

## Research Article

# Effect of Voriconazole on Tacrolimus Blood Concentration in Renal Transplant Recipients after Voriconazole Discontinuation

Lijuan Feng <sup>1</sup>, Guiyi Liao <sup>2</sup>, Hui Liu <sup>3</sup>, Yihou Luo <sup>3</sup>, Chunlan Yang <sup>1</sup>, Yan Du <sup>1</sup>,  
Dujuan Xu <sup>1</sup> and Su Yong <sup>1,3</sup>

<sup>1</sup>Department of Pharmacy, The First Affiliated Hospital of Anhui Medical University, Hefei, China

<sup>2</sup>Department of Urology, The First Affiliated Hospital of Anhui Medical University, Hefei, China

<sup>3</sup>School of Pharmacy, Anhui Medical University, Hefei, China

Correspondence should be addressed to Dujuan Xu; [xudujuan6365@163.com](mailto:xudujuan6365@163.com) and Su Yong; [272726955@qq.com](mailto:272726955@qq.com)

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*What is Known and Objective.* Voriconazole (VRC) increases the blood concentration of Tacrolimus (TAC). However, the patterns of changes in TAC trough concentration (TAC C<sub>0</sub>) and dose-adjustment regimens after VRC discontinuation have not been reported. We aimed to explore the changing pattern of TAC C<sub>0</sub> after VRC discontinuation and provide strategies for TAC dose adjustment and blood concentration monitoring in renal transplant recipients. *Methods.* The clinical data of 46 renal transplant patients pre- and during VRC medication and VRC discontinuation were retrospectively recorded, including doses and concentrations of TAC and VRC; biochemical indicators such as liver and kidney function; and CYP3A5, CYP3A4, and CYP2C19 gene types. *Results and Discussion.* After discontinuing VRC for 2–4 days, 81% of the patients returned to their initial TAC dose, although TAC C<sub>0</sub> and TAC dose-adjusted trough concentration (C/D) were 2.43-fold and 3.35-fold higher, respectively, than pre-VRC administration. After 5–7 days, TAC C<sub>0</sub> and C/D gradually recovered. TAC C/D was significantly higher after VRC discontinuation when the VRC trough concentration (VRC C<sub>0</sub>) was greater than 2.43 mg/L; CYP3A5, CYP3A4, and CYP2C19 genotypes and the administration of erythromycin did not affect the change in TAC C/D. *What is New and Conclusion.* TAC C/D remains elevated 2–4 days after discontinuing VRC compared to pre-VRC administration, with gradual recovery observed 5–7 days after VRC discontinuation. To avoid excessive blood TAC C<sub>0</sub>, the initial TAC dose should not be immediately reinstated upon VRC discontinuation for 2–4 days. VRC C<sub>0</sub> are a critical factor influencing the change in TAC C/D ratio after VRC discontinuation.

## 1. Introduction

Kidney transplantation is the preferred treatment for end-stage renal disease owing to its positive impact on quality of life. According to the Global Observatory on Donation and Transplantation, more than 100,000 kidney transplants are performed annually worldwide [1]. Clinical guidelines recommend Tacrolimus (TAC or FK506) as a first-line immunosuppressant for renal transplantation. It effectively reduces organ rejection, leading to improved graft survival and patient quality of life [2]. However, TAC has a narrow therapeutic window, with underexposure increasing the risk of acute rejection and overexposure

leading to serious adverse effects such as nephrotoxicity, neurotoxicity, and hyperglycemia. Therefore, the guidelines recommend routine monitoring of tacrolimus trough concentrations (TAC C<sub>0</sub>). In triple therapy regimens with TAC, mycophenolate mofetil, and glucocorticoid, maintaining TAC C<sub>0</sub> at 8–15 ng/ml is recommended for 0–3 months postsurgery [3]. Inpatient variability reflects fluctuations in trough levels over a specific time interval [4]. Inpatient variability of TAC C<sub>0</sub> increases the risk of poor prognosis after transplantation [5, 6]. Thus, maintaining TAC C<sub>0</sub> in the therapeutic window and minimizing the fluctuation of TAC C<sub>0</sub> in clinical treatment is crucial.

Intra- and interindividual variations in pharmacokinetics (PK) complicate the clinical application of TAC. This is attributed to the combination of multiple factors, such as demographic factors and drug-drug interactions [7]. Furthermore, there is a considerable genetic basis for interindividual variability in TAC PK, with TAC being a substrate for CYP3A4/5. CYP 3A5 nonexpressors (CYP 3A5 \*3/\*3) have 1.5-2-fold higher TAC dose-adjusted trough concentrations (C/D) and lower TAC dose requirements compared to CYP 3A5 expressors (CYP3A5 \*1/\*1 or CYP 3A5 \*1/\*3) [8]. CYP3A4\*1G (rs2242480, 20239G > A) has a relatively high frequency in the Chinese population and is significantly associated with TAC C/D in liver and kidney transplant recipients [9].

Owing to long-term immunosuppressive treatment, renal transplant recipients are at an increased risk for invasive aspergillosis [10]. In a case-control study using United States Renal Data System Data, invasive aspergillosis was associated with a 5.02 times greater risk of 1-year mortality and a 3.37 times greater risk of 1-year allograft failure [11]. Voriconazole (VRC) is the first-line treatment for invasive aspergillosis [12]. It is an inhibitor of CYP3A4 and therefore inevitably leads to drug-drug interactions with TAC in patients [13]. According to FDA drug labeling, when VRC is used concomitantly with TAC, the TAC dose should be reduced to 1/3 or lower, and blood concentration monitoring should be performed more frequently. However, the recovery of metabolic enzyme activity may take longer. Itraconazole, which acts on TAC metabolism similarly to VRC, continued to have effects on metabolic enzymes for more than 10 days after discontinuation [14]. A similar effect of VRC on metabolic inhibition was observed. Vanhove et al. showed that after 90 days of combined treatment with TAC and VRC, TAC doses were increased immediately upon VRC discontinuation; however, it took one week after VRC discontinuation for TAC C<sub>0</sub> to return to precombination levels [15]. This suggests the possibility of TAC C<sub>0</sub> exceeding the upper limit of the therapeutic window and fluctuations in TAC C<sub>0</sub> after VRC discontinuation, of which our understanding is still lacking.

Herein, we aimed to investigate TAC dose adjustment, fluctuations in TAC C<sub>0</sub> after VRC discontinuation, and the factors influencing these changes in renal transplant patients. Our study provides a basis for TAC dose adjustments and blood concentration monitoring after VRC discontinuation.

## 2. Methods

**2.1. Study Design and Population.** This study was conducted using data from the Department of Kidney Transplantation of the First Affiliated Hospital of Anhui Medical University and was approved by the Ethics Committee of our hospital (approval number: Quick-PJ-2023-8-43). None of the kidney donors were executed prisoners.

The clinical and laboratory data were collected between September 2019 and June 2021. The inclusion criteria were as follows: (1) after the first kidney transplantation; (2) treatment with a triple immunosuppression therapy regimen of

TAC, mycophenolate mofetil/macrolide sodium enteric tablets, and glucocorticoids; (3) concomitant administration of TAC and VRC for at least 5 days; and (4) availability of at least two TAC concentrations within 1 week of voriconazole discontinuation. The exclusion criteria were as follows: (1) concomitant with other drugs that could significantly influence the PK of tacrolimus (such as fluconazole, posaconazole, diltiazem, and Wuzhi preparations) except erythromycin after VRC discontinuation. (2) Those with abnormal liver function test results. Demographic and clinical data were collected using the Laboratory Information System and Hospital Information System. We retrospectively collected clinical data on 200 renal transplant patients treated with a combination of TAC and VRC. Most patients were excluded due to a lack of TAC concentrations within one week of voriconazole discontinuation, and only 46 patients were ultimately included.

