

Article

Predictors of Voriconazole Trough Concentrations in Patients with Child-Pugh Class C Cirrhosis: A Prospective Study

Yi-Chang Zhao^{1,2}, Jing-jing Hou^{1,2*}, Yi-Wen Xiao^{1,2}, Feng Wang^{1,2}, Bi-Kui Zhang^{1,2}, Min Zhang^{1,3}, Yong-fang Jiang^{1,3}, Jia-Kai Li^{1,2}, Guo-zhong Gong^{1,3}, Da-Xiong Xiang^{1,2}, Miao Yan^{1,2*}

¹ The Second Xiangya Hospital, Central South University, Changsha, Hunan, P.R. China 410011;

² Institute of Clinical Pharmacy, Central South University, Changsha, Hunan, P.R. China 410011;

³ Department of Infection, Central South University, Changsha, Hunan, P.R. China 410011;

* Co-author: Yi-chang Zhao and Jing-jing Hou contributed equally to the study. Author order was determined on the basis of seniority.

* Corresponding author: the Second Xiangya Hospital, Central South University; Institute of Clinical Pharmacy, Central South University, Changsha, China; Tel: +86-073185292098; Fax: +86-073184436720; E-mail addresses: yanmiao@csu.edu.cn.

Abstract: This prospective observational study aimed to describe voriconazole administrations and trough concentrations in patients with Child-pugh class C clinically, and to investigate the variability of trough concentration. A total of 144 voriconazole trough concentrations from 43 Child-pugh class C patients were analyzed. The majority of patients (62.8%) received adjustments. The repeated measured trough concentration was higher than the first and final ones generally (median, 4.33 vs. 2.99, 3.90 mg/L). Eight patients with ideal initial concentration later got supratherapeutic with no adjusted daily dose, implying accumulation. There was a significant difference in concentrations among the six groups by daily dose ($P=0.006$). The bivariate correlation analysis showed that sex, CYP2C19 genotyping, daily dose, prothrombin time activity, international normalized ratio, platelet, and Model for end-stage liver disease score were significant factors for concentration. Subsequently, the first four factors mentioned above entered into a stepwise multiple linear regression model (variance inflation factor < 5), implying that CYP2C19 testing makes sense for precision medicine of Child-pugh class C cirrhosis patients. The equation fits well and explains the 34.8% variety of concentrations ($R^2 = 0.348$). In conclusion, it needs more cautious administration clinically due to no recommendation for Child-pugh class C patients in the medication label. The adjustment of the administration regimen should be mainly based on the results of repeated therapeutic drug monitoring.

Keywords: Child-pugh C cirrhosis; voriconazole; trough concentrations; administration; CYP2C19

1. Introduction

Patients with Child-pugh class C (CP-C) cirrhosis, the most terrible class of liver cirrhosis in the Child-Pugh classification[1-3], are at a high risk of invasive fungal infections (IFIs)[4,5]. IFIs in CP-C patients are probably caused by the immunocompromised state and application of a large number of steroids and broad-spectrum antimicrobial agents, as well as invasive procedures[6]. It takes a heavy toll on outcome with high in-hospital mortality rates if not promptly recognized and treated[7,8], ascribed to the rapid progression with a lung infection and even septic shock[6,9,10].

Voriconazole is a broad-spectrum triazole antifungal agent with good in-body activity against fungal pathogens[11]. It is commonly used clinically to prevent and treat IFIs and is recommended as a first-line drug for invasive aspergillosis by Infectious Diseases Society of America (IDSA) and the Clinical Pharmacogenetics Implementation Consortium (CPIC) [12,13]. Targeted concentrations were recommended between 1.0-5.5 mg/L by British guidelines and other studies[14,15].

Voriconazole is metabolized primarily by the cytochrome P450 (CYP) 2C19, also a substrate for CYP3A4 and CYP2C9 enzyme[16]. The gene polymorphism of CYP2C19 and its mediated metabolism can be saturated within the therapeutic concentration range, resulting in the greater individual differences of pharmacodynamic parameters[17]. Voriconazole displays nonlinear pharmacokinetic (PK) in adults and manifests extreme inter- and intra-individual PK variability in all patient populations, which are also associated with liver dysfunction, drug-drug interaction and so on[18-21]. Although under the standard administration scheme, the voriconazole trough concentration[18], related to clinical efficacy, is distributed in the range of less than 0.2 mg/L to 12 mg/L, even exceptionally high (17.5 mg/L), leading to adverse reactions[22,23]. The medication label of voriconazole recommends that individuals with mild to moderate cirrhosis (Child-Pugh Class A and B) receive the same loading dose as individuals with hepatic function, but half the maintenance dose, while no recommendation is given for individuals with CP-C cirrhosis. The voriconazole terminal half-life ($t_{1/2}$) of CP-C patients in ICU were reported to be increased to about 5 times, resulting in high plasma exposure[24]. All in all, it is particularly urgent and vital to describe the use and trough concentration of voriconazole in CP-C patients.

This prospective observational study aimed to describe voriconazole administrations and trough concentrations in patients with Child-pugh class C clinically and investigate the variability of trough concentration.

2. Results

2.1. Patient characteristics

43 CP-C Asian patients were eligible to participate in the study (Figure 1a). Clinical characteristics were summarized in Table 1. The female-to-male ratio was 4/39. The mean patient age and weight were 49.35 ± 11.65 years (range, 32 to 89 years) and 61.27 ± 12.87 kg (range, 36 to 99 kg). The median duration of treatment with voriconazole was 12 days (range, 5 to 45 days). The median sampling time of C_0 was 5 days (interquartile range (IQR), 3 to 5 days; range, 3 to 11 days). 15, 23, 5 out of 43 patients (34.9%, 53.5%, 11.6%) took voriconazole orally, intravenously or in sequential therapy before measuring C_0 , respectively. Moreover, a total of 23.3% (10/43) of patients received transform from intravenously to orally. During the voriconazole therapy period, a total of 37 (86.0%) patients concomitantly used CYP2C19 inhibitors (31/43, 72.1%), antimicrobial agents (25/43, 58.1%), or CYP3A4 inhibitors (1/43, 2.3%), including proton pump inhibitors, antibacterial and antifungal agents. Adverse events identified as voriconazole related were reported in 20 patients (46.5%), including dizziness, hallucinations and visual disturbance such as altered colour discrimination, blurred vision and photophobia. The median duration from voriconazole initiation to onset of adverse events was 2 days (range, 1 to 12 days).

