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ARTICLE

Predictors of Adverse Events and Determinants of the Voriconazole Trough Concentration in Kidney Transplantation Recipients

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Voriconazole is the mainstay for the treatment of invasive fungal infections in patients who underwent a kidney transplant. Variant CYP2C19 alleles, hepatic function, and concomitant medications are directly involved in the metabolism of voriconazole. However, the drug is also associated with numerous adverse events. The purpose of this study was to identify predictors of adverse events using binary logistic regression and to measure its trough concentration using multiple linear modeling. We conducted a prospective analysis of 93 kidney recipients cotreated with voriconazole and recorded 213 trough concentrations of it. Predictors of the adverse events were voriconazole trough concentration with the odds ratios (OR) of 2.614 (P = 0.016), cytochrome P450 2C19 (CYP2C19), and hemoglobin (OR 0.181, P = 0.005). The predictive power of these three factors was 91.30%. We also found that CYP2C19 phenotypes, hemoglobin, platelet count, and concomitant use of ilaprazole had quantitative relationships with voriconazole trough concentration. The fit coefficient of this regression equation was R^2 = 0.336, demonstrating that the model explained 33.60% of interindividual variability in the disposition of voriconazole. In conclusion, predictors of adverse events are CYP2C19 phenotypes, hemoglobin, and voriconazole trough concentration. Determinants of the voriconazole trough concentration were CYP2C19 phenotypes, platelet count, hemoglobin, concomitant use of ilaprazole. If we consider these factors during voriconazole use, we are likely to maximize the treatment effect and minimize adverse events.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Voriconazole demonstrates wide interpatient variability in serum concentrations, due in part to variant CYP2C19 alleles. Individuals who are CYP2C19 ultrarapid metabolizers have decreased trough voriconazole concentrations, delaying achievement of target blood concentrations. In comparison, poor metabolizers have increased trough concentrations and are at increased risk of adverse drug events. However, CYP2C19 genotyping cannot replace therapeutic drug monitoring, as other factors (i.e., drug interactions, hepatic function, renal function, site of infection, and comorbidities) also influence the use of voriconazole. Besides, this association is markedly less visible in kidney transplantation recipients. Further studies are required to ensure the intelligent use of voriconazole.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This study identified predictors of the occurrence of adverse events and determinations of the magnitude of serum voriconazole trough concentration in kidney transplantation recipients.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This paper adds to the evidence that the CYP2C19 genotype serves as a mediator for voriconazole associated adverse events. Notably, it was seldom reported that the concentration of hemoglobin could statistically significantly influence the occurrence of adverse events and trough concentration of voriconazole.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Attention should be given not only to the genotype of CYP2C19 but also to other predictors, such as hemoglobin, platelet count, and drug interactions, during therapy with voriconazole in kidney transplant recipients.

Invasive fungal infections are a feared complication in kidney transplant recipients, occurring in 0.1–3.5% of solid organ recipients.[1] Its 12-week survival rates were only 60.7%, and 22.1% of the survivors experienced graft loss because of invasive fungal infections.[2] Kidney transplantation, in conjunction with calcineurin inhibitors, is regarded as the best

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option for patients with end-stage kidney disease. However, immunosuppression increases the risk of opportunistic infections and induces the occurrence of secondary fungal infections with high mortality rates (40–60%).[1,3]

Voriconazole is the first available second-generation triazole. Experts recommend voriconazole as primary therapy for invasive aspergillosis.[4] Clinicians also use it prophylactically to avoid severe infections in immunosuppressed organ transplant recipients. However, voriconazole exhibits nonlinear pharmacokinetics. With increasing dose, it shows a super-proportional increase in area under the plasma concentration-time curve; therefore, there is limited predictability of its accumulation or elimination. Maximum concentration and area under the plasma concentration-time curve also increase disproportionately with the dose.[5] Based on data from healthy individuals, voriconazole is rapidly absorbed within 2 hours after oral administration. The oral bioavailability of voriconazole is over 90%, allowing switching between oral and intravenous formulations. The protein binding is 58% and it is independent of dose or plasma concentrations. The mean elimination half-life of voriconazole is generally about 6 hours. The time to reach steady-state plasma concentrations is approximately 5 days with a maintenance dose. If administrated with a loading dose, it reaches a steady-state within 24 hours. The volume of distribution of voriconazole is 2–4.6 L/kg.[5–7]

Metabolism is hepatic, mediated by the CYP isoenzymes CYP2C9, CYP2C19, and CYP3A4 via N-oxidation, predominantly by CYP2C19.[8] Furthermore, it is both a substrate and an inhibitor of CYP2C19.[4] Like other CYP450 superfamily members, CYP2C19 is highly polymorphic with 35 defined variant star (*) alleles. A gene summary of CYP2C19 is available online.[9] Of note, the CYP2C19 genotype is a significant determinant of the wide pharmacokinetics variability for voriconazole.[4,10] Voriconazole is also associated with numerous adverse events, such as neurotoxicity, hepatotoxicity, and visual disturbances; adverse events correlated with concentration.[11,12] Nevertheless, the risk factors of adverse events in kidney transplantation recipients require further study. It is worth remembering that the ideal target trough concentration is not uniform, ranging from 0.5 mg/L to 6.0 mg/L. Simultaneously, voriconazole concentrations are affected by variant CYP2C19 alleles, age, hepatic function, concomitant medications, and inflammation.[13–15] Generally, voriconazole concentrations demonstrate wide interpatient variability.[16] Further studies are required to determine its variability in pathological states. Furthermore, most studies used classical population pharmacokinetics, which are not well-suited for clinicians. The purpose of this study was to identify predictors of the occurrence of adverse events and to determine the magnitude of serum voriconazole trough concentration in kidney transplantation recipients.

METHODS

Study design and population

This study was conducted in the Department of Urological Organ Transplantation, the Second Xiangya Hospital of Central South University. It was prospective and observational. The Ethics Committee of the Second XiangYa Hospital of Central South University (yxlb-lays-2015001) approved this study. We obtained informed consent from the patients for samples and data collection.

From January 1, 2016, to December 31, 2019, hospitalized kidney transplant recipients were eligible to enroll in the study. Inclusion criteria were the following: (i) age at least 18 years; (ii) administration of voriconazole for either prophylaxis or treatment; and (iii) availability of voriconazole trough concentration during therapy. The blood sample should be collected at least 3 days after initiation of a loading dose or a maintenance dose of 5 days[17]; (iv) availability of genotyping of CYP2C19; and (v) availability of physiological and biochemical indicators. Exclusion criteria were as follows: (i) pregnancy or lactation; (ii) concomitant use of rifampin, amobarbital, phenobarbital, efavirenz, and ritonavir; and (iii) incomplete dosing information and clinical data.

Demographic data, voriconazole trough concentration, and physiological indicators were recorded. We analyzed the concomitant administration of methylprednisolone, tacrolimus, cyclosporine, antibiotics, and proton pump inhibitors. Clinicians evaluated adverse events according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.[18]

Investigators recorded trough concentrations and other laboratory values once episodes of toxicity occurred. A standard case report form was used during the study. Remaining blood samples were collected to analyze the genotype of CYP2C19 alleles.

