A new perspective on variation of voriconazole steady-state valley concentration in Chinese population: CYP2C19 DNA methylation

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Abstract

Background: Voriconazoleis(VRC) often used in complex therapeutic environments for the treatment and prevention of invasive fungal infections. The steady-state valley concentration (Cminss) of VRC not only varies between individuals, but also within individuals, which is difficult to fully explain by pharmacogenomic theory. It is necessary to propose a new perspective to explain the variation of voriconazole steady-state valley concentration. Objectives: Based on the regulation of ADME gene expression by DNA methylation, this study aimed to explore the effect of CYP2C19 DNA methylation level on the VRC Cminss. Methods: In this study, 116 concentration points were divided into low concentration group (Cminss<1.0mg/L), standard concentration group (Cminss =1.0-5.5mg/L) and high concentration group (Cminss>5.5mg/L) according to Voriconazole Cmin standard range of 1.0-5.5 mg/L. The effect of CYP2C19 DNA methylation was highlighted by predisposition score matching to exclude other confounding factors. Results: The CYP2C19 CpG25 methylation level was different between low concentration group and standard concentration group (p=0.047). There was no difference in the CYP2C19 DNA methylation between the high concentration group and the standard concentration group, but there were significant differences in CRP (p<0.001), Alb (p=0.007) and T-BIL (p=0.024) between the high concentration group and the standard concentration group. Conclusions: The VRC Cminss in the low concentration group may be related to the methylation degree of CYP2C19 CpG25 site, while the VRC Cminss in the high concentration group may be unrelated to the methylation degree of CYP2C19 but related to the levels of CRP, Alb and T-BIL.

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A new perspective on variation of VRC C_{min}ss:*CYP2C19* DNA methylation

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The authors confirm that the Principal Investigator for this paper is Lin Huang and that she had direct clinical responsibility for patients.

Data availability statement

The datasets analysed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest Disclosure

All authors declare no conflicts of interest.

What is already known about this subject?

The expression of several key ADME genes has been shown to be regulated by DNA methylation, potentially affecting individual differences in medical treatment. Voriconazole exposure was associated with *CYP2C19* expression. It is unclear whether *CYP2C19* DNA methylation interferes with voriconazole steady-state valley concentration (C_{min}ss) variation.

What this study adds?

Voriconazole C_{min}ss in the low concentration group (C_{min}ss<1.0mg/L) may be associated with *CYP2C19* DNA methylation, which was not found in the high concentration group (C_{min}ss>5.5mg/L). CYP2C19 DNA methylation is a new perspective for interpreting voriconazole C_{min}ss variation between/within individuals.

Abstract

Background: Voriconazoleis(VRC) often used in complex therapeutic environments for the treatment and prevention of invasive fungal infections. The steady-state valley concentration (C_{min}ss) of VRC not only varies between individuals, but also within individuals, which is difficult to fully explain by pharmacogenomic theory. It is necessary to propose a new perspective to explain the variation of voriconazole steady-state valley concentration.

Objectives: Based on the regulation of ADME gene expression by DNA methylation, this study aimed to explore the effect of *CYP2C19* DNA methylation level on the VRC C_{min}ss.

Methods : In this study, 116 concentration points were divided into low concentration group (C_{min}ss<1.0mg/L), standard concentration group (C_{min}ss =1.0-5.5mg/L) and high concentration group (C_{min}ss>5.5mg/L) according to Voriconazole Cmin standard range of 1.0-5.5 mg/L. The effect of *CYP2C19* DNA methylation was highlighted by predisposition score matching to exclude other confounding factors.

Results: The *CYP2C19* CpG25 methylation level was different between low concentration group and standard concentration group (p=0.047). There was no difference in the *CYP2C19* DNA methylation between the high concentration group and the standard concentration group, but there were significant differences

in CRP (p<0.001), Alb (p=0.007) and T-BIL (p=0.024) between the high concentration group and the standard concentration group.