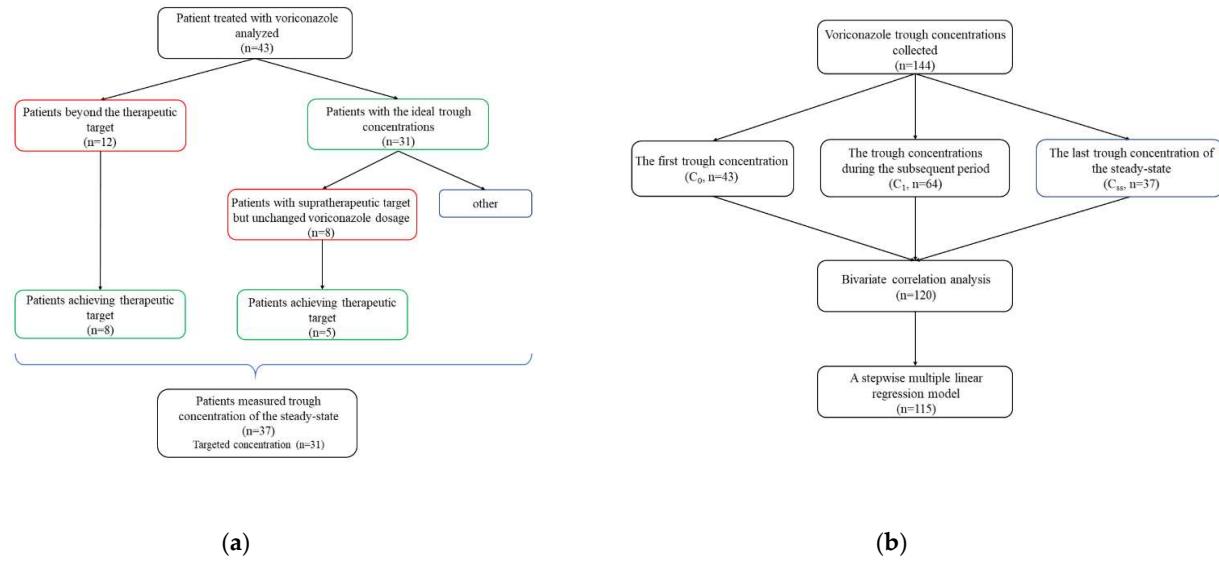


Figure 1. The flow charts (a) changes of voriconazole trough concentrations in 43 patients during treatment clinically; (b) statistical analysis of 144 measured voriconazole trough concentrations.

Table 1. Demographic, clinical data and voriconazole regimen for 43 CP-C patients with therapeutic drug monitoring of voriconazole.

Variable	Value
Mean±S (range) age (years)	49.35±11.65 (32-89)
Sex (no. [%] of males)	39 (90.7)
Mean±S (range) weight (kg)	61.27±12.87 (36-99)
No. (%) Genotype of CYP2C19	
*1/*1	20 (46.5)
*1/*17	1 (2.3)
*1/*2	13 (30.2)
*1/*3	3 (7.0)
*2/*2	6 (14.0)
No. (%) route of administration before C_0	
intravenously	15 (34.9)
orally	23 (53.5)
in sequential therapy	5 (11.6)
No. (%) transform from intravenously to orally throughout the treatment	10 (23.3)
Median sampling time of C_0 (IQR, range)	5 (3-5, 3-11days)
Concentrate (mg/mL)	
Median (IQR, range) voriconazole trough level (mg/L)	3.745 (2.485-5.6425, 0.32-14.08) n=144
C_0	2.99 (1.61-5.00, 0.32-14.08) n=43
C_1	4.33 (3.0775-6.1000, 1.86-11.83) n=64
C_{ss}	3.90 (2.51-4.84, 0.60-10.70) n=37
Proportion (%) of targeted C_{minS} (mg/L) ¹	
C_0	31/43 (72.1)
C_{ss}^2	31/37 (83.8)
C_{ss1}^3	8/12 (66.6)

C_{ss2}^4	5/8 (62.5)
No. (%) of adjustment times	
0	16 (37.2)
1	11 (25.6)
2	7 (16.3)
3-7	9 (21.9)
No. (%) of concomitant medication ⁵	
CYP2C19 inhibitors	31 (72.1)
Antimicrobial	25 (58.1)
CYP3A4 inhibitors	1 (2.3)

¹Targeted concentrations were recommended between 1.0-5.5 mg/L.

² C_{ss} was available from 37 patients with CP-C cirrhosis, while the remaining six patients have no C_{ss} .

³ C_{ss1} was evaluated in 12 patients with untargeted C_0 .

⁴ C_{ss2} was evaluated in 8 patients with supratherapeutic concentration, whereas no adjusted daily dose was applied in their subsequent therapy.

⁵Concomitant medication was applied to 37 out of 43 CP-C patients.

CYP, cytochrome P450; C_{minS} , voriconazole trough concentration; C_0 , the first trough concentration after voriconazole therapy; C_{ss} , the final trough concentration; $C1$, the repeated measured trough concentration except C_{ss} ; IQR, interquartile range

2.2. CYP2C19 genotyping

*CYP2C19*1/*1* was the most commonly identified genotype (20 [46.5%]), followed by *CYP2C19*1/*2* (13 [30.2%]), *CYP2C19*2/*2* (6 [14.0%]), *CYP2C19*1/*3* (3 [7.0%]) and *CYP2C19*1/*17* (1 [2.3%]) (Table 1). For subsequent analysis, *CYP2C19 *1/*17* was analysis appropriately together with *CYP2C19*1/*1* so that *CYP2C19* phenotypes were classified into four categories, **1/*1*, **1/*2*, **1/*3* and **2/*2*.

2.3. Administration dosage of voriconazole

Table 2 showed that the loading dosage were given in 51.2% patients and 400mg/12h, 200mg/12h or 200mg/24h were the most common administrations (5/22 [22.7%], 4/22 [18.2%], 4/22 [18.2%], respectively). On the other hand, the maintenance dosage of 100 mg/24h was the major regimen (10, 23.3%). As for the adjustment, the majority of patients (27/43 [62.8%]) received adjusted administration, especially 25.6% once (11/43), followed by three or more times (9, 21.9%) and two times (7, 16.3%).

Table 2. Administration dosage of voriconazole in 43 CP-C patients²

Regimen	Value
No. (%) of Loading dosage (mg)a	22 (51.2)
400 /12 h	5 (22.7)
200 /12 h	4 (18.2)
200 /24 h	4 (18.2)
No. (%) of Maintain dosage (mg)	
200 /12 h	8 (18.6)
200 /24 h	8 (18.6)
100 /24 h	10 (23.3)
100 /12 h	9 (20.9)
No. (%) of final steady-state administration (mg)	
200 /24h	8 (18.6)
100 /24h	15 (34.9)

50 /12h	8 (18.6)
---------	----------

¹Loading dosage was applied to 22/43 patients.

²Three most widely applied regimens in 43 patients with CP-C cirrhosis were showed.

2.4. Voriconazole trough concentrations

During the therapy period of voriconazole, a total of 144 plasma concentrations from 43 patients were analyzed in the present study in Table 1 and Figure 1b, and showed by scatterplot and box plot in Figure 2. The first trough concentration after voriconazole therapy (C_0), the repeated measured one (C_1) and the final one (C_{ss}) were 2.99 mg/L (IQR, 1.61-5.00 mg/L; range, 0.32-14.08 mg/L, n=43), 4.33 mg/L (IQR, 3.0775-6.1000 mg/L; range, 1.86-11.83 mg/L, n=64), 3.90 mg/L (IQR, 2.51-4.84 mg/L; range, 0.60-10.70 mg/L, n=37), respectively. Median sampling time of C_0 was five days (IQR, 3 to 5 days; range, 3 to 11 days) after voriconazole initial therapy, respectively.

A total of 12 patients did not achieve the ideal target trough concentration with 75% of C_0 over the upper target concentration, whose adjusting progress (range, 2-10 days; mode, two days) with 50 C_{min} s totally was showed in Figure 3. On the other hand, for thirty-one (72.1%) patients with the targeted C_0 , C_{min} s showed an upward trend. Among them, eight patients (8/31, 25.8%) were supratherapeutic levels for concentration, whereas no adjusted daily dose in the subsequent therapy (Figure 4). Besides, only four patients (4/43, 9.3%) always got the targeted C_{min} s, while a total of 27 patients (62.8%) appeared unideal ones once or more and then accepted adjustment, in which 23 patients were cut the dosage and the remaining 4 patients experienced dose fluctuations. Finally, there were 66.6% (8/12), 62.5% (5/8), and 83.8% (31/37) patients with C_{ss} achieving the therapeutic target after adjustment, respectively.