**2.2. Administration of Immunosuppressants and VRC.** Oral TAC (Astellas Ireland Co., Ltd., Kerry, Ireland) was administered approximately 2 days after transplantation surgery at a dose of 2.5 mg every 12 h to achieve an target range (8–15 ng/mL) in the early postoperative months (0–3). Patients with suspected or confirmed fungal infections initially received intravenous administration of VRC (Jincheng Hess Pharmaceutical Co., Shanxi, China), followed by a switch to oral VRC (Yangtze River Pharmaceutical Group Co., Ltd., Jiangsu, China) at a dose of 200 mg every 12 h. After the concomitant administration of VRC, the TAC dose was reduced and adjusted according to the TAC C<sub>0</sub>. The data were divided into four groups according to the chronological order of VRC administration: before VRC coadministration (pre-VRC), concomitant with VRC (Co-VRC), 2–4 days after VRC discontinuation (Post-VRC 2–4 d), and 5–7 days after VRC discontinuation (Post-VRC 5–7 d). Missing drug dose values were not imputed for TAC C/D statistical analysis.

**2.3. Tacrolimus and Voriconazole Plasma Concentration Measurement.** TAC C<sub>0</sub> and VRC trough concentration (VRC C<sub>0</sub>) of were determined using a Viva-E EMIT® blood concentration analyzer (Siemens, Germany) and a 2D liquid chromatograph (Demeter, China), respectively. Samples were collected on day 3 after TAC dose adjustment and on day 5 after VRC administration, allowing both blood concentrations to reach homeostasis. To account for TAC C<sub>0</sub> exceeding the upper limit of detection (30 ng/mL), we substituted a value of 30 ng/mL for those instances in the analysis.

**2.4. Genotyping of CYP3A4/5 and CYP2C19.** Genomic DNA was extracted from the blood using the QIAGEN genomic DNA extraction kit (Cat#69504, QIAGEN, Dusseldorf, Germany) and stored at –20°C. An improved multiplex-ligase detection reaction was used for genotyping. The following single nucleotide polymorphisms were detected: CYP3A4\*1G (rs2242480,20239G > A), CYP3A5\*3

(rs776746, 6986A > G), CYP2C19\*2 (rs4244285, 681G > A), and CYP2C19\*3 (rs4986893, 636G > A). The metabolic types were specified according to genotype. CYP3A5 metabolites are divided into CYP3A5 expressors (\*1/\*1 or \*1/\*3) and CYP3A5 nonexpressors (\*3/\*3). CYP3A4 metabolic types are divided into fast (\*1G/\*1G or \*1/\*1G) and slow metabolism (\*1/\*1). CYP2C19 metabolic types are divided into extensive (CYP2C19\*1/\*1), intermediate (IMs, \*1/\*2, \*1/\*3), and slow metabolic (PMs, \*2/\*2, \*2/\*3, or \*3/\*3) types.

**2.5. Statistical Analysis.** The Kolmogorov–Smirnov test was used to test the normality of numerical variables. According to their normality, numerical statistical variables were presented as mean  $\pm$  standard deviation (SD) or median and interquartile range (IQR). The paired *t*-test, Wilcoxon paired signed-rank test, Mann–Whitney *U* test, and Kruskal–Wallis *H* test were chosen according to each applicable condition. The test level  $\alpha = 0.05$  and bilateral  $P < 0.05$  were considered statistically significant. All analyses were performed using the IBM SPSS Statistics version 26 (IBM, New York, NY, USA). Figures were generated using GraphPad Prism software (version 8.0; San Diego, CA, USA).

### 3. Results

**3.1. Patient Characteristics.** This study included 46 kidney transplant patients. The demographic and genotypic distributions of the study population are shown in Table 1. The frequencies of the CYP3A5\*3, CYP3A4\*1G, CYP2C19\*2, and CYP2C19\*3 alleles were distributed in concordance with the Hardy–Weinberg equilibrium. As shown in Table 2, no significant differences were found between the Co-VRC and Post-VRC groups with respect to liver function, kidney function, routine blood tests, or CRP levels ( $P > 0.05$ ).

**3.2. Effect of VRC Discontinuation on TAC Daily Dose (D),  $C_0$ , C/D.** The initial daily dose of TAC was 5 mg in all patients. TAC  $C_0$  was maintained within the target range (8–15 ng/ml) in 32.6% (15/46) of patients; 6.5% (3/46) of patients exceeded 15 ng/ml. The mean TAC  $C_0$  was 7.15 ng/ml. Following concomitant administration of VRC, the daily dose of TAC was reduced to 0–2 mg in 73.9% (34/46) of patients, to 2–4 mg in 19.6% (9/46) of patients, and remained at 4–5 mg in only three patients. TAC  $C_0$  was maintained within the target range in 26.1% (12/46) of patients; 67.4% (31/46) of patients exceeded 15 ng/ml (two cases exceeded the upper limit of detection). The mean  $C_0$  of TAC was 17.72 ng/ml, which was 2.40-fold higher than that in the pre-VRC treatment group ( $P < 0.01$ ). This is consistent with previous findings [16]. After discontinuation of VRC for 2–4 days, the TAC dosage returned to 4–5 mg/d in 81.1% of patients (30/37; nine patients had missing dose data). However, a higher percentage of patients (73.0%, 27/37) had TAC  $C_0 > 15$  ng/ml; 10 patients exceeded the upper limit of detection. The mean  $C_0$  of TAC was 18.80 ng/ml, which was 2.73-fold higher than that in the pre-VRC treatment group ( $P < 0.01$ ). Owing to excessive blood concentration,

TABLE 1: Characteristics and genotype distribution of renal transplant recipients.

Characteristics	Value
Gender ( <i>n</i> , male/female)	32/14
Age (years)	39.22 $\pm$ 11.94
Body weight (kg)	56.64 $\pm$ 10.53
Genotype, <i>n</i> (%)	
CYP3A5*1/*1 or *1/*3	15 (35.7%)
CYP3A5*3/*3	27 (64.3%)
CYP3A4*1/*1	26 (61.9%)
CYP3A4*1/*1G or *1G/*1G	16 (38.1%)
CYP2C19*1/*1	22 (52.4%)
CYP2C19*1/*2 or *1/*3	14 (33.3%)
CYP2C19*2/*2 or *2/*3	6 (14.3%)

Note. Age and weight represented as mean  $\pm$  SD.

the dose of TAC returned to the initial dose in only 60% (18/30; 16 patients had missing dose data) of the patients after discontinuation of VRC for 5–7 days. The remaining 40% of patients returned to half of the initial dose or even lower, while maintaining TAC  $C_0$  within the target range (Figures 1(a) and 1(b)). Subsequently, we compared TAC  $C_0$  before and after VRC concomitant treatment in two and four groups using paired analysis, and the results were consistent with those shown in Figure 1(b); However, the amount of data was lower when four groups were compared ( $n = 21$ ) (Figures 1(c) and S1A).

We further compared trends in TAC C/D before and after concomitant VRC treatment. Concomitant VRC treatment significantly elevated TAC C/D by nearly 18-fold compared to pre-VRC treatment. After discontinuation of VRC for 2–4 d, TAC C/D values decreased but remained more than three times higher than with concomitant VRC treatment ( $P < 0.01$ ). After discontinuation of VRC for 5–7 d, TAC C/D gradually returned to normal levels (Figure 2(a)). Similar conclusions were obtained from the paired tests between two and four groups (Figures 2(b) and S1B). Although the sample size was small ( $n = 21$ ) due to the exclusion of patients with missing values when the four groups were paired for comparison, the same trends in TAC  $C_0$  and C/D were obtained. Our data indicate that even after 2–4 days of VRC discontinuation, VRC continues to have a strong inhibitory effect on TAC metabolism. Thus, immediate resumption of TAC dosing following VRC discontinuation may result in TAC  $C_0$  exceeding the upper limit of the therapeutic window and increased  $C_0$  fluctuations.

