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Effect of metronidazole use on tacrolimus concentrations in transplant patients treated for *Clostridium difficile*

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Running title: Early et al: Metronidazole and tacrolimus concentrations

Abstract: *Background.* Two case reports suggest that metronidazole treatment for *C. difficile* infections (CDI) increases tacrolimus (TAC) trough levels. The primary objective of this study was to determine the clinical significance of this potential interaction in transplant patients receiving CDI treatment. Currently, no robust literature exists to estimate a magnitude of pharmacokinetic interaction between metronidazole and TAC.

Methods. In this retrospective study, the effects of CDI and metronidazole treatment on TAC levels in 52 adult solid organ transplant patients were investigated. The primary outcome was to determine the difference in dose-normalized TAC levels between baseline and symptom resolution in patients treated with metronidazole or vancomycin. The secondary outcome was to determine the difference in dose-normalized TAC levels at baseline and CDI diagnosis.

Results. The average change in log-transformed dose-normalized TAC levels from baseline to symptom resolution was 0.99 for metronidazole (n = 35) and 1.04 for vancomycin (n = 17) treatment. The mean difference between the groups was 0.96 (95% confidence interval: 0.74–1.24). No significant difference was found between dose-normalized TAC levels at CDI diagnosis and baseline (P = 0.37).

Conclusion. CDI treatment with metronidazole was not associated with a > 30% increase in TAC levels compared to vancomycin. Both treatment groups required TAC dose adjustments to maintain goal TAC levels and those treated with metronidazole did not require a significantly greater dose adjustment.

Key words: tacrolimus; metronidazole; *Clostridium difficile;* diarrhea; vancomycin; trough concentration; CYP3A4

Tacrolimus (TAC) is an immunosuppressive medication used in patients who have undergone solid organ transplantation. Because of its narrow therapeutic index, TAC requires routine drug monitoring in order to maintain trough levels within a therapeutic range. TAC is metabolized through the CYP3A4 isoenzyme and is also a substrate for P-glycoprotein (Pgp) transporter, which puts it at high risk for drug-drug interactions. In addition, TAC trough levels are known to increase during episodes of diarrhea, likely because of intestinal damage resulting in a decrease in gut metabolism and secretion of TAC by epithelial cells (1–3).

In the general population, metronidazole is the first-line agent for treatment of mild-to-moderate *Clostridium difficile* infections (CDI). Because of the association between oral vancomycin exposure and vancomycin-resistant enterococci colonization, as well as its high cost, oral vancomycin is typically reserved for more severe or recurrent infections. In the transplant patient population, 2 case reports have documented increased TAC levels when metronidazole was used to treat CDI (4, 5). Because TAC is both a CYP3A4 and Pgp substrate, inhibition of either CYP3A4 or Pgp was proposed as a mechanism for the observed increase in trough levels. However, pharmacokinetic data do not support that metronidazole is a strong CYP3A4 or Pgp inhibitor (6, 7). Furthermore, the change in TAC levels observed with concurrent use of metronidazole is not always predictable and a publication bias may exist. Nonetheless, a combination of metronidazole and TAC is identified as a major pharmacokinetic interaction in commonly used tertiary drug information resources (8, 9) and oral vancomycin is often selected for this reason to treat CDI in patients taking TAC, regardless of CDI severity.

The primary objective of this study was to determine whether concomitant metronidazole therapy resulted in at least a 30% increase in TAC levels in transplant patients receiving treatment for CDI. We considered an increase in TAC levels by 30% or greater to be clinically significant as the interaction would warrant TAC dosing adjustments at initiation of the offending drug or avoidance of concomitant use. The results of this study will aid us in the decision-making of whether or not this interaction warrants an empiric dosage adjustment in TAC upon initiation of metronidazole or complete avoidance of metronidazole. Currently, no robust literature exists to estimate a magnitude of pharmacokinetic interaction between metronidazole and TAC.

Methods

We investigated the effects of CDI and metronidazole treatment on TAC levels in this retrospective analysis of clinical data at the University of Michigan Health System (UMHS). The protocol was approved by the institutional review board prior to beginning data collection.

