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ORIGINAL ARTICLE

Prolonged immunosuppression does not improve risk of sensitization or likelihood of retransplantation after kidney transplant graft failure

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SUMMARY

The optimum approach towards immunosuppression withdrawal following kidney transplant failure is unclear. Prolonged weaning may be associated with reduced sensitization, less graft nephrectomy and greater likelihood of retransplantation, but conversely increased risk of infection, malignancy and death. We conducted a single-centre retrospective analysis of patients experiencing graft failure between 2007 and 2017, comparing rates of sensitization, retransplantation, nephrectomy, infection, malignancy and death between patients who had immunosuppression weaned over <90 vs. 90–180 vs. >180 days. Patient survival after immunosuppression withdrawal over <90 vs. 90–180 vs. >180 days was 73.3%, 72.1% and 80.4%, respectively ($P = 0.35$), with no differences in cPRA (80.06 vs. 81.21 vs. 85.42, $P = 0.66$) or retransplantation rate [24/31 (77.4%) vs. 21/35 (60.0%) vs. 22/36 (61.1%), $P = 0.13$]. There was significantly less nephrectomy after late immunosuppression cessation [10/42 (23.8%) vs. 7/42 (16.7%) vs. 3/43 (7.0%), $P = 0.01$] but no differences in infections or malignancy. On competing risk regression (death as competing risk) controlling for cofactors including age, nephrectomy and rejection, prolonged immunosuppression did not predict likelihood of retransplantation (SHR 1.000, $P = 0.88$). Prolonged immunosuppression withdrawal does not reduce sensitization or improve retransplantation rates but is associated with less nephrectomy. Immunosuppression withdrawal should be tailored to individual circumstances after graft failure.

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Key words
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Introduction

Kidney transplantation is the optimal treatment for appropriately selected patients with end-stage kidney disease (ESKD) and is associated with better survival and quality of life compared with remaining on dialysis [1]. Developments in immunosuppressive medications have resulted in significant reductions in rates of acute rejection [2]; however, long-term outcomes have not improved commensurately. A substantial proportion of

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transplant recipients will eventually experience graft failure and therefore need to return to dialysis [3,4]. Management of immunosuppression in these patients remains challenging with a paucity of evidence available to guide clinicians.

Patients who re-commence dialysis after experiencing graft failure are at significantly greater risk of death compared with wait-listed dialysis patients who have yet to be transplanted [5]. Retransplantation can significantly abrogate this increase in mortality [6], but the opportunity to be retransplanted is often limited by the development of anti-human leukocyte antigen (HLA) antibodies as a result of exposure to allo-antigen from the previous transplant. Some studies have reported that continuation of immunosuppression after allograft failure is associated with reduced rates of allo-immunization and requirement for graft nephrectomy [7,8]. Conversely, prolonged exposure to immunosuppression may be associated with adverse events such as increased risks of infection [9], malignancy [10] and death [11]. Despite limited evidence, it has become common practice for immunosuppression to be withdrawn after graft failure although there is a universal lack of consensus on the optimal way to achieve this [12]. Some centres have advocated rapid withdrawal of immunosuppression [9], while others have suggested more gradual tapering regimens [13]. Ideally, withdrawal protocols should be designed to optimally balance the competing risks of sensitization against those of infection and malignancy.

Few studies have examined the relative benefits and risks associated with prolonged immunosuppression after graft failure, with regard to sensitization and likelihood of retransplantation. Overall, studies that have been published suggest an association between prolonged immunosuppression withdrawal and reduced risk of allo-immunization [7,8]. However, most of these have been limited by heterogeneous methodology, restrictive inclusion criteria, small sample sizes and reliance upon older antibody detection techniques. Further data are required to confirm these early findings.

We therefore conducted a single-centre retrospective cohort study of unselected kidney transplant recipients who experienced death-censored graft failure between January 2007 and December 2017, comparing outcomes between patients weaned from immunosuppression over different periods of time. We aimed to determine whether prolonged immunosuppression withdrawal after graft failure conferred a sensitization and retransplantation advantage, with sensitization being measured by modern solid-phase assay techniques used in organ allocation protocols. We also sought to evaluate the risks associated with such prolonged withdrawal.

Materials and Methods

Approval for this retrospective cohort study was obtained from the Royal Melbourne Hospital Human Research Ethics Committee (QA2019125). The study was conducted using data from the Nephrology departmental database, institution electronic medical record and institution admission coding record. Study participants consisted of kidney transplant recipients aged ≥18 years, who experienced death-censored graft failure between 1 January 2007 and 31 December 2017. The date of graft failure was defined as the date of commencement of haemodialysis or peritoneal dialysis. The duration of immunosuppression withdrawal was defined as the time between the date of graft failure to the date of the last prescription record of any noncorticosteroid immunosuppressive medication, death, or retransplantation (whichever came first), confirmed by review of the medical record. Subjects were analysed according to their duration of immunosuppression withdrawal: <90 days vs. 90–180 days vs. >180 days. Patients who transferred their care to other institutions, who had incomplete data or who had graft failure due to primary nonfunction were excluded from analysis.