**3.3. Effect of Coadministration of Erythromycin after VRC Discontinuation on Daily Dose,  $C_0$ , and C/D of TAC.** Erythromycin weakly inhibits CYP3A4 expression [17]. For some patients with low TAC concentrations, coadministration of erythromycin is a common therapeutic strategy to increase TAC  $C_0$  [18]. In our study data, some patients received a combination therapy with erythromycin immediately after VRC discontinuation. To further clarify whether the inhibition of TAC metabolism after VRC discontinuation was related to the coadministration of

TABLE 2: Baseline laboratory test results before and after VRC discontinuation.

Parameter	Co-VRC	Post-VRC	<i>t</i> / <i>Z</i>	<i>P</i>
ALB(g/L) <sup>a</sup>	36.23 ± 5.12	37.88 ± 6.39	-1.310	0.197
TBIL(μmol/L) <sup>b</sup>	9.70 (7.95, 13.40)	10.00 (8.10, 13.13)	-0.920	0.358
ALT (U/L) <sup>b</sup>	20.00 (16.00, 32.25)	24.50 (17.00, 49.25)	-1.559	0.119
AST (U/L) <sup>b</sup>	18.00 (14.00, 28.00)	20.50 (15.75, 44.25)	-0.961	0.337
CRE (μmol/L) <sup>b</sup>	210 (130.05, 383.65)	180.75 (107.40, 329.35)	-1.797	0.072
CCR (mL/min) <sup>b</sup>	32.50 (14.75, 57.25)	41.00 (19.00, 72.50)	-1.338	0.181
u-hsCRP (mg/L) <sup>b</sup>	4.09 (1.93, 10.38)	4.19 (1.67, 28.77)	-0.845	0.398
HB (g/L) <sup>a</sup>	88.65 ± 13.82	87.69 ± 15.14	0.371	0.713
HCV (%) <sup>a</sup>	26.92 ± 4.17	26.87 ± 4.60	-0.053	0.958

Albumin (ALB), total bilirubin (TBIL), glutamic aminotransferase (ALT), glutamic oxalacetic aminotransferase (AST), CRE (blood creatinine), CCR (creatinine clearance), C-reactive protein (u-hsCRP), hemoglobin (HB), and erythrocyte specific volume (HCV). <sup>a</sup>the paired *t*-tes, mean ± SD; <sup>b</sup>the Wilcoxon signed-rank test, median (IQR).

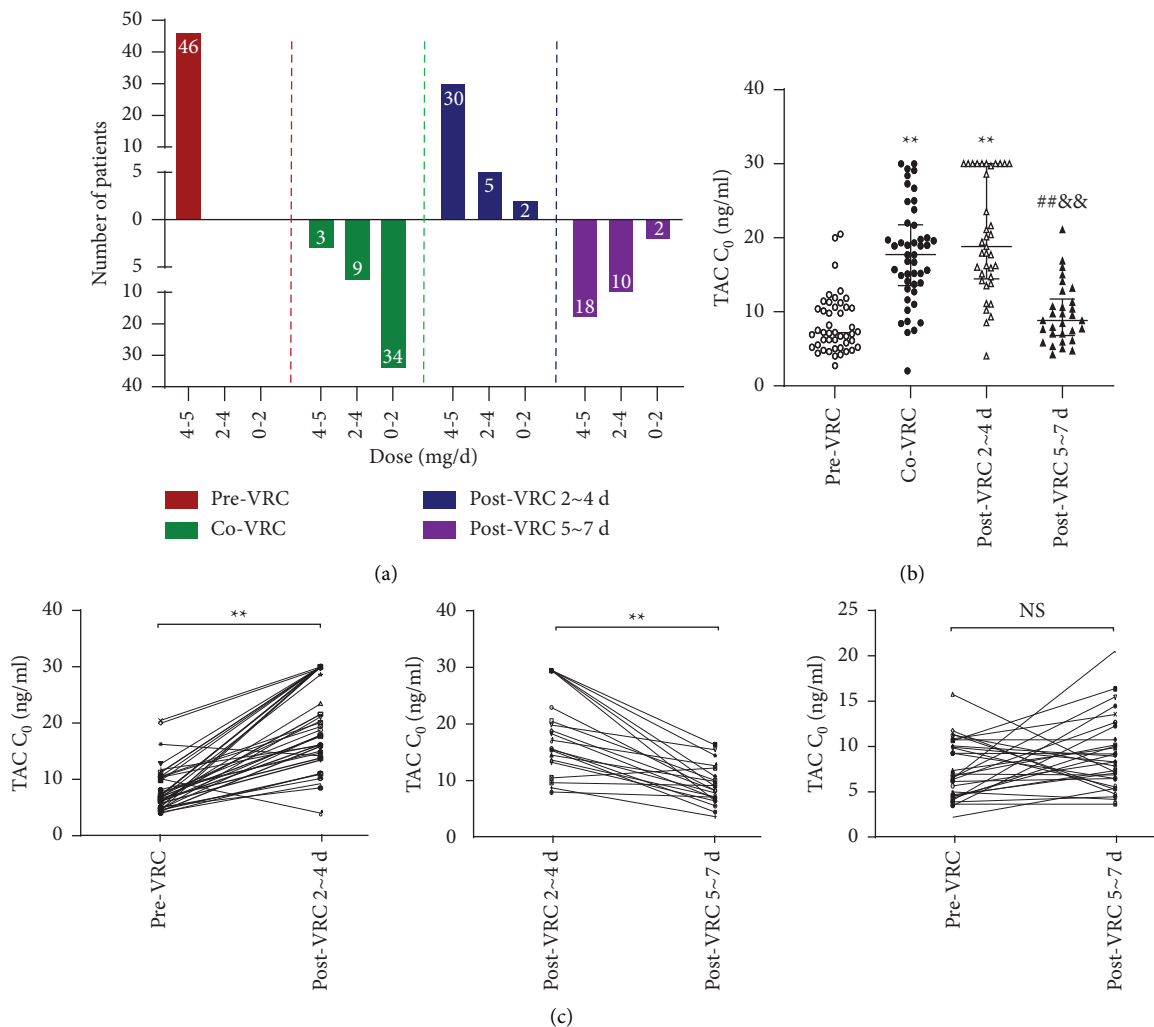


FIGURE 1: (a) Frequency distribution of TAC dose before and after concomitant VRC treatment. (b) Comparison of TAC C<sub>0</sub> between different groups before and after concomitant VRC treatment,  $P^{**} < 0.01$  compared with the Pre-VRC treatment,  $P^{##} < 0.01$  compared with the Co-VRC treatment, and  $P^{\&\&} < 0.01$  compared with the Post-VRC 2-4d. (c) Comparison of TAC C<sub>0</sub> between each two groups before and after concomitant VRC treatment using paired analysis,  $P^{**} < 0.01$ .

erythromycin, we divided the 46 patients into two groups: patients who were ( $n=24$ ) and were not ( $n=22$ ) coadministered with erythromycin after VRC discontinuation for further subgroup analysis.

