Analysis of Tacrolimus Trends and Trough Levels < 5 ug/L Preceding Development of Biopsy Proven Acute Rejection in Renal Transplant Patients

R. Shen[1]*, C. Cheung[2], B. Strijack[3]

[1]*University of British Columbia, British Columbia, Canada*
[2]*Fraser Health, British Columbia, Canada*
[3]*Department of Nephrology, University Of British Columbia, British Columbia, Canada*

ABSTRACT

British Columbia Transplant (BCT) modified the tacrolimus (TAC) target guideline for patients beyond 6 months post-transplant from 4–6 µg/L to 5–7 µg/L in 2021. Although TAC is the mainstay medication used in the prevention of allograft rejection in kidney transplants, optimal target concentration is still being actively researched and guidelines differ locally. This retrospective case series as a part of a QI project aims to explore the relationship between TAC values < 5 µg/L and the occurrence of biopsy proven acute rejections (BPAR). TAC and serum creatinine values one-year leading to BPAR were collected from 6 patients (PT1–6) in total. One out of the six patients showed TAC trough mean significantly < 5 µg/L (P = 0.0002) while four out of six patients experienced levels < 5 µg/L on at least one occasion. It is observed that baseline-altering drops in TAC were consistently followed by measurable renal function decline which is a potential determinant of BPAR. Further, TAC variability in patients is suggested as a contributing factor for rejections. The previous guideline of 4 µg/L minimum is seen to be effective in some patients and insufficient in others. In result, targeting > 5 µg/L may be beneficial to a wider range of patients when used as a general guideline.

KEYWORDS: Tacrolimus; Acute rejection; Biopsy, Renal transplant

INTRODUCTION

Since clinical use, calcineurin inhibitors brought about significant decrease in acute rejection rates for renal transplants. The administration of tacrolimus (TAC) in the prevention of acute kidney graft rejection is widely accepted [1]. However, clear consensus has not been established behind target trough levels in various patient populations. Insufficient TAC dosing risks higher probability of developing de-novo donor specific antibodies (dnDSA) which is the hallmark of allograft rejection [2]. Whereas overdosing of TAC could result in nephrotoxicity, allograft damage, opportunistic infections, diabetes, and malignancies [3-5]. The complex balance for optimal dosing depends on a variety of factors such as the degree of HLA mismatch, concomitant drugs, patient genetics, adherence, immune function, and others alike [3].

Due to these nuances, clinical guidelines revolving around TAC target vary between countries and health authorities. A recent change was adopted by British Columbia Transplant (BCT) to increase the maintenance TAC trough target level from 4–6 µg/L to 5–7 µg/L beyond 6 months post-transplant. This change may have been inspired by evidence suggesting a lowered risk of biopsy-proven acute rejection (BPAR) at 7-12 months with trough above 5 µg/L [6]. Another study also alludes to the positive association between TAC levels below 5 µg/L and dnDSA development [7]. Although previous studies mostly conclude that maintenance TAC target plays a significant part in reducing rejection rate, optimal dosing strategy remains unclear [6-10].

*Correspondence: Randi Shen, PharmD
University of British Columbia, British Columbia, Canada
ORCID: 0000-0002-2078-1882
E-mail: randis@alum.ubc.ca

Table 1: Individual demographics of patients with biopsy proven acute rejections (BPAR).

Patients	PT1	PT2	PT3	PT4	PT5	PT6
Sex	F	F	F	F	F	F
Age	52	66	70	77	63	41
Transplant date	March 17, 2019	October 11, 2018	January 28, 2019	March 27, 2018	January 06, 2010	July 06, 2013
Confirmed rejection date	March 18, 2021	June 3, 2020	March 24, 2021	December 13, 2019	July 16, 2021	June 25, 2021
cPRA%	99.84	99.00	0.00	39.00	22.00	99.96
Immuno-suppression regimen	TAC, MMF, PRED	TAC, MYF	TAC, MMF	TAC, AZA, PRED	TAC, MMF	TAC, MMF, PRED
Donor Status	Deceased	Deceased	Deceased	Living	Deceased	Deceased

Abbreviations: cPRA; calculated panel reactive antibody, TAC; Tacrolimus, MMF; Mycophenolate mofetil, MYF; Mycophenolate Sodium, AZA; Azathioprine, PRED; Prednisone

Moreover, most known literature studying the concentration-effect relationship of TAC uses cohorts with patients on maintenance steroid therapy, which does not apply to those patients managed with steroid sparing protocols in Vancouver General Hospital (VGH) and St. Paul's Hospital. It is unknown how this may affect the proposed TAC target needed for optimal immunosuppression [11].