The result of the Student's t-test showed that there were no significant differences in the corresponding concentration between 50 mg and 100 mg ($P = 0.129$), 150 mg and 200 mg ($P = 0.400$), so we incorporated them into the same group. Further results showed the significant difference among the three groups by daily dose approximately ($P = 0.003$) (Figure 4). Compared to those of 200 mg, the concentrations were significantly lower of 100 mg ($P = 0.041$) and higher of 400 mg or more ($P = 0.003$) (Figure 5).

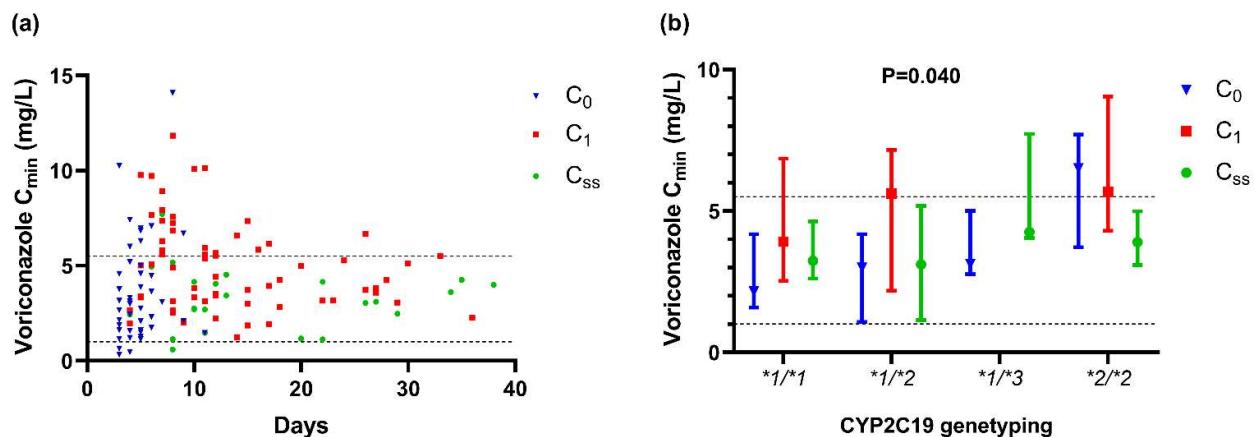


Figure 2. For 12 patients started beyond the therapeutic target (a) Distinction of voriconazole trough concentration in different CYP2C19 phenotype groups (n=50, $P=0.021$). The Kruskal-Wallis test was used to compare C_{min} s. (b) Distinction of voriconazole trough concentration in different groups of C_0 , C_1 , C_{ss} (n=50), and daily dose (n=27). Data are expressed as the median \pm interquartile range. (c) the mean concentration of such 12 patients changed by days. (d) every dosage adjustment (n=247) and the mode dose (n=45) by days (It showed the highest one if there are more than one mode).

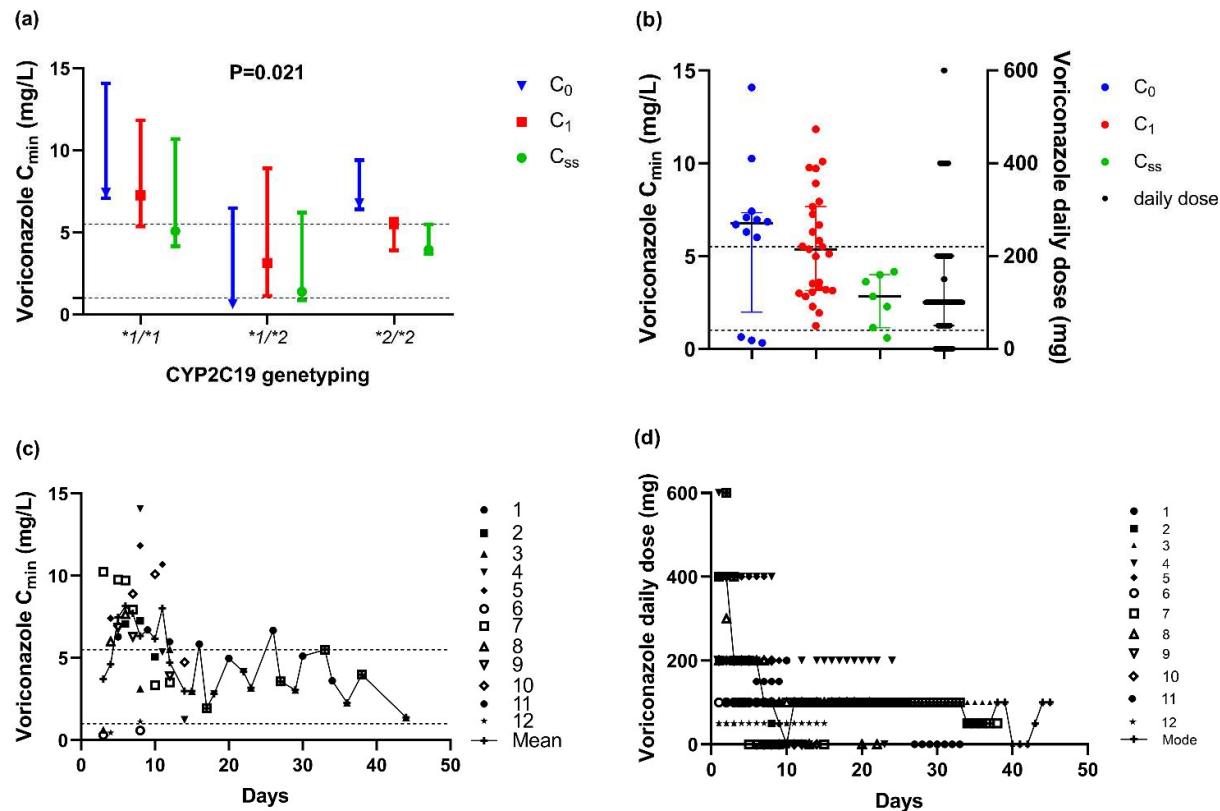


Figure 3. Distinction of voriconazole trough concentration of 43 patients (a) as the therapeutic progress by days (n=144); (b) in different CYP2C19 phenotype groups (n=103, P=0.040). The Kruskal–Wallis test was used to compare C_{min} s of the four groups. Data are expressed as the median \pm interquartile range.

C_{min} , voriconazole trough concentration; CYP2C19, cytochrome P450 2C19; C_0 , the first trough concentration after voriconazole therapy; C_{ss} , the final trough concentration except C_{ss} .