Administration of voriconazole and its trough concentration

Voriconazole was administered as an initial loading dose of 6 mg/kg i.v. or 400 mg p.o. every 12 hours on day 1 followed by 4 mg/kg i.v. or 200 mg p.o. every 12 hours for maintenance. Thereafter, the subsequent dose was adjusted by clinicians based on clinical reactions and results of therapeutic drug monitoring. Nurses would collect the blood sample within half an hour before the subsequent administration.

Genotype of CYP2C19

DNA of CYP2C19 was isolated from whole blood samples using commercially available EZNA SQ Blood DNA Kit II. Subsequently, we used the Sanger dideoxy DNA sequencing method for CYP2C19 genotyping using the ABI3730xl fully automatic sequencing instrument (ABI; Boshang Biotechnology, Shanghai, China). CYP2C19 phenotypes were classified into five categories[4]: ultra-rapid metabolizer (CYP2C19*17/*17), rapid metabolizer (CYP2C19*1/*17), normal metabolizer (CYP2C19*1/*1), intermediate metabolizer (CYP2C19*1/*2, CYP2C19*1/*3, and CYP2C19*2/*17), and poor metabolizer (CYP2C19*2/*2, CYP2C19*2/*3, and CYP2C19*3/*3).

Statistical analysis

The normality of quantitative data was tested using the Shapiro–Wilk test. According to the result of normality, the statistical approach chosen was either the Student's *t*-test or the Mann–Whitney test to select statistically significant factors of adverse events. The statistical description of enumeration data adopted the method of percentiles. Simultaneously, enumeration data were analyzed using the crosstab χ^2 test or Fisher exact test depending on varying

conditions. A two-tailed test with a P value < 0.05 was considered statistically significant. Results were given as point estimates or 95% confidence intervals. Subsequently, the predictors of statistical significance affecting the occurrence of the adverse events were analyzed using binary logistic regression.

Receiver operating characteristic curve analysis was used to test the power of prediction. Subsequently, the determinations of voriconazole trough concentration were then analyzed using multiple linear regression. For the regression analysis, the phenotype of CYP2C19 was set as a dummy variable.

A variance inflation factor (VIF) of > 5 was considered indicative of multicollinearity. We calculated the spearman correlations and point-biserial correlation analyses and also selected factors correlated to trough concentration. In subsequent analysis, we filtered out statistically significant factors to participate in the subsequent multiple linear regression. We conducted all analyses using IBM SPSS Statistics version 25 (IBM, Armonk, NY) and drew the figures using GraphPad Prism version 8 (San Diego, CA) and MedCalc version 19 (MedCalc Software Ltd, Ostend, Belgium).

RESULTS

Study population and adverse events caused by voriconazole

We included a total of 93 eligible patients, and we collected 213 voriconazole trough concentrations. A total of 77 (82.80%) patients suffered adverse events. Demographics and primary physiological indicators are summarized in **Table 1**. There were no statistical differences among groups with respect to sex, voriconazole trough concentration, and other factors (listed in **Table 1**). However, the concomitant use of tacrolimus, cyclosporin, and ilaprazole gave statistical differences. Importantly, the concentration of hemoglobin was significantly higher in the group without adverse events.

Among the 93 kidney transplantation recipients, 68 (73.12%) patients took voriconazole for a suspected fungal infection, whereas 25 (26.88%) took voriconazole for prophylaxis, referring to the guidelines.[19] The efficacy of the drug was evaluated in the therapeutic group. We found that the drug was ineffective in 14 (20.59%) patients, whereas 54 (79.41%) showed an apparent clinical effect. Subsequently, we collected additional information about the time of adverse reactions in 56 patients and found that 91.07% of adverse reactions occurred within 3 days. Only 8.93% appeared symptoms 3 days later after the administration of voriconazole. Hallucinations (63.64%), insomnia (55.84%), and visual impairment (44.16%) were common adverse events. Significantly, 50 (64.94%) of the 77 kidney transplantation recipients showed only one symptom, whereas 27 (30.06%) patients showed two or more symptoms. Further analysis demonstrated that hallucination was statistically related to voriconazole trough concentration.

Table 1 Patient characteristics in AEs and non-AEs cohorts

Parameters	Non-AEs cohort (n = 16; 17.20%)	AEs cohort (n = 77; 82.80%)	P value
Demographic variable			
Sex (male), *N* (%)	13 (17.60%)	61 (82.40%)	0.58
Age,[a] year, median (IQR)	32.00 (29.25 ~ 41.50)	35.50 (28.00 ~ 44.00)	0.934
Weight, kg, mean ± SD	57.09 ± 8.93	57.39 ± 10.93	0.917
Postoperative time,[a] months	4.05 (1.03 ~ 14.10)	3.87 (0.34 ~ 8.36)	0.421
Concomitant drug use (yes), *N* (%)			
Tacrolimus	6 (10.30%)	52 (89.70%)	0.024[b]
Cyclosporine	9 (45.00%)	11 (55.00%)	<0.001[b]
Levofloxacin	0 (0.00%)	1 (100.00%)	0.828
Moxifloxacin	10 (25.60%)	29 (74.40%)	0.067
Ceftriaxone	2 (16.70%)	10 (83.30%)	0.661
Lansoprazole	2 (11.80%)	15 (88.20%)	0.727
Ilaprazole	7 (35.00%)	13 (65.00%)	0.04[b]
Methylprednisolone	11 (15.70%)	59 (84.30%)	0.521
Other numerical variables			
Voriconazole C_{trough},[a] median (IQR)	1.89 (1.40 ~ 2.81)	2.54 (1.49 ~ 3.71)	0.200
Total,[a] median (IQR)	6.98 (4.86 ~ 9.49)	7.36 (4.86 ~ 9.40)	0.955
Hemoglobin, mean ± SD	120.13 ± 25.53	103.21 ± 22.49	0.009[b]
Platelet, mean ± SD	212.81 ± 84.80	190.36 ± 68.01	0.254
Alanine transaminase,[a] median (IQR)	16.05 (8.30 ~ 21.17)	13.30 (9.10 ~ 23.80)	0.757
Aspartate aminotransferase,[a] median (IQR)	19.85 (12.20 ~ 24.53)	16.00 (11.40 ~ 21.78)	0.446
Albumin, mean ± SD	33.78 ± 3.31	33.26 ± 3.35	0.578
Total bilirubin,[a] median (IQR)	8.30 (5.40 ~ 9.75)	7.15 (5.15 ~ 8.88)	0.370
Direct bilirubin,[a] median (IQR)	2.70 (1.92 ~ 3.90)	2.60 (1.83 ~ 3.37)	0.585
Creatinine,[a] median (IQR)	109.95 (99.55 ~ 130.6)	137.55 (104.35 ~ 177.45)	0.121

AEs, adverse events; C_{trough}, trough concentration; IQR, interquartile range.
[a]Shows that the variable is non-normal distribution analyzed by Shapiro–Wilk normal test.
[b]The distinction was statistically significant, at the level of 0.05 (double tail).