Outcomes

The primary outcomes of this study were as follows: patient survival, sensitization postgraft failure [as defined by calculated panel reactive antibody (cPRA) results] and rate of retransplantation. Secondary outcomes included the following: cancer diagnosis, graft nephrectomy, hospital admission and infection episodes. Infection episodes were further categorized as episodes of sepsis, urinary tract infection, pneumonia, skin infection and dialysis catheter-related infection.

Baseline demographics, clinical outcomes and pathology results were determined using data from the departmental database, institution electronic medical record and institution admission coding record. Infection episodes were identified from the hospital admissions databases (ICD-10 AM codes: sepsis A40XX or A49XX, septic shock R572, severe sepsis R651; infection with bacterial, viral or other agents B950-B978 or candida B374 and B477; urinary tract infections N390; pneumonia J1XX, J8XX and J9XX; skin infections L03XX or infection in setting of type 1 or type 2 diabetes E106; or infection related to catheter used for dialysis T8XX

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assigned as principal or complicating discharge diagnoses). Patients who were diagnosed with malignancies and those who underwent graft nephrectomy were identified from medical records.

Antibody detection

Detection of anti-HLA antibodies and determination of their specificity after graft failure was conducted using One Lambda Luminex single-antigen bead assays (OLISAG), with results expressed as mean fluorescence intensity (MFI). Serum tested by OLISAG was pretreated using hypotonic dialysis. cPRA was determined for each subject based upon the cumulation of anti-HLA antibodies with MFI >4000, as donor-specific antibodies (DSA) above this are used for determination of unacceptable antigen for use in virtual crossmatch. Luminex assays were not used at our centre prior to 2007; therefore, pretransplant sensitization was determined by the peak panel reactive antibody (PRA) as determined by complement-dependent cytotoxicity (CDC) screening. Subjects who were screened for anti-HLA antibodies after graft failure were deemed to have been worked up for retransplantation. Screening was performed at the time of referral and then annually thereafter.

Statistical analyses

For baseline characteristics, categorical variables are reported as counts and proportions with differences assessed by the chi-square test. Continuous data are summarized as means with standard deviation (SD) or median and interquartile range (IQR) where appropriate and compared using the one-way ANOVA. Outcome measures between cohorts were compared on a time-to-event basis using the log-rank test. Associations between outcomes and continuous variables were analysed using Cox regression and the Pearson correlation coefficient. Competing risk regression using a Fine and Gray model was conducted to identify factors predictive of retransplantation likelihood with death as the competing risk. Statistical analyses were conducted using GRAPHPAD PRISM, STATA and SPSS software, with two-sided P-values <0.05 considered to be significant.

Results

Patient population and baseline characteristics

A total of 168 patients experienced nondeath-related kidney allograft failure at our centre between 1 January 2007 and 31 December 2017. Of these, 23 patients who transferred their care to other centres, 10 patients with incomplete follow-up data and 1 patient with primary nonfunction were excluded, leaving 134 patients for inclusion in this analysis (Fig. 1). The majority of subjects were male (n = 85, 63.4%) and were recipients of deceased donor kidney transplants (n = 74, 55.2%). The most common reason for graft failure was chronic rejection (n = 107, 79.9%), while the median duration of immunosuppression withdrawal was 130 days (IQR 76.75–218.8). The majority had experienced a single allograft failure (n = 109, 81.3%), with 23 (17.2%) having had failure of two grafts and 2 (1.5%) having had failure of three or more grafts. Immunosuppression after graft failure consisted of: tacrolimus (n = 74, 55.2%), cyclosporine (n = 22, 16.4%), mycophenolate mofetil (n = 102, 76.1%), mycophenolate sodium (n = 17, 12.7%), azathioprine (n = 10, 7.5%), everolimus (n = 4, 3.0%) and sirolimus (n = 5, 3.7%). Pretransplant PRA was available in 127 (94.8%) of patients. Within this cohort, 45 patients had immunosuppression withdrawn in less than 90 days, 43 patients had immunosuppression withdrawn at between 90 and 180 days, while the remaining 46 patients had immunosuppression weaned over more than 180 days. The baseline characteristics between the three groups were similar (Table 1).

