International Journal of Antimicrobial Agents 47 (2016) 124–131

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

# Impact of *CYP2C19* genetic polymorphisms on voriconazole dosing and exposure in adult patients with invasive fungal infections

Fabien Lamoureux[a,b,*,1], Thomas Duflot[a,b,1], Jean-Baptiste Woillard[c], David Metsu[c], Tony Pereira[a], Patricia Compagnon[a], Hélène Morisse-Pradier[d], Mona El Kholy[d], Luc Thiberville[d], Jana Stojanova[e], Christian Thuillez[a,b]

[a] *Department of Pharmacology and Toxicology, University Hospital, Rouen, France*
[b] *INSERM U1096, University of Rouen, School of Medicine, Rouen, France*
[c] *INSERM UMR 850, University of Limoges, CHU Limoges, Limoges, France*
[d] *Department of Pneumology, University Hospital, Rouen, France*
[e] *Laboratory of Chemical Carcinogenesis and Pharmacogenetics, Molecular and Clinical Pharmacology Program, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile*

ARTICLE INFO

*Article history:*
Received 13 August 2015
Accepted 1 December 2015

*Keywords:*
Voriconazole
Pharmacogenomics
*CYP2C19*
Antifungals
Drug monitoring

ABSTRACT

Voriconazole (VCZ) use is limited by its narrow therapeutic range and significant interpatient variability in exposure. This study aimed to assess (i) the impact of *CYP2C19* genotype on VCZ exposure and (ii) the doses required to achieve the therapeutic range in adult patients with invasive fungal infections (IFIs). Therapeutic drug monitoring (TDM) of VCZ, based on trough concentration measurement, and *CYP2C19* genotyping were used to guide VCZ dosing in Caucasian patients with IFIs. The two common polymorphisms in Caucasians (*CYP2C19*2* and **17*), associated with decreased or increased CYP2C19 activity, respectively, were correlated with the daily VCZ dose, pharmacokinetic parameters and concentration-to-dose ratio. In total, 111 trough concentration measurements from 35 genotyped patients were analysed using linear mixed-effect models. The mean VCZ doses required to achieve target concentrations were significantly higher in *CYP2C19*17* carriers compared with *CYP2C19*1/*1* individuals ($P < 0.001$): $2.57 \pm 0.25$ mg/kg twice daily in *CYP2C19*1/*1* patients versus $3.94 \pm 0.39$ mg/kg and $6.75 \pm 0.54$ mg/kg in **1/*17* and **17/*17* patients, respectively. In addition, exposure to VCZ correlated with the *CYP2C19*17* variant. Indices of exposure for *CYP2C19*2* carriers were in line with the functional effect of this polymorphism compared with *CYP2C19*1/*1* individuals, however comparisons of doses required to achieve target concentrations were not statistically different. The *CYP2C19*17* allele predicted both VCZ exposure and dose required to achieve effective and non-toxic concentrations. *CYP2C19* genotyping appears useful to guide VCZ initial dosing when coupled with TDM and to explain subtherapeutic concentrations frequently observed in clinical practice.



## 1. Introduction

Voriconazole (VCZ) is a second-generation triazole oral antifungal with potent broad-spectrum activity that has become a drug of choice for the treatment of a wide variety of fungal infections, including invasive aspergillosis, candidaemia and fluconazole-resistant invasive *Candida* infections [1,2]. VCZ inhibits the conversion of lanosterol into ergosterol in fungal membranes, when the N-3 nitrogen (triazole) moiety binds to the heme cofactor of fungal CYP450. VCZ is extensively metabolised by hepatic cytochrome P450 (CYP) enzymes, principally by CYP2C19 and, to a lesser extent, by CYP3A4 and CYP2C9, giving rise to *N*-oxide and hydroxyl metabolites [3]. Despite its potent activity, a narrow therapeutic range and significant interpatient variability in drug exposure complicate its use. Interpatient variability in VCZ exposure is mainly due to non-linear pharmacokinetics, patient characteristics (e.g. age, weight and liver function), co-medications and genetic polymorphisms in the *CYP2C19* gene [4]. VCZ plasma trough concentrations have been correlated with efficacy and toxicity, thus therapeutic drug monitoring (TDM) represents a useful tool to optimise VCZ dosing regimens and clinical outcomes [5–7].

* Corresponding author. Present address: Laboratoire de Pharmacologie–Toxicologie, Pharmacogénétique, CHU–Hôpitaux de Rouen, 76000 Rouen, France. Tel.: +33 2 32 88 90 34; fax: +33 2 32 88 90 49.
*E-mail address:* fabien.lamoureux@chu-rouen.fr (F. Lamoureux).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijantimicag.2015.12.003
0924-8579/

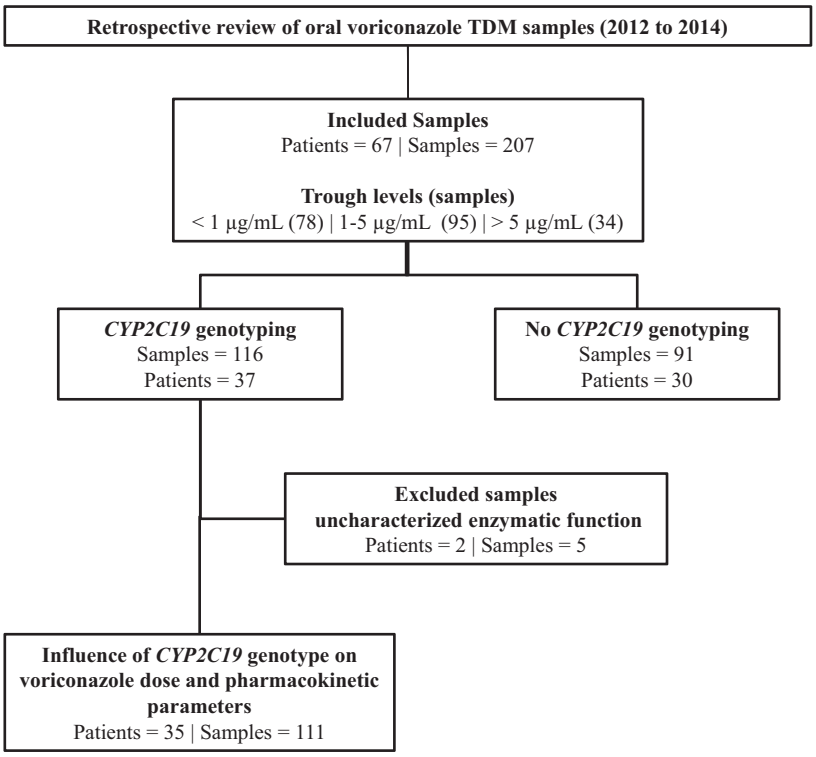


**Fig. 1.** Subject inclusion, specifying individuals receiving voriconazole therapeutic drug monitoring (TDM) and *CYP2C19* genotyping.