Conclusions: The VRC C_{min}ss in the low concentration group may be related to the methylation degree of CYP2C19 CpG25 site, while the VRC C_{min}ss in the high concentration group may be unrelated to the methylation degree of CYP2C19 but related to the levels of CRP, Alb and T-BIL.

Key words: Voriconazole, *CYP2C19* , DNA methylation, C_{min}ss, Chinese population, CRP, Alb,T-BIL

Introduction

Invasive fungal infections (IFI) mostly occur in people with low immunity and have high morbidity and mortality[1]. Voriconazole (VRC) is a second-generation triazole broad-spectrum antifungal drug, which has been recommended as a first-line drug for the treatment and prevention of IFI by many guidelines[2,3]. However, VRC is often in a complex therapeutic environment[4]. VRC steady-state valley concentration(C_{min}ss) not only varies between individuals, but also within individuals, which is difficult to be fully explained by pharmacogenomics theories. Previous studies have found that in the case of CYP2C19 and CYP3A4 genotyping, high levels of inflammation may slow down voriconazole metabolism in adult patients, resulting in higher C_{min}ss [5,6]. It is necessary to explore the new factors affecting VRC in order to promote the personalized medicine of VRC.

Epigenetic regulation is a reversible, heritable change in gene function that ultimately leads to phenotypic change without changing the DNA sequence. More and more studies have found that epigenetic factor is also important reasons for individual differences in drugs[7]. DNA methylation is a key epigenetic mechanism[8]. The DNA methylation level of specific pharmacokinetic gene will affect the its mRNA and protein expression, and affect the disposal and effect of corresponding drugs[9]. CYP2C19 DNA methylation is associated with its mRNA expression[10,11]. However, whether this epigenetic regulation is responsible for individual differences in VRC C_{min}ss has not been studied. The DNA methylation level of pharmacokinetic genes may also be influenced by some individual factors (e.g. parental exposure, environmental pollutants exposure, obesity and diet, drug, etc.)[12]. This study investigated the effect of *CYP2C19* DNA methylation on the VRC C_{min}ss by excluding the interference of other factors through propensity score matching (PSM), which is expected to provide a new perspective for the personalized administration of voriconazole.

Materials and methods

Study design

This study was approved by the Ethics Committee of Peking University People's Hospital (No. 2019PHB064-01). Patients receiving VRC for the prevention or treatment of IFD aged at least 18 years who had at least one VRC trough concentration determined at our institute between June 2019 and May 2022 were included. Patients were excluded if they were pregnant, were using potentially interacting drugs described in the drug package, had prior severe liver dysfunction, had a *CYP2C19*metabolic phenotype of ultrafast and slow metabolism, and were co-using DNA methyltransferase inhibitors (azacytidine, decitabine). The trough concentration of VRC (C_{min}ss) is defined as 2 doses after the first loading dose was administered, and 5 doses if no loading dose was given. A loading dose was considered as patients treated with two 400 mg of VRC on the first day and followed 200 mg every 12 h daily. Blood samples were collected just prior to the subsequent dosage when the plasma concentration would be at a steady state.

Voriconazole and voriconazole N-oxide assay

Blood samples from patients were centrifuged at 4000 rpm for 10 minutes at 4°C, and separated plasma was stored at -20°C until analysis. The methodology was established with blank plasma of patients and rats. Plasma Voriconazole and voriconazole *N* -oxide concentrations were measured by HPLC-MS/MS. The linear range of VRC and VNO was 0.01-10.00 μg/ml. The calibration curve showed good linearity, with $R^2 > 0.99$. The intra- and inter- day accuracy values, expressed as percent CV, were all within ± 15 %, and precision

values (as percent CV) were all less than 10 % in each calibration curve. Accuracy, method recovery and stability were all met the requirements.

Chromatographic conditions: Column Hypersil Gold C18 (1.9μm, 2.1×50 mm, Thermo Scientific, USA); Mobile phase: deionized water (0.1% formic acid, 10mM ammonium formate, solvent A), Acetonitrile (0.1% formic acid, solvent B); Elution conditions: 0.0-0.5 min, 90% A; 0.5-1.0 min, 90%-5% A; 1.0-3.5 min, 5% A; 3.5-3.6 min, 5%-90% A; 3.6-6.0 min, 90% A; Flow rate: 0.5mL*min^{-1}, Column temperature: 50, Automatic sampler temperature: 4, Sample size: 2μL.