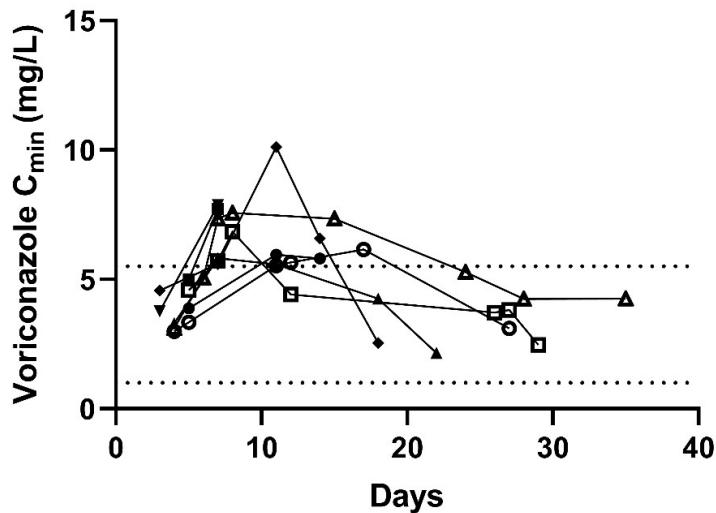


Figure 4. The voriconazole trough concentrations by days of 8 patients who had targeted C_0 but supratherapeutic C_1 during the constant unchanged therapy dosage of voriconazole (n=38).

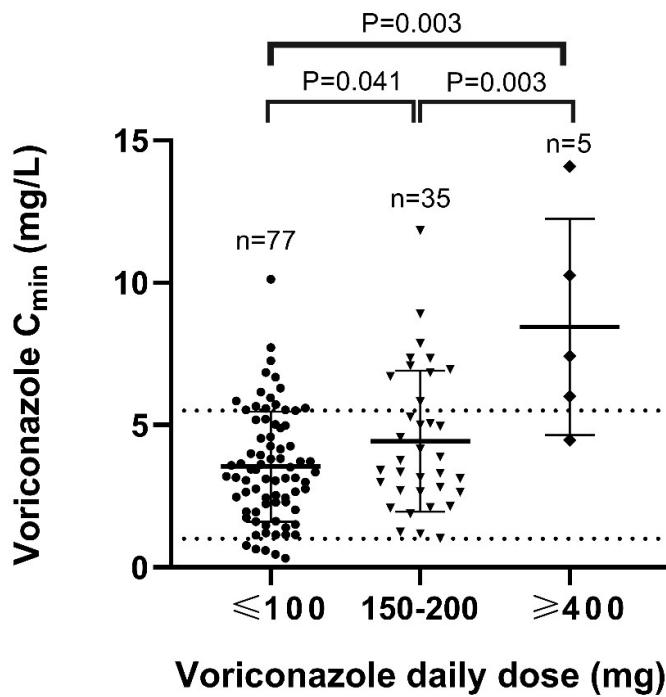


Figure 5. Distribution of voriconazole trough levels over daily dosages. The numbers of measurements for each daily dose are reported.

2.5. Factors affecting voriconazole trough concentration

It might be adjusted several times or withdrawn when the voriconazole trough concentration was not within the therapeutic target, and 120 C_{min} s were selected while the remaining of 144 C_{min} s were excluded because of admeasurement after withdrawal or lack of the corresponding key values of clinical or laboratory data. The results of bivariate correlation analysis show that sex, the genotype of CYP2C19, daily dose, prothrombin time activity (PTA), international normalized ratio (INR), platelet, and Model for end-stage liver disease (MELD) score are significant factors. Further, daily dose and platelet are closely correlated with voriconazole concentration (absolute value of coefficient >0.3) (Table 3).

Artificial extracorporeal liver support, CYP2C19 inhibitors, and antimicrobial agents, including proton pump inhibitors, antibacterial and antifungal agents, did not affect subsequent voriconazole trough concentrations ($P = 0.540, 0.962$, and 0.775 , respectively). Similarly, there were no significant associations between patient age or weight and voriconazole trough levels (Table 3).

Table 3. Multivariate bivariate correlation analysis of factors associated with voriconazole plasma concentration.

Variable	Coefficient	P value
Age	-0.064	0.485
Sex	0.221	0.015*
Weight	0.001	0.993
Daily dose	0.329	<0.001*
CYP2C19 genotyping ¹		
*1/*2	-0.068	0.417
*1/*3	0.196	0.018*

*2/*2	0.216	0.009*
Platelet	-0.302	0.001*
INR	0.184	0.047*
PTA	-0.278	0.002*
MELD score	0.184	0.048*
Hemoglobin	-0.063	0.518
Alanine aminotransferase	-0.037	0.696
Aspartate aminotransferase	0.051	0.588
Total bilirubin	0.104	0.265
Direct bilirubin	0.092	0.324
Bile acid	0.016	0.862
Albumin	0.153	0.101
Blood urea nitrogen	0.058	0.532
Creatinine	0.044	0.638
Creatinine Clearance	0.010	0.915
Prothrombin time	0.153	0.100
Artificial extracorporeal liver	0.044	0.635
Concomitant agents ²		
CY2C19 inhibitors	0.048	0.609
Antimicrobial agents	-0.010	0.919

¹CYP2C19 genotyping was dealt with the operation of dummy variables compared to CYP2C19*1/*1. We considered the patient with CYP2C19 *1/*17 (n=1) within the same group as the CYP2C19*1/*1.

²CYP2C19 inducers was not analyzed as only one patient applied.

IQR, interquartile range; PTA, prothrombin time activity; INR, international normalized ratio; MELD, Model for end-stage liver disease

Subsequently, the statistically significant factors entered into a stepwise multiple linear regression model of 115 C_{mins}, specifically, daily dose, CYP2C19 genotyping, sex, and PTA. Moreover, these factors were not collinear with one another (VIF < 5) considered independent and not confounding. Daily dose contributed the most to the voriconazole concentration, followed by PTA, sex, and genotype of CYP2C19 (Table 4). The final results showed that the model fitted well (R² = 0.348) and was statistically significant (P < 0.001).

On average, compared to the concentration of patients in CYP2C19*1/*1, the concentration tended to be 1.308mg/L higher in CYP2C19*2/*2 patients (P=0.014), whereas no significant change in other genotyping (P=0.535, 0.09). Moreover, the C_{mins} would increase by 1.3 mg/L as the dosage increase by 100 mg. By contrast, male patients would decrease 1.651 mg/L compared to the female. And PTA count was implicated with the voriconazole trough concentration as well. The concentration would decrease by 0.035 mg/L with a one-unit increase in PTA count (Table 4).

Variable	Unstandardized Coefficients		Standardized Coefficients	t	P value	VIF
	B	Std. Error				
Intercept	1.760	0.849	-	2.072	0.041	-
Daily dose	0.012	0.002	0.439	5.236	0.000	1.069
PTA	-0.036	0.012	-0.238	-3.139	0.002	1.038
Sex	1.602	0.598	-0.217	2.645	0.009	1.064
CYP2C19 genotyping ¹						
*1/*2	0.266	0.428	0.053	0.622	0.535	1.192

*1/*3	1.252	0.733	0.140	1.708	0.090	1.121
*2/*2	1.492	0.596	0.209	2.503	0.014	1.166
F=9.686						<0.001

Table 4. The multiple linear regression model about voriconazole trough concentration².¹Compared to *CYP2C19*1/*1*, dealt with the operation of dummy variables.²R² = 0.331; Durbin-Watson test value =1.801; 115 voriconazole trough concentrations was selected and analyzed.The multiple linear regression equation¹ was as follows:

$$C_{\min} = 0.012^* \text{ daily does} + CYP2C19^* (0.266^* A + 1.252^* B + 1.492^* C) + 1.602^* \text{gender}^* - 0.036^* \text{PTA} + 1.760 \quad (1)$$

("A=1, B=1 or C=1" if the patient is identified as *CYP2C19 *1/*2, *1/*3, *2/*2*, respectively, otherwise "A=0, B=0 or C=0"; "D=1" if the patient is male, otherwise "D=0")¹Compared to *CYP2C19*1/*1*, dealt with the operation of dummy variables.