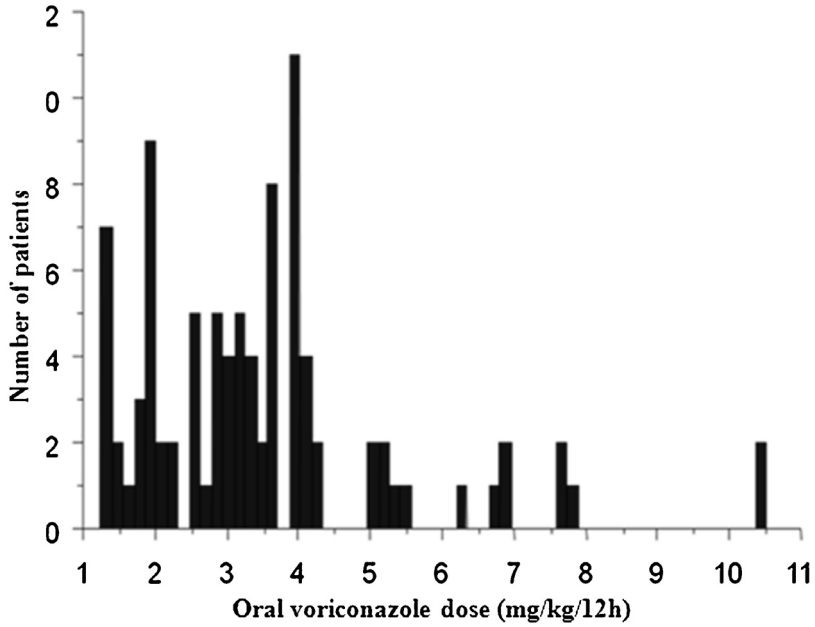


**Fig. 2.** Distribution of oral doses of voriconazole required to reach the target trough level at steady-state ($n = 95$, Shapiro–Wilk normality test $P$-value = 8.256e−08).

**Table 1**
Demographic data of individuals receiving routine voriconazole therapeutic drug monitoring and *CYP2C19* genotyping ($n = 35$).

| Demographic characteristic | *CYP2C19* genotype | | | | |
|---|---|---|---|---|---|
| | **1/*1* | **1/*17* | **17/*17* | **1/*2* | **2/*2* |
| *N* (%) | 11 (31) | 13 (37) | 4 (11) | 6 (17) | 1 (3) |
| $C_0$ samples ($n$) | 41 | 36 | 14 | 16 | 4 |
| Age (years) (mean ± S.D.) | 45 ± 18 | 46 ± 20 | 38 ± 18 | 47 ± 17 | 68 |
| Sex ratio (M/F) ($n$) | 8/3 | 8/5 | 2/2 | 2/4 | 1/0 |
| Weight (kg) (mean ± S.D.) | 61 ± 14 | 60 ± 15 | 53 ± 14 | 63 ± 17 | 67 |
| Concomitant PPI use ($n$) | 7 | 4 | 1 | 0 | 0 |
| Concomitant corticoid use ($n$) | 1 | 4 | 0 | 2 | 0 |

$C_0$, trough concentration at steady-state; S.D., standard deviation; PPI, proton pump inhibitor.

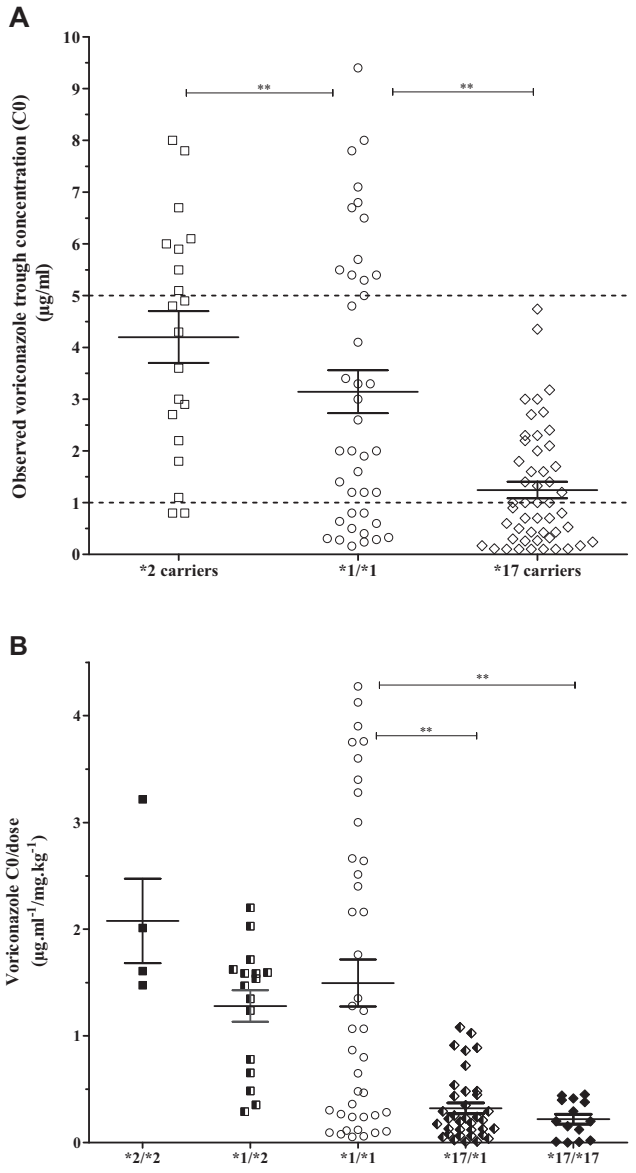


**Fig. 3.** Influence of *CYP2C19*17* (c.-806C>T) and *CYP2C19*2* (c.681G>A) on voriconazole trough plasma concentrations: (A) distribution of voriconazole trough concentrations; and (B) dose-adjusted trough concentrations, grouped according to *CYP2C19* genotype in 111 samples from 35 patients. Data are expressed as the mean ± standard error of the mean (SEM). $^{*}P < 0.05$; $^{**}P < 0.01$. (Multivariate analysis is presented in Table 2.)

Presently, there is no consensus for a VCZ target range or guidelines regarding dosing [8]. Poor treatment outcome has been reported in patients with trough concentrations lower than 1 µg/mL [1], whereas trough concentrations higher than 4.5–5.5 µg/mL have been associated with adverse events such as vision disturbances, rash and hepatotoxicity [5,7,9]. Favourable outcomes have been observed in the range 2–4 µg/mL [1,9–11].

Polymorphisms in *CYP2C19* influence drug metabolism and VCZ variability in exposure [1,12–14]. Two *CYP2C19* alleles, rs4244285 (*CYP2C19*2*, c.681G>A) and rs4986893 (*CYP2C19*3*, c.636G>A), lead to an mRNA splicing defect and stop codon, respectively, resulting in loss-of-function in the enzyme. A common allele, rs12248560 (*CYP2C19*17*, c.-806C>T), is associated with increased CYP2C19 activity owing to a promoter region variation thought to enhance the binding of nuclear proteins, resulting in increased transcription. This allele has been associated with therapeutic failure with VCZ and other substrates [12,15,16]. *CYP2C19*2* and *CYP2C19*17* are relatively frequent among Caucasians (15% and 22%, respectively), whilst *CYP2C19*3* is relatively rare (<1%).

