Impact of the CYP2C19 genotype on voriconazole exposure in adults with invasive fungal infections

Issam S. Hamadeh, Kenneth P. Klinker, Samuel J. Borgen, Ashley I. Richards, Wenhui Li, Naveen Mangal, John W. Hiemenz, Stephan Schmidt, Taimour Y. Langae, Charles A. Peloquin, Julie A. Johnson and Larisa H. Cavallari

Objectives Voriconazole, a first-line agent for the treatment of invasive fungal infections (IFIs), is metabolized by CYP2C19. A significant proportion of patients fail to achieve therapeutic trough concentrations with standard weight-based voriconazole dosing, placing them at increased risk for treatment failure, which can be life threatening. We sought to test the association between the CYP2C19 genotype and subtherapeutic voriconazole concentrations in adults with IFIs.

Patient and methods Adults receiving weight-based voriconazole dosing for the treatment of IFIs were genotyped for the CYP2C19*2, *3, and *17 polymorphisms, and CYP2C19 metabolizer phenotypes were inferred. Steady-state voriconazole trough plasma concentrations and the prevalence of subtherapeutic troughs (< 2 mg/l) were compared between patients with the CYP2C19*17/*17 (ultrarapid metabolizer, UM) or *1/*17 (rapid metabolizer, RM) genotype versus those with other genotypes. Logistic regression, adjusting for clinical factors, was performed to estimate the odds of subtherapeutic concentrations.

Results Of 70 patients included (mean age 52.5 ± 18 years), 39% were RM and UM. Compared with patients with the other phenotypes, RM/UM had a lower steady-state trough concentration (4.26 ± 2.2 vs. 2.86 ± 2.3, P = 0.0093) and a higher prevalence of subtherapeutic troughs (16 vs. 52%, P = 0.0028), with an odds ratio of 5.6 (95% confidence interval: 1.64–19.24, P = 0.0044).

Conclusion Our findings indicate that adults with the CYP2C19 RM or UM phenotype are more likely to have subtherapeutic concentrations with weight-based voriconazole dosing. These results corroborate previous findings in children and support the potential clinical utility of CYP2C19 genotype-guided voriconazole dosing to avoid underexposure in RM and UM patients.

Keywords: CYP2C19*17, invasive fungal infections, rapid and ultrarapid metabolizer phenotypes, subtherapeutic trough plasma concentration, voriconazole

Introduction Invasive fungal infections (IFIs) are one of the most feared complications of prolonged and profound neutropenia, with mortality rates approaching 90% if left untreated [1,2]. Hence, early diagnosis coupled with timely initiation of optimal doses of effective antifungal agents is critical for favorable patient outcomes [3]. Voriconazole is a broad-spectrum, second-generation triazole used widely for the treatment of life-threatening fungal infections. The Infectious Diseases Society of America recommends it as a first-line agent for the treatment of invasive aspergillosis [4]. This is because of its unequivocal efficacy compared with other agents such as amphotericin B, the former gold standard for IFI treatment [5,6]. There is a large body of evidence showing that therapeutic success with voriconazole is contingent on achieving a therapeutic trough plasma concentration at steady state [7–9], and response rates approach 100% when trough concentrations of at least 2 mg/l are attained early in the course of therapy [3,10,11]. However, the recommended weight-based voriconazole dosing for the treatment of IFIs, which consists of a loading dose of 6 mg/kg every 12 h for the first 24 h, followed by a maintenance dose of 4 mg/kg every 12 h, is associated with wide interindividual variability in voriconazole exposure [7,12], with reported troughs ranging from 0.2 to 13.5 mg/l [12,13]. Voriconazole undergoes extensive hepatic metabolism, which is predominantly mediated by the cytochrome P450 (CYP) 2C19 enzyme [14,15]. The gene encoding
CYP2C19 is highly polymorphic, with more than 34 variant alleles identified (http://www.cypalleles.ki.se) [16]. The CYP2C19*17 allele is a gain-of-function variant arising from a single nucleotide polymorphism (SNP) in the gene promoter region. The CYP2C19*1/*17 and *17/*17 genotypes confer the rapid metabolizer (RM) and ultrarapid metabolizer (UM) phenotypes, respectively, with increased enzyme activity compared with the normal metabolizer (NM) phenotype (*1/*1 genotype) [17]. Conversely, the *2 and *3 alleles are loss-of-function variants. Intermediate metabolizers (IMs) have a single loss-of-function variant and significant reduction in enzyme activity compared with NMs, whereas poor metabolizers (PMs), with two loss-of-function variants, have no enzyme activity [18,19].