Mass spectrometry conditions: Mass Ionization was performed using Electrospray Ionization (ESI) and Multiple Reaction Monitoring (MRM) modes. Ion transitions (in m/z) of VRC, VNO, and Voriconazole d3 (internal standard) were 350.2 to 224.2, 366.2 to 224.2 and 353.2 to 224.2, respectively. The declustering voltage and the collision energy were 75V and 25V respectively.

2.3.Genotyping

Genomic DNA was extracted from peripheral blood leukocytes according to standard procedures using a commercially available kit (E.Z.N.A.TM SQ Blood DAN Kit) following the manufacturer's instructions. Amplification and extension were carried out on the Veriti® PCR System (Applied Biosystems). The amplified PCR product was purified using the shrimp alkaline phosphatase (Fermentas Life Sciences) and ExoI (New England Biolabs). SNaPshot single base extension of the genetic polymorphisms was performed using ABI Prism® SNaPshot Multiplex Kit (Applied Biosystems). The re-purified products underwent capillary electrophoresis in a 96-well plate using an ABI 3730XL Genetic Analyzer (Applied Biosystems). Three SNPs within the CYP2C19 were determined. Detailed SNP and primer information is shown in Supplementary Table 1.

2.4 .DNA methylation assay

Genomic DNA was extracted from peripheral blood leukocytes according to standard procedures using a commercially available kit (E.Z.N.A.TM SQ Blood DAN Kit) following the manufacturer's instructions. Genomic DNA is treated with bisulfite using EZ DNA Methylation-Gold Kit. Multiplex PCR reaction was performed on the DNA after methylation. Primer information was shown in supplementary Table 2. Products were purified using AMPure XP beads. The Qubit 3.0 was used to determine the concentration of the library; Agilent 2100 Bioanalyzer system was used to determine the length of library fragments. Qualified libraries would be sequenced on an Illumina platform. Thirty-nine CpG loci on *CYP2C19* gene were detected, and the GRCh38.p14 location information of each loci was summarized as shown in supplementary Table 3. The level of methylation (%methylation) is expressed as the beta value, which = methylated cytosine/(methylated cytosine+unmethylated cytosine).

2.5 Data collection

Information collected from patients' electronic medical records includes demographic information, medical history, and laboratory parameters. Information collected from a patient's electronic medical record includes demographic information, medical history, and laboratory parameters. Demographic characteristics include age, sex, BMI. The medical history information included the diagnosis and treatment level of IFD, the underlying disease, the route and dose of voriconazole administration, and the combination of medications within one week. Laboratory tests include markers of inflammation (C-reaction protein (CRP), procalcitonin (PCT)), blood routine parameters (white blood cell count (WBC), neutrophil count (NEUT), red blood cell count (RBC), hemoglobin (RBC), and blood cell count (WBC) respectively. HB), hematocrit (HCT), and platelet count (PLT); Liver function indicators (alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin, T-BIL), albumin (Alb), total protein (TP), and alkaline phosphatase (ALP); Indicators of renal function (serum creatinine (SCr), Urea, estimated glomerular filtration rate (GFR)).Methylation degree of*CYP2C19* CpG site, voriconazole and voriconazole *N* -oxide concentration were recorded.

2.6. Statistical analysis

The Kolmogorov-Smirnov tests was used to assess the normality of continuous variables. Descriptive statistics of normal data were expressed as mean +- standard deviation (SD), and non-normal data were expressed as median and interquartile range (IQR). Independent sample T test or Mann-Whitney U test were used to determine the difference in continuous values between the two groups, and one-way ANOVA or Kruskal-Wallis H test is used to detect the difference of continuous variables between three groups or more. Categorical data were expressed as frequency and percentage, using $\chi 2$ tests. The Hardy-Weinberg equilibrium (HWE) was evaluated on each SNPs using $\chi 2$ tests. Propensity score matching was used by caliper matching method (caliper value 0.05) to exclude confounding factors other than methylation, and propensity matching score was used to evaluate the balance between the two groups after matching. SPSS 22.0 software was used for statistical analysis, STATA17.0 software was used for PSM, and GraphPad Prism 9 software was used for mapping.. The results with P value < 0.05 were considered statistically significant.