Effect of CYP2C19 genotype

All genotypes were in Hardy–Weinberg equilibrium. Detailed analysis was performed in various genetic groups. A detailed list of phenotype and genotype of CYP2C19 are presented in **Table S1**. There were six CYP2C19 alleles included. We performed either the χ^2 test or Fisher exact test to calculate pairwise comparisons. The result implicated that different phenotypes appeared to have no effect on the occurrence of adverse events. Compared with the unexpressed genotype of CYP2C19*2/3*, the occurrence of adverse events was statistically different in the group with the alleles of CYP2C19*1/*2 ($P = 0.009$) and CYP2C19*1/*1 ($P = 0.008$). Unfortunately, there was only one patient with a genotype of *1*17. Because of the limitation of sample size, we could not conduct any statistical analysis for this individual. Although doses were titrated to the therapeutic range during the whole therapy, the voriconazole trough concentration still showed statistically significant differences across CYP2C19 genotype groups (**Figure 1a**). Further analysis of the daily dose of various genotype groups showed statistically significant differences as well (**Figure 1b**).

Results of binary logistic regression

Based on the results shown in **Table 1**, these statistically significant factors, the genotype of CYP2C19, and voriconazole trough concentration, were entered into the subsequent binary logistic regression model to identify independent influencing factors of adverse events (**Table 2**). The results of binary logistic regression indicated that voriconazole trough concentration was an independent risk factor for adverse events (odds ratio (OR) 2.614, $P = 0.016$), suggesting that the risk of adverse events would increase by 2.614-fold if the concentration increased by 1 mg/L. The level of hemoglobin was an independent protective factor for adverse events (OR 0.181, $P = 0.011$), suggesting that the possibility of adverse events decreased in patients with high levels of hemoglobin. Compared with normal metabolizers, the risk of adverse events in poor metabolizers increased considerably (OR 111.614, $P = 0.002$), whereas the intermediate metabolizes indicated no statistical distinction.

The concomitant use of tacrolimus, cyclosporin, ilaprazole, and moxifloxacin demonstrated no evident impact, although they were statistically significant in the univariate analysis. $P > 0.05$ was considered statistically significant according to the Hosmer–Lemeshow test. The final result showed that the model fitted well and was statistically significant.

Receiver operating characteristic curve analysis

The independent influencing factors we obtained in the binary logistic regression were united to form the subsequent joint predictor. According to the results of logistic regression (**Table 2**), an equation for the joint predictor was built:

$$\text{Logit}\,P = 1.669 + 4.715 \times \text{CYP2C19poor - metabolizer} - 1.710 \times \text{Hemoglobin} + 0.961 \times C_{\text{trough}}$$

Afterward, we drew the receiver operating characteristic curves to determine the predictive power of the joint predictor. The area under the curve was 0.913 (95% confidence interval (CI) 0.836–0.962, $P < 0.001$). The Youden index was 0.7730, with a sensitivity of 0.9605 and a specificity of 81.25% (**Figure 2**). All these factors indicated good predictive power.

Taken together, these findings suggest that the joint predictor, consisting of the phenotype of CYP2C19, hemoglobin,

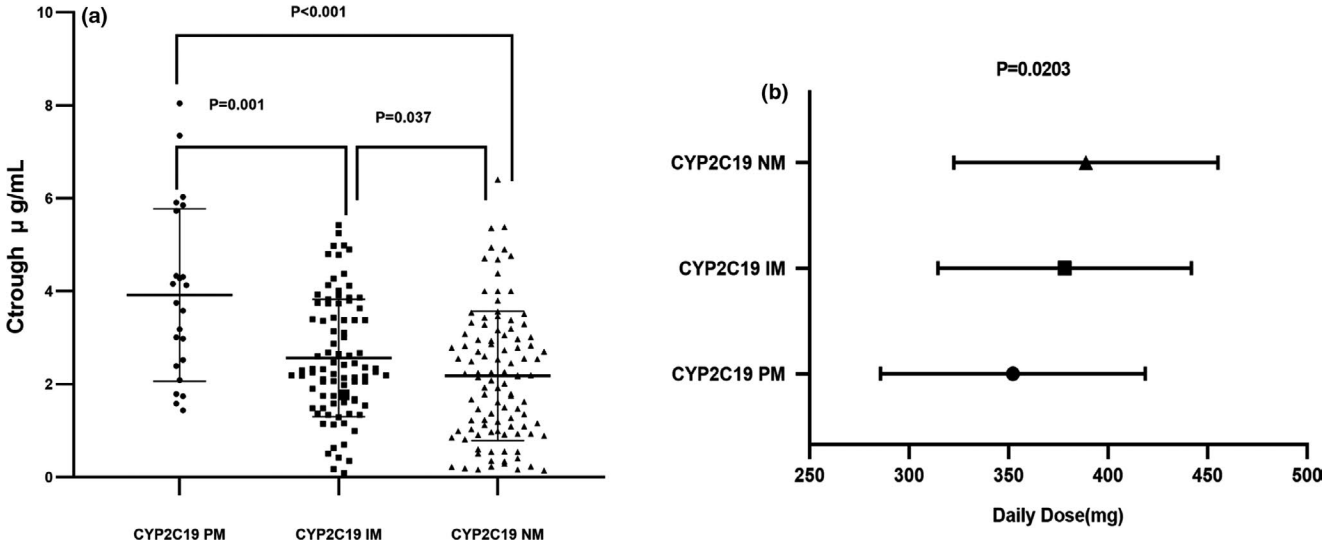

Figure 1 Distinction of voriconazole trough concentration and daily dose in different CYP2C19 phenotype groups. On average, the magnitude of voriconazole trough concerntration is highest in CYP2C19 PM group, while its dose of voriconazole is lowest compared to the other two group. The Kruskal–Wallis test was used to conduct the univariate analyses. Data are expressed as the median ± IQR. C_{trough}, trough concentration; CYP2C19, cytochrome P450 2C19; IM, intermediate metabolizer; IQR, interquartile range; NM, normal metabolizer; PM, poor metabolizer.

Table 2 Binary logistic regression analysis of adverse events predictors

Parameter	*B*	SE	Wald	df	*P* value	OR	95% CI
Concomitant medication							
Tacrolimus use	–1.495	1.186	1.589	1	0.207	0.224	0.022–2.292
Cyclosporine use	1.887	1.236	2.331	1	0.127	6.598	0.585–74.374
Moxifloxacin use	1.391	0.932	2.227	1	0.136	4.018	0.647–24.970
Ilaprazole use	1.030	0.978	1.108	1	0.293	2.800	0.412–19.047
CYP2C19 phenotypes	10.407	2	0.005				
Poor metabolizer	4.715	1.493	9.972	1	0.002[a]	111.614	5.981–2082.787
Intermediate metabolizer	–0.251	0.941	0.071	1	0.790	0.778	0.123–4.919
Classified hemoglobin[b]	–1.710	0.605	7.996	1	0.005 [a]	0.181	0.055–0.592
C_{trough}	0.961	0.397	5.855	1	0.016 [a]	2.614	1.200–5.694
Constant value	1.699	1.793	0.898	1	0.343	5.469	
F						12.537	
P value						0.129[c]	

CI, confidence interval; C_{trough}, trough concentration; CYP2C19, cytochrome P450 2C19.
[a]The variables was significant, at the level of 0.05 (double tail).
[b]In order to facilitate the interpretation of clinical significance, the variables were converted and defined as 3 grade: "1" means the concentration of hemoglobin is below 100; "2" between 100 and 120, and "3" means the concentration of hemoglobin is above 120. The above classification is based on the value of hemoglobin obtained in **Table 1**.
[c]Hosmer–Lemeshow test; $P > 0.05$, indicating that the model fits well and statistic significantly.

and voriconazole trough concentration, had a robust predictive capacity for adverse events.