The CYP2C19 UM phenotype has been associated with subtherapeutic voriconazole trough concentrations in children with IFIs [20]. There are important differences in voriconazole pharmacokinetics between children and adults, with more rapid drug clearance in children [21,22], limiting the ability to extrapolate findings to adults. Thus, studies in adults are needed. Several single-dose pharmacokinetic studies in healthy adults and a small retrospective study of fixed voriconazole dosing in adults with IFIs have shown that voriconazole disposition differed significantly according to the CYP2C19 genotype [23–26]. Nonetheless, these findings may not be generalizable to patients with IFIs receiving weight-based voriconazole dosing, the current standard of care for patients with IFIs [4]. Therefore, the primary objective of this study was to test the association between the CYP2C19 genotype and voriconazole plasma concentrations in adults receiving standard weight-based dosing for the treatment of IFIs.

**Patient and methods**

**Patient population selection and procedures**

This was a prospective cohort study of patients aged 18 years or older with probable or definite IFI on the basis of the European Organization for Research Criteria and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycosis Study Group criteria [27]. All patients were started on standard weight-based voriconazole dosing, consisting of a loading dose of 6 mg/kg every 12 h for the first 24 h, followed by a maintenance dose of 4 mg/kg every 12 h [4]. Individuals receiving voriconazole for prophylaxis of fungal infection or with a history of liver transplantation were excluded. Trough plasma concentration was measured on day 5–7 after voriconazole initiation (i.e. at steady state). The dose was subsequently adjusted if the trough concentration was beyond the therapeutic range (2–6 mg/l) recommended for critically ill patients [10–12,28]. The study protocol was approved by the institutional review board at the University of Florida and all participants provided written informed consent.

**DNA sample collection and isolation**

Genomic DNA was collected from each patient for genetic analysis by either mouthwash collection or buccal swabs, and isolated using the Puregene kit (Qiagen, Valencia, California, USA) according to the manufacturer’s protocol.

**CYP2C19 genotyping**

The CYP2C19*17 (c. –806C>T; rs12248560), *2 (c.681G>A; rs4244285), and *3 (c.636G>A; rs4986893) alleles were determined by PCR and pyrosequencing (Supplementary Table 1, Supplemental digital content 1, http://links.lww.com/FPC/B194) as described previously [29]. The CYP2C19*1 allele was assigned if the *2, *3, or *17 allele was not detected.

**CYP2C19 phenotype assignment**

According to nomenclature by the Clinical Pharmacogenetics Implementation Consortium [30], patients with the *1/*17 genotype were classified as RMs, and those with *17/*17 genotype were classified as UMs. Patients with one copy of a *2 or a *3 allele (e.g. *1/*2, *1/*3, *2/*17) were assigned the IM phenotype, and carriers of two copies (e.g. *2/*2) were assigned the PM phenotype. The NM phenotype was assigned by default to patients without a *2, *3, or *17 allele.