Therapeutic failure with VCZ is potentially life-threatening. Despite being dosed according to drug label recommendations, subtherapeutic and supratherapeutic VCZ trough concentrations are commonly observed during TDM [1,12]. Pharmacogenetic data may facilitate dosing decisions, especially at an early stage of treatment. The aim of this study was to assess (i) the impact of *CYP2C19* genotype on VCZ exposure following oral administration and (ii) the doses required to achieve the therapeutic range in adult patients with invasive fungal infections (IFIs).

## 2. Materials and methods

### 2.1. Study design and patient population

A single-centre retrospective analysis of data from adult patients receiving oral VCZ for the treatment of suspected or proven IFI was performed. Data from routine TDM of VCZ, taken as trough concentrations at steady-state ($C_0$), conducted between 2012 and 2014 at University Hospital of Rouen (Rouen, France) were considered. Patients were considered to be at steady-state after 2 days of treatment following a loading dose of 400 mg orally every 12 h for the first 24 h or after 6 days without a loading dose. Patients receiving intravenous (i.v.) VCZ were not included unless they were switched to an oral formulation for ≥2 days before the trough concentration was measured. The initial recommended VCZ maintenance dose was 200 mg twice daily in accordance with the drug label. Subsequent dosage adjustment following TDM and/or *CYP2C19* genotyping was at the discretion of each physician. TDM was performed in 67 patients, and 207 $C_0$ plasma samples were assessed (Fig. 1). For 27 patients TDM was based solely on $C_0$; in 37 patients TDM included $C_0$ and additional peak level 2 h after oral intake ($C_2$) to evaluate absorption; in 3 patients presenting difficulties in achieving target levels despite dosage adjustment, additional samples including $C_0$ and at 2, 4, 6, 8 and 10 h were performed to estimate the VCZ area under the concentration–time curve (AUC) and to understand the underlying mechanisms.

Genotyping for *CYP2C19*17* and **2* alleles was proposed to clinicians as complementary information to help guide dosing. Determination of genotype was realised before the first dose or within the first week of initiating therapy to predict the potential risk of overexposure or underexposure to VCZ. Assessment of genotype after the first plasma measurement and/or after the first week from initiation at steady-state was performed in order to explain extreme levels or adverse events.

Additional variables collected from medical records included age, sex, weight, VCZ indication, VCZ dose and plasma concentration. The daily dose of VCZ was systematically recorded and the weight-adjusted dose was calculated (mg/kg/12 h). Trough concentrations were dose-normalised using the concentration-to-dose ratio ($mg{\cdot}L^{-1}/mg{\cdot}kg^{-1}$). Written informed consent was obtained from each participant undergoing *CYP2C19* genotyping and additional sampling for pharmacokinetic analysis. A target range of 1–5 µg/mL was considered based on literature reports [7,10]. A dose increase or reduction of 50 mg to 100 mg (or 0.5–1 mg/kg) every 12 h was typically proposed if the trough concentration was <1 µg/mL or >5 µg/mL, respectively. Discontinuation of therapy was proposed in patients with trough levels >5 µg/mL when associated with adverse events related to VCZ.

This study was conducted respecting the Declaration of Helsinki and was approved by the Local Ethics Board for Observational Health Research [Comité d'éthique pour la recherche non interventionnelle (CERNI) N°E-2014-20, Centre de Protection des Personnes Nord-Ouest-I, Rouen University Hospital, Rouen, France].

**Table 2**
Univariate and multivariate linear mixed-effects regression analyses investigating the influence of covariates on voriconazole exposure ($C_0$, $C_0$/dose/kg) and dose (mg/kg/12 h) required to achieve the therapeutic range (1–5 μg/mL).

| Covariate | Category | Univariate analysis | | Multivariate final model | |
|---|---|---|---|---|---|
| | | $\beta$ coefficient (±S.D.) | *P*-value[a] | Adjusted $\beta$ coefficient (±S.D.) | Adjusted *P*-value[a] |
| $C_0$ (μg/mL) | | | | | |
| *CYP2C19*17* (rs12248560) | Intercept | 3.985 ± 0.571 | | −0.357 ± 0.772 | |
| | **1/*17* vs **1/*1* | −1.575 ± 0.405 | **0.000178**** | −1.437 ± 0.385 | **0.000306**** |
| | **17/*17* vs **1/*1* | −1.520 ± 0.559 | **0.007690**** | −0.984 ± 0.560 | 0.081565 |
| *CYP2C19*2* (rs4244285) | **1/*2* vs **1/*1* | 1.880 ± 0.523 | **0.000495**** | 2.018 ± 0.497 | **9.20e−05**** |
| | **2/*2* vs **1/*1* | 0.641 ± 0.913 | 0.484822 | | |
| Time | Intercept | 0.844 ± 0.358 | | | |
| | Intervisit delay | 0.626 ± 0.116 | **4.07e−07**** | 0.594 ± 0.098 | **1.77e−08**** |
| CYP450 inducer combination | Intercept | 3.832 ± 0.707 | | | |
| | Y vs N | −0.448 ± 0.513 | 0.384138 | | |
| CYP450 inhibitor combination | Intercept | 2.881 ± 0.597 | | | |
| | Y vs N | 0.899 ± 0.419 | **0.03412**† | | |
| Weight | Intercept | 0.898 ± 0.940 | | | |
| | Per kg increase | 0.037 ± 0.013 | **0.004880**** | 0.025 ± 0.011 | **0.024000**† |
| Total plasma proteins | Intercept | 9.592 ± 1.782 | | | |
| | Per g/L increase | −0.080 ± 0.023 | 0.100847 | | |
| Age | Intercept | 1.721 ± 0.799 | | | |
| | Per year increase | 0.027 ± 0.012 | **0.029200**† | | |
| $C_0$/dose ratio (μg·mL$^{-1}$/mg·kg$^{-1}$) | | | | | |
| *CYP2C19*17* (rs12248560) | Intercept | 1.510 ± 0.145 | | 0.401 ± 0.305 | |
| | **1/*17* vs **1/*1* | −1.166 ± 0.207 | **1.65e−07**** | −0.984 ± 0.198 | **2.44e−06**** |
| | **17/*17* vs **1/*1* | −1.291 ± 0.289 | **2.16e−05**** | −1.064 ± 0.275 | **0.000184**** |
| *CYP2C19*2* (rs4244285) | **1/*2* vs **1/*1* | −0.204 ± 0.268 | 0.4480 | | |
| | **2/*2* vs **1/*1* | 0.584 ± 0.475 | 0.2220 | | |
| Time | Intercept | 0.382 ± 0.178 | | | |
| | Intervisit delay | 0.220 ± 0.058 | **0.000236**** | 0.141 ± 0.052 | **0.007909**** |
| CYP450 inducer combination | Intercept | 0.734 ± 0.235 | | | |
| | Y vs N | 0.287 ± 0.259 | 0.26930 | | |
| CYP450 inhibitor combination | Intercept | 0.915 ± 0.204 | | | |
| | Y vs N | 0.290 ± 0.212 | 0.17426 | | |
| Total plasma proteins | Intercept | 2.616 ± 0.950 | | | |
| | Per g/L increase | −0.020 ± 0.013 | 0.13334 | | |
| Age | Intercept | −0.227 ± 0.304 | | | |
| | Per year increase | 0.023 ± 0.006 | **8.52e−05**** | 0.012 ± 0.005 | **0.021237**† |
| Dose required to achieve the therapeutic range (mg/kg/12 h) | | | | | |
| *CYP2C19*17* (rs12248560) | Intercept | 2.574 ± 0.246 | | 2.5742 ± 0.2456 | |
| | **1/*17* vs **1/*1* | 1.373 ± 0.389 | **0.000741**** | 1.3732 ± 0.3890 | **0.000741**** |
| | **17/*17* vs **1/*1* | 4.185 ± 0.537 | **4.30e−11**** | 4.1846 ± 0.5370 | **4.30e−11**** |
| *CYP2C19*2* (rs4244285) | **1/*2* vs **1/*1* | 0.829 ± 0.416 | 0.0511 | | |
| | **2/*2* vs **1/*1* | 0.785 ± 0.678 | 0.2508 | | |
| Time | Intercept | 3.235 ± 0.395 | | | |
| | Intervisit delay | 0.066 ± 0.114 | 0.5650 | | |
| CYP450 inducer combination | Intercept | 3.745 ± 0.468 | | | |
| | Y vs N | −0.398 ± 0.522 | 0.4481 | | |
| CYP450 inhibitor combination | Intercept | 3.427 ± 0.369 | | | |
| | Y vs N | 3.95e−05 ± 4.53e−01 | 0.9999 | | |
| Total plasma proteins | Intercept | 3.390 ± 1.666 | | | |
| | Per g/L increase | −0.001 ± 0.024 | 0.9709 | | |
| Age | Intercept | 5.716 ± 0.766 | | | |
| | Per year increase | −0.041 ± 0.013 | **0.00268**† | | |

