
Diabetes as 1^{o} cause of ESRD	0.504	0.059	4.337	0.5325
Allograft nephrectomy	0.604	0.224	1.628	0.3188
Induction, LDA v. other	2.268	0.696	7.391	0.1742
Black race recipient	4.934	0.410	59.323	0.2084
Acute rejection	1.463	0.526	4.073	0.4661
Female recipient	1.030	0.368	2.884	0.9546
Deceased donor	0.501	0.123	2.036	0.3338
Nonsensitized prior to 1^{o} graft	1.997	0.694	5.744	0.1996

IS=immunosuppression. ESRD=end stage renal disease. LDA=lymphocyte depleting agent

Table 3-7. Time dependent Cox model for 3-year infection related mortality in subjects not referred for retransplantation

Parameter	Adjusted odds ratio	95% confidence interval		*p* value
		Lower limit	Upper limit	
IS withdrawal, 1-3 months v ≤ 1 month	1.554	0.335	7.203	0.5732
IS withdrawal, >3 months v ≤ 1 month	0.348	0.046	2.644	0.3075
Diabetes as 1^{o} cause of ESRD	0.203	0.015	2.803	0.2342
Allograft nephrectomy	1.731	0.427	7.012	0.4422
Induction, LDA v. other	2.776	0.470	16.391	0.2598
Black race recipient	n/a	n/a	n/a	0.9983
Acute rejection	4.010	0.909	17.702	0.0667
Female recipient	0.717	0.139	3.705	0.6918
Deceased donor	0.697	0.128	3.800	0.6763
Nonsensitized prior to 1^{o} graft	n/a	n/a	n/a	0.9951

IS=immunosuppression. ESRD=end stage renal disease. LDA=lymphocyte depleting agent. n/a=not applicable

Table 3-8. Time dependent Cox model for 3-year mortality in all subjects

Parameter	Adjusted odds ratio	95% confidence interval		*p* value
		Lower limit	Upper limit	
IS withdrawal, 1-3 months v ≤ 1 month	1.768	0.643	4.859	0.2695
IS withdrawal, >3 months v ≤ 1 month	1.361	0.458	4.042	0.5793
Not referred for retransplantation	2.944	1.159	7.476	0.0231*
Diabetes as 1^{o} cause of ESRD	1.300	0.355	4.766	0.6920
Allograft nephrectomy	0.524	0.226	1.217	0.1327
Induction, LDA v. other	1.902	0.749	4.832	0.1763
Black race recipient	2.302	0.955	5.549	0.0634
Acute rejection	1.406	0.640	3.087	0.3964
Female recipient	1.419	0.620	3.247	0.4073
Deceased donor	0.555	0.145	2.134	0.3918
Nonsensitized prior to 1^{o} graft	1.695	0.685	4.192	0.2535

IS=immunosuppression. ESRD=end stage renal disease. LDA=lymphocyte depleting agent

*p value < 0.05

Table 3-9. Time dependent Cox model for 3-year infection related mortality in all subjects

Parameter	Adjusted odds ratio	95% confidence interval		*p* value
		Lower limit	Upper limit	
IS withdrawal, 1-3 months v ≤ 1 month	1.560	0.395	6.167	0.5260
IS withdrawal, >3 months v ≤ 1 month	0.343	0.053	2.243	0.2644
Not referred for retransplantation	10.272	1.231	85.714	0.0314*
Diabetes as 1^{o} cause of ESRD	0.648	0.101	4.156	0.6469
Allograft nephrectomy	1.489	0.444	4.991	0.5193
Induction, LDA v. other	1.796	0.397	8.127	0.4470
Black race recipient	1.449	0.397	5.287	0.5747
Acute rejection	2.754	0.754	10.054	0.1253
Female recipient	1.011	0.262	3.898	0.9876
Deceased donor	0.778	0.150	4.046	0.7651
Nonsensitized prior to 1^{o} graft	n/a	n/a	n/a	0.9923

IS=immunosuppression. ESRD=end stage renal disease. LDA=lymphocyte depleting agent. n/a=not applicable
*p value < 0.05