Determinants of voriconazole trough concentration

The results of normality showed that most of the variables adopted abnormal distribution. Spearman correlation and point-biserial correlation were used in the univariate analysis of voriconazole trough concentration.

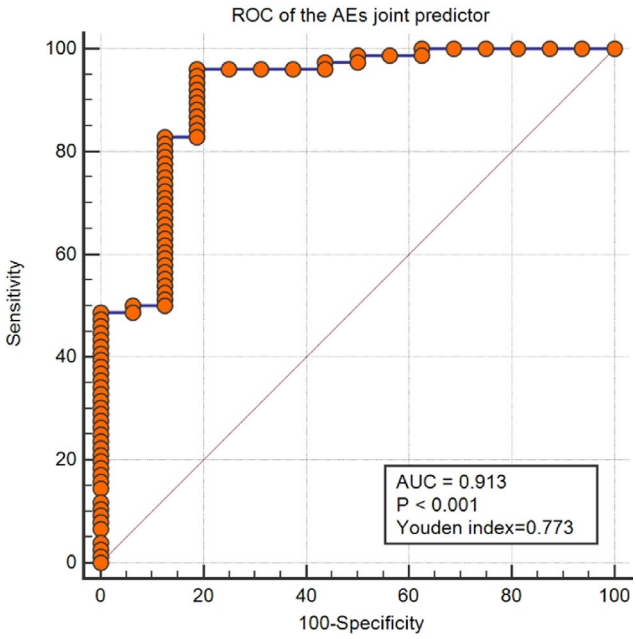

Figure 2 Receiver operating characteristic (ROC) curve for predicting adverse events. Hemoglobin, voriconazole trough concentration, and the CYP2C19 phenotypes together can predict the occurrence of adverse events (AEs) more accurately than any of them alone. AUC, area under the curve.

Correlation results are presented in **Table S2**, sex, age, weight, postoperative time, hemoglobin, platelet count, aspartate aminotransferase, direct bilirubin, creatinine, and CYP2C19 phenotype, including the concomitant use of tacrolimus ($P = 0.046$; **Figure 3c**) and ilaprazole ($P < 0.001$) all correlated with the value of voriconazole trough concentration. By contrast, other factors, including the administration route ($P = 0.883$) and dosage ($P = 0.527$), appeared not to correlate. In addition, the concomitant use of cyclosporine ($P = 0.521$) and lansoprazole ($P = 0.904$) were also uninfluential.

Depending on these correlation results, we used the Kruskal–Wallis test to identify distinction of voriconazole trough concentration in the groups of different sexes, CYP2C19 phenotypes, and concomitant use of tacrolimus and ilaprazole (**Figure 3**). Voriconazole trough concentration was higher women and the poor metabolizers of CYP2C19. On the contrary, the group with concomitant use of tacrolimus or ilaprazole appeared to have a lower trough concentration. Further analysis of dosage distinction in different CYP2C19 phenotypes was conducted. Normal metabolizers appeared to have the highest dosage.

Subsequently, the statistically significant factors were entered into a stepwise multiple linear regression model. Then, collinearity was diagnosed using the VIF. These factors were not collinear with one another (VIF < 5).

Predictors of voriconazole trough concentration are presented in **Table 3**. The final calculation demonstrated that the metabolism of voriconazole was affected by CYP2C19 phenotypes, the hemoglobin level, platelet count, and the concomitant use of ilaprazole. On average, compared with the poor metabolizer group, the concentration tended to be 1.23 mg/L lower in the intermediate metabolizer group and 1.521 mg/L lower in the normal metabolizer group. The voriconazole concentration would decrease by 0.805 due to the use of the ilaprazole. Hemoglobin and platelet count were implicated with the voriconazole trough concentration

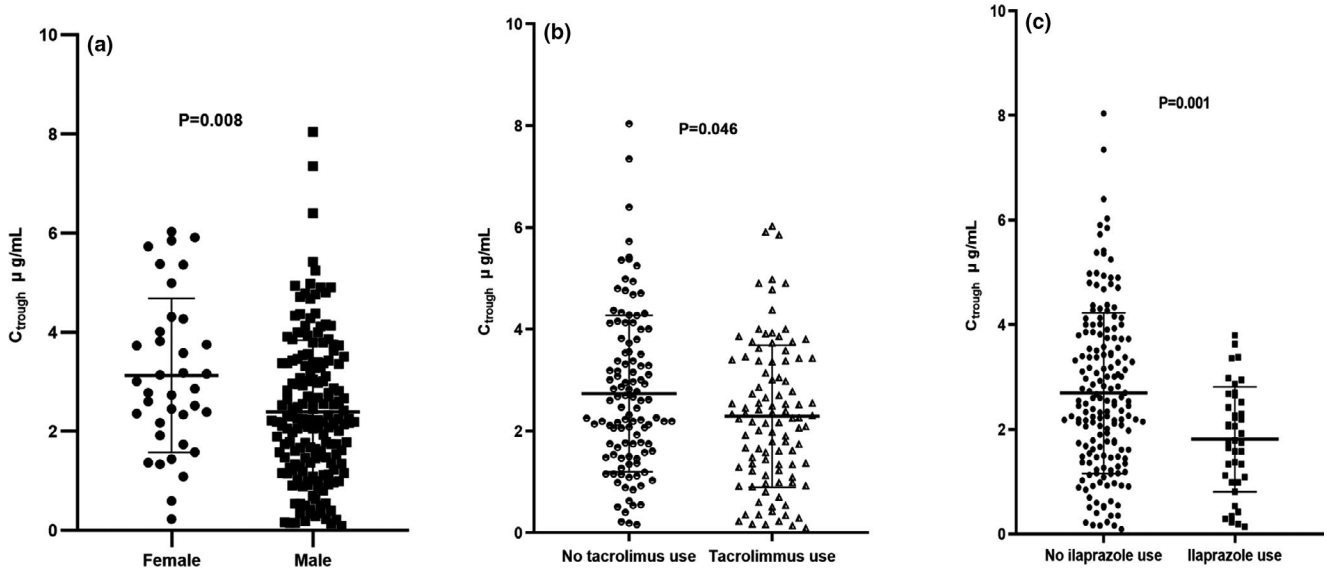

Figure 3 Distinction of voriconazole trough concentration in different groups [Gender groups (**a**), Tacrolimus use (**b**), Ilaprazole use (**c**)]. The Kruskal–Wallis test was used to conduct the univariate analyses. Data are expressed as the median ± IQR. C_{trough}, trough concentration; IQR, interquartile range.

as well. If hemoglobin increased by 1 g/L, the concentration of voriconazole would increase by 0.021 mg/L.