After discontinuation of VRC for 2–4 days, regardless of whether erythromycin was coadministered, TAC C<sub>0</sub> was significantly higher than pre-VRC treatment ( $P < 0.01$ ). Following VRC discontinuation for 5–7 days, TAC C<sub>0</sub>

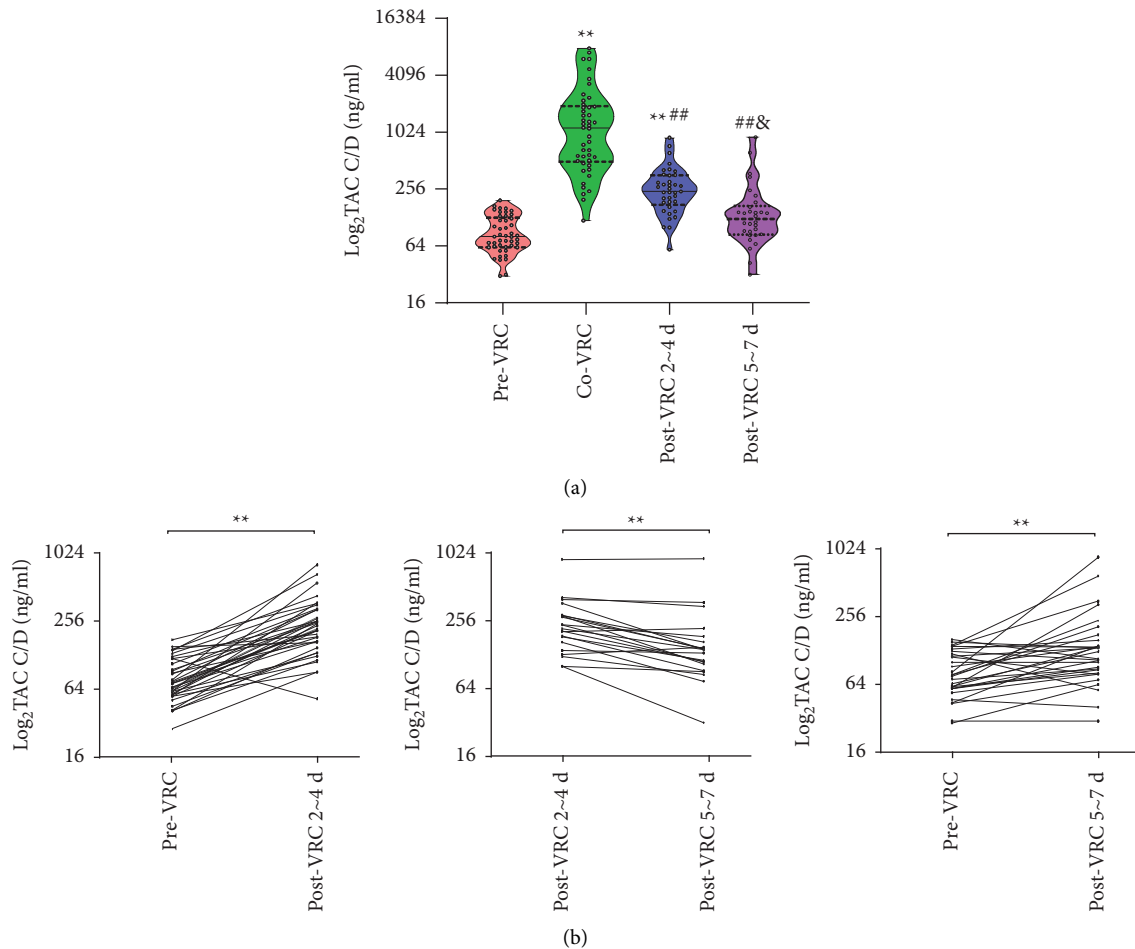


FIGURE 2: (a) Comparison of TAC C/D between different groups before and after concomitant VRC treatment,  $P^{**} < 0.01$  compared with the Pre-VRC treatment,  $P^{##} < 0.01$  compared with the Co-VRC treatment, and  $P^{\&} < 0.05$  compared with the Post-VRC 2-4d. (b) Comparison of TAC C/D between each two groups before and after concomitant VRC treatment using paired analysis,  $P^{**} < 0.01$ . Note: The vertical axis is taken as  $\log_2$  logarithm.

gradually returned to the pre-VRC treatment level in patients not coadministered erythromycin but was still higher than pre-VRC treatment in patients coadministered erythromycin ( $P < 0.05$ ) (Figure 3(a)). We further compared TAC  $C_0$  before and after VRC concomitant treatment in two and four groups by paired analysis, and the results were consistent with those in Figure 3(a); the amount of data was lower when four groups were compared (patients not coadministered erythromycin,  $n = 11$ ; patients coadministered erythromycin,  $n = 10$ ) (Figures 3(b) and S2A).

Subsequently, we compared the trends in TAC C/D before and after concomitant VRC treatment. After discontinuation of VRC for 2–4 days, regardless of whether erythromycin was coadministered, TAC C/D was significantly higher than pre-VRC treatment ( $P < 0.05$ ). After VRC discontinuation for 5–7 days, TAC C/D decreased further and gradually returned to normal levels in patients not coadministered erythromycin but was still higher than the pre-VRC treatment in patients coadministered erythromycin, with no statistically significant difference (Figure 4(a)). However, after VRC discontinuation for 5–7 days, TAC C/D was significantly higher than pre-VRC treatment in patients

coadministered with erythromycin ( $P < 0.01$ ) in the two-group paired analysis (Figure 4(b)). Similar conclusions were obtained from the paired tests between the four groups (Figure S2B).

**3.4. Effect of CYP3A4/5 Genotypes on C/D of TAC.** To investigate the potential effect of the CYP3A4/5 genotype on TAC C/D after VRC discontinuation, 42 patients were determined to have the CYP3A4/5 genotype and divided into different groups based on the different genotypes. We compared trends in TAC C/D before and after VRC treatment based on different genotypes. The results showed similar trends in TAC C/D in patients with different genotypes of CYP3A4/5. TAC C/D was significantly higher 2–4 days after VRC discontinuation than pre-VRC treatment, whereas TAC C/D recovered gradually after VRC discontinuation for 5–7 d (Figure 5(a)).

Further analysis revealed no significant differences in TAC C/D between CYP3A5-expressers and non-expressers before and after concomitant VRC treatment (Figure 5(a)). However, TAC C/D was significantly lower in the

