

Voriconazole metabolism is influenced by severe inflammation: a prospective study

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Background: During an infection or inflammation, several drug-metabolizing enzymes in the liver are down-regulated, including cytochrome P450 iso-enzymes. Since voriconazole is extensively metabolized by cytochrome P450 iso-enzymes, the metabolism of voriconazole can be influenced during inflammation via reduced clearance of the drug, resulting in higher voriconazole trough concentrations.

Objective: To investigate prospectively the influence of inflammation on voriconazole metabolism and voriconazole trough concentrations.

Methods: A prospective observational study was performed at the University Medical Center Groningen. Patients were eligible for inclusion if they were ≥ 18 years old and treated with voriconazole. Voriconazole and voriconazole-N-oxide concentrations were determined in discarded blood samples. To determine the degree of inflammation, C-reactive protein (CRP) concentrations were used. Subsequently, a longitudinal data analysis was performed to assess the effect of inflammation on the metabolic ratio and voriconazole trough concentration.

Results: Thirty-four patients were included. In total 489 voriconazole trough concentrations were included in the longitudinal data analysis. This analysis showed that inflammation, reflected by CRP concentrations, significantly influenced the metabolic ratio, voriconazole trough concentration and voriconazole-N-oxide concentration (all $P < 0.001$), when corrected for other factors that could influence voriconazole metabolism. The metabolic ratio was decreased by 0.99229^N and the voriconazole-N-oxide concentration by 0.99775^N , while the voriconazole trough concentration was increased by 1.005321^N , where N is the difference in CRP units (in mg/L).

Conclusions: This study shows that voriconazole metabolism is decreased during inflammation, resulting in higher voriconazole trough concentrations. Therefore, frequent monitoring of voriconazole serum concentrations is recommended during and following severe inflammation.

Introduction

Severe infections are commonly seen in hospitalized patients, particularly in patients in ICUs. The risk of infection even seems to increase with longer admission to an ICU. Additionally, the incidence of severe sepsis continues to increase.^{1,2} Multiple *in vitro* and *in vivo* studies have shown that during an infection or inflammation several drug-metabolizing enzymes in the liver are down-regulated, including cytochrome P450 (CYP) iso-enzymes. This can

result in reduced metabolism of drugs that are metabolized by these enzymes and hence higher drug concentrations.^{3,4}

Inflammation could also contribute to the pharmacokinetic variability of voriconazole. Voriconazole is a broad-spectrum triazole that is used for the treatment and prevention of invasive fungal infections.⁵ The clinical effect and the occurrence of adverse events with voriconazole are associated with its serum concentration. Therefore, therapeutic drug monitoring is indicated for voriconazole to improve treatment outcome and to reduce

toxicity.^{6–8} However, voriconazole serum concentrations are highly variable in clinical practice. This variability is not only seen between patients, but also within patients over time.^{9,10} Several factors are known to influence the serum concentration, including age, liver function, CYP2C19 genotype and co-medication,^{11–14} though these factors do not completely explain the observed variability. Since voriconazole is extensively metabolized by CYP isoenzymes,¹² the metabolism of voriconazole can be influenced during inflammation or an infection via reduced clearance of the drug, resulting in higher voriconazole serum concentrations. Therefore, if C-reactive protein (CRP) or IL-6 concentrations are significantly increased, a higher voriconazole serum concentration may be observed. Recently, a retrospective study showed that inflammation could influence the voriconazole serum concentration.¹⁵ In that study, higher voriconazole trough concentrations were observed during severe inflammation, as reflected by high CRP concentrations. Additionally, multiple voriconazole trough concentrations with corresponding CRP concentrations were analysed to determine the association between inflammation, reflected by CRP, and the voriconazole trough concentration over time.¹⁶ Both studies indicated that inflammation plays a significant role in the pharmacokinetic variability of voriconazole. However, voriconazole-N-oxide concentrations were not measured in both studies. Another retrospective study, where voriconazole-N-oxide concentrations were included, showed a changed metabolic ratio of voriconazole and the main metabolite of voriconazole, voriconazole-N-oxide, during inflammation.^{16,17} A limitation of that study was that a limited number of patients were included. In addition, only a limited number of samples per patient were available for analysis. Although these studies showed a trend that voriconazole serum concentrations were influenced during severe inflammation, methodological limitations hampered firm conclusions. To overcome these limitations, the aim of this study was to investigate prospectively the influence of inflammation on the metabolism of voriconazole by daily sampling during voriconazole treatment.

Patients and methods

Study design

This was a prospective observational study. Patients were eligible for inclusion if they were ≥ 18 years old and treated with intravenous or oral voriconazole at the University Medical Center Groningen, the Netherlands, between January 2014 and August 2014. Patients were excluded if they concomitantly used a strong inhibitor or inducer of CYP3A4 as described in the summary of product characteristics.