Outcomes of immunosuppression withdrawal over <90 days vs. 90–180 days vs. >180 days

To determine whether prolonged immunosuppression withdrawal was associated with improvement in sensitization or retransplantation rate, we compared outcomes between subjects who were weaned over <90 days with those who were weaned over 90–180 days or >180 days. Over a median follow-up period of 1286, 1327 and 1523 days, respectively (P = 0.87), no difference in patient survival was observed between the cohorts with immunosuppression withdrawal over <90 days and those who had immunosuppression withdrawn over 90–180 days or over >180 days [33/45 (73.3%) vs. 31/43 (72.1%) vs. 37/46 (80.4%), P = 0.35; Table 2]. In the cohort with the most rapid immunosuppression withdrawal, 31/45 (68.9%) were referred for retransplantation at a median of 567 days vs. 35/43 (81.4%) at a median of 430 days in the intermediate group and 36/46 (78.3%) at a median of 649 days of those in the gradual withdrawal group (P = 0.73). Of those undergoing a Luminex screen, there was no significant difference in the mean cPRA between subjects weaned over

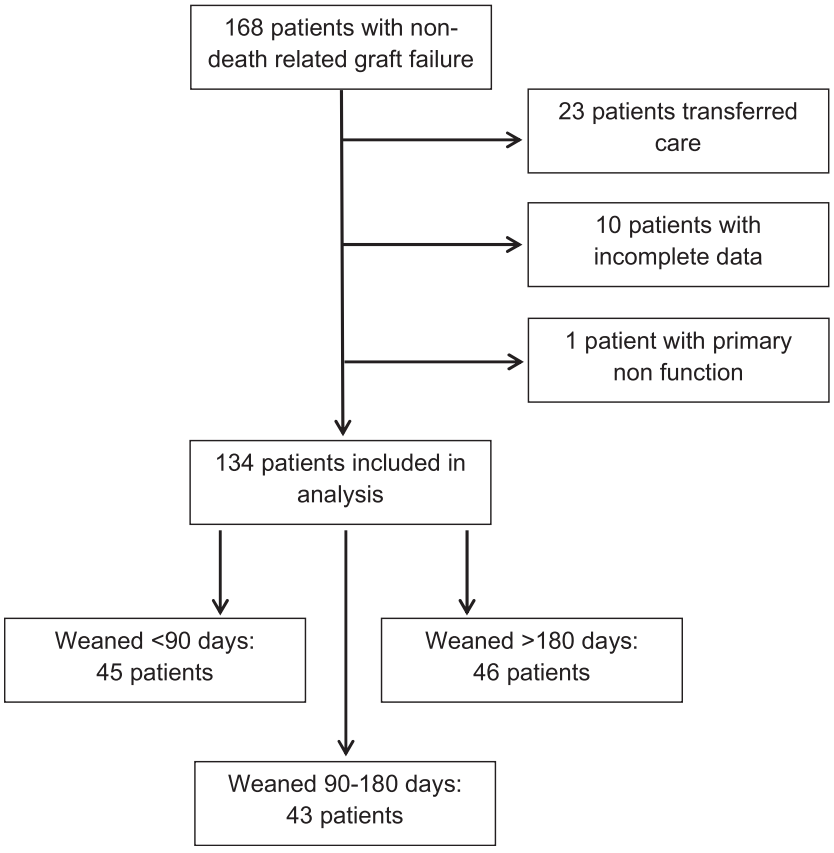

Figure 1 Immunosuppression after death-censored graft failure at our institution 2007–2017: patient population.

<90 days versus those weaned over 90–180 days or those weaned over >180 days [80.06 vs. 81.21 vs. 85.42, (P = 0.66; Fig. 2)]. The likelihood of retransplantation between patients referred for assessment in the three groups was also similar with 24/31 (77.4%) of those weaned over <90 days, 21/35 (60.0%) of those weaned over 90–180 days and 22/36 (61.1%) of those weaned over >180 days being retransplanted (P = 0.13). Among patients who were retransplanted, the median time to retransplantation was 687 days (IQR 289–1079) with higher cPRA being significantly associated with longer time to retransplantation (Pearson correlation coefficient R = 0.41, P = 0.001). Additional analysis showed no association in immunosuppression wean duration as a continuous variable and death, assessment for retransplantation, sensitization or retransplantation (Table S1). Five patients experienced graft failure within the first year post-transplant (3 in the <90 day cohort, 1 in the 90–180 day cohort and 1 in the >180 day cohort). Exclusion of these patients from analysis had no significant effect on outcomes (Table S2).