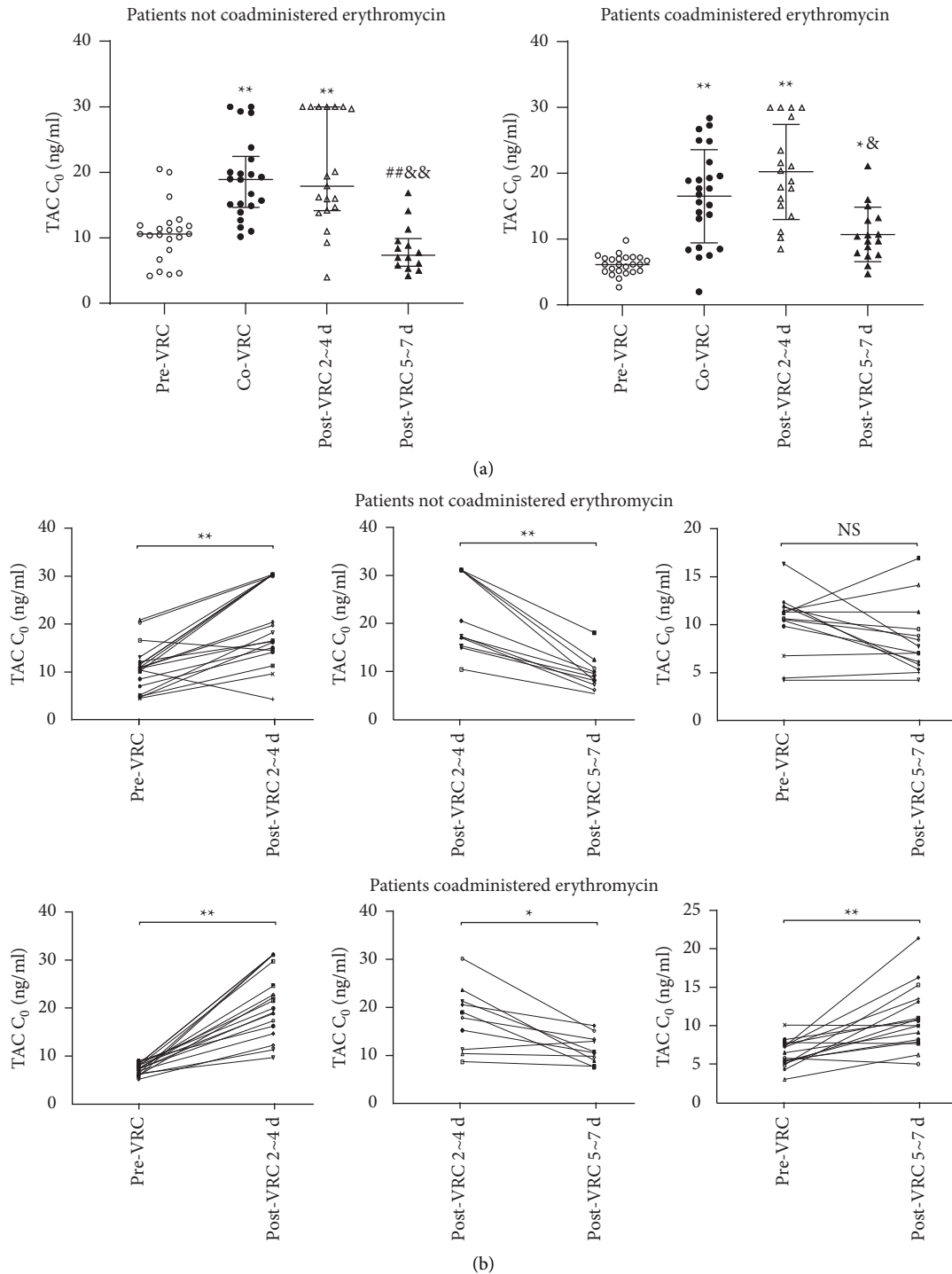


FIGURE 3: (a) Comparison of TAC C<sub>0</sub> between different groups before and after concomitant VRC treatment,  $P^* < 0.05$  or  $P^{**} < 0.01$  compared with the Pre-VRC treatment,  $P^{\#\#} < 0.01$  compared with the Co-VRC treatment, and  $P^{\&\&} < 0.05$  or  $P^{\&\&\&} < 0.01$  compared with the Post-VRC 2~4d. (b) Comparison of TAC C<sub>0</sub> between each two groups before and after concomitant VRC treatment using paired analysis,  $P^* < 0.05$  or  $P^{**} < 0.01$ .

CYP3A4\*1G allele than in the CYP3A4\*1/\*1 genotype group before VRC coadministration ( $P < 0.05$ ; Figure 5(a)). This suggested that the sustained inhibitory effect on TAC metabolism after VRC discontinuation was independent of the CYP3A4/5 genotype.

**3.5. Effect of CYP2C19 Genotypes and VRC Concentration on C/D of TAC.** The CYP2C19 genotype influences the TAC metabolism when VRC and TAC are combined; therefore, we further analyzed the role of the CYP2C19 genotype on the metabolism of TAC after VRC

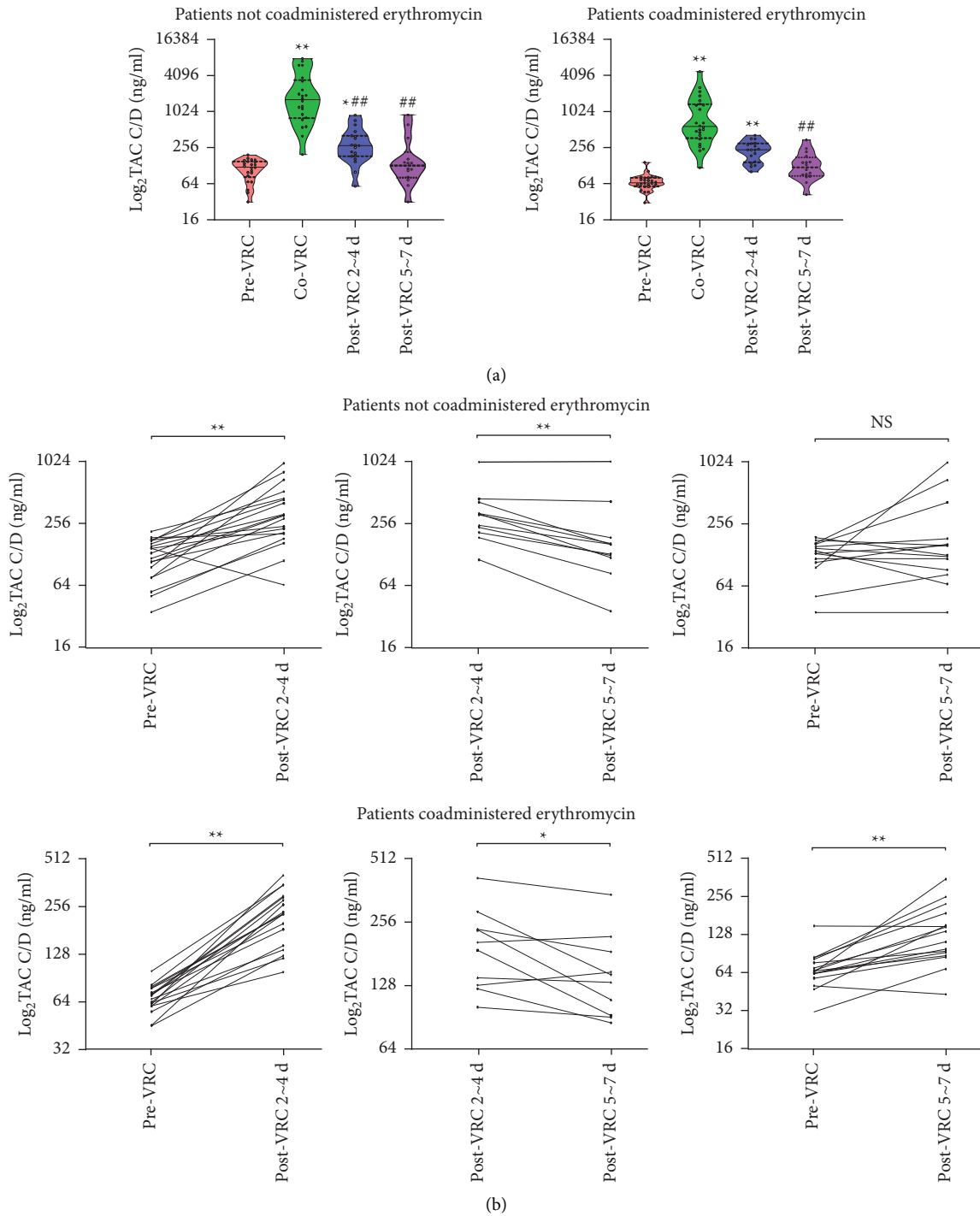


FIGURE 4: (a) Comparison of TAC C/D between different groups before and after concomitant VRC treatment,  $P^* < 0.05$  or  $P^{**} < 0.01$  compared with the Pre-VRC treatment,  $P^{##} < 0.01$  compared with the Co-VRC treatment, and  $P^{\&} < 0.05$  or  $P^{\&\&} < 0.01$  compared with the Post-VRC 2-4d. (b) Comparison of TAC C/D between each two groups before and after concomitant VRC treatment using paired analysis,  $P^* < 0.05$  or  $P^{**} < 0.01$ . Note: the vertical axis is taken as log2 logarithm.

discontinuation. Owing to the small number of CYP2C19 PMs, we placed them together with IMs for further analysis. We compared the trends in TAC C/D before and after VRC treatment for both CYP2C19 genotypes. Similar trends in TAC C/D in patients with different CYP2C19 genotypes were observed (Figure 5(b)). There

were also no significant differences in TAC C/D between the CYP2C19 PM/IM and CYP2C19EM groups before and after concomitant VRC treatment (Figure 5(b)). Hence, the sustained inhibitory effect on TAC metabolism after VRC discontinuation is independent of the CYP2C19 genotype.