Results

Patients characteristics

In this study, 116 concentration points were included, which were divided into three groups according to the standard range of voriconazole C_{min}ss (1.0-5.5mg/L) . There were 31 concentration points in the low concentration group (C_{min}ss<1.0mg/L), 55 concentration points in the standard concentration group (C_{min}ss =1.0-5.5mg/L), and 30 concentration points in the high concentration group (C_{min}ss>5.5mg/L). The *CYP2C19*phenotype of the included patients was normal metabolizer (NM) or intermediate metabolizer (IM). The daily dose of voriconazole is 400mg. There were no significant differences in age, sex, BMI, route of administration, purpose of administration, and information related to drug combination among the three groups. In laboratory examination, CRP, Abl and T-BIL were different between the high concentration group and the standard concentration group, while the other indexes were not statistically different between the three groups.The patient characteristics are detailed in Table.1.

Distribution and intergroup differences of CYP2C19 activity in NM/IM population

VNO is the main product of voriconazole metabolism by CYP2C19. The ratio of VNO to VRC concentration indicates the activity of CYP2C19. In the NM/IM population, the distribution of CYP2C19 activity is relatively wide (as shown in Fig.1A), and there may be incompatibility between*CYP2C19* genotype and phenotype.

As shown in Fig.1B, the activity of CYP2C19 in the low concentration group (C_{min}ss<1.0mg/L) was significantly higher than that in the standard concentration group (C_{min}ss=1.0-5.5mg/L), and that in the high concentration group (C_{min}ss >5.5mg/L) was significantly lower than that in the standard concentration group (C_{min}ss=1.0-5.5mg/L). This suggests that *CYP2C19*gene polymorphism may not be used as a factor to explain the VRC C_{min}ss in all patients in the low concentration group or high concentration group, and there may be other factors affecting the VRC C_{min}ss in patients.

Effect of *CYP2C19* DNA methylation on VRC C_{min}ss

Effect of CYP2C19 DNA methylation on VRC C_{min}ss in low concentration group

There was no significant difference in patient characteristics between the low concentration group (C_{min}ss<1.0mg/L) and the standard concentration group (C_{min}ss =1.0-5.5mg/L). The methylation level of CpG site of *CYP2C19*between the two groups is shown in Table2, where the methylation level of *CYP2C19* CpG25 is significantly different between the two groups. The methylation level of *CYP2C19* CpG25 in the low concentration group was higher than that in the standard concentration group, as shown in Fig.2.

Effect of CYP2C19 DNA methylation on VRC C_{min}ss in high concentration group

There were statistical differences in CRP, Alb and T-BIL between the high concentration group (C_{min}ss>5.5mg/L) and the standard concentration group (C_{min}ss =1.0-5.5mg/L) (as shown in Table.1). CRP, Alb and T-BIL may influence the VRC C_{min}ss in high concentration group. In order to exclude the above confounding factors and highlight the effect of*CYP2C19* DNA methylation, Propensity score matching

was performed by caliper matching method. There were no significant differences in CRP, Alb and T-BIL between the two groups after PSM (as shown in Table.3). As shown in Table2.7, there was no significant difference in methylation levels at CpG sites between the two groups after PSM(as shown in Supplementary Table4). This suggests that *CYP2C19* DNA methylation levels may not be a factor in VRC C_{min}ss in the high concentration group.