Weaning of immunosuppression over longer periods of time was associated with significantly less nephrectomy than weaning over shorter periods. In those weaned over <90 days, 13/45 (28.9%) underwent nephrectomy compared with 8/43 (18.6%) in the 90–180 day cohort and 6/46 (13.0%) of those weaned over >180 days (P = 0.04; Table 3). Seven of these patients underwent nephrectomy prior to the completion of their immunosuppression wean. After excluding these patients from analysis, postweaning nephrectomy occurred in 10/42 (23.8%) patients weaned over <90 days compared with 7/42 (16.7%) of those weaned over 90–180 days and 3/43 (7.0%) of those weaned over >180 days (P = 0.01). There were no differences in the incidence of new cancer diagnoses in patients who were weaned over <90 days compared with those weaned over 90–180 days or those weaned over >180 days [2/45 (4.4%) vs. 4/43 (9.3%) vs. 3/46 (6.5%), P = 0.35], nor were there any significant differences in the numbers of patients requiring hospital admission [24/45 (53.3%) vs. 23/43 (53.5%) vs. 25/46 (54.3%), P = 0.77] or those experiencing infection [24/45 (53.3%) vs. 24/43 (55.8%) vs. 26/46 (56.5%), P = 0.86], sepsis [14/45 (31.1%) vs. 11/43 (22.6%) vs. 11/46 (23.9%), P = 0.65], urinary tract infections [4/45

Table 1. Baseline characteristics of renal allograft recipients.

	<90 days (*n* = 45)	90–180 days (*n* = 43)	>180 days (*n* = 46)	*P*-value
Male (*n*, %)	27 (60.0%)	32 (74.4%)	26 (56.5%)	0.18
Age at transplantation (median, IQR)	37 (26.5, 47)	41 (34, 50)	37 (27, 50.3)	0.48
Age at graft failure (median, IQR)	46 (42, 57)	53 (43, 62)	47.5 (38.5, 59.3)	0.17
Year of transplantation				
1980–1999	13 (28.9%)	13 (30.2%)	13 (28.3%)	
2000–2009	21 (46.7%)	24 (55.8%)	23 (50.0%)	
2010–2016	11 (24.4%)	6 (14.0%)	10 (21.7%)	
Diabetes (*n*, %)	11 (24.4%)	11 (25.6%)	8 (17.4%)	0.60
Transplant type				
Deceased donor (*n*, %)	25 (55.6%)	24 (55.8%)	25 (54.3%)	0.99
Living donor (*n*, %)	20 (44.4%)	19 (44.2%)	21 (45.7%)	
Duration of functioning transplant (mean days ± SD)	3595 ± 2869	4093 ± 2670	3705 ± 2601	0.67
Graft number (*n*, %)				
1	36 (80.0%)	36 (83.7%)	37 (80.4%)	0.91
2	8 (17.8%)	7 (16.3%)	8 (17.4%)	
≥3	1 (2.2%)	0	1 (2.2%)	
Number of HLA mismatches (mean, SD)*	3.50 ± 1.5	3.26 ± 1.6	2.88 ± 1.5	0.20
PRA pretransplant (mean, SD)†	4.58 ± 12.5	5.55 ± 16.5	9.43 ± 23.7	0.86
Number of immunosuppressive medications				
1	3 (6.7%)	1 (2.3%)	4 (8.7%)	
2	17 (37.8%)	19 (44.2%)	13 (28.3%)	
3	25 (55.6%)	23 (53.5%)	29 (63.0%)	
Immunosuppressive medications (*n*, %)				
Tacrolimus	26 (57.8%)	20 (46.5%)	28 (60.9%)	
Cyclosporin	8 (17.8%)	9 (20.9%)	5 (10.9%)	
Mycophenolate mofetil	35 (77.8%)	30 (69.8%)	37 (80.4%)	
Mycophenolate sodium	6 (13.3%)	5 (11.6%)	6 (13.0%)	
Azathioprine	0	7 (16.3%)	3 (6.5%)	
Everolimus	0	1 (2.3%)	3 (6.5%)	
Sirolimus	2 (4.4%)	3 (7.0%)	0	
Prednisolone	35 (77.8%)	33 (76.7%)	35 (76.1)	
Wean duration (days, median, IQR)	56 (23.5, 77.5)	129 (108, 152)	290.5 (215.3, 393.3)	<0.0001
Reason for graft failure (*n*, %)				
Subacute rejection	0	0	1 (2.2%)	
Acute rejection	4 (8.9%)	2 (4.7%)	1 (2.2%)	
Chronic rejection	36 (80%)	35 (81.4%)	36 (78.3%)	
Glomerulonephritis	2 (4.4%)	1 (2.3%)	0	
Noncompliance	1 (2.2%)	0	2 (4.3%)	
Vascular	1 (2.2%)	0	1 (2.2%)	
Drug toxicity	1 (2.2%)	0	0	
Recurrent disease	0	3 (7.0%)	2 (4.3%)	
Cholesterol emboli	0	1 (2.3%)	0	
BK nephropathy	0	1 (2.3%)	0	
Other	0	0	1 (2.2%)	
Unknown	0	0	2 (4.3%)	

*Data available in 38 patients in <90 days group, 35 patients in 90–180 days group and 40 patients in >180 days group.