Discarded blood samples, drawn for clinical reasons, were collected during treatment with voriconazole, starting at steady state. Steady state was assumed to be achieved within 24 h after administration of two loading doses of voriconazole or after 10 doses if no loading dose was given.¹² A loading dose was defined as two intravenous doses of 6 mg/kg or two oral doses of 400 mg on the first day, followed by an intravenous dose of 4 mg/kg or an oral dose of 200 mg twice daily.

Ethics

This study was evaluated and allowed by the local ethics committee (Institutional Review Board 2013-511) and registered at ClinicalTrials.gov under registration number NCT02074462. Informed consent was obtained from each patient included.

Voriconazole and voriconazole-N-oxide assay

The voriconazole and voriconazole-N-oxide concentrations were measured with a validated LC-MS/MS method.¹⁸ The validation of voriconazole showed a within-run coefficient of variation (CV) ranging from 1.9% to 2.3%, and a between-run CV ranging from 0.0% to 3.1%. For voriconazole-N-oxide the within-run CV ranged from 3.6% to 10.8% and the between-run CV ranged from 0.0% to 7.7%. The limit of quantification for both voriconazole and voriconazole-N-oxide was 0.1 mg/L.

For the voriconazole analysis, we participated in an international proficiency testing programme for the measurement of antifungal drug concentrations and obtained good results.¹⁹

Data collection

Trough concentrations were used for the statistical analysis and the metabolic ratio was determined by dividing the voriconazole-N-oxide concentration by the corresponding voriconazole concentration.

Furthermore, CYP2C19 genotype was determined. Based on CYP2C19 genotype, patients were divided into four different categories: poor metabolizers were described as CYP2C19*2/*2, CYP2C19*2/*3 or CYP2C19*3/*3; intermediate metabolizers as CYP2C19*1/*2 or CYP2C19*1/*3; extensive metabolizers as CYP2C19*1/*1; and ultra-rapid metabolizers as CYP2C19*1/*17.²⁰

Demographic data were obtained from the medical chart of the patient and included age, sex, weight and underlying disease. Information about the voriconazole treatment included the dose (mg/kg/day), and time and route of administration. Furthermore, information about potentially interacting CYP450 co-medication was collected.

To determine the degree of inflammation, CRP concentrations were used, which were measured routinely or determined in discarded blood samples. The validation of CRP showed a CV ranging from 1.2% to 9.7%. In addition, other routine laboratory parameters that could influence the voriconazole and possibly the voriconazole-N-oxide concentration were collected, including alkaline phosphatase, ALT, AST, GGT and total bilirubin.

Statistical analysis

Numerical variables were summarized as medians with IQR, and categorical variables were summarized with frequencies and percentages. The longitudinal data of the voriconazole, voriconazole-N-oxide concentrations and metabolic ratios were analysed with a linear mixed model. A transformation was performed if the data were not normally distributed. A random additive effect was selected for patients to address different concentrations between patients. A first-order autoregressive correlation between voriconazole trough concentrations, voriconazole-N-oxide concentrations or metabolic ratios over time was selected to correct for differences in intervals between observations. To investigate the effect of inflammation on the metabolic ratio, voriconazole trough concentration and voriconazole-N-oxide concentration, the Wald type III test was conducted after correcting for gender, age, voriconazole dose and route of administration, liver enzymes (alkaline phosphatase, ALT, AST, GGT and total bilirubin) and the use of interacting co-medication. This correction means that we studied the contribution of inflammation on the metabolic ratio, voriconazole trough concentration and voriconazole-N-oxide concentration when we had eliminated the possible influence of the other variables first. Thus, we wanted to estimate the direct effect of inflammation on the metabolic ratio, voriconazole trough concentration and voriconazole-N-oxide concentration. The analysis was performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Results

Thirty-six patients were eligible for inclusion. Two patients were excluded because serum concentrations were not measured at

steady state. Therefore, 34 patients were included in the study and discarded blood samples were collected for these patients. The median duration with voriconazole treatment was 19 days (range 5–110 days). The baseline patient characteristics are shown in Table 1. The proton pump inhibitors (PPIs) esomeprazole, omeprazole or pantoprazole were the only potential interacting drugs that were used during treatment with voriconazole.

For 20 patients CYP2C19 genotype was determined; nine were extensive metabolizers, six intermediate metabolizers and five ultra-rapid metabolizers. In 14 patients, CYP2C19 genotype was not determined because they received allogeneic stem cell transplantation and therefore blood samples were not representative for the genotype. Due to the observational character of this study, no other methods for determining the genotype were used.