By contrast, the concentration would decrease by 0.004 mg/L with one unit increase in platelet count. The linear regression equation was as follows:

$$Y = 1.646 - 0.805 * \text{ilaprazole} * A + 0.021 * \text{Hemoglobin} - 0.004 * \text{platelet} - 1.23 * \text{intermediate metabolizer} * B - 1.521 * \text{normal metabolizer} * C$$

("A = 1" if ilaprazole is used, "A = 0" if ilaprazole is not used; "B = 1" if the patient is classified as CYP2C19-intermediate metabolizer, otherwise "B = 0"; "C = 1" if the patient is classified as CYP2C9-normal metabolizer; otherwise "C = 0").

Diagnosis of the multiple linear models

Model diagnosis was performed during the analysis in terms of three aspects: goodness of fit test, the test of linearity, and evaluation of the residual. The fit coefficient of this regression equation was R^2 = 0.336, which demonstrated that the equation could explain 33.60% of interindividual variability in the disposition of voriconazole. The F-value of this regression was 9.267 with a Pvalue of < 0.001, suggesting that there was a linear regression relationship among these factors. Dataset and steps of data analysis can be found in the supplymental materials.

We finally evaluated the residuals. As can be seen from the histogram and residual plot (**Figure 4**), the residual of the regression equation established obeyed the normal distribution and conformed to the precondition of the regression equation. This is further illustration of the accuracy and reliability of the operation result.

DISCUSSION

This prospective analysis of the occurrence of the adverse events in 93 kidney transplantation recipients is the first attempt to identify the independent influencing factors of adverse events. The multiple linear regression of 213 voriconazole trough concentrations is the first systematic assessment of factors governing the magnitude of voriconazole trough concentration, which is more acceptable and readable for clinicians than the classic population pharmacokinetics analysis.

Voriconazole is metabolized by the human hepatic cytochrome P450 enzymes, CYP2C19, CYP2C9, and CYP3A4.[8,20–22] There is substantial evidence linking CYP2C19 phenotypic variability in voriconazole pharmacokinetics. Studies *in vivo* indicated that CYP2C19 was significantly involved in the metabolism of voriconazole. This enzyme exhibits genetic polymorphism.[23] According to previous studies, 15–20% of the Asian population were likely to be poor metabolizers, whereas for whites and Blacks, the prevalence of poor metabolizers was 3–5%.[4,7] Studies have shown that poor metabolizers have fourfold higher voriconazole exposure than their homozygous extensive metabolizer counterparts on average.[7,24] Subjects who are heterozygous extensive metabolizers have, on average, had fourfold higher voriconazole exposure than their homozygous extensive metabolizer counterparts. Furthermore, the major metabolite of voriconazole is the N-oxide, which accounts for 72% of the circulating radiolabeled metabolites in plasma.[7,25] The ratio of voriconazole trough concentration to voriconazole-N-oxide concentration was also higher in poor metabolizers.[4,25,26] These results are consistent with those of our study. Those with phenotype of CYP2C19 poor metabolizers might have a higher risk of adverse events, in line with what has been previously observed.[21,27–29]

The allele frequencies of CYP2C19 were consistent with the results in Asians reported by Mikus *et al*.[24] That is why regulatory agencies include CYP2C19 as the only major pharmacogenetic biomarker in their dosing guidelines.[17] In addition, there are still many other genotypes, such as

Table 3 Multiple linear regression analysis of voriconazole trough concentration determinants

	Coefficient	*t*	*P* value	VIF
Demographic variable				
Sex	0.572	1.867	0.063	1.841
Age[a]	0.019	1.838	0.067	1.138
Weight[a]	4.58E−05	0.005	0.996	1.512
Postoperative time, months[a]	0	−0.276	0.783	1.276
Concomitant medication				
Tacrolimus use	−0.231	−1.173	0.242	1.32
Ilaprazole use	−0.805[b]	−3.426	0.001	1.173
Physiological and biochemical indexes				
Hemoglobin	0.021[b]	4.457	<0.001	1.532
Platelet	−0.004[b]	−2.929	0.004	1.228
Alanine transaminase	0.003	1.046	0.297	1.254
Direct bilirubina	−0.012	−0.418	0.676	1.314
Creatinine[a]	0	0.266	0.79	1.295
CYP2C19 phenotypes[c]				
Poor metabolizers	0			
Intermediate metabolizers	−1.23[b]	−3.881	<0.001	3.316
Normal metabolizers	−1.521[b]	−4.765	<0.001	3.475
Constant value	1.646	1.546	0.124	
F			9.267	
P			<0.001	
R^2			0.336[d]	
Dependent variable: voriconazole trough concentration				

CYP2C19, cytochrome P450 2C19; VIF, variance inflation factor.
[a]Shows that the variable is non-normal distribution obtained by Shapiro–Wilk normal test.
[b]The variables was significant, at the level of 0.05 (double tail).
[c]Dealt with the operation of dummy variables.
[d]$R^2 = 0.336$, $N = 213$; ($P < 0.001$).

FMO3, NR1I2, POR, CYP2C9, and CYP3A4,[30,31] that are related to the variability of voriconazole trough concentration. However, we did not analyze the effect of these genotypes in this study.

Other than genotypes, a previous retrospective analysis[27] of this particular population found that aspartate aminotransferase levels significantly affected voriconazole clearance. It was believed to be a determinant of the pharmacokinetic variability in kidney transplantation recipients. Nevertheless, we analyzed the liver function tests, such as alanine aminotransferase, aspartate aminotransferase, direct bilirubin, and total bilirubin, and found no statistically significant relationships. By contrast, a prospective study conducted by Lin *et al*.[28] reported that the clearance of voriconazole was 2.88 L/hour; this value was lower than other patients with invasive fungal infection.[32,33] The lower clearance possibly results from the unrecovered kidney function; but this phenomenon should be further evaluated.

The platelet count was also found to be a predictive factor for voriconazole trough concentration. *In vitro* data from Perkhofer *et al*.[34] indicated that voriconazole exhibited statistically significant effects with platelets for all tested Aspergillus species. However, their interactions *in vivo* remained unknown. Interestingly, other members of our research team[18] reached the same conclusion that platelet count was statistically significantly associated with voriconazole pharmacokinetic parameters. The difference was that they concluded the target population of patients with liver dysfunction.

They also found that CYP2C19 polymorphisms did not affect voriconazole disposition in patients with liver dysfunction.[18] Different populations are likely to have different physiological and pathological statuses. This is the reason for the large inter and individual variability in voriconazole metabolism.

Another interesting phenomenon is the concomitant drug use of proton pump inhibitors. Voriconazole is metabolized by the human hepatic CYP2C19, CYP2C9, and CYP3A4. Results of *in vitro* metabolism studies indicate that the affinity of voriconazole was highest for CYP2C19, followed by CYP2C9, and was appreciably lower for CYP3A4.[7] Inhibitors or inducers of these three enzymes may increase or decrease voriconazole systemic exposure (plasma concentrations), respectively. Omeprazole has two-way interactions with voriconazole as it is both an inhibitor and a substrate of CYP2C19.[35] Proton pump inhibitors are metabolized to a varying degree by CYP2C19, and the primary hepatic metabolism is the CYP2C19 enzyme pathway, except for rabeprazole. Omeprazole and esomeprazole are inhibitors of CYP2C19, whereas lansoprazole and pantoprazole are not. The pharmacokinetic characteristics of omeprazole and esomeprazole are nonlinear, whereas pharmacokinetics characteristics of lansoprazole, pantoprazole, and rabeprazole are linear.[35–38] As a result, proton pump inhibitors exhibit varied influences on the metabolism of voriconazole metabolism. For patients with available CYP2C19 genotyping results, adverse events might be avoided by choosing alternative agents in different metabolizers, respectively.