Effect of other factors on VRC C_{min}ssin high concentration group

There were statistical differences in CRP, Alb and T-BIL between the high concentration group (C_{min}ss>5.5mg/L) and the standard concentration (C_{min}ss=1.0-5.5mg/L) before PSM (as shown in Table.1 and Table.3). These results suggest that CRP, Alb and T-BIL may be the factors influencing the VRC C_{min}ss in the high concentration group. Compared with the standard concentration group, CRP in the high concentration group was significantly higher (as shown in Fig.3A), Alb was significantly lower in the high concentration group (as shown in Fig.3B), and T-BIL was significantly higher in the high concentration group (as shown in Fig.3C).

Discussion

In this study, patients with CYP2C19 normal metabolizer (NM) and intermediate metabolizer (IM) were included, and the effect of CYP2C19 gene polymorphism on voriconazole Cminss was excluded. In the NM/IM population, CYP2C19 activity is widely distributed, and the predicted phenotype of CYP2C19 genotype may not be completely consistent with the measured phenotype. Burns et al. reported that 1/3 of gastrointestinal tumor patients have "dynamic changes" in liver CYP2C19 function, and the enzyme activity loss of the seemingly "poor metabolizer" phenotype is inconsistent with its genotype (*CYP2C19*2, CYP2C19*3*)[13]. This divergence between genotype and phenotype of *CYP2C19* has also been reported in solid tumors such as breast cancer, lung cancer, kidney cancer, and ovarian cancer[14] and hematological malignancies[15]. Our research is consistent with that. Compared with standard concentration group (C_{min}ss=1.0-5.5mg/L), CYP2C19 activity was significantly higher in patients with low concentration group (C_{min}ss<1.0mg/L) and significantly lower in patients with high concentration group (C_{min}ss>5.5mg/L). In summary, CYP2C19 activity may be determined by more than its metabolic function.

The expression of *CYP2C19* is moderately associated with its activity[16]. The DNA methylation level of specific ADME gene affects its gene expression[9].This study detected the *CYP2C19* DNA methylation level to explore whether it is a factor for the VRC C_{min}ss. DNA methylation at*CYP2C19* CpG25 site was significantly down-regulated in low concentration group (C_{min}ss<1.0mg/L) compared with standard concentration group (C_{min}ss=1.0-5.5mg/L). In previous studies, the CYP2C19 mRNA expression in primary hepatocytes[10], HepG2 cells, and HCT116 cells[11] was up-regulated after treatment with DNA methyltransferase inhibitor 5-aza-DC. This suggests that there may be a negative correlation between *CYP2C19* DNA methylation and mRNA expression. In this study, the decrease of VRC C_{min}ss in the low concentration group may be due to the down-regulation of DNA methylation at *CYP2C19* CpG25 site, which may lead to the up-regulation of *CYP2C19* expression and increase of activity. This is consistent with the relationship between *CYP2C19* DNA methylation and its expression in previous studies. A clinical study[17] reported the relationship between*CYP2C19* methylation and clopidogrel resistance. When*CYP2C19* is hypomethylated, clopidogrel resistance may be due to the reduction of products metabolized by CYP2C19, which seems to be inconsistent with our study. This may be because the CYP2C19 methylation site detected in this study is located in the gene body, while the CYP2C19 methylation site detected in this study is located in the promoter. There was no significant difference in the CYP2C19 DNA methylation level in the high concentration group (C_{min}ss>5.5mg/L) compared with the standard concentration group (C_{min}ss=1.0-5.5mg/L) after PSM. This suggests that *CYP2C19* DNA methylation may not be a factor affecting the VRC C_{min}ss in the high-concentration group.

There were significant differences in CRP, Alb and T-BIL between the high concentration group (C_{min}ss>5.5mg/L) and the standard concentration group (C_{min}ss=1.0-5.5mg/L) before PSM. CRP is a biomarker of inflammatory state[18]. Compared with CRP [31.40 (10.40,71.80) mg/L] in the standard con-