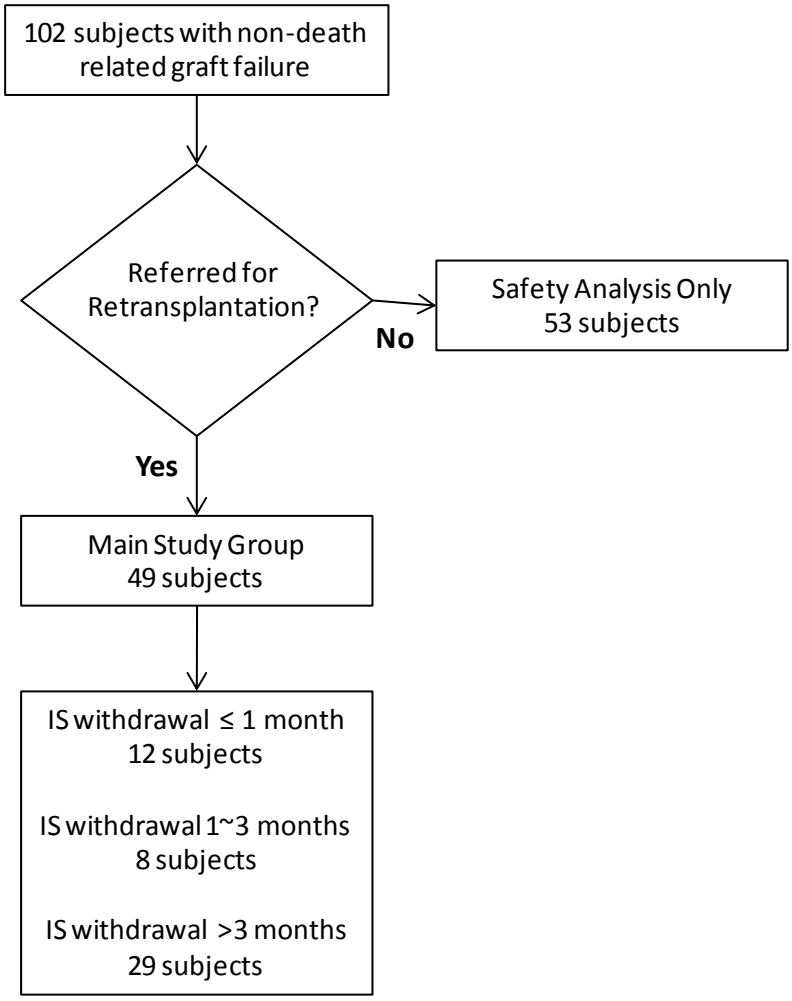

Figure 3-1. Subject categorization. IS=immunosuppression

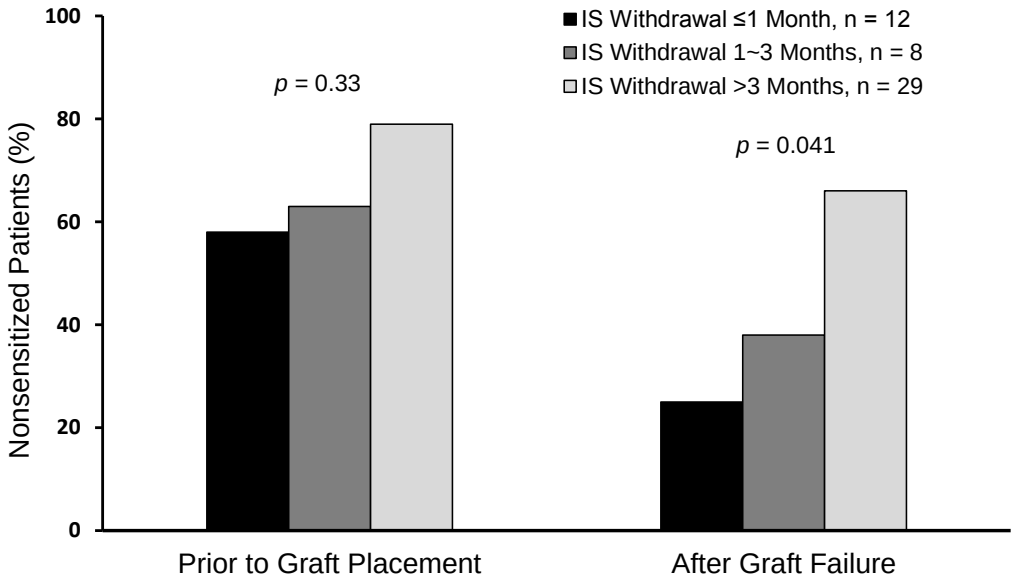

Figure 3-2. Nonsensitization stratified by immunosuppression withdrawal duration. IS=immunosuppression