Hashemizadeh *et al*.,[39] found that voriconazole trough levels were significantly higher for individuals receiving proton pump inhibitors. The proton pump inhibitors likely contributed to the reduced hepatic clearance of voriconazole.[11,40] Nevertheless, it is puzzling that this study indicated that the concomitant use of the ilaprazole tended to have lower voriconazole trough concentrations, whereas there was no distinction with concomitant use of lansoprazole. Another study[36] showed concomitant use of ilaprazole, omeprazole, and esomeprazole statistically significantly increased the plasma voriconazole trough level ($P < 0.05$) but found no statistically significant association with ilaprazole, which is a neutral conclusion. It is notable that the previous studies showed some limitations. For instance, confounding factors, such as the effect of administration dosage, CYP2C19 gene polymorphism, and other interactions on voriconazole trough concentration, were not analyzed. Furthermore, few studies have reported the interactions of ilaprazole and voriconazole. For these reasons, further study is needed to determine the specific mechanism of this drug-interaction.

Hashemizadeh *et al*.[39] also reported that oral administration of voriconazole and concomitant use of glucocorticoids would reduce voriconazole blood concentrations statistically significantly. Dolton *et al*.[41] drew

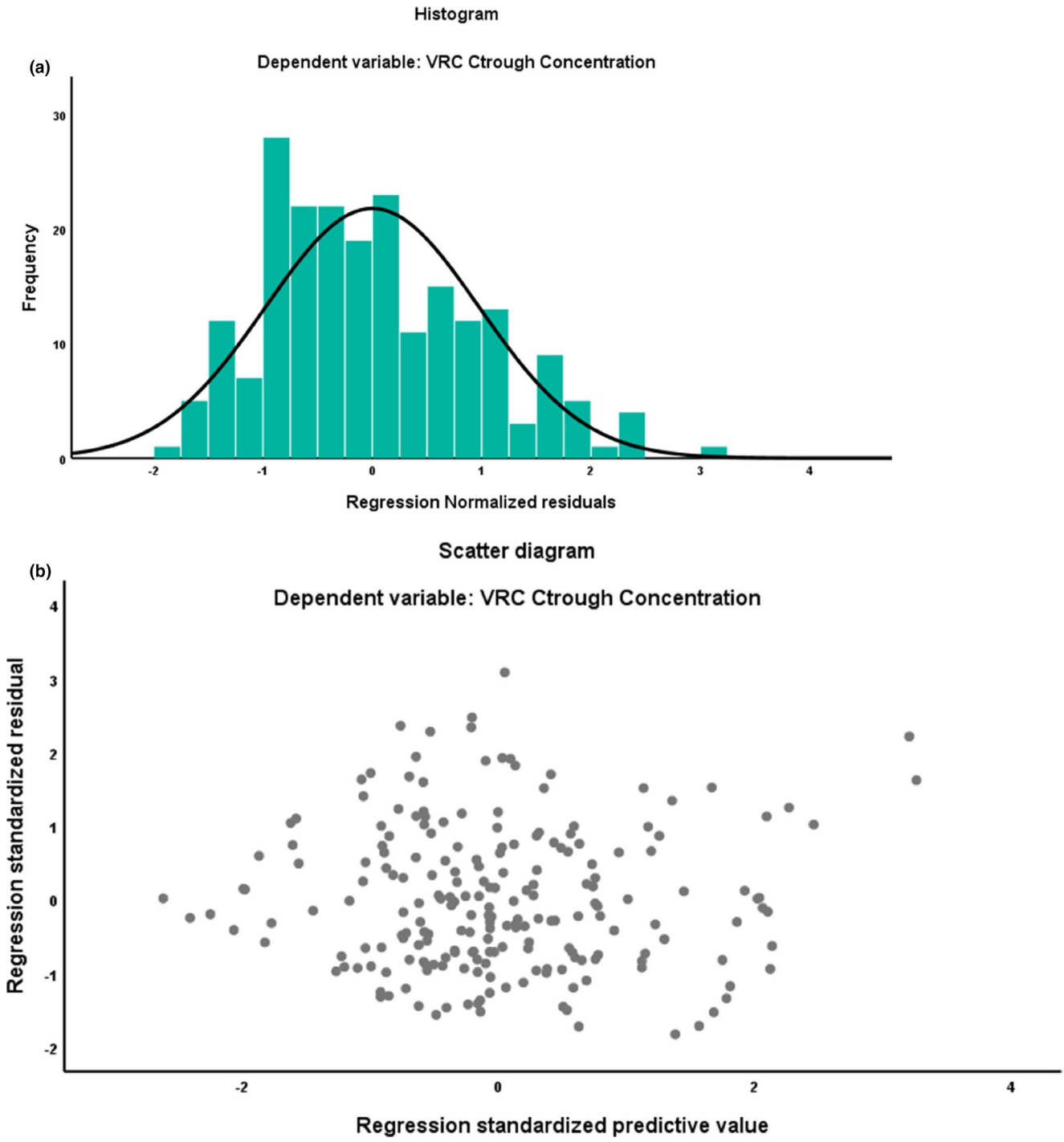

Figure 4 Model fitting test diagram. Histogram of residual distribution (**a**) and Scatter diagram of residual distribution (**b**). Residuals are normally distributed; the residual distribution is between –2 and 2. Both demonstrate that the linear regression model fits well. C_{trough}, trough concentration.

the same conclusion. Nevertheless, neither of them was statistically significant in our study. Regarding the drug-drug interaction of tacrolimus, it was correlated with the voriconazole trough concentration, but it did not enter into the final multiple linear regression model. The conclusion was consistent with those of Hashemizadeh *et al*.[39] The drug-drug interaction of tacrolimus and voriconazole was complicated. Several studies[30,42,43] reported changes in tacrolimus levels after the administration of voriconazole; but they seldom studied the changes of voriconazole levels. Overall, the nature and extent of drug-drug interactions between these drugs are affected by numerous modulators. To reach these conclusions, objective and systematic evaluations are needed.

With respect to the occurrence of adverse events, our model had higher correlativity, better stability, and more precise predictability than other models.[39,44] It is also more reasonable than the adverse events are affected by not only one factor.

The concentration of hemoglobin was a statistically significant factor, which affected both adverse events and voriconazole trough concentration. Hemoglobin is a protein with multiple functions, acting as an O2 transport protein, and having peroxidase and oxidase activities with xenobiotics that lead to substrate radicals.[45] Studies on the influence of this factor are rare. Nevertheless, we identified some studies that reached similar conclusions in the study of tacrolimus. Han *et al*. demonstrated that hemoglobin and hematocrit were associated with the distribution of tacrolimus using a simple linear regression model, and that hemoglobin was the most reliable clinical marker.[46] Coincidentally, hematocrit reached a nadir around the time of azole initiation, and the dose-corrected trough concentration was more significant with higher hematocrit values.[47] Although it is closely related to hematocrit, and hemoglobin makes up 90% of red blood cells.