**Measurement of voriconazole trough plasma concentration**

Plasma samples were stored at –80°C until assayed at the University of Florida Infectious Disease Pharmacokinetics Laboratory, a Clinical Laboratory Improvement Act/College of American Pathologists-certified laboratory, using a validated high-performance liquid chromatography (HPLC) assay. Briefly, concentration was determined using a system consisting of a ThermoFinnegan P4000 HPLC pump (San Jose, California, USA) with UV2000 ultraviolet detector, a Gateway Series E computer (Poway, California, USA), and the Chromquest HPLC data management system. The plasma standard curve for voriconazole ranged from 0.05 to 10.0 mg/l. The absolute recovery of voriconazole from plasma was 94%. The within-sample precision (percent coefficient of variation) of validation of a single standard concentration was 1.82% and the overall validation precision across all standards was 0.55–3.71%. No interferences were observed with the measurement of voriconazole with 90 different commonly used medications. Before the assay, we planned to reanalyze any samples that did not fulfill quality control criteria (per Standard Operating Procedure).
Data analysis
The χ²-test with one degree of freedom was used to test for genotype deviation from Hardy–Weinberg Equilibrium. The primary endpoint was the prevalence of subtherapeutic voriconazole trough plasma concentrations at steady state between the CYP2C19 RM/UM phenotype and other phenotypes. A subtherapeutic voriconazole plasma concentration was defined as a trough plasma concentration less than 2 mg/l on day 5–7 of therapy on the basis of evidence that supports targeting a trough of at least 2 mg/l for treatment success, particularly for critically ill patients [3,10,11]. The secondary endpoints were the mean voriconazole trough plasma concentration, prevalence of supratherapeutic trough concentrations (>6 mg/l), and prevalence of trough concentrations less than 1 mg/l [31]. The prevalence of subtherapeutic trough concentrations was compared between CYP2C19 RM/UMs and other phenotypes using the χ²-test. Simple and multiple logistic regression analyses were carried out to estimate the odds of having a subtherapeutic voriconazole trough plasma concentration at steady state with the RM/UM phenotype after adjusting for other covariates such as age, route of voriconazole administration, sex, race, weight, and concomitant medications. Additional comparisons between phenotype groups were performed using the χ²-test for categorical data or Student’s unpaired t-test or analysis of variance for continuous data. Statistical significance was set at a P value of less than 0.05. The inclusion of at least 70 patients, with 14 expected to have the CYP2C19 RM/UM phenotype on the basis of reported phenotype frequencies [16], was estimated to provide 80% power to detect a 30% difference [32] in the prevalence of subtherapeutic trough plasma concentrations between groups with an α of 0.05. All statistical analyses were carried out using SAS (version 9.3; SAS Institute, Cary, North Carolina, USA).

Results
A total of 81 patients were enrolled. However, voriconazole was discontinued in 11 patients before day 5–7 (steady state), and thus analysis was limited to 70 patients with trough concentrations drawn at steady state. Their characteristics are summarized in Table 1. The majority were men, White, and had a recent history of hematopoietic stem cell transplant or induction chemotherapy for hematologic malignancy. Most patients were also receiving pantoprazole, the only proton pump inhibitor (PPI) on formulary, and none was receiving other medications known to induce or inhibit the CYP2C19 enzyme (e.g. rifampicin, carbamazepine, phenytoin, protease inhibitors, or omeprazole).

CYP2C19 allele frequencies were 0.11 for *2 and 0.22 for *17. The CYP2C19*3 allele was not detected. The distributions of CYP2C19 genotypes and inferred metabolizer phenotypes are shown in Table 2. None of the genotypes deviated from Hardy–Weinberg Equilibrium. In all, 39% had the RM or the UM phenotype.

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.5 ± 18.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.6 ± 16</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>42 (60)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>57 (81)</td>
</tr>
<tr>
<td>African American</td>
<td>11 (16)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Voriconazole through the intravenous route</td>
<td>44 (63)</td>
</tr>
<tr>
<td>Voriconazole dose (mg/day)</td>
<td></td>
</tr>
<tr>
<td>Loading dose</td>
<td>880 ± 161</td>
</tr>
<tr>
<td>Maintenance dose</td>
<td>553 ± 120</td>
</tr>
</tbody>
</table>

Comorbidities
- Hematopoietic stem cell transplant: 21 (30)
- Hematologic malignancies: 22 (31)
- Solid organ transplant: 12 (17)
- Other*: 15 (21)
- Concomitant pantoprazole: 50 (71)

Mean ± SD or n (%).
*Central nervous system fungal infections, fungal endocarditis, inflammatory bowel disease, and connective tissue disorders.