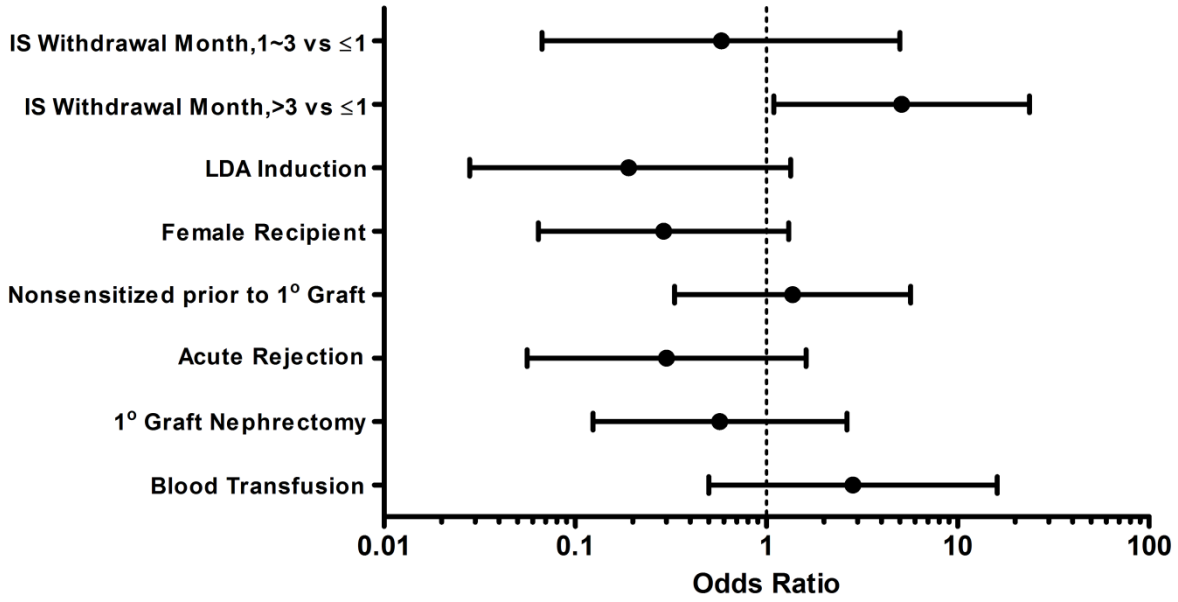

Figure 3-3. Multivariate analysis of nonsensitization after transplant failure. IS=immunosuppression. LDA=lymphocyte depleting agent

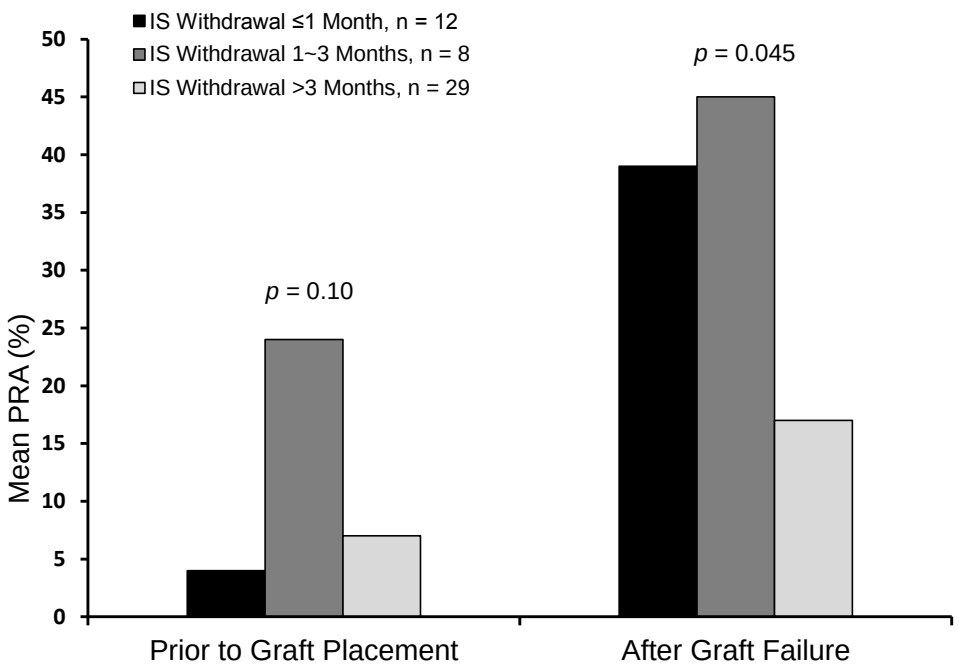

Figure 3-4. Mean PRA stratified by immunosuppression withdrawal duration. IS=immunosuppression