Patient population

The study cohort included solid organ transplant patients who were admitted to UMHS between January 1, 2009 and March 31, 2014. Adult patients, 18 years of age and older were eligible for

inclusion if they had a positive *C. difficile* assay and were taking TAC as part of maintenance immunosuppression during the study period. CDI treatment with either vancomycin or metronidazole, in addition to documentation of TAC levels at UMHS from 4 weeks pre-CDI through symptom resolution, was also required for inclusion. Only levels that resulted from a stable TAC dose, defined as no dosage change in the past 48 h, were included. Any patient with record of inappropriately drawn TAC levels (<10 h after the previous dose was administered), who was treated for CDI with both metronidazole and vancomycin, or who had an addition, discontinuation, or dosage change in concomitant CYP3A4 or Pgp inducers or inhibitors that were known to affect TAC levels from 4 weeks pre-admission until the end of CDI treatment, was excluded. If a patient experienced multiple episodes of CDI, the first eligible episode of CDI was included. Patients were divided into 2 groups based on CDI treatment with metronidazole or vancomycin.

TAC trough levels

TAC levels were collected at 3 time points (Fig. 1) and normalized based on the patient's daily TAC dose (mg) to determine the dose-normalized trough level (ng/mL/mg) (1). A level documented within 4 weeks prior to the positive *C. difficile* assay was deemed the baseline level (t1); a second level was obtained prior to the initiation of antibiotic treatment and TAC dosage adjustment, but after CDI documentation (t2); and the third level was obtained after documented symptom resolution or hospital discharge without ongoing diarrhea, while antibiotic therapy was ongoing (t3). If multiple levels were drawn after symptom resolution, the last level drawn during the course of treatment, without record of TAC dosage change for at least 2 days prior, was chosen.

Statistical analysis

The primary hypothesis was that metronidazole is noninferior to vancomycin with respect to affecting TAC levels in transplant patients being treated for CDI. Because the observed changes in dose-normalized TAC levels were calculated in ratios, we transformed the dose-normalized TAC levels to the log scale by calculating the natural log of the data. The effect of antibiotic therapy on TAC levels was estimated by calculating the difference in log-transformed dose-normalized TAC levels after antibiotic treatment from baseline (Δt3–t1). A difference of 30%

or greater in Δt3-t1 between the metronidazole and vancomycin groups was determined to be clinically significant *a priori* by the investigative group. The criteria for noninferiority required that the upper limit of the 95% confidence interval (CI) was below the prespecified margin for the difference in Δt3-t1 between the groups (< 1.3). Noninferiority would therefore be established if the change in the log-transformed dose-normalized TAC level after metronidazole treatment, from baseline, was no more than 30% higher than the change seen with vancomycin.

To evaluate the impact of diarrhea alone on TAC trough levels, we also examined differences in log-transformed dose-normalized TAC levels from baseline to the time of CDI diagnosis, prior to initiation of antibiotic therapy and TAC dosage adjustment (Δt2–t1), for patients with available levels by using a paired *t*-test. Because two logarithmic values were subtracted for data analysis, Δt3–t1 and Δt2–t1, we followed the rules of logarithms [ln(x)-ln(y) = ln(x/y)] and calculated the ratio of the values in order to better describe the relationship between the 2 dose-normalized levels. Normal distribution of continuous variables was verified using the Shapiro–Wilk test. A *P*-value < 0.05 was considered significant. All statistical analyses were performed in SPSS version 22 (IBM, New York, New York USA).