S.D., standard deviation.
[a] Significant values are shown in bold: †$P < 0.05$; **$P < 0.01$.

### 2.2. Measurement of voriconazole plasma levels

VCZ trough plasma levels were determined using a two-dimensional high-performance liquid chromatography–tandem mass spectrometry (2D LC-MS/MS) detection method developed in our laboratory. The LC-MS/MS system comprised a quaternary and a binary pump (LC-20AD/AB; Shimadzu, Kyoto, Japan), an auto sampler (SIL-20ACHT; Shimadzu), a column compartment and a 4000QTRAP tandem mass spectrometer equipped with a Turbo Ion Spray® source (AB Sciex, Les Ulis, France). Sample preparation included protein precipitation by mixing 100 μL of plasma with 200 μL of reagent containing a structural analogue of VCZ as an internal standard (VCZ-$d_5$ 1 μg/mL in acetonitrile). One microlitre of the deproteinised supernatant was directly injected into the chromatographic system. The LC-integrated online sample clean-up was performed using a perfusion column (POROS R2/20, 2.1 mm × 30 mm; Applied Biosystems, Waltham, MA) and a loading phase composed of 15 mM ammonium acetate in water. Chromatographic separation was achieved on an Alltima$^{TM}$ HP C18 HL (3 μm, 50 mm × 2.1 mm; Alltech, Grace Discovery Sciences, Columbia, MD) at 60 °C and the mobile phase consisted of a mixture methanol/ammonium acetate 10 mM buffer + 0.1% acetic acid (97/3, v/v, respectively) at an isocratic flow rate of 0.2 mL/min. Following optimisation of the MS/MS system parameters, VCZ detection and quantification were performed in the multiple reaction monitoring mode (MRM) using the protonated $[M+H]^+$ VCZ and VCZ-$d_5$ as precursor ions (m/z 350 and 355, respectively). The ion transitions monitored were m/z 350.0 → 126.9 and m/z 350.0 → 281.0 for quantitation and confirmation of VCZ, respectively. The analytical assay was validated according to the US Food

and Drug Administration (FDA) guidelines for bioanalytical method validation and met all of the required quality criteria, including: selectivity; between- and within-assay accuracy and precision; linearity; upper/lower limits of quantification assessment; matrix effect evaluation; and stability. The lower limit of quantification of the assay was 0.1 μg/mL and the upper limit of quantification was 10 μg/mL.

### 2.3. CYP2C19 *genotyping*

Genomic DNA from whole blood was isolated using a QIAamp® DNA Blood Mini Kit (QIAGEN, Les Ulis, France) according to the manufacturer's instructions. *CYP2C19* genotyping for rs4244285 G>A (*CYP2C19*2*) and rs12248560 C>T (*CYP2C19*17*) was carried out by *Taq*Man® allelic discrimination assays (C__25986767_70 and C__469857_10, respectively) on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Direct sequencing was used to validate internal quality controls corresponding to genomic DNA of each genotype. Amplification of sequences containing the target alleles was conducted using the following primers pairs: forward 5′-TCTCTTGTCAGAATTTTCTTTCTCAA-3′/reverse 5′-TGAATCACAAATACGCAAGCA-3′ for the **2* allele; and forward 5′-ATCTCTGGGGCTGTTTTCCT-3′/reverse 5′-ACGTGAAGGCAGGAATTGTT-3′ for the **17* allele. Whilst there are other loss-of-function alleles in *CYP2C19*, we did not genotype for these due to their rare minor allele frequencies. The wild-type form of the gene lacking **2* and **17* alleles is represented by *CYP2C19*1*.

### 2.4. *Statistical analysis*

The impact of *CYP2C19* genotype on VCZ dose and exposure (dose per kg, $C_0$/dose/kg and $C_0$) was investigated by linear mixed-effect regressions using the lme4 R package (R Foundation for Statistical Computing; http://www.r-project.org). Random effects were included on both the intercept and the time (visit) covariate to take into account variability over time. Covariates included age, *CYP2C19*17*, *CYP2C19*2*, per intervisit delay, total plasma proteins, weight and concomitant use of CYP450 inducers and/or inhibitors. In a first step, each covariate was tested as a fixed effect in univariate analysis and those characterised by a *P*-value of <0.1 were included in intermediate models. The final model was selected using a backward stepwise process based on the likelihood ratio test. The Shapiro–Wilk normality test was used to assess the distribution of VCZ doses required to achieve the targeted plasma concentrations. Statistical analyses were performed with R software v.3.2.1 (R Foundation for Statistical Computing).