3. Discussion

Patients with CP-C cirrhosis are susceptible to fungal infection, a major cause of morbidity and mortality in cirrhosis[25]. Voriconazole is a broad-spectrum triazole anti-fungal agent with good in-body activity against *Aspergillosis* bacteria, fluconazole-resistant *Candida*[11]. It is commonly used clinically to prevent and treat IFIs, recommended as a first-line drug for invasive aspergillosis by IDSA and CPIC[12,13].

The oral bioavailability of voriconazole was 91.6%, approach to that of intravenously, and the intravenously and orally showed similar efficacy and safety in patients with severe hepatic dysfunction[26], whereas the quality of evidence was inferior and not for cirrhosis patients[27]. Although we observed 23.3% of patients received transform from intravenously to orally, the present study did not study the effect of the administration route for CP-C patients.

Targeted concentrations were recommended between 1.0-5.5 mg/L by British guidelines and other studies[14,15]. In this study, the C₁ was higher than C₀ and C_{ss} generally (4.33 vs. 2.99, 3.90 mg/L; IQR, 1.61-5.00 vs. 3.0775-6.1000, 2.51-4.84 mg/L). Only 9.3% (4/43) patients always got the targeted trough concentrations after antifungal therapy, and 62.8% of patients (27/43) applied to adjustment. It is noteworthy that among the 31 (72.1%) patients with targeted C₀, the subsequent concentrations of 25.8% (8/31) patients went upper the target (>5.5 mg/L) on the unchanged regimen, implying accumulation. It was consistent with the results on voriconazole median terminal t_{1/2} and clearance of CP-C patients in ICU (60.7 h and 2.04 L/h, respectively)[24]. These results mean that repeated TDM is necessary. While among the 12 patients, 66.6% (8/12 patients) achieved the therapeutic target after adjusting. The majority got timely withdrawal or the targeted final C_{minS}, whether for 12 patients with unideal C₀, 8 patients with unideal C₁, or 37 CP-C patients measured C_{ss} (66.6%, 62.5%, or 83.3%, respectively). Above all, we conclude that voriconazole is prone to vary even in the same patients with CP-C. Voriconazole displays high inter-individual variability, and the administration in therapeutic doses leads to extremely varied serum levels from patient to patient and even in the same patient[28]. Voriconazole concentrations were closely related to efficacy and safety. Therefore, TDM is essential to provide patient-specific dosing recommendations, leading to more effective antifungal regimens to increase clinical efficacy and reduce adverse drug reactions[29]. We recommend more cautious administration for CP-C patients and more timely and appropriate adjustments in the clinic.

Voriconazole is metabolized primarily by CYP2C19, and the previous research reported on the low frequency of the *CYP2C19*17* allele, an ultrarapid metabolizer genotype, in Chinese subjects (< 5%) relative to Ethiopians and Swedes (18% in both)[30,31].

Similarly, this study only collected one patient (2.3%) with *CYP2C19* *1/*17, and for the sake of simplicity, we considered him within the same group as the wild-type *CYP2C19**1 homozygote, similar to the classification of Sugimoto *et al.*[32]. Simultaneously, the *CYP2C19**17 allele's clinical relevance should not yet be discounted because the available evidence suggests that it predicted both voriconazole exposure and the dose required to achieve effective and non-toxic concentrations[33,34]. The present study investigated the significant factors, sex, genotype of *CYP2C19*, daily dose, PTA, INR, platelet, and MELD score associated with the variability of voriconazole trough concentrations (n=117). Interestingly, the MELD score is another score of end-stage liver disease and is got by PTA value, related to INR, affecting our result. Shi C drew the same conclusion of the correlation between the genotype of *CYP2C19* and concentration[20]. The other research team members have drawn a similar conclusion for platelet count, that platelet count was statistically significantly associated with voriconazole pharmacokinetics[35]. Except for the above factors, the variability of concentrations can be partly explained by non-linear kinetics, weight, liver function, concomitant medications[20], clinical and laboratory data such as C-reactive protein[17,36], albumin[37], and hemoglobin[38]. The different findings from these studies and the influence of sex found by this one may be due to the limited sample size and the large proportion of male patients (90.7%). Although, we didn't find the effect of artificial extracorporeal liver support and *CYP2C19* inhibitors in this specific population, further research is needed, considering that *CYP2C19* inhibitors can influence voriconazole exposure to a great extent theoretically[39] and the effect on drug metabolism and excretion of artificial extracorporeal liver support is still unclear[40].

Furthermore, the multiple linear regression model (1) is meaningful ($p < 0.001$, Durbin-Watson test value = 1.801). Daily dose contributed the most to the voriconazole concentration, followed by PTA, sex, and genotype of *CYP2C19* according to the standardized coefficients, without collinear with each other ($VIF < 5$). *CYP2C19**2/*2, the poor metabolizer, is related to the higher concentration, implying that *CYP2C19* testing is still meaningful for precision medicine of Child-pugh class C cirrhosis patients. Though there is insufficient evidence on the relationship between *CYP2C19* genotypes and clinical outcomes, there is a great potential for the initial voriconazole dose selection to be guided by the *CYP2C19* genotype[41]. Furthermore, more and larger studies, including multiple doses, are needed to confirm the impact of pharmacogenetics on voriconazole. The equation fits well and explains the 34.8% variety of voriconazole trough concentrations ($R^2 = 0.348$), which means remaining relatively high unexplained variability and demanding further improvement.

The results showed significant differences in the three groups' corresponding concentration by daily dose ($P = 0.003$). Administration dosage is a major essential factor contributing to the highly variable concentrations of voriconazole. However, the medication label of voriconazole did not recommend for individuals with CP-C cirrhosis, which means the experienced regimen is the common choice in the clinic, and it is urgent and vital to find a rational administration in such patients. The present study results imply that the administration regimen may be inappropriate in patients with CP-C cirrhosis, concretely, a loading dose regimen of 400 mg and maintenance dose of 100 mg twice daily or 200 mg daily orally or intravenously because of the higher voriconazole trough concentration[42]. We recommend the half loading dose and far less maintenance dose than that of the patients with Child-pugh class A or B, and 100 mg or less daily orally or intravenously is a better choice. Dialetically, considering the wide range of C_{minS} in each genotypic group and overlapping concentrations (Fig 1 and Fig 2a) and poorer liver function of CP-C patients than others, we doubt that dosing based on *CYP2C19* polymorphism. It is suggested that the adjustment of the administration regimen should be mainly based on the results of TDM, while the genotype is only a reference. Clinically, meticulous monitoring voriconazole trough concentration constantly during therapy is highly needed in patients with Child-Pugh class C cirrhosis to increase clinical efficacy and avoid adverse reactions.

4. Materials and Methods

4.1. Patients

Researchers conducted the prospective observational study and collected the hospital information and laboratory information at one tertiary hospital from January 2018 to December 2019. Patients with CP-C treated by voriconazole for either prophylaxis or treatment of a suspected fungal infection were identified and then screened by applying inclusion criteria of (1.) diagnosed with liver dysfunction, such as liver failure or liver cirrhosis according to the Child-Pugh classifications, (2.) performed therapeutic drug monitoring (TDM) during voriconazole therapy at least three days after initiation of a loading dose or maintenance or an adjusted dose of four days and retest about every three days[26], (3.) aged 18 years and older, and exclusion criteria of (1.) pregnancy or lactation and (2.) missing the key values of voriconazole-related information, clinical data, or laboratory data. The adjustment of administration regimen is mainly under empirical treatment regimens based on the results of TDM. This study was approved by the Ethics Committee of The Second XiangYa Hospital of Central South University with an approved NO. yxlb-lays-2018012.