Moreover, anemia is defined as the decrease of hematocrit, however, it is often replaced by hemoglobin. Notably, hemoglobin tends to be the modulator of voriconazole trough concentration. Voriconazole is primarily metabolized in the liver, and hemoglobin may be involved in the formation of its major metabolite, N-oxide voriconazole.[48] Nevertheless, the mechanism of interaction remains unknown. Pharmacokinetic studies of detailed metabolism need to be performed.

We expected that the concentration of albumin might be a statistically significant factor by effecting its plasma protein blinding rate,[49] but this was not the case.

There are several limitations to this study. First, the sample size was not very large, and only trough concentration was analyzed. Specifically, due to the limitation of the genotyped polymorphism in various races, the number of patients with the genotype of CYP2C19*1*17 was limited. Other genotypes could also be considered, such as CYP3A4 and POR.[30,31] Second, we did not analyze infection or inflammation indexes, such as C-reaction protein or procalcitonin in our study, which were reported to modulate the reactions of cytochrome P450 iso-enzymes.[50] Finally, the results of potential drug-drug interactions were different because of different research methods; further system assessment is necessary, and we also need to verify the results in further prospective studies.

Drug combinations should also be recorded in detail and assessed thoroughly. Above all, the adverse events were reported by patients, which was likely affected by several factors, not least recall bias.

The results of our analysis demonstrated the pharmacokinetic variability of voriconazole in kidney transplantation recipients. Depending on the joint predictor we established, we can predict 91.3% of the adverse events, which is meaningful during voriconazole therapy. Hemoglobin, platelet count, and concomitant use of ilaprazole deserve further study to verify their influence and to explore the specific mechanisms, which might be clinically significant. In conclusion, CYP2C19 phenotypes, hemoglobin, and trough concentration can be united together to predict the occurrence of adverse events. Moreover, CYP2C19 phenotypes, hemoglobin, platelet, and concomitant use of ilaprazole are modulators of the magnitude of voriconazole trough concentration. Voriconazole dosing adjustment can be directed using these results to maximize the treatment effect of voriconazole and to minimize adverse events.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

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1. Robin, C. *et al*. Mainly post-transplant factors are associated with invasive aspergillosis after allogeneic stem cell transplantation: a study from the surveillance Des Aspergilloses Invasives en France and Societe Francophone de Greffe de Moelle et de Therapie Cellulaire. *Biol. Blood Marrow. Transplant.* **25**, 354–361 (2019).
2. Lopez-Medrano, F. *et al*. Risk factors associated with early invasive pulmonary aspergillosis in kidney transplant recipients: results from a multinational matched case-control study. *Am. J. Transplant.* **16**, 2148–2157 (2016).
3. Balcan, B., Ozcelik, U., Ugurlu, A.O., Aydin, M., Nalcaci, S. & Yarbug Karakayali, F. Increased mortality among renal transplant patients with invasive pulmonary aspergillus infection. *Prog. Transplant.* **28**, 349–353 (2018).
4. Moriyama, B. *et al*. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP2C19 and voriconazole therapy. *Clin. Pharmacol. Ther.* **102**, 45–51 (2017).
5. Purkins, L., Wood, N., Ghahramani, P., Greenhalgh, K., Allen, M.J. & Kleinermans, D. Pharmacokinetics and safety of voriconazole following intravenous- to oral-dose escalation regimens. *Antimicrob. Agents Chemother.* **46**, 2546–2553 (2002).
6. Karlsson, M.O., Lutsar, I. & Milligan, P.A. Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies. *Antimicrob. Agents Chemother.* **53**, 935–944 (2009).
7. Labe, P.I.VFEND® Tablets(voriconazole) and VFEND® I.V. (voriconazole) for injection instruction book USPI Esoph Cand Nov 1003 ver 23 (2009).
8. Mikus, G. *et al*. Potent cytochrome P450 2C19 genotype-related interaction between voriconazole and the cytochrome P450 3A4 inhibitor ritonavir. *Clin. Pharmacol. Ther.* **80**, 126–135 (2006).
9. Scott, S.A. *et al*. PharmGKB summary: very important pharmacogene information for cytochrome P450, family 2, subfamily C, polypeptide 19. *Pharmacogenet. Genomics* **22**, 159–165 (2012).
10. Lee, S. *et al*. Effect of CYP2C19 polymorphism on the pharmacokinetics of voriconazole after single and multiple doses in healthy volunteers. *J. Clin. Pharmacol.* **52**, 195–203 (2012).
11. Lopez, J.L. & Tayek, J.A. Voriconazole-induced hepatitis via simvastatin- and lansoprazole-mediated drug interactions: a case report and review of the literature. *Drug Metab. Dispos.* **44**, 124–126 (2016).
12. Rosanova, M.T., Bes, D., Serrano Aguilar, P., Sberna, N. & Lede, R. Efficacy and safety of voriconazole in immunocompromised patients: systematic review and meta-analysis. *Infect. Dis.* **50**, 489–494 (2018).
13. Andes, D. *et al*. Drug-drug interaction associated with mold-active triazoles among hospitalized patients. *Antimicrob. Agents Chemother.* **60**, 3398–3406 (2016).