centration group, CRP [100.75 (44.50,175.28) mg/L] in the high concentration group was increased. Most patients were in a state of moderate to severe inflammation (CRP[?]40mg/L)[19]. A large number of inflammatory factors (such as IL-6, IL-1β, TNF-α, etc.) are usually produced in inflammatory states[20]. Some studies have reported that increasing the inflammatory factors concentration will reduce the expression and activity of drug metabolic enzymes[21-23]. Therefore, the VRC C_{min}ss may be caused by the inflammatory state affecting the expression and activity of VRC metabolic enzymes such as CYP2C19 in the high concentration group. The plasma protein binding rate of VRC is 58%[24], and the main binding protein is albumin[25]. Compared with the standard concentration group (34.27±4.539), the Alb in the high concentration group (31.61±5.088) was significantly decreased, and the free concentration of VRC in the high concentration group may be increased. In addition, it has been reported that the change of Alb may affect the clearance rate of VRC[26]. Therefore, the VRC C_{min}ss may also be due to the effect of Alb level on the distribution and elimination of voriconazole in the body in the high concentration group. T-BIL is one of the indicators indicating liver function. Compared with the T-BIL of the standard concentration group [9.60 (6.20, 14.10) μmol/L], the T-BIL of the high concentration group [11.45 (8.80, 23.80) μmol/L] was significantly higher. The liver function of the high concentration group may be worse than that of the standard concentration group. It has been reported that decreased liver function may affect the expression of drug metabolic enzymes[27]. In addition, T-BIL was found to be related to VRC clearance[28]. Therefore, the VRC C_{min}ss in the high concentration group may also be due to the effect of T-BIL level on the metabolism and elimination of VRC. In addition, the down-regulation of Alb and up-regulation of T-BIL may also be affected by inflammatory states[29]. In summary, the VRC C_{min}ss may be related to the up-regulation of CRP, down-regulation of Alb and up-regulation of T-BIL in the high-concentration group.

In this study, factors influencing the VRC C_{min}ss in the low concentration group (C_{min}ss<1.0mg/L) and the high concentration group (C_{min}ss>5.5mg/L) were investigated, and the relationship between *CYP2C19* DNA methylation and the VRC C_{min}ss was investigated for the first time, providing a new perspective for exploring the individual administration of voriconazole. The limitation of this study may be that *CYP2C19*DNA methylation levels in blood are used to replace *CYP2C19* DNA methylation levels in liver, and there may be some differences in DNA methylation levels between blood and liver[30]. At the same time, some studies[17,31,32]used blood to measure the DNA methylation level of ADME gene to explore its role in pharmacokinetics. Due to the rarity of liver samples and the limitation of detection techniques, the use of blood samples instead of liver to explore the role of DNA methylation in pharmacokinetics is also an option to be considered.

Conclusion

In this study, the VRC C_{min}ss in patients in the low concentration group (C_{min}ss<1.0mg/L) may be related to methylation levels at the *CYP2C19* CpG25 site . Based on the above possible qualitative relationships, it seems possible to obtain a better therapeutic effect of voriconazole by adjusting certain lifestyles associated with DNA methylation. More studies are expected to establish a quantitative relationship between DNA methylation and VRC C_{min}ss, and it is possible to optimize the dosing regimen by adjusting the degree of DNA methylation in the future. The VRC C_{min}ss in high concentration group (C_{min}ss>5.5mg/L) may not be related to*CYP2C19* DNA methylation level. The VRC C_{min}ss in patients in high concentration group may be related to the levels of CRP, Alb and T-BIL of patients. *CYP2C19* DNA methylation may be influenced by CRP, Alb, and T-BIL, and it is more meaningful to explore the interaction between DNA methylation and some non-genetic factors in the future.

Ethics approval

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the People's Hospital of Peking University (2019PHB064-01).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Availability of data and material

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Lin Huang and Yufei Feng made contributions to conception and design. Yinyu Zhao and Nan Guo helped with the acquisition of data. Boyu Liu and Lei Hu performed the extraction of genomic DNA and genotyping. Xu Hao performed the statistical analysis, interpreted the data and wrote the manuscript. Yinyu Zhao drew the figures and tables. Nan Guo helped to revise the manuscript. Fang Liu provided guidance on statistical analysis. Xiaoyan Nie and Feng Yu were involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published.