The difference in approach from site to site may call into question the generalizability of literature findings for the care of Fraser Health patients. Thus, it is critical to approach this issue from a site-specific perspective. This study sets out to evaluate BCT's decision to increase TAC target levels to 5–7 µg/L by investigating the mean TAC and TAC level trends of those patients who experienced BPAR at Fraser Health in the past 3 years.

MATERIALS AND METHODS

Study Design

A retrospective patient chart review was conducted on 6 adult patients treated in the Fraser Health Post Transplant Clinic in Surrey, BC, Canada, as a quality improvement project. Enrolled patients followed the defined inclusion criteria: (1) experienced biopsy-proven acute rejection of the kidneys in the last 3 years (2019-2021) at Fraser Health, (2) used TAC asa part of their anti-rejection regimen following BC Fraser Health guidelines. Diagnosis of BPAR was determined adhering to the Banff classification system.

Data Collection

Patient demographics, laboratory values, medical and medication history were collected from electronic medical records on PROMIS® and paper-based charts. TAC trough levels within 1 year leading up to kidney graft rejection were gathered along with the regimens of other co-administered immunosuppressant drugs. Any trough levels above 10 µg/L were cross-referenced with clinical notes to ensure pre-dose conditions were met; otherwise, the data point was discarded from statistical analysis. Serum creatinine values were tracked in conjunction as an important biomarker of kidney function.

Statistical Analysis

Demographic data were represented as mean and standard deviations for continuous variables and percentages for categorical variables. Descriptive statistics performed on sample TAC data points yielded mean, confidence interval and coefficient of variance. Comparison of means to theoretical value were done using a one sample t-test (one tailed). Statistical analyses were performed using GraphPad Prism version 9.0.0 with significance level set at $P< 0.05$.

Table 2: Summarized patient characteristics.

Variables	Total
Sex (Female), n (%)	6 (100.0)
Steroid Regimen, n (%)	3 (50.0)
Living Donor, n (%)	1 (16.7)
Age, mean (SD)	61.50 (13.00)
cPRA%, mean (SD)	59.97 (45.14)

Abbreviations: SD; standard deviation, cPRA; calculated panel reactive antibody

RESULTS

A total of six BPAR patients were enrolled from Fraser Health Post Transplant Clinic in Surrey, BC, Canada. Individual demographic data were reported in Table 1 and summarized in Table 2. The patient sample was 100% female and had an average age of 61.5 ± 13 years. Their kidney transplant date ranged widely from 2010 to 2019 and all experienced BPAR in the past 3 years (2019–2021). Calculated panel reactive antibody (cPRA) of enrolled patients showed a mean of 59.97 ± 45.14%. Concomitant use of immunosuppression regimen varied with 3 (50%) patients on prednisone. Five out of the six patients received cadaveric donor kidney and only one received living donor kidney.

Analyses were made on the patient's mean TAC trough level one-year preceding confirmed rejection. PT1–4 and 6 recorded TAC values of (mean ± 95% CI) 8.1 ± 1.4, 7.5 ± 1.4, 7.6 ± 0.8, 6.8 ± 1.4, 6.2 ± 1.3 µg/L respectively and showed no significance in mean trough < 5 µg/L (P≥ 0.98). PT5 had a mean trough level of 3.9 ± 0.5 µg/L that was significantly below 5 µg/L (P= 0.0002). TAC variability within each patient is captured by the coefficient of variance 25.6, 30.0, 16.2, 27.9, 23.0, 29.5% for PT 1–6 in Table 3.

In Fig. 1, patient TAC values were plotted in tandem with their serum creatinine within one year before BPAR. This allows for the qualitative analysis of trends. Notably, a drop in TAC trough levels can be seen preceding an increase in serum creatinine around September 2020 for PT1, October 2019 for PT2, February 2021 for PT3, August 2019 for PT4, June 2021 for PT5, and April 2021 for PT6. Patients 2, 4, 5, 6 experienced TAC level of < 5 µg/L on at least one occasion.