Results

A total of 159 adult patients on TAC-based immunosuppression at the time of CDI diagnosis were screened for eligibility. Then 107 were excluded because of receiving treatment with both metronidazole and vancomycin, concomitant drug-drug interactions, changing TAC dosages prior to drawing trough levels, or inappropriately timed trough levels (Fig. 2). A total of 53 were included for study analysis: 35 patients were treated with metronidazole and 17 with vancomycin. Demographic and clinical characteristics were similar for the 2 groups, including age and white blood cell count (WBC) at CDI diagnosis, and serum creatinine (Scr) at baseline and at CDI diagnosis (Table 1). Patients in the vancomycin group were treated for an average of 21 days compared to 14 days for those in the metronidazole group ($P = 0.03$). Most patients were treated in the inpatient setting and did not meet criteria for severe infection (WBC ≥ 15,000 or an increase in Scr by ≥ 50% from baseline) (10). The majority of patients included were kidney transplant recipients, and all heart transplant patients were treated with vancomycin, reflecting the current practice at our center.

The average change in dose-normalized TAC levels from baseline (t1) to CDI treatment with metronidazole or vancomycin (t3) was similar between the two treatment groups (Table 2). Patients in both groups required dosage changes to maintain therapeutic TAC trough levels while receiving antibiotic treatment for CDI. Although not statistically significant between any 2 time points, there was a trend toward an increase in dose-normalized level for each group at the time of CDI diagnosis before treatment compared to baseline (Table 2). Patients treated with vancomycin had a wider range of dose-normalized TAC levels at t3, and on average these levels remained closer to the t2 levels compared to patients treated with metronidazole whose levels at t3 were closer to baseline (Fig. 3). Despite some variation in dose-normalized levels throughout treatment, the ratio of log-transformed dose-normalized TAC levels from baseline for patients treated with either metronidazole or vancomycin was approximately 1, indicating the dose patients required at the time of symptom resolution to achieve a specific trough level was very similar to their requirement at baseline (metronidazole: 0.99; vancomycin: 1.04). One patient, for example, who was treated with metronidazole was receiving a daily dosage of 16 mg at baseline to achieve a TAC trough level of 12.2 ng/mL (dose-normalized level: 0.76 ng/mL/mg), and a daily dosage of 14 mg at the time of symptom resolution to achieve a trough level of 10.8 ng/mL (dose-normalized level of 0.77 ng/mL/mg). Although this patient's daily dose decreased by 2 mg over the course of treatment, the patient's dose-normalized level did not change significantly. Similarly, 1 patient who was treated with vancomycin was receiving a daily dosage of 5 mg at baseline to achieve a TAC trough level of 6.0 ng/mL (dose-normalized level: 1.2 ng/mL/mg), and a daily dosage of 6 mg at the time of symptom resolution to achieve a trough level of 8.1 ng/mL (dose-normalized level of 1.35 ng/mL/mg). The change in the dose-normalized levels from baseline to the time of CDI treatment in the metronidazole group was not > 30% compared to the change in the vancomycin group (0.96; 95% CI: 0.74–1.24). Given that the upper limit of the 95% CI was <1.3, metronidazole demonstrated noninferiority with respect to its impact on TAC levels when compared to vancomycin.

Regarding the impact of CDI alone on TAC levels, no significant difference was found when comparing dose-normalized TAC levels from baseline (t1) to the time of CDI diagnosis before initiating antibiotic therapy (t2) in the entire study group (Table 3). In the 35 patients with a level drawn at the time of CDI diagnosis, the ratio of log-transformed dose-normalized TAC levels compared to baseline was 0.94 (95% CI: 0.83, 1.07; P = 0.37). Patients with a

history of prior CDI had higher mean dose-normalized TAC levels at every time point compared to those with no prior history of CDI (Table 4), although there was no significant difference in the change in dose-normalized TAC levels from baseline to symptom resolution between the 2groups (P = 0.15).

Discussion

The wide variability of both TAC bioavailability and impact of CDI that exists between patients was further emphasized in this study. The results of this study support that CDI can lead to unpredictable TAC levels, but demonstrate that the use of metronidazole does not further potentiate these effects.