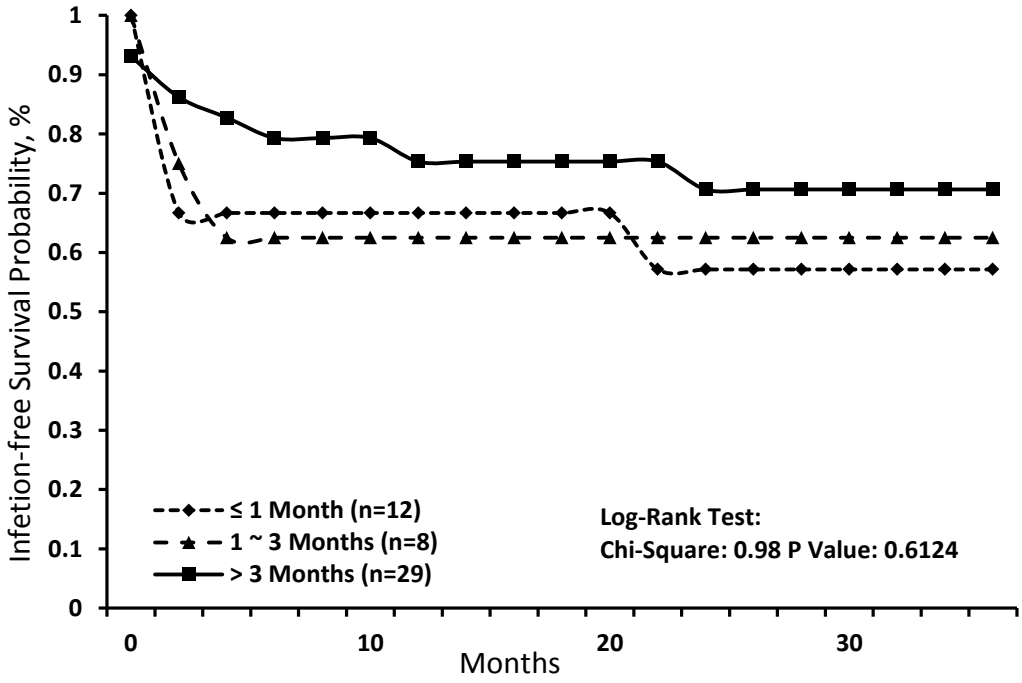

Figure 3-5. Three year infection-free survival stratified by immunosuppression withdrawal duration (death censored)