4.2. Sample collection, storage, and bio-analysis

Venous blood samples (2 mL) were collected into anticoagulant tubes. In addition, the steady-state of C_{min} of VRZ is recommended to be monitored, so samples were collected at least three days after initiation of a loading dose or maintenance and an adjusted dose of four days as well as within 2 h before any maintenance doses[26]. Monitoring continued every three days. All voriconazole plasma concentrations were analysed by automatic two-dimensional liquid chromatography (2D-HPLC, Demeter Instrument Co., Ltd., Hunan, China)[35]. The two-dimensional separation conditions consisted of FRO C18 (100 mm×3.0 mm, 5 μ m, ANAX) and ASTON HD C18 (150 mm×4.6 mm, 5 μ m, ANAX) with the same flow rate 1.0 mL/min. The intra-day and inter-day precisions were 1.94% to 2.22% and 2.15% to 6.78%, respectively. The absolute and relative recovery ranged from 88.2% to 93.6% and 94.2% to 105.3%. This method is being commonly applied in the clinical and passed the external quality assessment (EQA) annually. The stability of blood sample at room temperature for 8 hr and at -20°C of three repeated freeze-thaw cycles was within $\pm 8\%$ and $\pm 10\%$, respectively. This laboratory participated in the annual national external quality assessment scheme.

4.3. Genotype of CYP2C19

Genomic DNAs Deoxyribonucleic acid (DNA) of CYP2C19 was extracted from whole blood samples (1-3 mL) using commercially available EZNA® SQ. Blood DNA Kit II. Subsequently, the Sanger dideoxy DNA sequencing method was used for CYP2C19 genotyping using the ABI3730xl fully automatic sequencing instrument (ABI Co.) from Boshang Biotechnology Co.Ltd (Shanghai, China).

4.4. Data collection

Data were extracted from medical records and laboratory information system, including voriconazole-related information (C_{min} , timing, amount, route), demographics, laboratory data, and concomitant medications (antimicrobial agents, CYP2C19 inducers, and inhibitors). The first trough concentration, the repeated measured ones and the last one of patients were recorded as C_0 , C_1 , C_{ss} , respectively. The Child-Pugh class and MELD score were calculated based on previous criteria.

4.5. Statistical analysis

Data were presented as the mean \pm standard (range) for continuous variables. The box plot showed by median and quartile.

The normality of quantitative data was tested using the Shapiro-Wilk test or Kolmogorov-Smirnov according to the sample size. On the basis of the result of normality, the statistical approach chosen was one of the four, the Student's t-test, the nonparametric

Kruskal-Wallis test, Mann-Whitney U test, or one-way ANOVA, to compare voriconazole trough concentrations, as appropriate.

The Pearson correlation and nonparametric Spearman correlation were used to study the relationship between clinical or laboratory data and C_{\min} s during the therapy, according to the type of variables. Then we identified the factors associated with variability of the voriconazole trough level. Statistically significant and independent factors correlated to voriconazole trough concentrations were filtered out to participate in the subsequent multiple linear regression and identified contributing to the variability in C_{\min} s. The C_{\min} s measured after withdrawal or lack of the corresponding key values of clinical or laboratory data were excluded in pairs in the bivariate correlation analysis or in rows in the multiple linear regression. For the regression analysis, the genotype of *CYP2C19* was set as a dummy variable. A variance inflation factor (VIF) of >5 was considered indicative of multicollinearity.

Data analysis was performed using IBM SPSS Statistics version 25 (IBM, New York, NY), and the figures were drawn using GraphPad Prism version 8 (San Diego, CA, USA). All tests were two-sided. P values of <0.05 were considered significant.

5. Conclusions

This study explored predictors related to voriconazole administration regimen clinically and the change of trough concentrations in different periods and their effects for individuals with Child-pugh class C cirrhosis in the prospective fashion.

- Sex, *CYP2C19* genotyping, daily dose, PTA, INR, platelet, and MELD score correlated with the measured trough concentrations.
- Daily dose, PTA, sex and genotype of *CYP2C19* entered into a stepwise multiple linear regression about concentrations, and daily dose contributed the most. *CYP2C19*2/*2* contributed high concentrations, meaning that *CYP2C19* testing for precision medicine of Child-pugh class C cirrhosis patients.
- Attach great importance to therapeutic drug monitoring constantly during voriconazole therapy. In conclusion, it needs more cautious administration clinically due to no recommendation for Child-pugh class C patients in the medication label. The adjustment of the administration regimen should be mainly based on the results of repeated therapeutic drug monitoring.

Author Contributions: Yi-chang Zhao and Jing-jing Hou contributed equally to the study. Jing-jing Hou wrote the main manuscript. Yi-chang Zhao reviewed the manuscript. Min Zhang, Yong-fang Jiang, Guo-zhong Gong, Bi-kui Zhang, Da-Xiong Xiang, Miao Yan, and Yi-chang Zhao designed research. Jing-jing Hou, Yi-wen Xiao, Jia-Kai Li and Feng Wang performed the statistic analysis.

Funding: This study was supported by Hunan Medical Association of China with a fund number [HMA202001002]

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: I declare that my research data is available. And I will share my research data with other researchers if they need. Especially, the data will include, (but not limited to): raw data, processed data, software, algorithms. If additional files are required, they will also be shared on request. Meanwhile, the data will become available since the date it was published. The researchers can send email to me if they are interested in the study and need the research data for analysis.

Acknowledgments: The authors would like to acknowledge Dan-Tang, Yang-Zou, Jia-Min Wu for assistance in collecting clinical data and blood samples. We also would like to thank Doctor Mou-Ze Liu for helping with the concentration analysis.

Conflicts of Interest: The authors have indicated that they have no conflicts of interest regarding the content of this article.

ABBREVIATION

CP-C, Child-pugh class C; CYP2C19, cytochrome P450 2C19; C_{mins} , voriconazole trough concentration; C_0 , the first trough concentration after voriconazole therapy; C_{ss} , the final trough concentration; C1, the repeated measured trough concentration except C_{ss} ; IQR, interquartile range; PTA, prothrombin time activity; INR, international normalized ratio; MELD, Model for end-stage liver disease; VIF, variance inflation factor