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Table.1 Characteristics of included patients

Parameters	Low concentration $C_{min}ss$<1.0mg/L (n=31)	Standard concentration $C_{min}ss$=1.0-5.5mg/L (n=55)	High concentration $C_{min}ss$>5.5mg/L (n=30)	*P* value
Voriconazole $C_{min}ss$ (n)				
Demographics				
Age[a] (yr)	56.00(43.00,62.00)	51.91±16.025	54.33±17.810	0.535
Sex				
Male[b]	26(83.9%)	38(69.1%)	21(70.0%)	0.296
Female[b]	5(16.1%)	17(30.9%)	9(30.0%)	
BMI[a]	22.84±3.727	23.71±3.381	23.12±4.103	0.548
Administration route[b]				
Intravenous	22(71.0%)	43(78.2%)	25(83.3%)	0.506
Oral	9(29.0%)	12(21.8%)	5(16.7%)	
Concomitant medications[b]				
PPI	9(29.0%)	14(25.5%)	12(40.0%)	0.372
GC	9(29.0%)	13(23.6%)	4(13.3%)	0.325
Carbapenems	15(48.4%)	29(52.7%)	21(70.0%)	0.187
SMZ/TMP	6(19.4%)	13(23.6%)	5(16.7%)	0.733
Indication for VRC[b]				
Therapy of probable	4(12.9%)	5(9.1%)	5(16.7%)	0.858
Diagnostic-driven therapy of undefined and possible	25(80.6%)	42(76.4%)	21(70.0%)	
Empirical antifungal therapy of febrile neutropenia	1(3.2%)	5(9.1%)	3(10.0%)	
Prophylaxis	1(3.2%)	3(5.5%)	1(3.3%)	
Laboratory parameter[a]				
CRP (mg/L)	20.13(7.80,59.00)	31.40(10.40,71.80)	115.35±82.967***	¡0.001
PCT (ng/ml)	0.23(0.17,2.00)	0.25(0.16,2.39)	0.34(0.16,6.28)	0.554

Parameters	Low concentration $C_{min}ss$<1.0mg/L (n=31)	Standard concentration $C_{min}ss$=1.0-5.5mg/L (n=55)	High concentration $C_{min}ss$>5.5mg/L (n=30)	*P* value
Alb (g/L)	34.36±4.069	34.27±4.539	31.85(28.10,33.83)**	0.007
TP (g/L)	66.84±7.174	63.87±8.865	62.95(59.76,67.83)	0.137
ALT (U/L)	30.10±30.486	19.00(12.00,34.00)	16.00(6.75,28.00)	0.264
AST (U/L)	18.00(10.25,30.75)	21.00(13.00,36.00)	24.00(13.75,35.50)	0.296
ALP(U/L)	116.00±65.077	102.00(78.00,151.00)	83.50(68.50,130.00)	0.318
T-BIL (umol/L)	9.00(7.50,11.00)	9.60(6.20,14.10)	11.45(8.80,23.80)*	0.024
SCr (umol/L)	60.00(49.00,77.00)	59.00(53.00,73.00)	67.00(52.50,77.75)	0.631
GFR (ml/(min*1.73m^2))	102.59±31.807	110.10(93.48,120.19)	103.15(95.06,120.78)	0.6

Table2. Methylation level of CYP2C19 CpG sitesin the low concentration group and the standard group