†Data available in 43 patients in <90 days group, 40 patients in 90–180 days group and 44 patients in >180 days group.

(8.9%) vs. 7/43 (16.3%) vs. 9/46 (19.6%), $P = 0.19$], pneumonia [4/45 (8.9%) vs. 9/43 (20.9%) vs. 9/46 (19.6%), $P = 0.25$], skin infections [1/45 (2.2%) vs. 1/43 (2.3%) vs. 1/46 (2.2%), $P = 0.99$] or dialysis catheter infections [3/45 (6.7%) vs. 6/43 (14.0%) vs. 6/46 (13.0%), $P = 0.12$].

A competing risk regression using a Fine and Gray model was employed to examine variables associated

Table 2. Clinical outcomes and sensitization postgraft failure with immunosuppression weaned <90 days vs. 90–180 days vs. >180 days.

	<90 days (n = 45)	90–180 days (n = 43)	>180 days (n = 46)	P-value
Follow-up time (days) (median, IQR)	1286 (572 - 2399)	1327 (730 – 2040)	1523 (953.8 – 2108)	0.87
Death (n, %)	12 (26.7%)	12 (27.9%)	9 (19.6%)	0.35
Assessed for retransplantation (n, %)	31 (68.9%)	35 (81.4%)	36 (78.3%)	0.73
Median time to screening for retransplantation (days)	567	430	649	
cPRA (mean, SD)	80.06 ± 29.4	81.21 ± 25.0	85.42 ± 23.9	0.66
Retransplanted (n, % of patients assessed for retransplantation)	24/31 (77.4%)	21/35 (60.0%)	22/36 (61.1%)	0.13

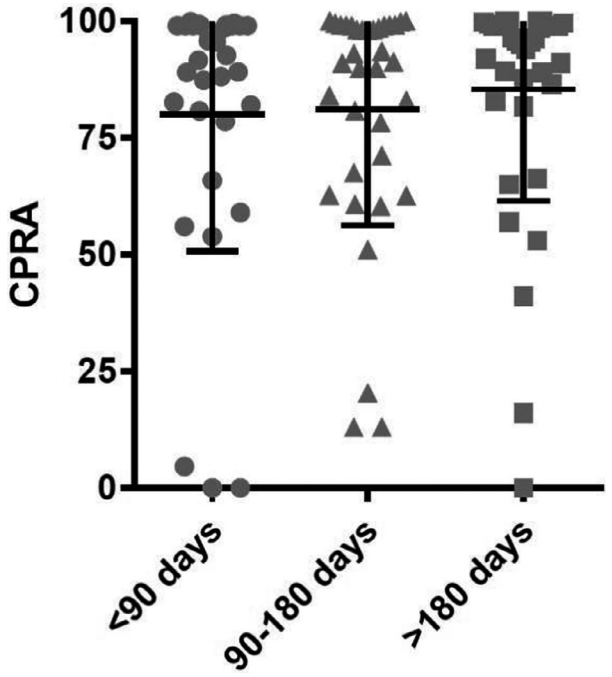

Figure 2 Sensitization after graft failure between patients weaned over <90 days and those weaned over 90–180 days or >180 days. cPRA was determined for each subject based upon anti-HLA antibodies with MFI >4000 detected by OLISAG. Error bars indicate mean ± SD.

with the likelihood of retransplantation after graft failure with death as the competing risk (Table 4). Cofactors included in the analysis included: duration of immunosuppression wean postgraft failure, age at transplant failure, female gender, deceased donor transplant, rejection as cause of graft failure and nephrectomy. Only age at transplant failure predicted for likelihood of retransplantation (SHR 0.999, 95% CI 0.998–0.999, P = 0.04). Duration of immunosuppression wean postgraft failure (SHR 1.000, 95% CI 0.999–1.001, P = 0.88), female gender (SHR 1.271, 95% CI 0.766–2.108, P = 0.35), deceased donor transplant (SHR 0.701, 95% CI 0.406–1.211, P = 0.20), rejection (SHR 0.848, 95% CI 0.428–1.682, P = 0.64) and nephrectomy (SHR 0.837, 95% CI 0.472–1.485, P = 0.54) did not predict for likelihood of eventual retransplantation.

Discussion

There are currently little data to guide clinicians in the management of immunosuppression following kidney allograft failure. The clinical challenge when considering immunosuppression withdrawal is to balance the risk of allo-immunization and acute allograft rejection with the risk of infection and malignancy associated with excessive exposure to immunosuppression. Relatively few studies and, to our knowledge, no prospective randomized control trials, have addressed this issue. As such, no consensus exists about how best to manage immunosuppression withdrawal, with some centres advocating rapid withdrawal [9] while others suggesting more gradual tapering [13].