DISCUSSION

A major goal of this study is exploring the potential relationship between TAC trough levels< 5 µg/L and BPAR incidences to provide evidence in lieu of the 2021 BCT TAC target guideline change from 4–6 µg/L to 5–7 µg/L for patients beyond 6 months post-transplant. There are 2 approaches to analyze from, (1) comparing patient's mean TAC trough values to the new guideline minimum and (2) analyzing TAC level trends with respect to kidney function prior to rejection.

It is observed that only one patient (PT5) showed a mean concentration significantly below 5 µg/L (P= 0.0002). The other subjects experienced rejection despite their average TAC level exceeding the new target minimum. Furthermore, two patients (PT1, 3) experienced rejection without any instance of TAC falling below 5 ug/L in the previous year. This is a strong indicator that extraneous factors outside of the patient's TAC levels were responsible for BPAR. On the other hand, four out of six patients (PT2, 4, 5, 6) had at least one occurrence where TAC trough were < 5 µg/L. These declines in TAC were consistently followed by a sharp or baseline-altering increase in serum creatinine indicating a decline in graft function (Fig. 1). With that in mind, it could be speculated that fluctuations and especially the drops < 5 µg/L played a role in BPAR even though the average trough levels were sustained above minimum. Comparably, Huang et al. found an increased risk of acute kidney rejections in patients with higher TAC trough variability [12]. This establishes the importance for achieving low TAC variability among transplant patients and may partly explain the rejections occurring despite exceeding target TAC mean.

It is difficult to determine the exact correlation between TAC and creatinine due to two

Table 3: Mean Tacrolimus trough level 1 year leading up to biopsy proven acute rejections (BPAR).

Patients	Mean trough TAC (μg/L)	95% CI	CV (%)	P-value
PT1	8.1	6.7–9.5	25.6	0.99
PT2	7.5	6.1–8.9	30.0	0.99
PT3	7.6	6.9–8.4	16.2	> 0.99
PT4	6.8	5.3–8.2	27.9	0.98
PT5	3.9	3.4–4.4	23.0	0.0002
PT6	6.2	5.1–7.2	29.5	0.98

One sample t-test (one tailed) for theoretical mean of < 5 μg/L

Abbreviations: CI; Confidence Interval, TAC; Tacrolimus, CV; Coefficient of Variation

reasons. Firstly, the effects of under immunosuppression from low TAC levels may not be immediately reflected. Secondly, TAC may directly cause afferent arteriole vasoconstriction and potential nephrotoxicity without being a sign for graft rejection [13]. Nonetheless, in the context of confirmed rejection, the trends in Fig. 1 become a valid tool to analyze the progression of each individual patient. Comparing TAC and creatinine values, it is recognized that the patients had varying baseline values prior to rejection. Patients with creatinine values above 100 μmol/L (PT1–4 and 6) averaged a TAC trough of 6–8 μg/L as baseline prior to decline. Whereas PT5, marked with relatively higher renal function (60–80 μmol/L creatinine baseline) maintained a lower TAC concentration of 4-5 μg/L.

Looking closer into PT5's trends, it is evident that their allograft stability was upheld even when the TAC concentrations were persistently between 4–5 μg/L. This could suggest that the target minimum of 4 μg/L in the past was an effective regimen in this particular patient. What is suspected as a primary contributor to allograft rejection in PT5 was the significant drop in TAC to 1.9 μg/L a month pre-BPAR. Predictively, this drop was immediately reflected in the increase in creatinine from 70 to 160 μmol/L. In contrast, for patients like PT6, a concentration < 5 μg/L maintained 7 months pre-BPAR may have resulted in an insufficient amount of immunosuppression, elevating creatinine from 91 to 116 μmol/L. This effect of graft function decline is also observed in PT4 with respect to their TAC level falling to 4.9 μg/L three months pre-BPAR. There seemingly exists a considerable amount of variability in the TAC maintenance levels required from patient to patient. One existing explanation from Wiebe *et al.*, [7] illustrates how individual class II eplet mismatch modulates the TAC levels required for the prevention of dnDSA. Relevantly in their analysis, they showed that patients with higher degrees of HLA-DR/DQ mismatch were more at risk for developing dnDSA when TAC concentrations are < 5 μg/L. Thus, it may be suggested that a higher general guideline minimum of 5 μg/L is more beneficial to patients with higher donor mismatch in place where 4 μg/L was not tolerable. In the same literature, it is demonstrated that standard level TAC (5–8 μg/L) achieved lower development of dnDSA when compared with low level TAC (2-5 μg/L), which is consistent with most trends observed in this study [7].