CpG sites	Low concentration ($C_{min}ss$ <1.0mg/L) (n=31)	Standard concentration ($C_{min}ss$ =1.0-5.5mg/L) (n=55)	Standard concentration ($C_{min}ss$ =1.0-5.5mg/L) (n=55)	*P* value
CpG1	96.74±1.670	96.48 (95.33,97.44)	0.331	0.331
CpG2	96.07±1.527	96.36 (95.01,97.00)	0.538	0.538
CpG3	93.04±2.956	92.81±2.338	0.711	0.711
CpG4	94.49±2.329	93.76±2.017	0.148	0.148
CpG5	33.35(31,71,35.44)	34.28(32.49,38.70)	0.147	0.147
CpG6	51.72 (50.74,52.30)	52.01(51.06,53.90)	0.315	0.315
CpG8	69.44±4.710	71.76±6.961	0.102	0.102
CpG9	47.83±12.476	49.57±12.043	0.529	0.529
CpG10	43.35±11.433	45.58±15.920	0.496	0.496
CpG11	96.34 (95.38,97.15)	96.20(95.38,96.66)	0.432	0.432
CpG12	84.24±7.747	87.39(81.95,89.39)	0.532	0.532
CpG13	97.18(95.70,97.49)	96.77(96.02,97.49)	0.848	0.848
CpG14	96.18±1.336	96.07(95.20,96.63)	0.422	0.422
CpG15	93.06(92.19,94.17)	92.96(91.87,94.00)	0.985	0.985
CpG16	92.19±2.674	92.31(90.77,93.74)	0.869	0.869
CpG17	47.80(47.22,49.03)	48.77±2.004	0.381	0.381
CpG18	46.77±8.351	48.75(47.11,52.78)	0.574	0.574
CpG19	45.21±7.266	45.39±8.758	0.924	0.924
CpG20	51.34±7.785	52.10(50.46,56.31)	0.624	0.624
CpG21	73.28(71.65,74.86)	73.71(71.01,75.60)	0.847	0.847
CpG22	63.26±6.653	63.95(61.08,66.91)	0.840	0.840
CpG23	93.89±2.482	93.57±4.182	0.710	0.710
CpG24	92.16(47.00,96.31)	91.26(47.77,95.54)	0.967	0.967

CpG sites	Low concentration ($C_{min}ss$ <1.0mg/L) (n=31)	Standard concentration ($C_{min}ss$ =1.0-5.5mg/L) (n=55)	Standard concentration ($C_{min}ss$ =1.0-5.5mg/L) (n=55)	*P* value
CpG25	43.75±24.311	60.55(36.05,73.57)	0.047	0.047
CpG26	83.28±7.006	84.67(79.42,87.35)	0.949	0.949
CpG27	78.40±8.888	78.58±8.532	0.925	0.925
CpG28	86.82±4.007	87.66(85.46,89.63)	0.837	0.837
CpG29	86.67±9.684	88.52(86.46,90.13)	0.862	0.862
CpG30	80.82±3.106	80.92±9.890	0.955	0.955
CpG31	63.86±1.736	63.24±3.168	0.323	0.323
CpG32	95.34(94.24,96.85)	94.89(91.83,96.29)	0.165	0.165
CpG33	96.06(95.44,96.83)	96.19±1.698	0.988	0.988
CpG34	95.05(92.89,95.81)	94.58±2.307	0.474	0.474
CpG35	85.31±6.200	83.72±9.092	0.411	0.411
CpG36	89.87±4.369	88.52±6.921	0.350	0.350
CpG37	68.25±12.300	69.23±10.440	0.712	0.712
CpG38	57.63±6.625	55.12(51.72,59.53)	0.170	0.170
CpG39	82.39±5.093	83.66(80.92,85.72)	0.395	0.395

Table.3 CRP, Alb and T-BIL were in the standard group and the high concentration group before and after propensity score matching.

Parameters Voriconazole $C_{min}ss$ (n)	before propensity score matching	before propensity score matching	*P* value	after propensity score matching	after propensity score matching	*P* value
	Standard concentration $C_{min}ss$=1.0-5.5mg/L (n=55)	High concentration $C_{min}ss$ >5.5mg/L (n=30)		Standard concentration $C_{min}ss$=1.0-5.5mg/L (n=42)	High concentration $C_{min}ss$ >5.5mg/L (n=22)	
CRP (mg/L)	31.40(10.40,71.80)	100.75(44.50,175.28)	<0.001	46.25 (22.92,77.40)	89.24±65.590	0.098
Alb (g/L)	34.27±4.539	31.61±5.088	0.003	33.02±3.544	32.10(29.80,33.83)	0.141
T-BIL(umol/L)	9.60(6.20,14.10)	11.45(8.80,23.80)	0.023	10.65 (6.88,14.90)	10.95(8.63,22.53)	0.333

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