Disease severity, as classified by our institutional guidelines, was similar in both groups reflecting the overuse of vancomycin in our transplant population with mild-moderate disease. Patients in both the vancomycin and metronidazole treatment groups experienced changes in dose-normalized TAC levels while they received CDI treatment. The magnitude of change in dose-normalized levels that was observed in the metronidazole group was not 30%, greater than that seen in the vancomycin group. This indicates that the variation in TAC levels that was observed in each group can most likely be attributed to the CDI itself rather than to a specific antibiotic effect. On average, almost no difference was seen in the dose-normalized TAC level in patients who were treated with either metronidazole or vancomycin at the time of symptom resolution compared to baseline. Patients therefore did not require significantly lower TAC doses to achieve therapeutic trough levels while taking metronidazole. This observation is contrary to what has been reported in prior case reports, which is that metronidazole leads to significantly elevated TAC levels (4, 5). However, our findings do support pharmacokinetic studies that suggest metronidazole is not a potent CYP3A4 or Pgp inhibitor, and therefore should not have a significant effect on the pharmacokinetics of TAC (6, 7). Considering that currently no mechanisms of significant drug interactions are known between vancomycin and TAC, the change in dosage requirement throughout the course of CDI seen in both groups is most likely a result of the variable impact of CDI on enterocyte integrity. Interestingly, the vancomycin group appeared to demonstrate a numerically higher mean dose-normalized trough level than the metronidazole group on treatment, although not statistically significant. Selection bias may have contributed, if more patients with severe CDI (based on criteria other than our institutional

guidelines) were treated with vancomycin. We were also unable to reliably quantify the amount of diarrhea patients were experiencing in the 2 groups which has been shown to have a significant impact on TAC levels (3). Patients who had suffered from a prior *C. difficile* infection had higher mean dose-normalized TAC levels at every time point, compared to those who were suffering from their first CDI. This finding may be associated with the effect of inflammatory cytokines on down-regulation of CYP3A4 hepatocyte activity. which has been previously described (11). No included cases met criteria for recurrent CDI, as all prior infections were diagnosed >4 weeks before the investigated CDI. This finding suggests that CDI may affect TAC bioavailability even after completion of antibiotic treatment and symptom resolution.

In contrast to the previous reports (1–3), our study did not show a significant increase in dose-normalized TAC levels at the time of CDI diagnosis compared to baseline. This comparison was done in an effort to show the impact diarrhea has on TAC bioavailability, which has previously been described as causing significant increases in trough levels. Although most patients in this study were considered to have mild-moderate disease, when analyzing only patients with severe disease, a significant difference also was not seen. This finding may have been a result of the inability to accurately quantify patient's diarrhea. Although surrogate markers, such as Scr, can be used to help identify the severity of a patient's dehydration, without quantifying the amount of diarrhea a patient is experiencing, we were unable to capture the true impact of diarrhea on TAC levels. In addition, only a small sample size was included in this analysis owing to a low number of appropriate trough levels drawn prior to initiating antibiotics, which could have contributed to the lack of significance seen.

This study had methodological limitations, including its retrospective design, which prevented us from being able to assess steady state TAC trough levels. In an effort to minimize this effect, dose-normalized trough levels were reported, and only trough levels that reflected at least 48 h of a new dosing regimen were included. In addition, medication adherence as outpatients could not be confirmed and it was assumed patients were taking TAC as prescribed in the medical record when calculating dose-normalized TAC levels. Also, diarrhea was not quantified in this study because of inconsistent and unreliable reporting of stool occurrences in the inpatient medical record and inclusion of outpatients. Finally, if a patient experienced CDI or

was treated with an interacting medication while at a facility outside of UMHS, this was unknown and thus could not be taken into account.