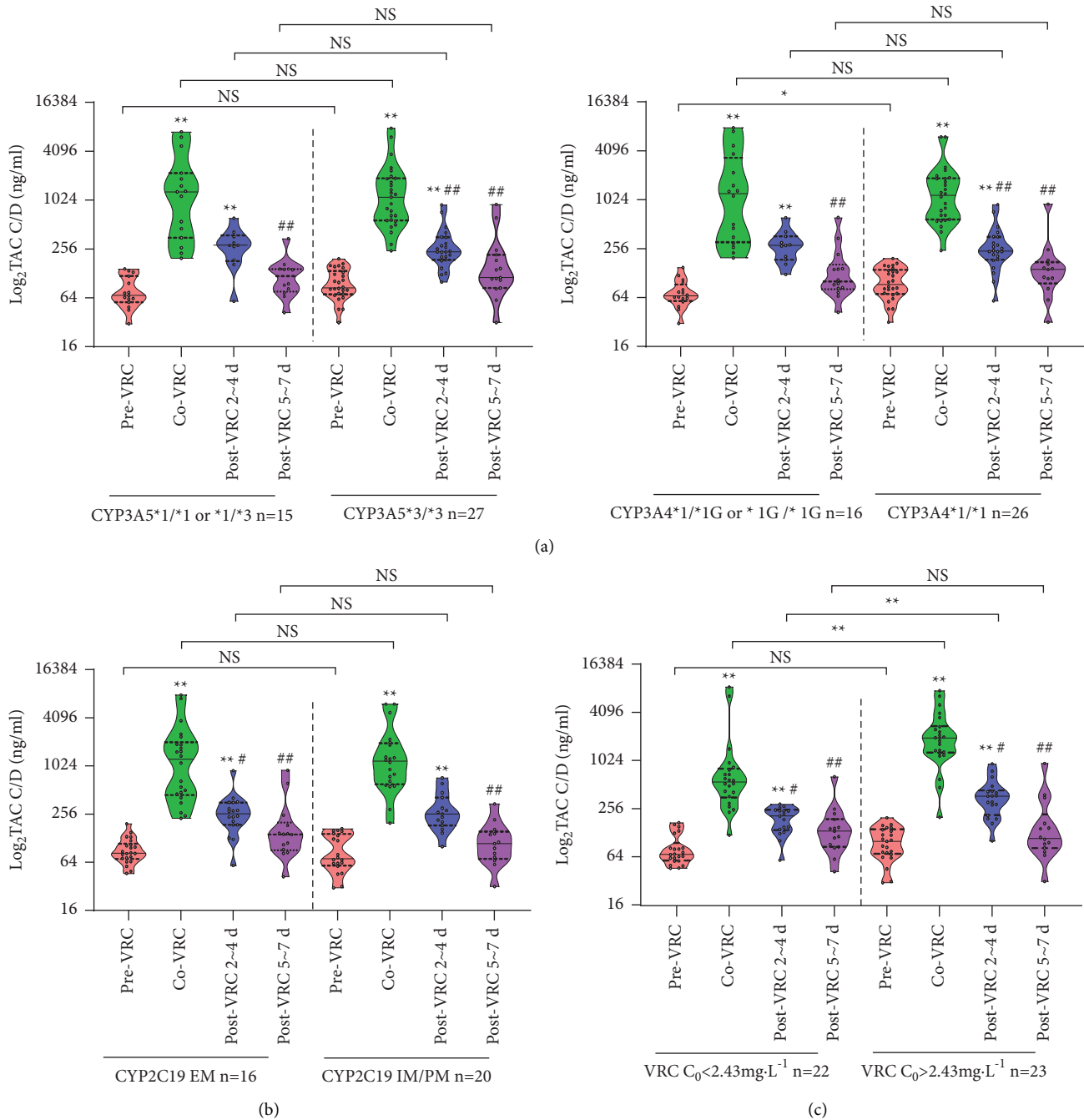


FIGURE 5: Comparison of TAC C/D in patients with different genotype of CYP3A4/5 (a) CYP2C19 (b) before and after concomitant VRC treatment. (c) Effect of VOR  $C_0$  on TAC C/D before and after VRC discontinuation. Note: Comparison of TAC C/D between each two groups,  $P^* < 0.05$  or  $P^{**} < 0.01$ ; comparison of TAC C/D between four groups before and after VRC discontinuation,  $P^{**} < 0.01$  compared with the Pre-VRC group,  $P^\# < 0.05$ ,  $P^{\#\#} < 0.01$  compared with the Co-VRC group, compared with the Post-VRC 2-4d group.

Finally, we analyzed the effect of VRC  $C_0$  on TAC metabolism after VRC discontinuation. Forty-five patients were tested for VRC  $C_0$ , and the individual variability of VRC  $C_0$  was large ( $0.14\text{--}9.4 \text{ mg}\cdot\text{L}^{-1}$ ) with a median of  $2.43$  ( $1.19, 4.51$ )  $\text{mg}\cdot\text{L}^{-1}$ . Patients were divided into the high (VRC  $C_0 > 2.43 \text{ mg}\cdot\text{L}^{-1}$ ) and low (VRC  $C_0 < 2.43 \text{ mg}\cdot\text{L}^{-1}$ ) concentration VRC groups according to the median VRC  $C_0$ .

The TAC C/D of the high concentration VRC group was significantly higher than that of the low concentration VRC group after concomitant VRC and after VRC discontinuation at 2-4 days, whereas there was no significant difference in TAC C/D between the two groups before VRC co-administration and after VRC discontinuation at 5-7 d (Figure 5(c)).



#### 4. Discussion

This retrospective study of 46 kidney transplant recipients demonstrated that patients with high TAC inpatient variability (coefficient of variation >30%) had a higher risk of de novo donor-specific antibody formation (hazard ratio, 5.35; 95% confidence interval, 2.45 to 11.68), which was associated with poor graft outcomes [4]. The Consensus on Modifiable Risk Management in Transplantation expert panel also recommended that large fluctuations in TAC levels and high exposure levels in the early post-transplant period should be avoided because they increase mortality caused by events related to excessive immunosuppression such as infections, cardiovascular events, and malignancies [19]. Maintaining TAC  $C_0$  within the appropriate range remains complex owing to genetic polymorphisms in CYP3A4/5 and a wide range of drug interactions. VRC is a strong CYP3A inhibitor, and when combined with TAC, the dose of TAC must be reduced to 1/3 or even lower to maintain TAC  $C_0$  in the appropriate range. However, the recovery of metabolic enzyme activity often takes longer than the onset of metabolic enzyme inhibition.

A study on the effect of fluoxetine on dextromethorphan metabolism showed that it took 2–3 weeks for CYP2D6 activity to return to baseline levels after the discontinuation of fluoxetine [20]. A similar phenomenon was also observed in CYP3A. Lilja et al. used Simvastatin as a probe of CYP3A metabolic capacity and clarified that CYP3A activity was gradually restored only 3–7 days after grapefruit juice administration [21]. Data from Vanhove et al. also suggested that TAC metabolism requires a longer time to return to pre-VRC levels after VRC discontinuation [15]. However, there is still a lack of knowledge about the changing pattern of TAC  $C_0$  and dose adjustment protocols after VRC discontinuation. Therefore, our findings can help clinicians to be more precise in the TAC treatment, not only to reduce the rejection reactions caused by dramatic fluctuations in TAC  $C_0$  but also to reduce the toxic reactions of immunosuppression. This is beneficial for prolonging survival after kidney transplantation.

Herein, we retrospectively analyzed the changes in TAC  $C_0$  in kidney transplant recipients approximately 1 week after VRC discontinuation. Only 46 patients were included, as per the inclusion and exclusion criteria and the lack of complete TAC  $C_0$  monitoring data in most patients. After concomitant VRC, the TAC dose was reduced to 93.5% in patients, but TAC  $C_0$  remained significantly elevated, which is consistent with previous studies [16]. After discontinuation of VRC for 2–4 d, 81.1% of patients had their TAC returned to the initial dose, but the TAC  $C_0$  was 2.40-fold higher than the pre-VRC treatment; 73.0% of patients exceeded the upper limit of the therapeutic window. After VRC discontinuation for 5–7 d, TAC  $C_0$  gradually returned to the pre-VRC treatment level, but TAC C/D was still higher than the pre-VRC treatment. There were still 40% of patients whose TAC dose returned to only half of the initial dose or even lower, but the TAC  $C_0$  remained in the therapeutic window.