In this study, we sought to determine whether delayed immunosuppression withdrawal in unselected patients with kidney allograft failure was associated with benefits in terms of degree of sensitization and associated increase in likelihood of retransplantation. We also sought to assess the impact of extended immunosuppression exposure on rates of adverse outcomes including infection, malignancy and need for nephrectomy. Our findings suggest that slower withdrawal of immunosuppression beyond >90 or 180 days postgraft failure is not associated with any significant reduction in sensitization, nor is it associated with an increase in the likelihood of retransplantation. Extended immunosuppression withdrawal is, however, associated with a significantly reduced risk of allograft nephrectomy.

Our results are in contrast with those of previous retrospective studies examining the effect of immunosuppression withdrawal on sensitization after kidney allograft failure. In a retrospective analysis of 119

Table 3. Immunosuppression complications postgraft failure with immunosuppression weaned <90 days vs. 90–180 days vs. >180 days.

	<90 days (n = 45)	90–180 days (n = 43)	>180 days (n = 46)	P-value
Nephrectomy (n, %)	13 (28.9%)	8 (18.6%)	6 (13.0%)	0.04
Nephrectomy post wean (n, %)	10/42 (23.8%)	7/42 (16.7%)	3/43 (7.0%)	0.01
Cancer (n, %)	2 (4.4%)	4 (9.3%)	3 (6.5%)	0.35
Admitted to hospital (n, %)	24 (53.3%)	23 (53.5%)	25 (54.3%)	0.77
Median time to first admission (days)	1741	885	681	
Infection (n, %)				
Any	24 (53.3%)	24 (55.8%)	26 (56.5%)	0.86
Sepsis	14 (31.1%)	11 (22.6%)	11 (23.9%)	0.65
Urinary tract infections	4 (8.9%)	7 (16.3%)	9 (19.6%)	0.19
Pneumonia	4 (8.9%)	9 (20.9%)	9 (19.6%)	0.25
Skin infections	1 (2.2%)	1 (2.3%)	1 (2.2%)	0.99
Dialysis catheter infection	3 (6.7%)	6 (14.0%)	6 (13.0%)	0.12

Table 4. Multivariate analysis of factors associated with retransplantation—competing risk regression (Fine and Gray).

	SHR	95% CI	P-value
Duration of immunosuppression wean	1.000	0.999–1.001	0.88
Age at transplant failure	0.999	0.998–0.999	0.04
Female gender	1.271	0.766–2.108	0.35
Deceased donor transplant	0.701	0.406–1.211	0.20
Rejection history	0.848	0.428–1.682	0.64
Nephrectomy	0.837	0.472–1.485	0.54

Outcome: retransplantation. Competing risk: death.

patients with low pretransplant PRA (median 0%) who experienced graft failure, Augustine *et al.* [7] reported that only 8% of patients who continued immunosuppression became highly sensitized (defined as PRA >80%) compared with 68% of those who were weaned from immunosuppression. Multivariate analysis suggested that immunosuppression withdrawal predicted the development of sensitization independently of HLA matching or graft nephrectomy. There were also non-significant trends towards greater likelihood of retransplantation and shorter time to retransplantation in the group who continued immunosuppression. It is important to note, however, that this earlier study was designed to compare outcomes between patients who continued immunosuppression indefinitely with those who ceased immunosuppression at any time rather than between patients who eventually ceased immunosuppression over defined periods of time. This difference in study design may account for some of the differences in the outcomes compared to our results. Importantly, the study by Augustine *et al.* did not report the incidence of immunosuppression related adverse outcomes such as infection but did report two malignancy-related deaths in the maintenance immunosuppression group (melanoma and central nervous system lymphoma) which may have been related to continuation of immunosuppression.

In another retrospective study examining the potential effect of prolonged immunosuppression withdrawal in preventing allo-immunization after allograft failure, Casey *et al.* reported that of 49 patients who experienced graft failure and who were subsequently referred for retransplantation, there was a beneficial effect seen in those whose immunosuppression was withdrawn over a period >3 months [8]. Specifically, this study found that 66% of patients who were weaned over more than 3 months remained unsensitized (PRA 0%) compared with 30% of patients who were weaned over <3 months. A secondary analysis of PRA results performed in 38 patients found mean PRA levels to be significantly lower in the prolonged withdrawal group compared with the early withdrawal group (12.5% vs. 40.6%). No excess in mortality, infection or malignancy was detected in the prolonged withdrawal group,

although event numbers were low. Of note, the median duration of immunosuppression in the early withdrawal groups in this previous study was lower than that in our current study (24 days vs. 56 days), raising the possibility that overly rapid withdrawal may be disadvantageous with regards to sensitization. This suggests that there might be some benefit in continuing immunosuppression for a very short period after graft failure, but that no additional benefit is derived from more extended exposure.