There was considerable interindividual variability in voriconazole trough concentrations at steady state, which ranged from 0.26 to 9.53 mg/l. Trough concentrations less than 2 mg/l or more than 6 mg/l (out of therapeutic range) were observed in 30% and 20% of the patients, respectively (Supplementary Fig. 1, Supplemental digital content 2, http://links.lww.com/FPC/B195). There was no difference in trough concentration between the NM (4.27 ± 2.4 mg/l) and the IM/PM (4.13 ± 1.6 mg/l) groups (P = 0.84, Supplementary Table 2, Supplemental digital content 3, http://links.lww.com/FPC/B196), supporting combining these groups for comparison with RMs/UMs. Trough concentrations were lower in RMs/UMs compared with patients with other CYP2C19 phenotypes (2.86 ± 2.3 vs. 4.26 ± 2.2, P = 0.0093). The mean steady-state trough concentrations were 1.35 ± 0.7, 2.97 ± 2.3, and 4.26 ± 2.2 mg/l in patients with the CYP2C19 *17/*17 (UMs), *1/*17 (RMs), and other genotypes, respectively (P = 0.02 for both the *17/*17 and *1/*17 genotypes compared with other genotypes, Fig. 1).

More patients with versus without the RM/UM phenotype had a subtherapeutic trough concentration (52 vs. 16%, P = 0.0028). All three UMs and 46% of RMs had a subtherapeutic trough (P ≤ 0.01 for each compared with other CYP2C19 phenotypes, Fig. 2). On univariate analysis, the RM/UM phenotype and weight were the only

### Table 2. Distribution of CYP2C19 genotypes and metabolizer phenotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>N (%)</th>
<th>Inferred phenotype</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*2/*2</td>
<td>1 (1.4)</td>
<td>Poor metabolizer</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>*1/*2</td>
<td>13 (18.6)</td>
<td>Intermediate Metabolizer</td>
<td>14 (20)</td>
</tr>
<tr>
<td>*2/*17</td>
<td>1 (1.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*17</td>
<td>28 (40)</td>
<td>Normal metabolizer</td>
<td>28 (40)</td>
</tr>
<tr>
<td>*1/*17</td>
<td>24 (34.3)</td>
<td>Rapid metabolizer</td>
<td>24 (34.3)</td>
</tr>
<tr>
<td>*17/*17</td>
<td>3 (4.3)</td>
<td>Ultrarapid metabolizer</td>
<td>3 (4.3)</td>
</tr>
</tbody>
</table>

No patient had the *3 variant.
variables associated with a subtherapeutic trough concentration (Table 3). The association between phenotype and trough concentration remained significant (Table 4) after including other covariates (age, race, sex, body weight, concomitant pantoprazole use, and route of administration) in the model, with an odds ratio of 5.6 (95% confidence interval: 1.64–19.24, \( P = 0.0044\)). The RM/UM phenotype was also more prevalent than other phenotypes among patients with a trough concentration less than 1 mg/l (27 vs. 7%, \( P = 0.031\)). In contrast, the likelihood of having a supratherapeutic trough concentration (>6 mg/l) at steady state was lower in RMs/UMs compared with NMs (7.4 vs. 35.7%, \( P = 0.03\), Fig. 3).

Although not an endpoint of the study, we also examined trough concentrations after dose adjustment in RMs and UM who initially had a subtherapeutic trough, given the paucity of data on appropriate doses with these phenotypes. Voriconazole was either discontinued or switched to an alternative antifungal agent in the majority of patients with a trough concentration less than 1 mg/l (27 vs. 7%, \( P = 0.031\)). In contrast, the likelihood of having a supratherapeutic trough concentration (>6 mg/l) at steady state was lower in RMs/UMs compared with NMs (7.4 vs. 35.7%, \( P = 0.03\), Fig. 3).

### Discussion
Consistent with previous reports [3,23,33–36], we observed wide interindividual variability in voriconazole exposure at steady state, with 30% of patients having a subtherapeutic trough concentration on treatment day 5–7 with recommended weight-based dosing. Given the severity of illness in patients with IFIs, it is critical that therapeutic voriconazole concentrations are attained rapidly to prevent adverse outcomes [7–9]. We found that the CYP2C19 genotype was a major contributor to risk for voriconazole underexposure, with 100% of those with the *17/*17 genotype (UM) and nearly 50% of those with the *1/*17 genotype (RM) failing to achieve therapeutic trough concentrations (2–6 mg/l) with weight-based dosing. The influence of the CYP2C19 genotype on voriconazole underexposure remained significant on logistic regression analysis that included route of administration and use of concomitant pantoprazole, a CYP2C19 inhibitor.