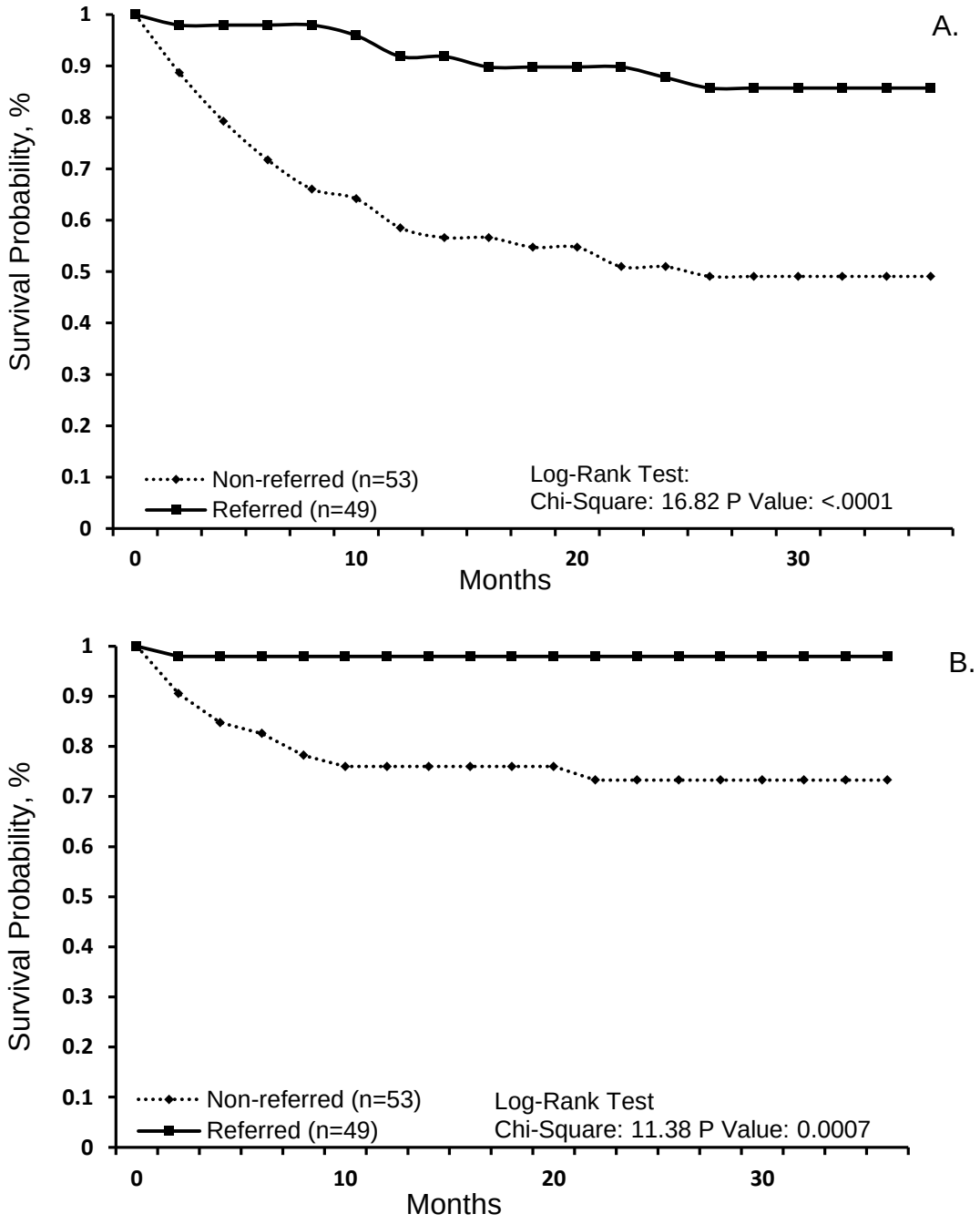

Figure 3-6. Three year survival in patients referred versus non-referred for kidney retransplantation. A) Overall patient survival, B) Survival from infection related death

CHAPTER 4
DISCUSSION AND CONCLUSION

Efficacy of Delayed Immunosuppression Withdrawal

Our study addresses the question of whether a strategy of delayed immunosuppression withdrawal after transplant failure may help preserve a patient's nonsensitization status. We chose to study nonsensitization (PRA = 0%) as the primary outcome because it is an unambiguous endpoint where there is a clear distinction between subjects with and without HLA alloantibodies. Since less sensitized patients are more likely to be transplanted sooner,[10,19] preserving a patient's nonsensitization status is a crucial component of care for those who hope to be retransplanted in the future. In addition, nonsensitized patients have the clinical advantages of decreased risk for acute rejection, better graft function, and increased patient and graft survival compared to sensitized patients.[20-21] In stepwise fashion, our study found that longer durations of immunosuppression withdrawal were associated with greater nonsensitization preservation after graft failure. After adjusting for other potential risk factors in the multivariate model, this phenomenon persisted in the > 3 months immunosuppression withdrawal group compared to the rapid ≤ 1 month immunosuppression withdrawal group.

Our results are consistent with recent single center studies that have suggested that immunosuppression withdrawal after transplant failure may be a risk factor for the emergence of HLA antibodies and sensitization.[14,22] In a previous small study from our transplant center[14], 23% of patients who did not undergo transplant nephrectomy developed antibodies, and 39% of patients who were not transfused became sensitized. However, 11 patients continued on immunosuppression and remained nonsensitized

despite 7 who underwent transplant nephrectomy or were transfused.[14] In a single center study by Augustine and colleagues,[22] 24 subjects who continued their immunosuppression after kidney graft failure (mainly due to a functioning pancreas transplant) were compared with subjects who had their immunosuppression weaned after graft failure. They observed that weaning of immunosuppression was associated with sensitization independent of transplant nephrectomy, but their study was limited by the absence of blood transfusion data—a significant risk factor for sensitization—and the absence of safety data on infection related outcomes.[22]

Similar to first-time kidney transplant recipients, retransplantation confers a significant survival benefit to patients who returned to dialysis after primary graft failure.[3,8] In our study, we identified a trend towards more retransplantations in the > 3 months immunosuppression withdrawal group (49%) compared to the early ≤ 1 month group warranting further investigation with larger samples. However, it is important to note that retransplantation rates observed in our single center study may not be generalizable due to the varied waiting times in regional retransplantation rates. In our 1-3 months immunosuppression withdrawal group, no retransplantations were observed probably because this group was less healthy than the other groups, as evidenced by a significantly lower percentage of relisted subjects.