Due to sample size and the nature of this retrospective case series, the contribution from co-administered immunosuppression drugs was not analyzed. This is particularly critical when considering the involvement of steroid regimen such as prednisone. Most studies published in TAC dosing optimization involve patients that are also on corticosteroids. However, BCT, pharmacy centres in VGH, and St. Paul's Hospital may opt for a steroid sparing management strategy in certain patients depending on their clinical evaluation. Among the patients investigated in this study, three out of the six were not on steroids, yet there are a lack of studies dictating ideal TAC dosing in these cases [14]. Future studies may aim to investigate outcomes from varying TAC levels in steroid absent regimens as these are now becoming increasingly prescribed [15].

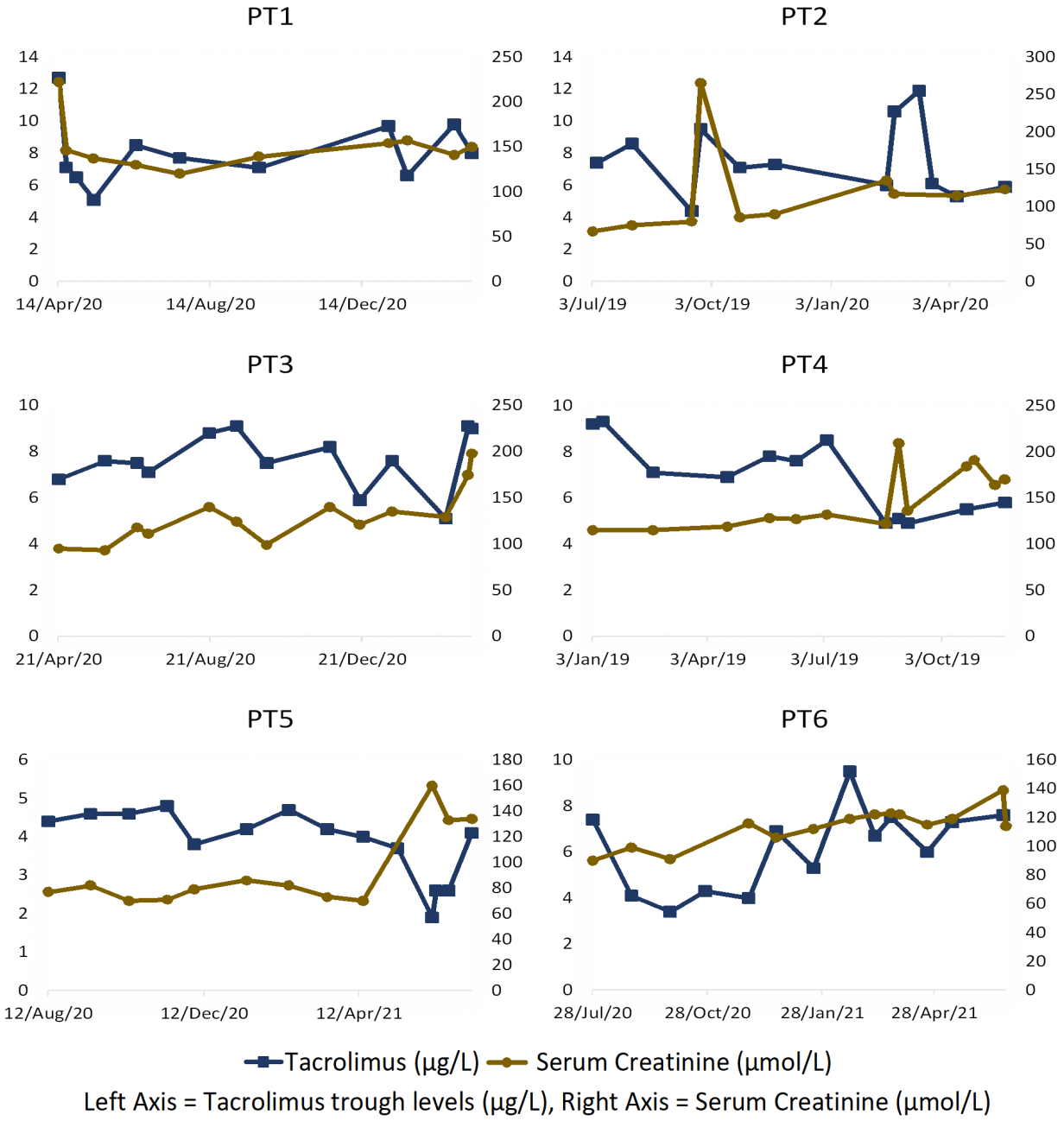

Figure 1: Creatinine and tacrolimus level trends in patients one-year leading up to biopsy proven acute rejections (BPAR).