Some of the patients in this study had a low initial TAC of  $C_0$ . To prevent a sudden drop in TAC  $C_0$  after VRC discontinuation, the patients received a combination of erythromycin and TAC. As a large number of patients received erythromycin in combination, they were not excluded from this study. Erythromycin, a hepatic drug enzyme inhibitor, inhibits TAC metabolism in the liver and small intestine by binding to the CYP3A4 enzyme to form an inactive complex, thereby increasing the blood concentration of TAC [22]. Our group has previously recommended a dose adjustment of TAC in combination with erythromycin to 3/4 of the original dose based on population pharmacokinetics. The results of the subgroup analysis suggested that whether erythromycin was coadministered, TAC C/D was significantly higher 2–4 d after VRC discontinuation than pre-VRC treatment, whereas TAC C/D recovered gradually after discontinuation of VRC for 5–7 d.

The degree of CYP3A inhibition by erythromycin depends on the duration of administration and a plateau in CYP3A inhibition is achieved only after 4 days or longer of erythromycin treatment [23]. Meanwhile, Vanhove et al. concluded that the proportional inhibition effect of adding azoles may be diminished if CYP3A4 expression/activity is already low (resulting in high Tac C/D) or inhibited by drugs such as diltiazem [15]. Therefore, theoretically, the inhibition of CYP3A by erythromycin is not yet maximal 2–4 days after VRC discontinuation and peaks at 5–7 days as the duration of erythromycin treatment is extended. However, the values of TAC C/D 2–4 days after VRC discontinuation remained significantly higher than those at 5–7 days. This phenomenon, contrary to the theory, suggests that the inhibition of CYP3A by VRC persists for 2–4 days after VRC discontinuation. After VRC discontinuation for 5–7 d, the persistent inhibition of VRC gradually disappeared, whereas the inhibition of erythromycin reached its maximum level. This also explains why TAC  $C_0$  and C/D gradually returned to pre-VRC administration levels in patients not coadministered erythromycin but remained higher than pre-VRC administration in patients coadministered erythromycin. Therefore, our data suggest that the metabolic enzyme activity takes 5–7 days to recover after VRC discontinuation and that the TAC dose should not be immediately restored to the initial dose to prevent toxic reactions due to high TAC  $C_0$ .

A semiphysiological population pharmacokinetic model showed that CYP3A inhibition persisted even when VRC  $C_0$  had approached zero, and CYP3A activity was predicted to recover completely to baseline levels only 4 days after the last dose of VRC [24]. Another population pharmacokinetic model based on semiphysiology in adult liver transplant recipients yielded a similar conclusion [25]. These results are consistent with the observed phenomena.

VRC inhibition of TAC metabolism was positively correlated with VRC  $C_0$  using in vitro human liver microsomal assays [26, 27]. Therefore, we hypothesized that the recovery of CYP3A activity after VRC discontinuation depends on the metabolic activity of CYP3A and VRC clearance metabolism, which are regulated by the CYP3A4/5 and CYP2C19 gene polymorphisms, respectively. Therefore,

we further explored the effects of CYP3A4/5 and CYP2C19 polymorphisms on TAC  $C_0$  and C/D after VRC discontinuation. This may be owing to the small sample size in our study; only one patient had the CYP3A5\*1/\*1 genotype, and the rest of the patients carried CYP3A5\*3, resulting in the CYP3A4-dominant metabolism of TAC. However, there was no significant difference in TAC C/D in patients with different genotypes of CYP3A4/5 after coadministration of VRC or VRC discontinuation. This may be attributed to the strong CYP3A inhibitory effect of VRC, which masks the potential effects of CYP3A4/5 gene polymorphisms on TAC metabolism [28].

CYP2C19 is the primary enzyme involved in VRC metabolism. A pharmacokinetic study in healthy volunteers suggested that the CYP2C19 genotype may serve as an influential factor in the interaction between TAC and VRC [29]. Another real-world study with a larger sample showed that the values of TAC C/D were significantly higher in the CYP2C19\*2/\*2 and 2/\*3 groups than in the other genotype groups [30]. In contrast, our study showed no significant difference in TAC C/D after coadministration of VRC or VRC discontinuation in patients with different CYP2C19 genotypes. This may be owing to the small sample size of the current study. However, a more probable reason is the difference between CYP2C19 genotypes and phenotypes. To verify this hypothesis, we explored the relationship between VRC  $C_0$  and TAC C/D after coadministration of VRC or VRC discontinuation. The patients were divided into high- and low-concentration VRC groups based on the median VRC  $C_0$  (2.43 mg/L). We found that the TAC C/D values in the high concentration group were significantly higher than those in the low concentration VRC group during VRC coadministration and 2–4 days after VRC discontinuation, whereas there was no significant difference in TAC C/D between the two groups before VRC coadministration and 5–7 days after VRC discontinuation. This may be because of the stronger inhibitory effect of higher VRC  $C_0$  on CYP3A, as well as its longer clearance time in vivo. This suggests a difference between the CYP2C19 genotype and phenotype. VRC  $C_0$  may serve as a biomarker for assessing the metabolic pattern of TAC after VRC discontinuation.

The present study had some limitations. As a retrospective study, we included only 46 patients, some of whom still had missing data such as dose, TAC  $C_0$ , and metabolic enzyme genotype. This affected the credibility of the study. In addition, the small sample size in this study precludes the performance of a statistically sound multivariate analysis, and future studies with a larger sample size are required. The authors observed adverse effects caused by high TAC  $C_0$  after VRC discontinuation in clinical practice, such as (elevated creatinine levels, hand tremors, and elevated blood glucose, etc.). However, owing to the large number of missing medical records, the data was insufficient to assess the adverse reactions caused by fluctuations in TAC  $C_0$  after VRC discontinuation. Therefore, larger cohort studies are needed to further explore TAC dose adjustment options after VRC discontinuation. In addition, VRCN-oxide, a metabolite of

VRC, is an important indicator of VRC metabolism. However, we only monitored VRCN-oxide concentrations in a small number of patients and therefore did not analyze them in this study.

## 5. Conclusions

Herein, we explore changes in TAC metabolism after VRC discontinuation and their impact factors. We found that the inhibitory effect on TAC metabolism persisted after VRC discontinuation and gradually recovered 5–7 days after discontinuation. Restoring the TAC dose to the initial dose immediately after VRC discontinuation resulted in the TAC  $C_0$  exceeding the upper limit of the therapeutic window, leading to concentration fluctuations. In some patients, an appropriate TAC  $C_0$  can be obtained by restoring the TAC dose to half the initial dose 5–7 d after VRC discontinuation. Genetic polymorphisms in CYP3A4/5 and CYP2C19 and the combination with erythromycin after VRC discontinuation did not affect the persistence of VRC inhibition. Instead, VRC trough concentration may be a biomarker for predicting changes in TAC metabolism after VRC discontinuation.

## Data Availability

The data that support the findings of this study are available on request from the corresponding author.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Authors' Contributions

Lijuan Feng and Guiyi Liao share first position authorship.