Unlike previous reported studies where PRA measurement was based on CDC or Flow-PRA, our results are based entirely on solid-phase assays using single-antigen beads and cPRA. Solid-phase assays are significantly more sensitive than CDC and can detect anti-HLA antibodies that may not otherwise be identified [14]. Additionally, single-antigen bead assays such as OLISAG may be more relevant than other solid-phase assays (such as Flow-PRA) that incorporate panels of representative HLA antigens and are actually used to define unacceptable antigens in current organ allocation protocols. These differences may account for the significantly higher sensitization postgraft failure seen in our study compared with previous cohorts. It may also partially explain the discordance between our results and that of previous reports with regard to the benefits of prolonged immunosuppression on sensitization. Of note, the average cPRA of patients on the waiting list for a first transplant at our centre is 13.9%.

Despite the lack of improvement in postgraft failure sensitization and retransplantation rates with prolonged immunosuppression withdrawal seen in this study, we did observe a significant reduction in requirement for graft nephrectomy in patients whose immunosuppression was weaned more slowly. Withdrawal of immunosuppression may precipitate acute on chronic rejection necessitating nephrectomy after graft failure. While 7.0% of patients weaned over >180 days still eventually required nephrectomy, this was significantly lower than the 16.7% and 23.8% of those weaned over 90–180 and <90 days, respectively, who underwent the same procedure. These findings are similar to those of Augustine *et al*. [7] who also reported a lower graft nephrectomy rate in the cohort continued on immunosuppression. In addition to the potential reduction in morbidity and mortality associated with graft nephrectomy, there is evidence that rates of sensitization may increase following this procedure. Several small studies have reported higher PRA scores and an associated prolonged time to retransplantation in those subjects undergoing graft nephrectomy [15–17]. The increase in sensitization after nephrectomy may be especially pronounced in patients who were previously unsensitized and in those who undergo nephrectomy within the first 6 months post-transplant [18]. The mechanism of increased sensitization after graft nephrectomy remains unclear, although a possibility might be loss of antibody binding to the allograft [15].

Notwithstanding this, the benefits of prolonged immunosuppression in reducing nephrectomy risk must be balanced against the potential adverse effects, particularly with regard to infection and malignancy. Previous studies have shown infection-related mortality after kidney allograft failure and return to dialysis to be significantly higher compared with transplant-naïve subjects [19]. Infection-related complications are the second most common cause of death in patients experiencing graft failure, with only cardiac disease being more prevalent [9,11]. Earlier analyses have shown maintenance immunosuppression after kidney allograft failure to be significantly associated with a greater incidence of infection compared with cessation [20]; however, our study did not find any significant differences in infection risk in the patients who underwent slower withdrawal of immunosuppression compared with those whose immunosuppression was withdrawn in shorter periods of time.

Cancer is a commonly seen complication of kidney transplant recipients and is a leading cause of death with a functioning graft [21]. Cumulative exposure to immunosuppression is a significant risk factor for the development of malignancy. A registry analysis of 8173 kidney transplant recipients found the incidence of some cancers to be higher during periods of immunosuppression exposure compared with the rate observed following allograft failure and associated withdrawal of immunosuppression [10]. This was particularly the case for malignancies associated with viral infections such as Kaposi sarcoma (human herpes virus 8) and non-Hodgkin lymphoma (Epstein-Barr virus), as well as lip cancer and melanoma. The incidence of renal tract cancers, lung cancers and leukaemia was not affected. While 6.7% of patients in our cohort had a new cancer diagnosis, we did not find any associations between cancer incidence and rate of immunosuppression withdrawal.

The strengths of this study include evaluation of data from patients over a prolonged period with significant follow-up time, as well as a uniform method of evaluating sensitization using modern solid-phase assay-based techniques that are currently standard of care for organ allocation in most jurisdictions. Conversely, our study is limited by sample size and the inherent issues involved with analysing retrospective data. In addition, only

patients being considered for retransplantation had their cPRA measured after allograft failure. Residual confounders such as hospital admissions, infections or blood transfusions in the community or at other healthcare centres were not captured by our data.

In conclusion, this single-centre, retrospective analysis of unselected patients with kidney allograft failure demonstrated no beneficial effect of prolonged immunosuppression withdrawal on likelihood of alloimmunization or time to retransplantation. Prolonged immunosuppression was associated with a significantly lower risk of nephrectomy. Future prospective control studies are required to definitively determine the benefits and risks of prolonged immunosuppression weaning. Until these studies are conducted, caution is warranted before subjecting patients to extended immunosuppression with the aim of increasing chances of retransplantation. Clinicians should carefully individualize weaning protocols to account for comorbidities, risk profile and patient preferences, aiming to minimize the adverse effects of immunosuppression without increasing sensitization and compromising prospects of retransplantation.