Therapeutic drug monitoring of voriconazole is routinely performed in our hospital, particularly for patients who receive voriconazole as treatment for invasive fungal infections. Thereby, serum concentrations were measured as routine care for 25 of the included patients during participation in this study. In general the therapeutic range applied from 1.5 up to 5 mg/L and for prophylaxis there was a lower limit of 1 mg/L.^{6,7} For 14 patients the voriconazole dose was adjusted after routine care measurement of the voriconazole serum concentration.

A total of 489 voriconazole trough concentrations were included in the analysis, with a median of 11 trough concentrations per patient (range 2–73). As well as the metabolic ratio, voriconazole and voriconazole-N-oxide trough concentrations were not normally distributed, so the data were log transformed. In Figure 1 a scatter plot is shown of all calculated metabolic ratios (panel a) and measured voriconazole trough concentrations

(panel b) with the corresponding CRP concentration. In this figure the metabolic ratio seems to decrease with increasing CRP concentration (panel a), while the voriconazole trough concentration seems to increase with increasing CRP concentration (panel b). Subsequently, a longitudinal data analysis was performed to determine the influence of CRP on the metabolic ratio, voriconazole trough concentration and voriconazole-N-oxide concentration where the repeated measurements were taken into account as well as other factors that could influence the metabolism of voriconazole. The longitudinal data analysis showed that after correcting for other factors that could influence voriconazole metabolism, which are mentioned in the Patients and methods section, the voriconazole trough concentration was significantly increased at higher CRP concentrations, while voriconazole-N-oxide concentrations and the metabolic ratio were significantly decreased at higher CRP concentrations (all $P < 0.001$). The metabolic ratio decreased by 0.99229^N and the voriconazole-N-oxide concentration by 0.99775^N , while the voriconazole concentration increased by 1.005321^N , where N is the difference in CRP units (expressed in mg/L). Besides CRP, the metabolic ratio, voriconazole concentration and voriconazole-N-oxide concentration were significantly associated with the voriconazole dose, ALT and AST concentrations (all $P < 0.05$). In addition, voriconazole-N-oxide concentrations were significantly associated with total bilirubin ($P < 0.05$).

The simulated expected voriconazole trough concentrations for the difference in CRP concentration from 5 up to 300 mg/L are shown in Figure 2, where an initial voriconazole trough concentration of 1, 2 and 3 mg/L is used. To obtain this figure the above-mentioned formula 1.005321^N is used, where N is the difference in CRP units. Others factors that could influence the voriconazole concentration are taken into account in this formula. Figure 2 shows that with an increase of the CRP concentration from, e.g. 5 to 205 mg/L ($n = 200$ mg/L), the initial voriconazole concentration of 2 mg/L is expected to increase to ~ 6 mg/L (calculated by $2 * 1.005321^{200}$).

To assess the influence of genotype on the metabolism of voriconazole and hence the voriconazole trough concentration, an analysis was performed on a subgroup of patients. The patient characteristics at baseline were comparable between the patients for whom genotyping was performed and the total population, as can be seen in Table 2. In total, 301 voriconazole trough concentrations were included in the longitudinal data analysis. The results of this analysis for the subgroup showed that the metabolic ratio was decreased and the voriconazole trough concentration was increased at higher CRP concentrations, after correcting for other factors that could influence voriconazole metabolism. The extent of decrease of the metabolic ratio varied between the different genotypes ($P < 0.001$). For extensive metabolizers the metabolic ratio decreased by 0.991972^N , for intermediate metabolizers by 0.986512^N and for ultra-rapid metabolizers by 0.994147^N , where N is the difference in CRP units (expressed in mg/L). For instance, if N is 200 mg/L, this results in a decrease of the metabolic ratio by 80% for extensive metabolizers, >90% for intermediate metabolizers and 70% for ultra-rapid metabolizers. The extent of increase in the voriconazole trough concentration also varied between the different genotypes ($P = 0.04$). For extensive metabolizers the voriconazole trough concentration increased by 1.004965^N , for intermediate metabolizers by 1.009365^N and for ultra-rapid metabolizers by 1.003685^N . For example, if a patient has an initial voriconazole trough concentration of 2 mg/L, with a

Table 1. Baseline patient characteristics ($n = 34$)

Characteristic	No. (%) of patients or median (IQR)
Demographics	
Admitted to an ICU	7 (21)
Sex	
male	23 (68)
female	11 (32)
Age (years)	60 (40–66)
Weight (kg)	79 (68–95)
Height (cm)	177 (168–185)
BMI (kg/m ²)	25.0 (22.3–28.8)
Underlying conditions	
Haematological malignancy	25 (74)
Solid organ transplantation	4 (12)
Other ^a	5 (14)
Voriconazole treatment	
Route of administration	
intravenous	25 (73.5)
oral	9 (26.5)
Daily dose (mg/kg)	5.7 (4.2–7.3)
Co-administered medication	
Proton pump inhibitor	23 (68)

^aOther diagnosed diseases included cystic fibrosis, chronic obstructive pulmonary disease and vasculitis.