### 2.5. *Pharmacokinetic modelling*

A population pharmacokinetic model was developed from data obtained in 40 patients from whom more than one sample was taken. $C_0$ and $C_2$ levels were obtained in 37 patients, whilst full pharmacokinetic profiles (trough, 2, 4, 6, 8 and 10 h levels) were performed in 3 individuals that represented different *CYP2C19* genotypes (**1/*1*, **1/*2* and **1/*17*). A simple one-compartment non-parametric population pharmacokinetic model with first-order absorption and elimination was developed using the Pmetrics package of the statistical software R v.3.2.1 [17]. Population pharmacokinetic parameters including apparent oral clearance (CL), elimination half-life ($t_{1/2}$), apparent volume of distribution ($V_d$), elimination rate constant (Ke) and area under the concentration–time curve (AUC) were compared between genotype groups. A combined error model was used to describe the residual variability. Median and interquartile range were calculated for CL.

## 3. Results

### 3.1. *Therapeutic drug monitoring of voriconazole*

Among 207 levels from 67 patients, the mean VCZ trough level was 2.50 ± 1.96 μg/mL and the mean VCZ dose was 210 ± 54 mg twice daily. Trough concentrations with respect to the defined range were subtherapeutic for 78 samples (37.7%), supratherapeutic for 34 samples (16.4%) and within the target range for 95 samples (45.9%). VCZ doses required for achieving target concentrations ranged from 1.1 mg/kg to 10.6 mg/kg twice daily. The frequency distribution of doses for the samples within the target range was not normally distributed, suggesting a multimodal distribution of drug disposition ($P < 0.0001$) (Fig. 2).

### 3.2. CYP2C19 *genotyping*

*CYP2C19* genotype was determined in 37 patients, corresponding to 116 TDM samples. The baseline characteristics of the five genotype groups are displayed in Table 1. The allele frequencies of the *CYP2C19*17* and **2* alleles were 49% and 20%, respectively. Two individuals (five samples), heterozygous for both *CYP2C19*2* and *CYP2C19*17*, were excluded from the analyses (Fig. 1).

### 3.3. *Voriconazole dose requirements and exposure according to CYP2C19 genotype*

Observed VCZ trough concentrations correlated with the *CYP2C19* polymorphic alleles (Fig. 3A). The gain-of-function allele *CYP2C19*17* was associated with significantly lower trough concentrations and more frequent subtherapeutic levels in *CYP2C19*1/*17* and **17/*17* versus *CYP2C19*1/*1* patients, respectively ($P < 0.001$) (Fig. 3; Table 2). The loss-of-function allele *CYP2C19*2* was associated with significantly higher trough levels in *CYP2C19*2/*2* carriers ($P = 0.00626$), but not for *CYP2C19*1/*2* in the final multivariate model (Fig. 3; Table 2). *CYP2C19*2* was associated with increased trough levels versus *CYP2C19*1/*1* (Fig. 3A).

Mean twice-daily VCZ doses required to achieve levels within the target range were higher in *CYP2C19*17* allele carriers and demonstrated an allelic dose effect: 2.57 ± 0.25 mg/kg for *CYP2C19*1/*1* compared with 3.94 ± 0.39 mg/kg for *CYP2C19*1/*17* ($P < 0.001$) and 6.75 ± 0.54 mg/kg for *CYP2C19*17/*17* ($P < 0.0001$), respectively (Fig. 4). The mean concentration-to-dose ratio in these patients was 1.51, 0.34 and 0.22 for *CYP2C19*1/*1*, *CYP2C19*1/*17* and *CYP2C19*17/*17*, respectively (Fig. 3B; Table 2). *CYP2C19*17* allele carriers required a mean VCZ dose of 4.76 ± 0.47 mg/kg twice daily compared with 3.03 ± 0.36 mg/kg for **2* carriers. No significant decrease was observed in doses needed to target the therapeutic range in *CYP2C19*2* allele carriers versus *CYP2C19*1/*1* following multivariate analysis (Table 2; Fig. 4).

### 3.4. *Voriconazole pharmacokinetics according to CYP2C19 genotype*

With respect to the population pharmacokinetic model, individual predicted plasma concentrations (iPRED) as a function of observed concentrations, normalised residues as a function of iPRED, and examples of individual fits showed that the developed model adequately fit the data with a bias of 1.50% and a root-mean-square error of 12.30% (Fig. 5).

The pharmacokinetic model was applied to the genotyped patients: parameter estimates from the non-compartmental-analysis and associated standard errors are shown in Table 3. Among the three groups, all pharmacokinetic parameters correlated with *CYP2C19* allele carriage. In the *CYP2C19*1*1* group, the mean VCZ pharmacokinetic parameters were: CL = 4.52 ± 1.16 L/h;

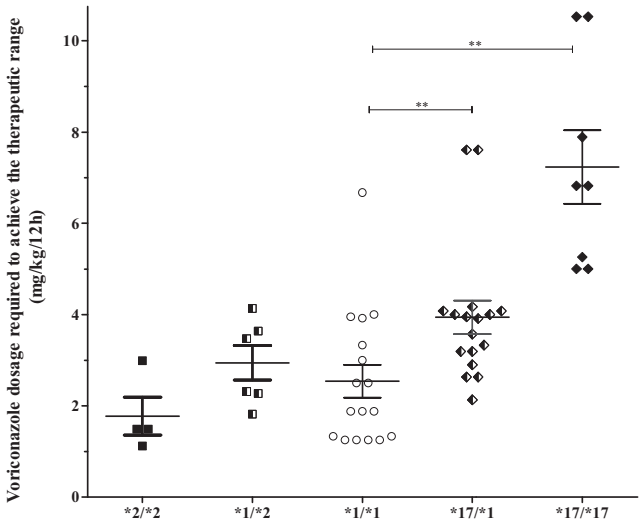


**Fig. 4.** Influence of *CYP2C19*17* (c.-806C>T) and *CYP2C19*2* (c.681G>A) on voriconazole doses required to achieve the therapeutic range (1–5 μg/mL) in mg/kg every 12 h, according to *CYP2C19* status. Data are expressed as the mean ± standard error of the mean (SEM). $^{*}P < 0.05$; $^{**}P < 0.01$. (Multivariate analysis is presented in Table 2.)

Ke = 0.07 ± 0.01 $h^{-1}$; and $t_{1/2}$ = 12.57 ± 0.80 h. *CYP2C19*17* carriers demonstrated increased VCZ CL and Ke and decreased $t_{1/2}$. Conversely, parameters for carriers of *CYP2C19*2* were in the direction of a loss-of-function effect (Table 3).

## 4. Discussion

Despite its efficacy, clinical use of oral VCZ is complicated by its narrow therapeutic range and considerable systemic interindividual variability. In the present work, we demonstrate that the *CYP2C19*17* allele is an important determinant of clearance, in line with the findings of other authors [3,12,14,18]. To our knowledge, this report is the first to correlate *CYP2C19* genotype with VCZ exposure in adults and to use this information to individualise dosing.

Poor outcomes are observed in patients with VCZ trough levels below 1 μg/mL [5,7,9,11]; this is a frequent occurrence and is a major contributor to treatment failure [12,19]. When genotyping was not performed, 47% of trough concentrations were within the therapeutic range. *CYP2C19* genotype influenced the dose required to achieve therapeutic concentrations up to 10-fold.