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## Supplementary Materials

Figure S1: Comparison of TAC  $C_0$  (A) and C/D (B) between multiple groups before and after concomitant VRC treatment using paired analysis. Figure S2: Multiple group comparisons to evaluate the impact of coadministration of erythromycin on TAC  $C_0$  (A) and C/D (B) before and after concomitant VRC treatment using paired analysis. (*Supplementary Materials*)

## References

- [1] L. S. M. Siger and D. W. Denning, "Invasive aspergillosis after renal transplantation," *Journal of Fungi*, vol. 9, no. 2, p. 255, 2023.
- [2] B. M. Shrestha, "Two decades of tacrolimus in renal transplant: basic science and clinical evidences," *Experimental and Clinical Transplantation*, vol. 15, no. 1, pp. 1–9, 2017.
- [3] W.-Q. Chen, "Expert consensus on tacrolimus individualized therapy for Solid organ transplantation in China," *Evaluation and Analysis of Drug-Use in Hospital of China*, vol. 21, pp. 1409–1424, 2021.
- [4] K. H. Piburn, V. K. Sigurjonsdottir, O. S. Indridason et al., "Patterns in tacrolimus variability and association with de novo donor-specific antibody formation in pediatric kidney transplant recipients," *Clinical Journal of the American Society of Nephrology*, vol. 17, no. 8, pp. 1194–1203, 2022.
- [5] A. Nuchjumroon, S. Vadcharavivad, W. Singhan et al., "Comparison of tacrolimus intra-patient variability during 6–12 Months after kidney transplantation between CYP3A5 expressers and nonexpressers," *Journal of Clinical Medicine*, vol. 11, no. 21, p. 6320, 2022.
- [6] A. Gold, B. Tonshoff, B. Dohler, and C. Susal, "Association of graft survival with tacrolimus exposure and late intra-patient tacrolimus variability in pediatric and young adult renal transplant recipients-an international CTS registry analysis," *Transplant International*, vol. 33, no. 12, pp. 1681–1692, 2020.
- [7] C. E. Staatz and S. E. Tett, "Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation," *Clinical Pharmacokinetics*, vol. 43, no. 10, pp. 623–653, 2004.
- [8] K. A. Birdwell, B. Decker, J. M. Barbarino et al., "Clinical pharmacogenetics implementation consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing," *Clinical Pharmacology and Therapeutics (St Louis)*, vol. 98, no. 1, pp. 19–24, 2015.
- [9] Y. Dong, Q. Xu, R. Li et al., "CYP3A7, CYP3A4, and CYP3A5 genetic polymorphisms in recipients rather than donors influence tacrolimus concentrations in the early stages after liver transplantation," *Gene*, vol. 809, Article ID 146007, 2022.
- [10] B. Balcan, U. Ozelcik, A. O. Ugurlu, M. Aydin, S. Nalcaci, and F. Yarbug Karakayali, "Increased mortality among renal transplant patients with invasive pulmonary Aspergillus infection," *Progress in Transplantation*, vol. 28, no. 4, pp. 349–353, 2018.
- [11] D. Z. P. Friedman, B. K. Johnson, E. Beam, W. K. Kremers, and P. Vergidis, "Risk factors and outcomes of invasive aspergillosis in kidney transplant recipients: a case-control study of United States renal data System data," *Clinical Infectious Diseases*, vol. 76, no. 8, pp. 1431–1439, 2023.
- [12] T. F. Patterson, G. R. Thompson 3rd, D. W. Denning et al., "Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the infectious diseases society of America," *Clinical Infectious Diseases*, vol. 63, no. 4, pp. e1–e60, 2016.
- [13] A. Mimura, S. Yamaori, N. Ikemura, Y. Katsuyama, N. Matsuzawa, and S. Ohmori, "Influence of azole antifungal drugs on blood tacrolimus levels after switching from intravenous tacrolimus to once-daily modified release tacrolimus in patients receiving allogeneic hematopoietic stem cell transplantation," *Journal of Clinical Pharmacy and Therapeutics*, vol. 44, no. 4, pp. 565–571, 2019.
- [14] D. Capone, A. Gentile, P. Imperatore, G. Palmiero, and V. Basile, "Effects of itraconazole on tacrolimus blood concentrations in a renal transplant recipient," *The Annals of Pharmacotherapy*, vol. 33, no. 10, pp. 1124–1125, 1999.
- [15] T. Vanhove, H. Bouwsma, L. Hilbrands et al., "Determinants of the magnitude of interaction between tacrolimus and voriconazole/posaconazole in solid organ recipients," *American Journal of Transplantation*, vol. 17, no. 9, pp. 2372–2380, 2017.
- [16] S. Cheng, M. Tang, J. Du, and T. Yin, "Effects of antifungal drugs on the plasma concentrations and dosage of tacrolimus in kidney transplant patients," *European Journal of Hospital Pharmacy*, vol. 29, no. 4, pp. 202–206, 2022.
- [17] C. Jensen, M. Jordan, R. Shapiro et al., "Interaction between tacrolimus and erythromycin," *The Lancet*, vol. 344, no. 8925, p. 825, 1994.
- [18] L. H. Chiang, T. H. Wu, T. C. Tsai, and W. C. Lee, "Co-administration of erythromycin to increase tacrolimus concentrations in liver transplant recipients," *Transplantation Proceedings*, vol. 51, no. 5, pp. 1439–1441, 2019.
- [19] J. M. Neuberger, W. O. Bechstein, D. R. Kuypers et al., "Practical recommendations for long-term management of modifiable risks in kidney and liver transplant recipients: a guidance report and clinical checklist by the consensus on managing modifiable risk in transplantation (commit) group," *Transplantation*, vol. 101, no. 4S, pp. S1–S56, 2017.
- [20] J. Amchin, L. Ereshefsky, W. Zarycranski, K. Taylor, D. Albano, and P. M. Klockowski, "Effect of venlafaxine versus fluoxetine on metabolism of dextromethorphan, a CYP2D6 probe," *The Journal of Clinical Pharmacology*, vol. 41, no. 4, pp. 443–451, 2001.
- [21] J. J. Lilja, K. T. Kivisto, and P. J. Neuvonen, "Duration of effect of grapefruit juice on the pharmacokinetics of the CYP3A4 substrate simvastatin," *Clinical Pharmacology and Therapeutics (St Louis)*, vol. 68, no. 4, pp. 384–390, 2000.
- [22] I. Desmond Padhi, P. Long, M. Basha, and J. V. Anandan, "Interaction between tacrolimus and erythromycin," *Therapeutic Drug Monitoring*, vol. 19, no. 1, pp. 120–122, 1997.
- [23] T. Okudaira, T. Kotegawa, H. Imai, K. Tsutsumi, S. Nakano, and K. Ohashi, "Effect of the treatment period with erythromycin on cytochrome P450 3A activity in humans," *The Journal of Clinical Pharmacology*, vol. 47, no. 7, pp. 871–876, 2007.
- [24] S. Frechen, L. Junge, T. I. Saari et al., "A semiphysiological population pharmacokinetic model for dynamic inhibition of liver and gut wall cytochrome P450 3A by voriconazole," *Clinical Pharmacokinetics*, vol. 52, no. 9, pp. 763–781, 2013.
- [25] Z. R. Li, C. H. Shen, R. D. Li et al., "Individual dose recommendations for drug interaction between tacrolimus and voriconazole in adult liver transplant recipients: a semi-physiologically based population pharmacokinetic modeling approach," *European Journal of Pharmaceutical Sciences*, vol. 184, Article ID 106405, 2023.
- [26] R. Venkataramanan, S. Zang, T. Gayowski, and N. Singh, "Voriconazole inhibition of the metabolism of tacrolimus in a liver transplant recipient and in human liver microsomes," *Antimicrobial Agents and Chemotherapy*, vol. 46, no. 9, pp. 3091–3093, 2002.
- [27] S. Zhang, V. C. Pillai, S. R. Mada, S. Strom, and R. Venkataramanan, "Effect of voriconazole and other azole antifungal agents on CYP3A activity and metabolism of tacrolimus in human liver microsomes," *Xenobiotica*, vol. 42, no. 5, pp. 409–416, 2012.
- [28] X. Huang, Y. Zhou, J. Zhang et al., "The importance of CYP2C19 genotype in tacrolimus dose optimization when concomitant with voriconazole in heart transplant recipients,"

*British Journal of Clinical Pharmacology*, vol. 88, no. 10, pp. 4515–4525, 2022.

- [29] C. K. Imamura, K. Furihata, S. Okamoto, and Y. Tanigawara, “Impact of cytochrome P450 2C19 polymorphisms on the pharmacokinetics of tacrolimus when coadministered with voriconazole,” *The Journal of Clinical Pharmacology*, vol. 56, no. 4, pp. 408–413, 2016.
- [30] Y. C. Zhao, C. L. Xiao, J. J. Hou et al., “The effect of voriconazole on tacrolimus in kidney transplantation recipients: a real-world study,” *Pharmaceutics*, vol. 14, no. 12, p. 2739, 2022.