All things considered; the state of current literature progressively indicates the importance of adequate TAC dosing in lowering kidney graft rejection risk [6-10]. Many factors are at play evident by some patients experiencing rejection despite exceeding optimal guideline target. The previous guideline minimum of 4 µg/L may have been effective in some patients but was not sufficient in others. TAC level variability and the intermittent drops below 5 µg/L may be a determinant of BPAR. It is recommended for future studies to take into account the risks (i.e., potential for CNI toxicity) involved from the higher target range. More robust studies are required to determine optimal TAC dosing on an individual basis with regards to recognizing specific BPAR risks.

CONFLICTS OF INTEREST: None declared.

REFERENCES

1. Kalluri HV, Hardinger KL. Current state of renal transplant immunosuppression: Present and future. *World J Transplant* 2012;**2**:51.
2. Sellarés J, de Freitas DG, Mengel M, *et al*. Understanding the causes of kidney transplant failure: The dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2011;**12**:388-99.
3. Pratschke J, Weiss S, Neuhaus P, Pascher A. Review of nonimmunological causes for deteriorated graft function and graft loss after transplantation. *Transplant Int* 2008;**21**:512-22.
4. Issa N, Kukla A, Ibrahim HN. Calcineurin inhibitor Nephrotoxicity: A review and perspective of the evidence. *Am J Nephrol* 2013;**37**:602-12.
5. Al-Adra D, Al-Qaoud T, Fowler K, Wong G. De Novo Malignancies after kidney transplantation. *Clin J Am Soc Nephrol* 2022;**17**:434-43.
6. Jung H-Y, Cho S-Y, Choi J-Y, *et al*. Comparison of Transplant Outcomes for Low-level and Standard-level Tacrolimus at Different Time Points after Kidney Transplantation. *J Korean Med Sci* 2019;**34**: e103.
7. Wiebe C, Rush DN, Nevins TE, *et al*. Class ii eplet mismatch modulates tacrolimus trough levels required to prevent donor-specific antibody development. *J Am Soc Nephrol* 2017;**28**:3353-62.
8. Gaynor JJ, Ciancio G, Guerra G, *et al*. Lower tacrolimus trough levels are associated with Subsequently HIGHER ACUTE Rejection risk during the first 12 months after kidney transplantation. *Transplant Int* 2015;**29**:216-26.
9. Arreola-Guerra JM, Serrano M, Morales-Buenrostro LE, *et al*. Tacrolimus trough levels as a risk factor for acute rejection in renal transplant patients. *Annal Transplant* 2016;**21**:105-14.
10. Størset E, Åsberg A, Skauby M, *et al*. Improved tacrolimus target concentration achievement using computerized dosing in renal transplant recipients—a prospective, randomized study. *Transplantation* 2015;**99**:2158-66.
11. Duijnhoven EM, Boots JMM, Christiaans MHL, *et al*. Increase in tacrolimus trough levels after steroid withdrawal. *Transplant Int* 2003;**16**:721-5.
12. Huang C-T, Shu K-H, Ho H-C, Wu M-J. Higher variability of tacrolimus trough level increases risk of acute rejection in kidney transplant recipients. *Transplant Proc* 2016;**48**:1978-80. \
13. Naesens M, Kuypers DR, Sarwal M. Calcineurin inhibitor Nephrotoxicity. *Clin J Am Soc Nephrol* 2009;**4**:481-508.
14. Steiner RW, Awdishu L. Steroids in kidney transplant patients. Seminars in Immunopathology. 2011;**33**:157-67.
15. Schold JD, Santos A, Rehman S, Magliocca J, Meier-Kriesche H-U. The success of continued steroid Avoidance after kidney transplantation in the US. Am J Transplant 2009;**9**:2768-76.