References

1. Wan, S.Z.; Nie, Y.; Zhang, Y.; Liu, C.; Zhu, X. Assessing the Prognostic Performance of the Child-Pugh, Model for End-Stage Liver Disease, and Albumin-Bilirubin Scores in Patients with Decompensated Cirrhosis: A Large Asian Cohort from Gastroenterology Department. *Disease markers* **2020**, *2020*, 5193028, doi:10.1155/2020/5193028.
2. Pugh, R.N.; Murray-Lyon, I.M.; Dawson, J.L.; Pietroni, M.C.; Williams, R. Transection of the oesophagus for bleeding oesophageal varices. *The British journal of surgery* **1973**, *60*, 646-649, doi:10.1002/bjs.1800600817.
3. Ampuero, J.; Simón, M.; Montoliú, C.; Jover, R.; Serra, M.; Córdoba, J.; Romero-Gómez, M. Minimal hepatic encephalopathy and critical flicker frequency are associated with survival of patients with cirrhosis. *Gastroenterology* **2015**, *149*, 1483-1489, doi:10.1053/j.gastro.2015.07.067.
4. Theocharidou, E.; Agarwal, B.; Jeffrey, G.; Jalan, R.; Harrison, D.; Burroughs, A.K.; Kibbler, C.C. Early invasive fungal infections and colonization in patients with cirrhosis admitted to the intensive care unit. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2016**, *22*, 189.e181-189.e187, doi:10.1016/j.cmi.2015.10.020.
5. Levesque, E.; Ait-Ammar, N.; Dudau, D.; Clavieras, N.; Feray, C.; Foulet, F.; Botterel, F. Invasive pulmonary aspergillosis in cirrhotic patients: analysis of a 10-year clinical experience. *Annals of intensive care* **2019**, *9*, 31, doi:10.1186/s13613-019-0502-2.
6. Bajaj, J.S.; Liu, E.J.; Kheradman, R.; Fagan, A.; Heuman, D.M.; White, M.; Gavis, E.A.; Hylemon, P.; Sikaroodi, M.; Gillevet, P.M. Fungal dysbiosis in cirrhosis. *Gut* **2018**, *67*, 1146-1154, doi:10.1136/gutjnl-2016-313170.
7. Lahmer, T.; Brandl, A.; Rasch, S.; Baires, G.B.; Schmid, R.M.; Huber, W.; Mayr, U. Prevalence and outcome of invasive pulmonary aspergillosis in critically ill patients with liver cirrhosis: an observational study. *Scientific reports* **2019**, *9*, 11919, doi:10.1038/s41598-019-48183-4.
8. Bajaj, J.S.; Reddy, R.K.; Tandon, P.; Wong, F.; Kamath, P.S.; Biggins, S.W.; Garcia-Tsao, G.; Fallon, M.; Maliakkal, B.; Lai, J.; et al. Prediction of Fungal Infection Development and Their Impact on Survival Using the NACSELD Cohort. *The American journal of gastroenterology* **2018**, *113*, 556-563, doi:10.1038/ajg.2017.471.
9. Bassetti, M.; Peghin, M.; Carnelutti, A.; Righi, E.; Merelli, M.; Ansaldi, F.; Trucchi, C.; Alicino, C.; Sartor, A.; Toniutto, P.; et al. Clinical characteristics and predictors of mortality in cirrhotic patients with candidemia and intra-abdominal candidiasis: a multicenter study. *Intensive care medicine* **2017**, *43*, 509-518, doi:10.1007/s00134-017-4717-0.
10. Arvaniti, V.; D'Amico, G.; Fede, G.; Manousou, P.; Tsochatzis, E.; Pleguezuelo, M.; Burroughs, A.K. Infections in Patients With Cirrhosis Increase Mortality Four-Fold and Should Be Used in Determining Prognosis. *Gastroenterology* **2010**, *139*, 1246-+, doi:10.1053/j.gastro.2010.06.019.
11. Patterson, T.F.; Thompson, G.R., 3rd; Denning, D.W.; Fishman, J.A.; Hadley, S.; Herbrecht, R.; Kontoyiannis, D.P.; Marr, K.A.; Morrison, V.A.; Nguyen, M.H.; et al. Executive Summary: Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2016**, *63*, 433-442, doi:10.1093/cid/ciw444.

12. Patterson, T.F.; Thompson, G.R., 3rd; Denning, D.W.; Fishman, J.A.; Hadley, S.; Herbrecht, R.; Kontoyiannis, D.P.; Marr, K.A.; Morrison, V.A.; Nguyen, M.H.; et al. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2016**, *63*, e1-e60, doi:10.1093/cid/ciw326.
13. Moriyama, B.; Obeng, A.O.; Barbarino, J.; Penzak, S.R.; Henning, S.A.; Scott, S.A.; Agúndez, J.; Wingard, J.R.; McLeod, H.L.; Klein, T.E.; et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP2C19 and Voriconazole Therapy. *Clinical pharmacology and therapeutics* **2017**, *102*, 45-51, doi:10.1002/cpt.583.
14. Ashbee, H.R.; Barnes, R.A.; Johnson, E.M.; Richardson, M.D.; Gorton, R.; Hope, W.W. Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the British Society for Medical Mycology. *The Journal of antimicrobial chemotherapy* **2014**, *69*, 1162-1176, doi:10.1093/jac/dkt508.
15. Lin, X.B.; Li, Z.W.; Yan, M.; Zhang, B.K.; Liang, W.; Wang, F.; Xu, P.; Xiang, D.X.; Xie, X.B.; Yu, S.J.; et al. Population pharmacokinetics of voriconazole and CYP2C19 polymorphisms for optimizing dosing regimens in renal transplant recipients. *British journal of clinical pharmacology* **2018**, *84*, 1587-1597, doi:10.1111/bcp.13595.
16. Theuretzbacher, U.; Ihle, F.; Derendorf, H. Pharmacokinetic/pharmacodynamic profile of voriconazole. *Clinical pharmacokinetics* **2006**, *45*, 649-663, doi:10.2165/00003088-200645070-00002.
17. van Wanrooy, M.J.; Span, L.F.; Rodgers, M.G.; van den Heuvel, E.R.; Uges, D.R.; van der Werf, T.S.; Kosterink, J.G.; Alffenaar, J.W. Inflammation is associated with voriconazole trough concentrations. *Antimicrobial agents and chemotherapy* **2014**, *58*, 7098-7101, doi:10.1128/aac.03820-14.
18. Kadam, R.S.; Van Den Anker, J.N. Pediatric Clinical Pharmacology of Voriconazole: Role of Pharmacokinetic/Pharmacodynamic Modeling in Pharmacotherapy. *Clinical pharmacokinetics* **2016**, *55*, 1031-1043, doi:10.1007/s40262-016-0379-2.
19. Tang, D.; Yan, M.; Song, B.L.; Zhao, Y.C.; Xiao, Y.W.; Wang, F.; Liang, W.; Zhang, B.K.; Chen, X.J.; Zou, J.J.; et al. Population pharmacokinetics, safety and dosing optimization of voriconazole in patients with liver dysfunction: A prospective observational study. *British journal of clinical pharmacology* **2021**, *87*, 1890-1902, doi:10.1111/bcp.14578.
20. Shi, C.; Xiao, Y.; Mao, Y.; Wu, J.; Lin, N. Voriconazole: A Review of Population Pharmacokinetic Analyses. *Clinical pharmacokinetics* **2019**, *58*, 687-703, doi:10.1007/s40262-019-00735-7.
21. Shen, J.; Wang, B.; Wang, S.; Chen, F.; Meng, D.; Jiang, H.; Zhou, Y.; Geng, P.; Zhou, Q.; Liu, B. Effects of Voriconazole on the Pharmacokinetics of Vonoprazan in Rats. *Drug design, development and therapy* **2020**, *14*, 2199-2206, doi:10.2147/dddt.S255427.
22. Wang, T.; Zhu, H.; Sun, J.; Cheng, X.; Xie, J.; Dong, H.; Chen, L.; Wang, X.; Xing, J.; Dong, Y. Efficacy and safety of voriconazole and CYP2C19 polymorphism for optimised dosage regimens in patients with invasive fungal infections. *International journal of antimicrobial agents* **2014**, *44*, 436-442, doi:10.1016/j.ijantimicag.2014.07.013.
23. Boyd, A.E.; Modi, S.; Howard, S.J.; Moore, C.B.; Keevil, B.G.; Denning, D.W. Adverse reactions to voriconazole. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2004**, *39*, 1241-1244, doi:10.1086/424662.
24. Lin, X.B.; Huang, F.; Tong, L.; Xia, Y.Z.; Wu, J.J.; Li, J.; Hu, X.G.; Liang, T.; Liu, X.M.; Zhong, G.P.; et al. Pharmacokinetics of intravenous voriconazole in patients with liver dysfunction: A prospective study in the intensive care unit. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* **2020**, *93*, 345-352, doi:10.1016/j.ijid.2020.02.041.
25. Bajaj, J.S.; O'Leary, J.G.; Tandon, P.; Wong, F.; Garcia-Tsao, G.; Kamath, P.S.; Biggins, S.W.; Lai, J.C.; Vargas, H.E.; Maliakkal, B.; et al. Nosocomial Infections Are Frequent and Negatively Impact Outcomes in Hospitalized Patients With Cirrhosis. *The American journal of gastroenterology* **2019**, *114*, 1091-1100, doi:10.14309/ajg.00000000000000280.