Safety of Delayed Immunosuppression Withdrawal

After transplant failure, infection is the second leading cause of death[23] and the risks of sepsis and infection related death are significantly higher compared to subjects with a functioning transplant.[5,7] Prior authors have suggested that significant risk was associated with continuing immunosuppression after transplant failure and that immunosuppression should be tapered off as quickly as feasible.[15,24] Other authors

have advocated a more gradual withdrawal to immunosuppression after noting few infection related complications.[17] In other words, there is no consensus on the optimal duration of immunosuppression withdrawal after graft failure.[16,25]

A unique feature of this study is that we independently studied subjects with graft failure who were subsequently evaluated for retransplantation (main study group). This is an important distinction from prior studies because we selected a cohort that was probably healthier and better suited to handle the additional risks of prolonged immunosuppression compared to the general dialysis population with transplant failure. It is easy to understand why many clinicians are wary of delaying immunosuppression withdrawal when rates of death and infection-related complications are high after transplant failure.[5,7,15] However, our study showed lower rates of death and infection related death in the main study group when compared to subjects not referred for retransplantation. Therefore not all patients with graft failure may be equally susceptible to the risks of delayed immunosuppression withdrawal and further exploration of this subgroup is needed.

The other aim of this study was to examine the risk associated with delayed immunosuppression withdrawal that could impact patient survival. In our study, no obvious safety signals were observed in the main study group to suggest that delayed immunosuppression withdrawal was overtly hazardous, but we were unable to draw any conclusions about patient death and infection-related death due to few event numbers. We were, however, able to study time to first infection (infection-free survival) and found no difference between the three immunosuppression withdrawal groups in patients referred for retransplantation.

The safety analysis for the subjects not referred for retransplantation showed higher rates of patient death and infection related death compared to the main study group. These findings were not surprising since these subjects were older and probably less healthy. Interestingly, our multivariate models did not show that delayed immunosuppression withdrawal was associated with patient death or infection related death in subjects not referred for transplantation (safety analysis only group) and in the total study population (main study group + safety analysis only group) . Our results differ from the study results of Smak Gregoor and colleagues who saw increased mortality and infections in “time periods” of low dose immunosuppression use after graft failure.[15] However, our study was adjusted for time-bias using a time dependent Cox model. Yet, our study’s limited sample size may be prone to the pitfall of insufficient power. Given the known risks of increased mortality and infection after transplant failure[5,7], it would be premature to declare that delayed immunosuppression withdrawal is uniformly safe, so further investigation with larger sample size is warranted. At the time of our study’s inception, a prospective study of this nature would not have been ethical because we knew that prolonged immunosuppression can be harmful and there was scant evidence for benefit. However, with these and other data, a prospective trial may be more realistic in the future.

Generalizability

In our single center study, generalizability is an important issue and our mortality rates appeared to be in line with other publications. In subjects not referred for retransplantation (safety analysis only), the 51% three-year mortality rate was almost identical to the mortality rate in incident United States dialysis patients.[26] Also, mortality rates in the main study group, safety analysis only group, and the combined groups

(102 subjects) were comparable to those seen in a prior large United States registry study of patients with failed kidney transplants.[8]

Study Strengths

There are several strengths to our study which also address some of the shortcomings from prior studies. First, our study has the largest cohort of patients on prolonged immunosuppression after transplant failure available for PRA analysis (29 subjects) and safety analysis (50 subjects). Second, it is a clinical based observational study where outcome, exposure, and covariates were ascertained using medical records. Third, unlike prior studies, we address the issue of optimal timing for immunosuppression withdrawal by comparing efficacy and safety outcomes across different durations of immunosuppression withdrawal after graft failure. Fourth, important potential confounders in the PRA analysis such as acute rejection,[27] induction medication[28], HLA matching[10,29], graft nephrectomy[11,30], and blood transfusions[31-35] are analyzed and controlled for in the multivariate models. Finally, we provide a more detailed safety analysis of delayed immunosuppression withdrawal including infection related outcomes when compared to prior studies. We believe these strengths allow for the most wide-ranging analysis of the risks and benefits of delayed immunosuppression withdrawal to date.

Study Limitations

Our study is subjected to the inherent limitations of a retrospective cohort study design where associations can be made but causality cannot be assigned. However as mentioned previously, a prospective study would have not been ethical due to the lack of prior safety data for delayed immunosuppression withdrawal. Limited sample size may cause insufficient power and an inconclusive death analysis in this study, however

significant differences in nonsensitization rates among different immunosuppression withdrawal groups were identified out of the small study cohort, which drives the results more conservative. Residual confounders may exist from unmeasurable events such as blood transfusions and infections that may have occurred in the subject's local community instead of the hospital lab and may not have been captured by the study. Finally, we were not able to investigate the effects of different immunosuppression regimens or the order of drug withdrawal.