Low drug concentrations were observed among *CYP2C19*17* carriers. The data presented led us to suggest that oral doses of VCZ initiated at 2.5, 4 and 6 mg/kg every 12 h might better achieve target levels in adult **1/*1*, **1/*17* and **17/*17* carriers, respectively. Early *CYP2C19* genotyping may help clinicians in initial dose selection and result in a higher proportion of concentrations within therapeutic targets.

High VCZ trough concentrations have been associated with an increased risk of neurological and hepatic adverse events [4,7,9]. Individuals carrying *CYP2C19* loss-of-function alleles exhibit elevated VCZ concentrations compared with wild-type and *CYP2C19*17* carriers [4,9]. A recent report described increased toxicity symptoms among intermediate metabolisers determined by genotype, which in the studied cohort represented *CYP2C19*2* and **3* carriers [20]. We likewise observed elevated VCZ trough concentrations among *CYP2C19*2* carriers compared with wild-type carriers. Nevertheless, the proportion of individuals with supratherapeutic levels and the doses required to achieve therapeutic levels were not significantly different compared with individuals expressing a functional enzyme. No adverse events such as visual disturbances or altered liver function were observed in the studied population. This might be due to insufficient power given relatively few *CYP2C19*2* carriers (six heterozygous and one homozygous individuals).

Similar results were recently reported in paediatric cancer patients. The authors suggest that starting doses based on age and *CYP2C19* genotype could facilitate dosing, especially in *CYP2C19*17* allele carriers [21]. Although there are marked differences in VCZ pharmacokinetics between children and adults [21–23], the data from this study are consistent with these findings.

Since its approval by the FDA and the European Agency for the Evaluation of Medicinal Products in 2002, VCZ has been commercially available orally and intravenously. The oral route is characterised by high bioavailability (>90%), without marked delay to peak concentrations, thus similar dose adjustments would be expected between the two formulations. Contrary to the i.v. route, labelling for the oral formulation does not recommend dosing based on weight in adults. As is proposed for children and for the i.v. route, we suggest weight-adjusted oral VCZ starting doses based on *CYP2C19* genotype to achieve adequate trough concentrations

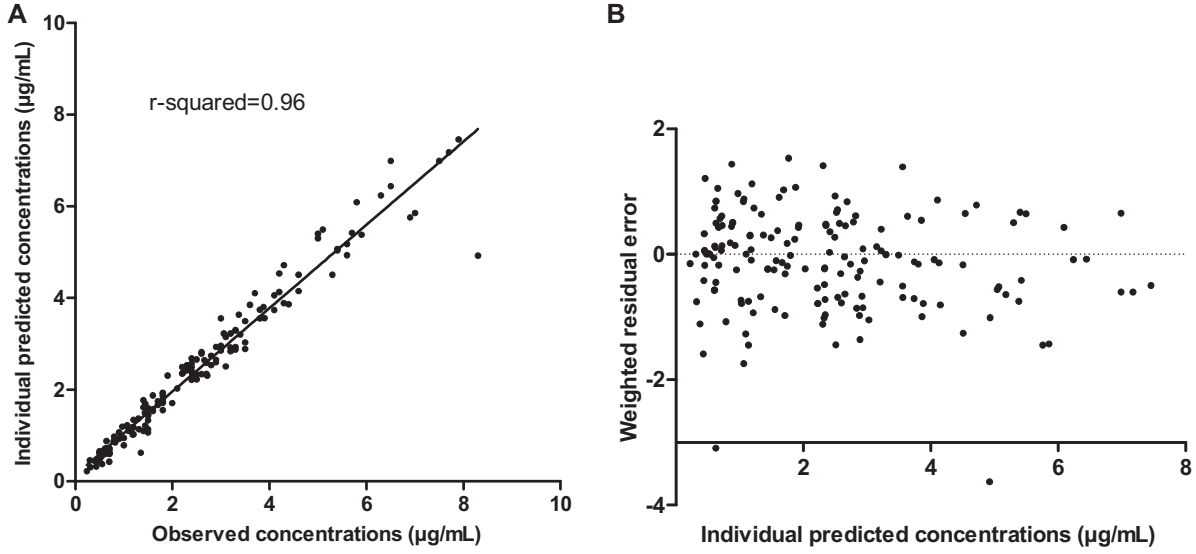


**Fig. 5.** Plots for the voriconazole population pharmacokinetic model including (A) the individual predicted plasma concentrations (iPRED) versus the observed concentrations and (B) individual-weighted residuals (iWRES) versus iPRED.

**Table 3**
Univariate and multivariate linear mixed-effects regression analyses investigating the influence of covariates on voriconazole pharmacokinetic parameters (CL, $t_{1/2}$ and Ke).