14. Zeng, G. *et al.* Effect of cyclosporine a and polymorphisms in CYP2C19 and ABCC2 on the concentration of voriconazole in patients undergoing allogeneic hematopoietic stem cell transplantation. *Xenobiotica* **50**, 614–619 (2020).
15. Zhou, P.Y. *et al.* The utility of voriconazole therapeutic drug monitoring in a multi-racial cohort in Southeast Asia. *J. Glob. Antimicrob. Resist.* **21**, 427–433 (2020).
16. Hicks, J.K. *et al.* Prospective CYP2C19-guided voriconazole prophylaxis in patients with neutropenic acute myeloid leukemia reduces the incidence of subtherapeutic antifungal plasma concentrations. *Clin. Pharmacol. Ther.* **107**, 563–570 (2020).
17. Chen, K., Zhang, X., Ke, X., Du, G., Yang, K. & Zhai, S. Individualized medication of voriconazole: a practice guideline of the division of therapeutic drug monitoring. Chinese Pharmacological Society. *Ther. Drug Monit.* **40**, 663–674 (2018).
18. Tang, D. *et al.* Identifying factors affecting the pharmacokinetics of voriconazole in patients with liver dysfunction: a population pharmacokinetic approach. *Basic Clin. Pharmacol. Toxicol.* **125**, 34–43 (2019).
19. Mellinghoff, S.C. *et al.* Primary prophylaxis of invasive fungal infections in patients with haematological malignancies: 2017 update of the recommendations of the Infectious Diseases Working Party (AGIHO) of the German Society for Haematology and Medical Oncology (DGHO). *Ann. Hematol.* **97**, 197–207 (2018).
20. Hyland, R., Jones, B.C. & Smith, D.A. Identification of the cytochrome P450 enzymes involved in the N-oxidation of voriconazole. *Drug Metabo. Dispos.* **31**, 540–547 (2003).
21. Miao, Q. *et al.* Correlation of CYP2C19 genotype with plasma voriconazole exposure in South-western Chinese Han patients with invasive fungal infections. *Medicine (Baltimore)* **98**, e14137 (2019).
22. Jeong, S., Nguyen, P.D. & Desta, Z. Comprehensive in vitro analysis of voriconazole inhibition of eight cytochrome P450 (CYP) enzymes: major effect on CYPs 2B6, 2C9, 2C19, and 3A. *Antimicrob. Agents Chemother.* **53**, 541–551 (2009).
23. Qu, H.L. *et al.* CYP2C19 genetic polymorphism and monitoring voriconazole plasma concentrations in the treatment and prevention of invasive fungal disease for hematological patients [in Chinese]. *Zhonghua Xue Ye Xue Za Zhi* **39**, 202–206 (2018).
24. Mikus, G., Scholz, I.M. & Weiss, J. Pharmacogenomics of the triazole antifungal agent voriconazole. *Pharmacogenomics* **12**, 861–872 (2011).
25. Chawla, P.K. *et al.* Correlation of CYP2C19 genotype with plasma voriconazole levels: a preliminary retrospective study in Indians. *Int. J. Clin. Pharm.* **37**, 925–930 (2015).
26. Wang, T. *et al.* Efficacy and safety of voriconazole and CYP2C19 polymorphism for optimised dosage regimens in patients with invasive fungal infections. *Int. J. Antimicrob. Agents* **44**, 436–442 (2014).
27. Li, Z.W. *et al.* Impact of CYP2C19 genotype and liver function on voriconazole pharmacokinetics in renal transplant recipients. *Ther. Drug Monit.* **39**, 422–428 (2017).
28. Lin, X.B. *et al.* Population pharmacokinetics of voriconazole and CYP2C19 polymorphisms for optimizing dosing regimens in renal transplant recipients. *Br. J. Clin. Pharmacol.* **84**, 1587–1597 (2018).
29. Kirbs, C. *et al.* High voriconazole target-site exposure after approved sequence dosing due to nonlinear pharmacokinetics assessed by long-term microdialysis. *Eur. J. Pharm. Sci.* **131**, 218–229 (2019).
30. Suetsugu, K. *et al.* Impact of CYP3A5, POR, and CYP2C19 polymorphisms on trough concentration to dose ratio of tacrolimus in allogeneic hematopoietic stem cell transplantation. *Int. J. Mol. Sci.* **20**, 2413 (2019).
31. Murayama, N., Imai, N., Nakane, T., Shimizu, M. & Yamazaki, H. Roles of CYP3A4 and CYP2C19 in methyl hydroxylated and N-oxidized metabolite formation from voriconazole, a new anti-fungal agent, in human liver microsomes. *Biochem. Pharmacol.* **73**, 2020–2026 (2007).
32. Pascual, A. *et al.* Challenging recommended oral and intravenous voriconazole doses for improved efficacy and safety: population pharmacokinetics-based analysis of adult patients with invasive fungal infections. *Clin. Infect. Dis.* **55**, 381–390 (2012).
33. Wang, T. *et al.* Identification of factors influencing the pharmacokinetics of voriconazole and the optimization of dosage regimens based on Monte Carlo simulation in patients with invasive fungal infections. *J. Antimicrob. Chemother.* **69**, 463–470 (2014).
34. Perkhofer, S., Trappl, K., Striessnig, B., Nussbaumer, W. & Lass-Florl, C. Platelets enhance activity of antimycotic substances against non-Aspergillus fumigatus Aspergillus species in vitro. *Med. Mycol.* **49**, 157–166 (2011).
35. Mangal, N. *et al.* Optimization of voriconazole therapy for the treatment of invasive fungal infections in adults. *Clin. Pharmacol. Ther.* **104**, 957–965 (2018).
36. Yan, M. *et al.* The impact of proton pump inhibitors on the pharmacokinetics of voriconazole in vitro and in vivo. *Biomed. Pharmacother.* **108**, 60–64 (2018).
37. Bernal, C.J. *et al.* CYP2C19 phenotype and risk of proton pump inhibitor-associated infections. *Pediatrics* **144**, e20190857 (2019).
38. Qi, F., Zhu, L., Li, N., Ge, T., Xu, G. & Liao, S. Influence of different proton pump inhibitors on the pharmacokinetics of voriconazole. *Int. J. Antimicrob. Agents* **49**, 403–409 (2017).
39. Hashemizadeh, Z., Badiee, P., Malekhoseini, S.A., Raeisi Shahraki, H., Geramizadeh, B. & Montaseri, H. Observational study of associations between voriconazole therapeutic drug monitoring, toxicity, and outcome in liver transplant patients. *Antimicrob Agents Chemother* **61**, e01211–e01217 (2017).
40. Chayakulkeeree, M., Poovipirom, N., Siengwattana, P. & Maneerattanaporn, M. Effect of proton pump inhibitor on plasma voriconazole concentration in Thai patients. *J. Med. Assoc. Thai.* **98**, 232–237 (2015).
41. Dolton, M.J., Ray, J.E., Chen, S.C., Ng, K., Pont, L.G. & McLachlan, A.J. Multicenter study of voriconazole pharmacokinetics and therapeutic drug monitoring. *Antimicrob. Agents Chemother.* **56**, 4793–4799 (2012).
42. Utano, T. *et al.* Tacrolimus blood concentration increase depends on administration route when combined with voriconazole in pediatric stem cell transplant recipients. *Pediatr. Transplant.* **24**, e13619 (2020).
43. Ota, R. *et al.* Relationship between the blood concentrations of tacrolimus and voriconazole in hematopoietic stem cell transplant recipients. *Int. J. Clin. Pharmacol. Ther.* **57**, 561–566 (2019).
44. Cheng, L. *et al.* Therapeutic drug monitoring and safety of voriconazole in elderly patients. *Int. Immunopharmacol.* **78**, 106078 (2020).
45. Spolitak, T., Hollenberg, P.F. & Ballou, D.P. Oxidative hemoglobin reactions: applications to drug metabolism. *Arch. Biochem. Biophys.* **600**, 33–46 (2016).
46. Han, N. *et al.* Prediction of the tacrolimus population pharmacokinetic parameters according to CYP3A5 genotype and clinical factors using NONMEM in adult kidney transplant recipients. *Eur. J. Clin. Pharmacol.* **69**, 53–63 (2013).
47. Vanhove, T. *et al.* Determinants of the magnitude of interaction between tacrolimus and voriconazole/posaconazole in solid organ recipients. *Am. J. Transplant.* **17**, 2372–2380 (2017).
48. Giri, P., Naidu, S., Patel, N., Patel, H. & Srinivas, N.R. Evaluation of in vitro cytochrome P450 inhibition and in vitro fate of structurally diverse n-oxide metabolites: case studies with clozapine, levofloxacin, roflumilast, voriconazole and zopiclone. *Eur. J. Drug Metab. Pharmacokinet.* **42**, 677–688 (2017).
49. Vanstraelen, K. *et al.* Impact of hypoalbuminemia on voriconazole pharmacokinetics in critically ill adult patients. *Antimicrob. Agents Chemother.* **58**, 6782–6789 (2014).
50. Veringa, A. *et al.* Voriconazole metabolism is influenced by severe inflammation: a prospective study. *J. Antimicrob. Chemother.* **72**, 261–267 (2017).