In conclusion, TAC levels varied significantly during treatment of CDI with either metronidazole or vancomycin and most patients required frequent dosage adjustments of TAC to maintain specific target levels. Regardless of the antibiotic used, TAC levels should be monitored closely and the dose should be adjusted accordingly. The results of this study suggest that concomitant treatment with metronidazole does not result in a > 30% increase in dose-normalized TAC levels compared to vancomycin treatment in patients experiencing CDI. Therefore, no reason exists to avoid the use of metronidazole in this patient population, solely for the purpose of avoiding a potential drug interaction with TAC. While TAC concentrations may increase throughout CDI, this may be related to the amount of diarrhea a patient is experiencing, which was not investigated directly in this study. The variability seen in TAC levels while patients were suffering from CDI was likely caused by the disease itself, rather than by antibiotic therapy. Patients with prior episodes of CDI may have greater absorption of TAC even after antibiotic treatment is completed. Therefore, close monitoring of TAC levels in these patients may be required to avoid supratherapeutic levels. To our knowledge, this is the largest study to date that has investigated the impact of CDI on TAC levels as well as the proposed interaction between metronidazole and TAC. A future study, investigating how often patients are in the goal therapeutic range when suffering from diarrhea or being treated with antibiotics, may be even more beneficial in this patient population than determining a specific difference between groups.

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Author contributions: C.R.E. contributed significantly to the data collection and analysis, study design, and critical revision of the article. J.M.P. contributed significantly to the study design, data analysis, and critical revision of the article. M.P. D. and K.T.P. contributed to the study design and critical revision of the article. S.M.H. contributed significantly to the study design, data collection and analysis, and critical revision of the article.

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Figure Legends:

Fig. 1. Tacrolimus trough collection. The 3 time points from which tacrolimus trough levels were utilized in this study, at baseline (t_1) prior to CDI infection, after the onset of diarrhea and prior to initiation of antibiotic therapy (t_2), and after symptom resolution while remaining on antibiotic therapy (t_3). CDI, *Clostridium difficile* infection.

Fig 2. Study design. The selection for patients into the study based on inclusion and exclusion criteria. CDI, *Clostridium difficile* infection.

Fig. 3. Dose-normalized tacrolimus trough levels. The median and interquartile range of dose-normalized tacrolimus trough levels (ng/mL/mg) in all patients included in the study based on antibiotic therapy with either metronidazole or vancomycin at each of the 3 time points compared in the study. CDI, *Clostridium difficile* infection.

Patient demographics

Characteristic	Metronidazole (N = 35)	Vancomycin (N = 17)
Age, years	55 ± 11	56 ± 14
Gender, n (%)		
Male	16 (46)	11 (65)
Female	19 (54)	6 (35)
Organ type, n (%)		
Kidney	23 (65.7)	6 (35.3)
Liver	9 (25.7)	6 (35.3)
Heart	0	4 (23.5)
Lung	3 (8.6)	0
Heart/Kidney	0	1 (5.9)
Time after transplant, days	118 (44–332)	233 (142–374)
Inpatient treatment, n (%)	26 (74)	14 (82)
Index CDI, n (%)	26 (74)	9 (53)
Baseline Scr, mg/dL	1.7 ± 1.2	1.6 ± 0.8
Scr at diagnosis, mg/dL	2.0 ± 1.2	1.8 ± 0.7
WBC at diagnosis, K/mm^3	7.6 ± 4.6	8.4 ± 4.5
Disease severity, n (%)		
Mild/moderate	27 (77)	14 (82)
Severe	8 (23)	3 (18)
Treatment duration, days	14.3 ± 5.2*	21.3 ± 17.4*

CDI, *Clostridium difficile* infection; Scr, serum creatinine;

WBC, white blood cell count.

Table 1

Dose-normalized tacrolimus trough levels

	Metronidazole (N = 35)	Vancomycin (N = 17)
Dose-normalized tacrolimus trough (ng/mL/mg)		
Baseline (t1)	1.4 ± 1.1	1.6 ± 0.8
CDI diagnosis before treatment (t2)	1.7 ± 1.3	1.9 ± 1.1
Symptom resolution on treatment (t3)	1.4 ± 0.9	1.8 ± 1.1
Difference from baseline (Δt3–t1)	0.99	1.04

Baseline (t_1) prior to *Clostridium difficile* infection (CDI); after the onset of diarrhea and prior to initiation of antibiotic therapy (t_2); and after symptom resolution while remaining on antibiotic therapy (t_3).