Authorship

KM and KC: participated in research design, performance of the research, data analysis and writing of the paper. LC: participated in performance of the research and data analysis. KB, ML, RM and PH: participated in the performance of the research.

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Conflicts of interest

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Association between immunosuppression wean duration (continuous variable) and outcomes.

Table S2. Clinical outcomes and sensitisation post graft failure with immunosuppression weaned <90 days vs. 90–180 days vs. >180 days in patients with transplant duration ≥1 year.

REFERENCES

1. Coresh J, Selvin E, Stevens LA, *et al*. Prevalence of chronic kidney disease in the United States. *JAMA* 2007; **298**: 2038.
2. Ekberg H, Tedesco-Silva H, Demirbas A, *et al*. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
3. Meier-Kriesche HU, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? *Am J Transplant* 2004; **4**: 1289.
4. Messa P, Ponticelli C, Berardinelli L. Coming back to dialysis after kidney transplant failure. *Nephrol Dial Transplant* 2008; **23**: 2738.
5. Rao PS, Schaubel DE, Jia X, Li S, Port FK, Saran R. Survival on dialysis post-kidney transplant failure: results from the scientific registry of transplant recipients. *Am J Kidney Dis* 2007; **49**: 294.
6. McCaughan JA, Patterson CC, Maxwell AP, Courtney AE. Factors influencing survival after kidney transplant failure. *Transplant Res* 2014; **3**: 18. eCollection 2014.
7. Augustine JJ, Woodside KJ, Padiyar A, Sanchez EQ, Hricik DE, Schulak JA. Independent of nephrectomy, weaning immunosuppression leads to late sensitization after kidney transplant failure. *Transplantation* 2012; **94**: 738.
8. Casey MJ, Wen X, Kayler LK, Aiyer R, Scornik JC, Meier-Kriesche HU. Prolonged immunosuppression preserves nonsensitization status after kidney transplant failure. *Transplantation* 2014; **98**: 306.
9. Smak Gregoor P, Zietse R, Van Saase J, *et al*. Immunosuppression should be stopped in patients with renal allograft failure. *Clin Transplant* 2001; **15**: 397.
10. van Leeuwen MT, Webster AC, McCredie MRE, *et al*. Effect of reduced immunosuppression after kidney transplant failure on risk of cancer: population based retrospective cohort study. *BMJ* 2010; **340**: c570.
11. Gill JS, Abichandani R, Kausz AT, Pereira BJ. Mortality after kidney transplant failure: the impact of non-immunologic factors. *Kidney Int* 2002; **62**: 1875.
12. Bayliss GP, Gohh RY, Morrissey PE, Rodrigue JR, Mandelbrot DA. Immunosuppression after renal allograft failure: a survey of US practices. *Clin Transplant* 2013; **27**: 895.
13. Morales A, Gavela E, Kanter J, *et al*. Treatment of renal transplant failure. *Transpl Proc* 2008; **40**: 2909.
14. Picascia A, Infante T, Napoli C. Luminex and antibody detection in kidney transplantation. *Clin Exp Nephrol* 2012; **16**: 373.
15. Martin L, Guignier F, Mousson C, Rageot D, Justrabo E, Rifle G. Detection of donor-specific anti-HLA antibodies with flow cytometry in eluates

and sera from renal transplant recipients with chronic allograft nephropathy. *Transplantation* 2003; **76**: 395.
16. Johnston O, Rose C, Landsberg D, Gourlay WA, Gill JS. Nephrectomy after transplant failure: current practice and outcomes. *Am J Transplant* 2007; **7**: 1961.
17. Wang K, Xu X, Fan M, Qianfeng Z. Allograft nephrectomy vs. no-allograft nephrectomy for renal transplantation: a meta-analysis. *Clin Transplant* 2016; **30**: 33.
18. Khakhar AK, Shahinian VB, House AA, *et al*. The impact of allograft nephrectomy on percent panel reactive antibody and clinical outcome. *Transpl Proc* 2003; **35**: 862.
19. Perl J, Zhang J, Gillespie B, *et al*. Reduced survival and quality of life following return to dialysis after transplant failure: the Dialysis Outcomes and Practice Patterns Study. *Nephrol Dial Transplant* 2012; **27**: 4464.
20. Woodside KJ, Schirm ZW, Noon KA, *et al*. Fever, infection, and rejection after kidney transplant failure. *Transplantation* 2014; **97**: 648.
21. ANZDATA Registry. 42nd Report, Chapter 7: Kidney Transplantation. Australia and New Zealand Dialysis and Transplant Registry, Adelaide, Australia. 2019. Available at: http://www.anzdata.org.au.