26. 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. *Therapeutic drug monitoring* **2018**, *40*, 663-674, doi:10.1097/ftd.0000000000000561.
27. Wen, A.P.; Chen, K.; Jin, H.Y.; Zhai, S.D.; Zhang, C. Efficacy and safety comparison between intravenous and oral voriconazole: a systematic review and meta-analysis. *Int. J. Clin. Exp. Med.* **2016**, *9*, 16006-+.
28. Trifilio, S.M.; Yarnold, P.R.; Scheetz, M.H.; Pi, J.; Pennick, G.; Mehta, J. Serial plasma voriconazole concentrations after allogeneic hematopoietic stem cell transplantation. *Antimicrobial agents and chemotherapy* **2009**, *53*, 1793-1796, doi:10.1128/aac.01316-08.
29. Zhong, X.; Tong, X.; Ju, Y.; Du, X.; Li, Y. Interpersonal Factors in the Pharmacokinetics and Pharmacodynamics of Voriconazole: Are CYP2C19 Genotypes Enough for Us to Make a Clinical Decision? *Current drug metabolism* **2018**, *19*, 1152-1158, doi:10.2174/1389200219666171227200547.
30. Li-Wan-Po, A.; Girard, T.; Farndon, P.; Cooley, C.; Lithgow, J. Pharmacogenetics of CYP2C19: functional and clinical implications of a new variant CYP2C19*17. *British journal of clinical pharmacology* **2010**, *69*, 222-230, doi:10.1111/j.1365-2125.2009.03578.x.
31. Sim, S.C.; Risinger, C.; Dahl, M.L.; Aklillu, E.; Christensen, M.; Bertilsson, L.; Ingelman-Sundberg, M. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clinical pharmacology and therapeutics* **2006**, *79*, 103-113, doi:10.1016/j.clpt.2005.10.002.
32. Sugimoto, K.; Uno, T.; Yamazaki, H.; Tateishi, T. Limited frequency of the CYP2C19*17 allele and its minor role in a Japanese population. *British journal of clinical pharmacology* **2008**, *65*, 437-439, doi:10.1111/j.1365-2125.2007.03057.x.
33. Dolton, M.J.; McLachlan, A.J. Clinical importance of the CYP2C19*17 variant allele for voriconazole. *British journal of clinical pharmacology* **2011**, *71*, 137-138, doi:10.1111/j.1365-2125.2010.03801.x.
34. Lamoureux, F.; Duflot, T.; Woillard, J.B.; Metsu, D.; Pereira, T.; Compagnon, P.; Morisse-Pradier, H.; El Kholy, M.; Thiberville, L.; Stojanova, J.; et al. Impact of CYP2C19 genetic polymorphisms on voriconazole dosing and exposure in adult patients with invasive fungal infections. *International journal of antimicrobial agents* **2016**, *47*, 124-131, doi:10.1016/j.ijantimicag.2015.12.003.
35. Tang, D.; Song, B.L.; Yan, M.; Zou, J.J.; Zhang, M.; Zhou, H.Y.; Wang, F.; Xiao, Y.W.; Xu, P.; Zhang, B.K.; et al. Identifying factors affecting the pharmacokinetics of voriconazole in patients with liver dysfunction: A population pharmacokinetic approach. *Basic & clinical pharmacology & toxicology* **2019**, *125*, 34-43, doi:10.1111/bcpt.13208.
36. Encalada Ventura, M.A.; van Wanrooy, M.J.; Span, L.F.; Rodgers, M.G.; van den Heuvel, E.R.; Uges, D.R.; van der Werf, T.S.; Kosterink, J.G.; Alffenaar, J.W. Longitudinal Analysis of the Effect of Inflammation on Voriconazole Trough Concentrations. *Antimicrobial agents and chemotherapy* **2016**, *60*, 2727-2731, doi:10.1128/aac.02830-15.
37. Dote, S.; Sawai, M.; Nozaki, A.; Naruhashi, K.; Kobayashi, Y.; Nakanishi, H. A retrospective analysis of patient-specific factors on voriconazole clearance. *Journal of pharmaceutical health care and sciences* **2016**, *2*, 10, doi:10.1186/s40780-016-0044-9.
38. Zhao, Y.C.; Lin, X.B.; Zhang, B.K.; Xiao, Y.W.; Xu, P.; Wang, F.; Xiang, D.X.; Xie, X.B.; Peng, F.H.; Yan, M. Predictors of Adverse Events and Determinants of the Voriconazole Trough Concentration in Kidney Transplantation Recipients. *Clinical and translational science* **2020**, doi:10.1111/cts.12932.
39. Mangal, N.; Hamadeh, I.S.; Arwood, M.J.; Cavallari, L.H.; Samant, T.S.; Klinker, K.P.; Bulitta, J.; Schmidt, S. Optimization of Voriconazole Therapy for the Treatment of Invasive Fungal Infections in Adults. *Clinical pharmacology and therapeutics* **2018**, *104*, 957-965, doi:10.1002/cpt.1012.
40. Messina, A.; Luce, E.; Hussein, M.; Dubart-Kupperschmitt, A. Pluripotent-Stem-Cell-Derived Hepatic Cells: Hepatocytes and Organoids for Liver Therapy and Regeneration. *Cells* **2020**, *9*, doi:10.3390/cells9020420.
41. Blanco-Dorado, S.; Maroñas, O.; Latorre-Pellicer, A.; Rodríguez Jato, M.T.; López-Vizcaíno, A.; Gómez Márquez, A.; Bardán García, B.; Belles Medall, D.; Barbeito Castiñeiras, G.; Pérez Del Molino Bernal, M.L.; et al. Impact of CYP2C19 Genotype and

Drug Interactions on Voriconazole Plasma Concentrations: A Spain Pharmacogenetic-Pharmacokinetic Prospective Multicenter Study. *Pharmacotherapy* **2020**, *40*, 17-25, doi:10.1002/phar.2351.

42. Wang, T.; Yan, M.; Tang, D.; Xue, L.; Zhang, T.; Dong, Y.; Zhu, L.; Wang, X.; Dong, Y. A retrospective, multicenter study of voriconazole trough concentrations and safety in patients with Child-Pugh class C cirrhosis. *J Clin Pharm Ther* **2018**, *43*, 849-854, doi:10.1111/jcpt.12724.