Table 2

Dose-normalized tacrolimus trough levels at CDI diagnosis

	Baseline (N = 35)	CDI diagnosis before treatment (N = 35)	Mean difference (95% CI)
Dose-normalized tacrolimus trough (ng/mL/mg)	1.4 ± 1.7	1.5 ± 1.9	0.94 (0.83–1.07)

CDI, *Clostridium difficile* infection; CI, confidence interval.

Table 3

Dose-normalized tacrolimus trough levels for patients with index and repeat *Clostridium difficile* infection (CDI)

	Index CDI (N = 35)	Repeat CDI (N = 17)
Dose-normalized tacrolimus trough (ng/mL/mg)		
Baseline (t_1)	1.2 ± 1.8	1.5 ± 1.7
CDI diagnosis before treatment (t_2)	1.3 ± 2.0	1.8 ± 1.7
Symptom resolution on treatment (t_3)	1.1 ± 1.8*	1.7 ± 1.9*
Difference from baseline (Δt3–t1)	0.95	1.14

** ? {Au: what does * signify)*

Baseline (t_1) prior to CDI infection; after the onset of diarrhea and prior to initiation of antibiotic therapy (t_2); and after symptom resolution while remaining on antibiotic therapy (t_3).

Table 4

Figure 1: Tacrolimus Trough Collection

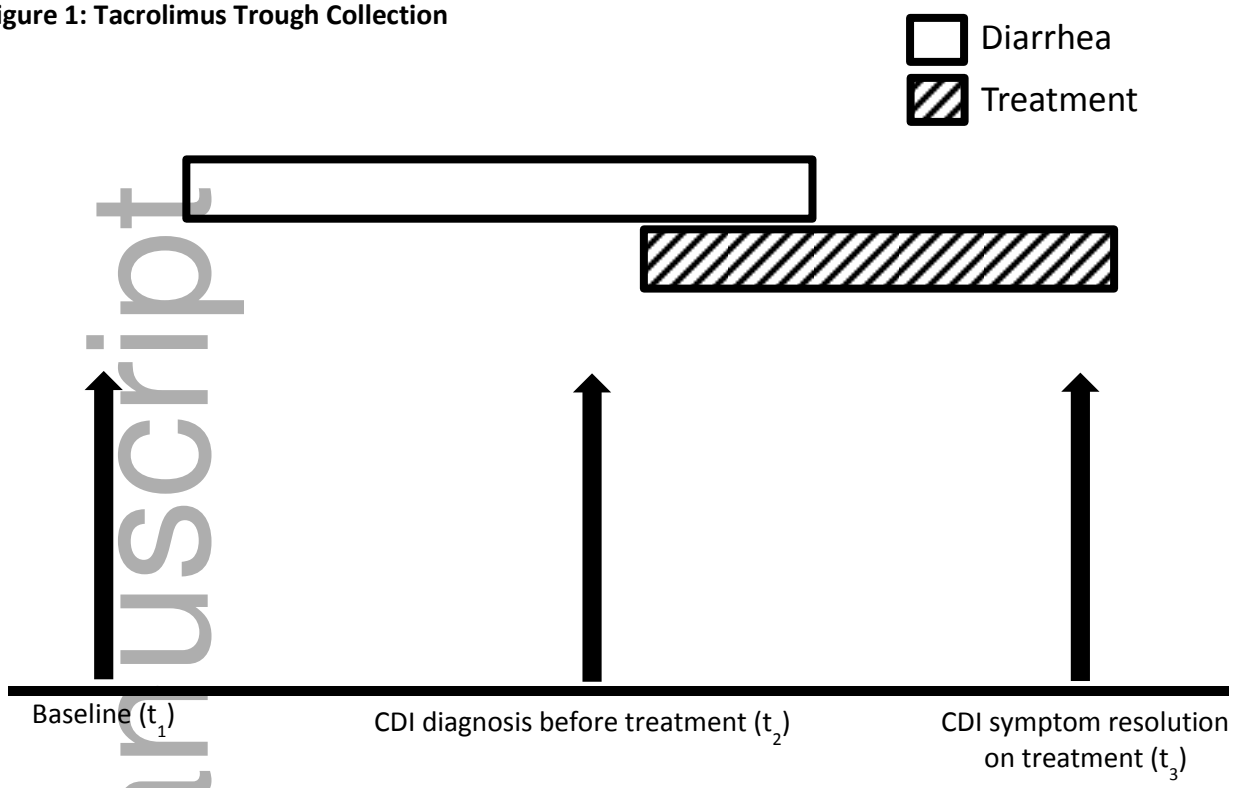

Figure 2: Study Design

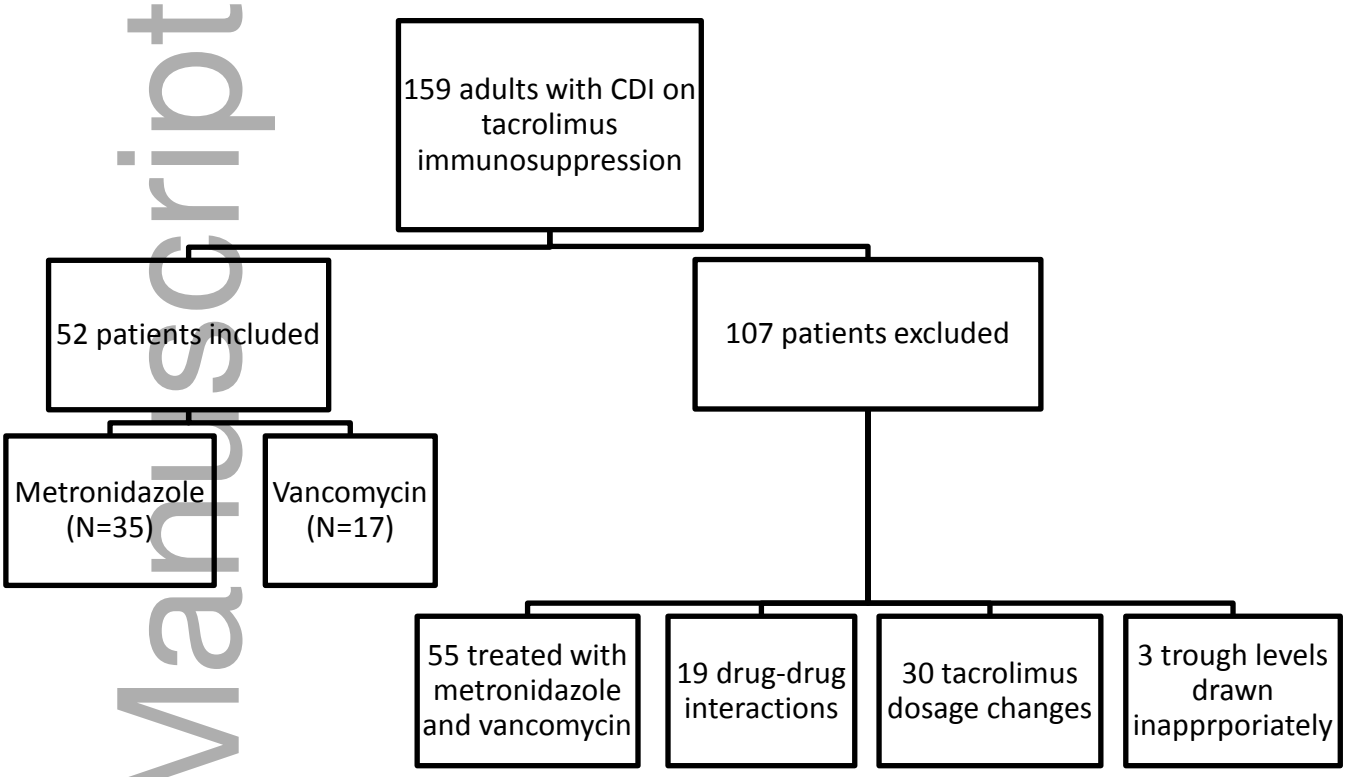

Figure 3: Dose-Normalized Tacrolimus Trough Levels