Conclusion

In this study, the strategy of delayed immunosuppression withdrawal appears to preserve nonsensitization status in patients with renal graft failure referred for retransplantation. In addition, we observed other potential benefits with this strategy such as lower mean PRA scores and a trend toward more retransplantations. Patient death, infection related death, and other safety signals were not associated delayed immunosuppression withdrawal in subjects referred for retransplantation (main study group) as well as subjects not referred for retransplantation (safety analysis only group) and the combined study population. However, further investigation with larger sample size is required for further verification. In summary, delayed immunosuppression withdrawal appears safe as a strategy to minimize sensitization in low risk retransplantation candidates such as those who anticipate a short stay on the waitlist or those with live donors.

LIST OF REFERENCES

1. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, Held PJ, Port FK. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N Engl J Med. 1999;341(23):1725-30.

2. Ojo AO, Hanson JA, Meier-Kriesche H, Okechukwu CN, Wolfe RA, Leichtman AB, Agodoa LY, Kaplan B, Port FK. Survival in recipients of marginal cadaveric donor kidneys compared with other recipients and wait-listed transplant candidates. J Am Soc Nephrol. 2001;12:589-97.

3. Meier-Kriesche HU, Schold JD, Srinivas TR, Reed A, Kaplan B. Kidney transplantation halts cardiovascular disease progression in patients with end-stage renal disease. Am J Transplant 2004;4:1662-1668.

4. Danovitch, GM ed. Handbook of Kidney Transplantation. 4th ed. Philadelphia, PA, USA: Lippincott Williams & Wilkins;2005, pp 19-20.

5. Kaplan B, Meier-Kriesche HU. Death after graft loss: an important late study endpoint in kidney transplantation. Am J Transplant. 2002; 2: 970-974

6. Knoll G, Muirhead N, Trpeski L, Zhu N, Badovinac K. Patient survival following renal transplant failure in Canada. Am J Transplant. 2005; 5(7): 1719-1724.

7. Johnston O, Zalunardo N, Rose C, Gill JS. Prevention of Sepsis during the Transition to Dialysis May Improve the Survival of Transplant Failure Patients. J Am Soc Nephrol 18: 1331–1337, 2007.

8. Ojo A, Wolfe RA, Agodoa LY, Held PJ, Port FK, Leavey SF, Callard SE, Dickinson DM, Schmouder RL, Leichtman AB. Prognosis after primary renal transplant failure and the beneficial effects of repeat transplantation: multivariate analyses from the United States Renal Data System. Transplantation 1998;66(12):1651-9.

9. U.S. Renal Data System, USRDS 2011 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2011. Table E.3.

10. Meier-Kriesche HU, Scornik JC, Susskind B, Rehman S, Schold JD. A lifetime versus a graft life approach redefines the importance of HLA matching in kidney transplant patients. Transplantation. 2009;88(1):23-9.

11. Adeyi OA, Girnita AL, Howe J, Marrari M, Awadalla Y, Askar M, Martell J, Zeevi A, Shapiro R, Nalesnik M, Randhawa P, Demetris AJ, Duquesnoy RJ. Serum analysis after transplant nephrectomy reveals restricted antibody specificity patterns against structurally defined HLA class I mismatches. Transpl Immunol. 2005;14(1):53-62.

12. Sumrani N, Delaney V, Hong JH, Daskalakis P, Sommer BG. The influence of nephrectomy of the primary allograft on retransplant graft outcome in the cyclosporine era. Transplantation 1992;53(1):52-5.

13. Khakhar AK, Shahinian VB, House AA, Muirhead N, Hollomby DJ, Leckie SH, McAlister VC, Chin JL, Jevnikar AM, Luke PP. The impact of allograft nephrectomy on percent panel reactive antibody and clinical outcome. Transplant Proc. 2003;35(2):862-3.

14. Scornik, JC; Meier-Kriesche, H-U. Human leukocyte antigen sensitization after transplant loss: timing of antibody detection and implications for prevention. Human Immunology 2011;72:398–401.

15. Smak Gregoor PJH, Zietse R, van Saase JLCM, op de Hoek CT, IJzermans JNM, Lavrijssen ATJ, de Jong GMTh, Kramer P, Weimar W. Immunosuppression should be stopped in patients with renal allograft failure. Clin Transplant 2001: 15: 397–401.

16. Kendrick EA, Davis CL. Managing the failing allograft. Semin Dial. 2005 Nov-Dec;18(6):529-39.

17. Morales A, Gavela E, Kanter J, Beltrán S , Sancho A, Escudero V, Crespo J, Pallardó LM. Treatment of Renal Transplant Failure. Transplantation Proceedings 2008; 40, 2909–2911.