| Covariate | Category | Univariate analysis | | Multivariate final model | |
|---|---|---|---|---|---|
| | | $\beta$ coefficient (±S.D.) | *P*-value[a] | Adjusted $\beta$ coefficient (±S.D.) | Adjusted *P*-value[a] |
| CL (L/h) | | | | | |
| *CYP2C19*17* (rs12248560) | Intercept | 4.516 ± 1.162 | | 8.137 ± 1.534 | |
| | **1/*17* vs **1/*1* | 5.006 ± 1.479 | **0.0010**** | 4.358 ± 1.490 | **0.00419**** |
| | **17/*17* vs **1/*1* | 6.985 ± 2.053 | **0.000954**** | 6.435 ± 2.027 | **0.00194**** |
| *CYP2C19*2* (rs4244285) | **1/*2* vs **1/*1* | −3.080 ± 1.911 | **0.0109905**† | −3.869 ± 1.922 | **0.04652**† |
| | **2/*2* vs **1/*1* | −3.914 ± 3.376 | 0.249046 | | |
| Time | Intercept | 9.725 ± 1.251 | | | |
| | Intervisit delay | −1.063 ± 0.406 | **0.0102**† | −1.015 ± 0.372 | **0.00747**** |
| CYP450 inducer combination | Intercept | 7.168 ± 1.585 | | | |
| | Y vs N | −0.222 ± 1.772 | 0.901 | | |
| CYP450 inhibitor combination | Intercept | 7.173 ± 1.175 | | | |
| | Y vs N | −0.287 ± 1.473 | 0.846 | | |
| Total plasma proteins | Intercept | 6.644 ± 6.025 | | | |
| | Per g/L increase | 0.178 ± 0.083 | **0.0359**† | | |
| Age | Intercept | 11.409 ± 2.172 | | | |
| | Per year increase | −0.085 ± 0.040 | **0.034**† | | |
| $t_{1/2}$ (h) | | | | | |
| *CYP2C19*17* (rs12248560) | Intercept | 12.572 ± 0.803 | | 9.227 ± 1.399 | |
| | **1/*17* vs **1/*1* | −3.365 ± 0.864 | **0.000171**** | −3.313 ± 0.853 | **0.000179**** |
| | **17/*17* vs **1/*1* | −3.394 ± 1.196 | **0.005454**** | −2.730 ± 1.210 | **0.026221**† |
| *CYP2C19*2* (rs4244285) | **1/*2* vs **1/*1* | 2.568 ± 1.116 | **0.023336**† | 2.541 ± 1.0991 | **0.022744**† |
| | **2/*2* vs **1/*1* | 3.266 ± 1.963 | 0.099201 | | |
| Time | Intercept | 8.572 ± 0.752 | | | |
| | Intervisit delay | 0.829 ± 0.244 | **0.00095**** | | |
| CYP450 inducer combination | Intercept | 11.651 ± 1.122 | | | |
| | Y vs N | −0.334 ± 1.063 | 0.7540 | | |
| CYP450 inhibitor combination | Intercept | 11.184 ± 0.879 | | | |
| | Y vs N | 0.301 ± 0.884 | 0.7340 | | |
| Total plasma proteins | Intercept | 22.672 ± 3.529 | | | |
| | Per g/L increase | −0.152 ± 0.048 | **0.00227**** | | |
| Age | Intercept | 6.521 ± 1.292 | | | |
| | Per year increase | 0.080 ± 0.024 | **0.000881**** | 0.055 ± 0.021 | **0.012256**† |
| Ke ($h^{-1}$) | | | | | |
| *CYP2C19*17* (rs12248560) | Intercept | 0.066 ± 0.007 | | 0.091 ± 0.008 | |
| | **1/*17* vs **1/*1* | 0.028 ± 0.008 | **0.000414**** | 0.025 ± 0.008 | **0.00161**** |
| | **17/*17* vs **1/*1* | 0.032 ± 0.011 | **0.003301**** | 0.029 ± 0.011 | **0.00699**** |
| *CYP2C19*2* (rs4244285) | **1/*2* vs **1/*1* | −0.021 ± 0.010 | **0.037443**† | −0.025 ± 0.010 | **0.01683**† |
| | **2/*2* vs **1/*1 | −0.026 ± 0.018 | 0.146351 | | |
| Time | Intercept | 0.098 ± 0.007 | | | |
| | Intervisit delay | −0.007 ± 0.002 | **0.00302**** | −0.006 ± 0.002 | **0.01034**† |
| CYP450 inducer combination | Intercept | 0.077 ± 0.009 | | | |
| | Y vs N | 5.19e−04 ± 0.009 | 0.956 | | |
| CYP450 inhibitor combination | Intercept | 0.079 ± 0.007 | | | |
| | Y vs N | −0.003 ± 0.008 | 0.743 | | |
| Total plasma proteins | Intercept | −0.013 ± 0.031 | | | |
| | Per g/L increase | 0.001 ± 4.31e−04 | **0.00674**** | | |
| Age | Intercept | 0.112 ± 0.012 | | | |
| | Per year increase | −5.87e−04 ± 2.10e−04 | **0.00621**** | | |

CL, apparent oral clearance; $t_{1/2}$, elimination half-life; Ke, elimination rate constant; S.D., standard deviation.
[a] Significant values are shown in bold: †$P < 0.05$; **$P < 0.01$.

[21–23]. Although the present drug information label includes instruction regarding *CYP2C19* loss-of-function alleles, information regarding the frequent *CYP2C19*17* allele is lacking.

Other proteins may influence VCZ disposition and clinical outcomes, however their genes have not received the same attention as *CYP2C19*. VCZ *N*-oxidation and 4-hydroxylation is partially catalysed by CYP3A4 and CYP3A5: the genes for these enzymes harbour frequent variants that may likewise impact VCZ pharmacokinetics [24,25]. The functional consequences of polymorphisms in these enzymes are not always clear and these do not necessarily impact the coding sequence. An example is the purported loss-of-function allele *CYP3A4*22*. CYP3A5 is expressed only in carriers of at least one *CYP3A5*1* allele: when expressed, this enzyme is expected to reduce systemic concentrations of substrates, including VCZ [25,26]. This allele exhibits a differential frequency between ethnic groups: ca. 65% in Africans, 25–40% in Asians and 5–30% in Caucasians. A three-fold higher increase in VCZ AUC is expected in *CYP3A5*3/*3* carriers, which lack a functional enzyme, compared with *CYP3A5*1* carriers, based on in vitro work [25].

Flavin-containing monooxygenase enzymes, namely FMO1 and 3, catalyse ca. 25% of the total VCZ metabolism [27,28]. At present, genetic polymorphisms of FMO are not well characterised and their impact on VCZ pharmacokinetics remains to be addressed by further studies, particularly in adults.

Variability in VCZ trough concentrations may be influenced by co-medications such as alternative co-substrates of metabolising enzymes, inducers or inhibitors. CYP inducers such as rifabutin, rifampicin or steroids can reduce VCZ levels [4]. CYP2C19 substrates and/or inhibitors, including proton pump inhibitors (PPIs), may increase VCZ concentrations. PPIs did not cause significantly decreased VCZ metabolism in a large series of VCZ samples, although this was a retrospective evaluation [4]. We retrospectively analysed the medical records of included patients and found no concomitant administration with potent CYP inducers or inhibitors,

including rifampicin, human immunodeficiency (HIV) antivirals drugs, carbamazepine or St John's Wort, nor evidence for a significant effect of PPIs and steroids between the distinct genotype groups.

## 5. Conclusions

Targeting an appropriate therapeutic range is challenging with VCZ, and the use of optimal TDM together with pharmacogenomic information could improve patient outcomes [29]. *CYP2C19*17* was found to be significantly associated with VCZ exposure and the dose required to achieve effective and non-toxic concentrations in patients with IFIs. We suggest that dose adjustment based on early *CYP2C19* genotyping, together with TDM and/or assessment of VCZ/*N*-oxide VCZ ratio may improve clinical outcomes and help to determine the cause of frequently observed subtherapeutic VCZ concentrations. Furthermore, genotyping costs are relatively accessible (ca. €75 or US$80) [30]. The prospective validation of predictive oral VCZ doses based on *CYP2C19* genotype, VCZ/metabolite ratio, weight and trough concentration is currently under way in a larger series of patients at our centre.

## Acknowledgments

The authors thank Caroline Haubreux, Germain Tinnière, Patricia Courchay and David Nunes-Ferreira for generous assistance in method design as well as collection and management of clinical data.

*Funding*: This work was supported by internal funding from the University Hospital of Rouen (Rouen, France).

*Competing interests*: None declared.

*Ethical approval*: This study was conducted respecting the Declaration of Helsinki and was approved by the Local Ethics Board for Observational Health Research [Comité d'éthique pour la recherche non interventionnelle (CERNI) N°E-2014-20, Centre de Protection des Personnes Nord-Ouest-I, Rouen University Hospital, Rouen, France]. Written informed consent was obtained from each participant undergoing *CYP2C19* genotyping and additional sampling for pharmacokinetic analysis.