To our knowledge, this is the first prospective study showing an association between the CYP2C19 genotype and subtherapeutic voriconazole trough concentrations in adults receiving weight-based dosing, the currently accepted dosing approach for the treatment of IFIs. Our results corroborate previous findings in healthy adults and in adults receiving fixed-dose (vs. weight-based) voriconazole [23,32,33,37,38]. Specifically, in a retrospective study of 35 patients receiving voriconazole...
with children with the voriconazole exposure in children [20,39]. Compared
the CYP2C19 phenotype, but not the *1/*17 genotype (RM phenotype). In
the UM phenotype in our study with a voriconazole dose
of 553 mg), we still observed a greater likelihood for
higher doses in our study (mean daily maintenance dose
of 4 mg/kg). These data suggest that using
a PPI, specifically pantoprazole. Although previous
in-vivo and in-vitro studies show that PPIs increase vor-
iconazole levels, we observed no association between
pantoprazole use and voriconazole disposition [16,42–45].
This finding is consistent with a previous study [20] and
may be secondary to the weak inhibitory effect of panto-
prazole on CYP2C19 [33,44,45]. Hence, our results should
not be extrapolated to other PPIs because of the varying
degrees of CYP2C19 enzyme inhibition that exist among
the members of this drug class.

The current guidelines from the British Society for
Medical Mycology recommend voriconazole trough plasma
concentrations of at least 2 mg/l in critically ill patients with
a poor prognosis [28]. Alternatively, the 2016 update of the
Infectious Diseases Society of America guidelines for the
diagnosis and treatment of aspergillosis recommend vor-
iconazole trough concentrations of more than 1–1.5 mg/l
[4]. Given that most of the patients enrolled in this study
were presumed to have impaired host defense mechanisms
subsequent to the conditioning chemotherapy regimens
and/or receipt of immunosuppressants, we opted to set
the therapeutic threshold at 2 mg/l. Nonetheless, we also
examined the association between CYP2C19 genotype and
trough concentrations less than 1 mg/l and found that the
likelihood of trough concentrations less than 1 mg/l was
significantly higher with the RM/UM phenotype.

Although there were only three patients with the RM or
the UM phenotype in our study with a voriconazole dose
adjustment in response to a subtherapeutic concentra-
tion, all three had trough levels that were therapeutic or
at a nearly therapeutic level following a 25% dose (i.e.
increase from 4–5 mg/kg). These data suggest that using

200 mg twice daily, Lamoureux et al. [23] reported lower
voriconazole trough concentrations in CYP2C19*17 allele
carriers compared with noncarriers. Despite the use of
higher doses in our study (mean daily maintenance dose
of 553 mg), we still observed a greater likelihood for
subtherapeutic troughs among RMs/UMs compared with
those with other phenotypes. These data show that the
use of weight-based dosing versus fixed dosing is not
sufficient to overcome the risk for subtherapeutic vor-
icazole exposure in RMs/UMs.

The CYP2C19 genotype has also been associated with
voriconazole exposure in children [20,39]. Compared
with children with the *1/*1 genotype, Hicks et al. [20]
higher voriconazole doses may be a viable option for patients known to have the CYP2C19 RM or UM phenotype to increase the likelihood of attaining therapeutic concentrations. However, further assessment of dose adjustments on the basis of the CYP2C19 genotype is warranted.

Conclusion

Our findings indicate that adult patients with IFIs and the CYP2C19 RM or UM phenotype are at increased risk for subtherapeutic trough plasma concentrations of voriconazole with currently recommended weight-based dosing. Hence, the current ‘one size fits all’ approach used in clinical practice for initiating voriconazole treatment is far from optimal. Alternatively, pre-emptive CYP2C19 genotyping may serve as a valuable tool to help identify patients at risk for voriconazole under-exposure with standard weight-based dosing regimens, who may require higher voriconazole doses or alternative therapy to effectively treat IFIs.

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Conflicts of interest

There are no conflicts of interest.

References