18. Jassal SV, Lok CE, Walele A, Bargman JM. Continued transplant immunosuppression may prolong survival after return to peritoneal dialysis: results of a decision analysis. Am J Kidney Dis. 2002;40(1):178-83.

19. Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR). OPTN / SRTR 2010 Annual Data Report. Rockville, MD: Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation; 2011: Table 5.2.

20. Dunn TB, Noreen H, Gillingham K, Maurer D, Ozturk OG, Pruett TL, Bray RA, Gebel HM, Matas AJ. Revisiting traditional risk factors for rejection and graft loss after kidney transplantation. Am J Transplant. 2011;11(10):2132-43.

21. Barama A, Oza U, Panek R, Belitsky P, MacDonald AS, Lawen J, McAlister V, Kiberd B. Effect of recipient sensitization (peak PRA) on graft outcome in haploidentical living related kidney transplants. Clin Transplant. 2000;14(3):212-7.

22. Augustine JJ, Woodside KJ, Padiyar A, Sanchez EQ, Hricik DE, Schulak JA. Independent of nephrectomy, weaning of immunosuppression leads to late sensitization after kidney transplant failure. Transplantation 2012 Sep 5. [Epub ahead of print]

23. Gill JS, Abichandani R, Kausz AT, Pereira BJ. Mortality after kidney transplant failure: the impact of non-immunologic factors. Kidney Int. 2002 Nov;62(5):1875-83.

24. Vanrenterghem Y, Khamis S. The management of the failed renal allograft. Nephrol Dial Transplant. 1996;11(6):955-7.

25. Bennett WM. The failed renal transplant: in or out? Semin Dial. 2005 May-Jun;18(3):188-9.

26. U.S. Renal Data System, USRDS 2011 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2011. Volume 2, Table 5a.

27. Katznelson S, Bhaduri S, Cecka JM. Clinical aspects of sensitization. Clin Transpl. 1997:285-96.

28. Dawson KL, Patel SJ, Xu J, Knight RJ, Gaber AO. Effect of immunosuppression for first kidney or kidney/pancreas transplant on sensitization at the time of second transplant. Transplantation. 2011 Apr 15;91(7):751-6.

29. Sanfilippo F, Goeken N, Niblack G, Scornik J, Vaughn WK. The effect of first cadaver renal transplant HLA-A, B match on sensitization levels and re-transplant rates following graft failure. Transplantation 1987;43:240–4.

30. Billen EVA, Christians MHL, Lee J, van den Berg-Loonen EM. Donor-directed HLA antibodies before and after transplantectomy detected by the Luminex single antigen assay. Transplantation 2009;87:563–9.

31. Fuller TC, Delmonico FL, Cosimi B, Huggins CE, King M, Russell PS. Impact of blood transfusion on renal transplantation. Ann Surg. 1978 Feb;187(2):211-8.

32. Scornik JC, Ireland J, Howard RJ, Pfaff WW, Fennell RS 3rd. Sensitization by blood transfusions in previously transplanted patients. Transplantation 1983;35:505–6.

33. Hyun J, Park KD, Yoo Y, Lee B, Han BY, Song EY, Park MH. Effects of different sensitization events on HLA alloimmunization in solid organ transplantation patients. Transplant Proc. 2012 Jan;44(1):222-5.

34. Scornik JC, Schold JD, Bucci M, Meier-Kriesche HU. Effects of blood transfusions given after renal transplantation. Transplantation 2009;87:1381–6.

35. Scornik JC, Brunson ME, Howard RJ, Pfaff WW. Alloimmunization, memory, and the interpretation of crossmatch results for renal transplantation. Transplantation 1992;54:389–94.

BIOGRAPHICAL SKETCH

Michael Casey is an assistant professor of medicine in the University of Florida's College of Medicine, Department of Medicine. After completing medical school at the University of North Carolina in 2001, he trained in internal medicine at California Pacific Medical Center in San Francisco, California. Subsequently, he completed a nephrology fellowship at Stanford University and a transplant nephrology fellowship at California Pacific Medical Center. In 2008, he joined the University of Florida as a transplant nephrologist in the Division of Nephrology, Hypertension, and Renal Transplantation. Dr. Casey has a strong interest in clinical transplant research and has presented his work at several national conferences as well as served as a co-investigator on many clinical trials. In 2012, he completed a Master of Science in Clinical and Translational Research while a scholar in the University of Florida CTSI's Advanced Postgraduate Program in Clinical Investigation.