## References

[1] Owusu Obeng A, Egelund EF, Alsultan A, Peloquin CA, Johnson JA. *CYP2C19* polymorphisms and therapeutic drug monitoring of voriconazole: are we ready for clinical implementation of pharmacogenomics? Pharmacotherapy 2014;34:703–18.
[2] Smith J, Andes D. Therapeutic drug monitoring of antifungals: pharmacokinetic and pharmacodynamic considerations. Ther Drug Monit 2008;30:167–72.
[3] Scholz I, Oberwittler H, Riedel KD, Burhenne J, Weiss J, Haefeli WE, et al. Pharmacokinetics, metabolism and bioavailability of the triazole antifungal agent voriconazole in relation to *CYP2C19* genotype. Br J Clin Pharmacol 2009;68:906–15.
[4] Dolton MJ, Mikus G, Weiss J, Ray JE, McLachlan AJ. Understanding variability with voriconazole using a population pharmacokinetic approach: implications for optimal dosing. J Antimicrob Chemother 2014;69:1633–41.
[5] Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. Antimicrob Agents Chemother 2009;53:24–34.
[6] Park WB, Kim NH, Kim KH, Lee SH, Nam WS, Yoon SH, et al. The effect of therapeutic drug monitoring on safety and efficacy of voriconazole in invasive fungal infections: a randomized controlled trial. Clin Infect Dis 2012;55:1080–7.
[7] Pascual A, Calandra T, Bolay S, Buclin T, Bille J, Marchetti O. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. Clin Infect Dis 2008;46:201–11.
[8] Karthaus M, Lehrnbecher T, Lipp HP, Kluge S, Buchheidt D. Therapeutic drug monitoring in the treatment of invasive aspergillosis with voriconazole in cancer patients—an evidence-based approach. Ann Hematol 2015;94:547–56.
[9] Matsumoto K, Ikawa K, Abematsu K, Fukunaga N, Nishida K, Fukamizu T, et al. Correlation between voriconazole trough plasma concentration and hepatotoxicity in patients with different *CYP2C19* genotypes. Int J Antimicrob Agents 2009;34:91–4.
[10] Smith J, Safdar N, Knasinski V, Simmons W, Bhavnani SM, Ambrose PG, et al. Voriconazole therapeutic drug monitoring. Antimicrob Agents Chemother 2006;50:1570–2.
[11] Troke PF, Hockey HP, Hope WW. Observational study of the clinical efficacy of voriconazole and its relationship to plasma concentrations in patients. Antimicrob Agents Chemother 2011;55:4782–8.
[12] Hassan A, Burhenne J, Riedel KD, Weiss J, Mikus G, Haefeli WE, et al. Modulators of very low voriconazole concentrations in routine therapeutic drug monitoring. Ther Drug Monit 2011;33:86–93.
[13] Mikus G, Scholz IM, Weiss J. Pharmacogenomics of the triazole antifungal agent voriconazole. Pharmacogenomics 2011;12:861–72.
[14] Weiss J, Ten Hoevel MM, Burhenne J, Walter-Sack I, Hoffmann MM, Rengelshausen J, et al. *CYP2C19* genotype is a major factor contributing to the highly variable pharmacokinetics of voriconazole. J Clin Pharmacol 2009;49:196–204.
[15] Helsby NA, Burns KE. Molecular mechanisms of genetic variation and transcriptional regulation of *CYP2C19*. Front Genet 2012;3:206.
[16] Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, et al. A common novel *CYP2C19* gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clin Pharmacol Ther 2006;79:103–13.
[17] Neely MN, van Guilder MG, Yamada WM, Schumitzky A, Jelliffe RW. Accurate detection of outliers and subpopulations with Pmetrics, a nonparametric and parametric pharmacometric modeling and simulation package for R. Ther Drug Monit 2012;34:467–76.
[18] Wang G, Lei HP, Li Z, Tan ZR, Guo D, Fan L, et al. The *CYP2C19* ultra-rapid metabolizer genotype influences the pharmacokinetics of voriconazole in healthy male volunteers. Eur J Clin Pharmacol 2009;65:281–5.
[19] Trifilio S, Pennick G, Pi J, Zook J, Golf M, Kaniecki K, et al. Monitoring plasma voriconazole levels may be necessary to avoid subtherapeutic levels in hematopoietic stem cell transplant recipients. Cancer 2007;109:1532–5.
[20] Trubiano JA, Crowe A, Worth LJ, Thursky KA, Slavin MA. Putting *CYP2C19* genotyping to the test: utility of pharmacogenomic evaluation in a voriconazole-treated haematology cohort. J Antimicrob Chemother 2015;70:1161–5.
[21] Hicks JK, Crews KR, Flynn P, Haidar CE, Daniels CC, Yang W, et al. Voriconazole plasma concentrations in immunocompromised pediatric patients vary by *CYP2C19* diplotypes. Pharmacogenomics 2014;15:1065–78.
[22] Neely M, Rushing T, Kovacs A, Jelliffe R, Hoffman J. Voriconazole pharmacokinetics and pharmacodynamics in children. Clin Infect Dis 2010;50:27–36.
[23] Walsh TJ, Driscoll T, Milligan PA, Wood ND, Schlamm H, Groll AH, et al. Pharmacokinetics, safety, and tolerability of voriconazole in immunocompromised children. Antimicrob Agents Chemother 2010;54:4116–23.
[24] Levin MD, den Hollander JG, van der Holt B, Rijnders BJ, van Vliet M, Sonneveld P, et al. Hepatotoxicity of oral and intravenous voriconazole in relation to cytochrome P450 polymorphisms. J Antimicrob Chemother 2007;60:1104–7.
[25] Yamazaki H, Nakamoto M, Shimizu M, Murayama N, Niwa T. Potential impact of cytochrome P450 3A5 in human liver on drug interactions with triazoles. Br J Clin Pharmacol 2010;69:593–7.
[26] Daly AK. Significance of the minor cytochrome P450 3A isoforms. Clin Pharmacokinet 2006;45:13–31.
[27] Yanni SB, Annaert PP, Augustijns P, Bridges A, Gao Y, Benjamin Jr DK, et al. Role of flavin-containing monooxygenase in oxidative metabolism of voriconazole by human liver microsomes. Drug Metab Dispos 2008;36:1119–25.
[28] Yanni SB, Annaert PP, Augustijns P, Ibrahim JG, Benjamin Jr DK, Thakker DR. In vitro hepatic metabolism explains higher clearance of voriconazole in children versus adults: role of CYP2C19 and flavin-containing monooxygenase 3. Drug Metab Dispos 2010;38:25–31.
[29] Chau MM, Kong DC, van Hal SJ, Urbancic K, Trubiano JA, Cassumbhoy M, et al. Consensus guidelines for optimising antifungal drug delivery and monitoring to avoid toxicity and improve outcomes in patients with haematological malignancy. Intern Med J 2014;44:1364–88.
[30] Calcagno A, Baietto L, Pagani N, Simiele M, Audagnotto S, D'Avolio A, et al. Voriconazole and atazanavir: a CYP2C19-dependent manageable drug–drug interaction. Pharmacogenomics 2014;15:1281–6.