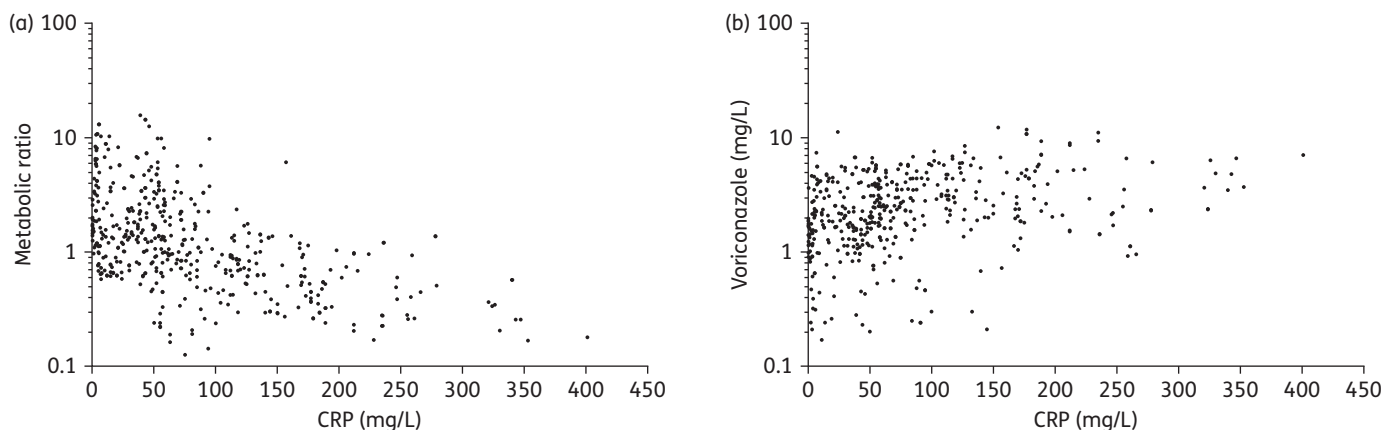


Figure 1. (a) Scatter plot of metabolic ratio versus CRP concentration (mg/L) for all calculated metabolic ratios. A trend of decreasing metabolic ratio with increasing CRP concentration can be observed. (b) Scatter plot of voriconazole trough concentration (mg/L) versus CRP concentration (mg/L) for all measured concentrations. A trend of increasing voriconazole trough concentration with increasing CRP concentration can be observed.

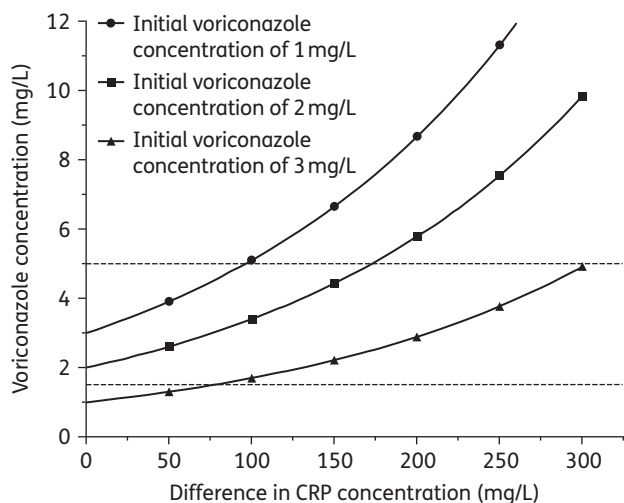


Figure 2. Simulated expected increase in voriconazole serum or plasma concentration versus difference in CRP concentration with an initial voriconazole trough concentration of 1, 2 or 3 mg/L. Horizontal dotted lines represent the therapeutic range of voriconazole.

corresponding CRP concentration of 5 mg/L, the voriconazole trough concentration will increase to ~5 mg/L for an extensive metabolizer if the CRP concentration increases to 205 mg/L. For the same increase in CRP and initial voriconazole trough concentration, the voriconazole trough concentration will increase to ~13 mg/L for an intermediate metabolizer and to ~4 mg/L for an ultra-rapid metabolizer.

Discussion

In this study, we show that the metabolism of voriconazole is influenced by the degree of inflammation as reflected by CRP concentration. The decreased drug metabolism during inflammation can be explained by the synthesis of pro-inflammatory cytokines during inflammation (e.g. TNF- α , IL-1, IL-6), resulting in a changed expression of specific transcription factors (e.g. NF- κ B). These

Table 2. Baseline patient characteristics with genotyping (n=20)

Characteristic	No. (%) of patients or median (IQR)
Demographics	
Admitted to an ICU	3 (15)
Sex	
male	13 (65)
female	7 (35)
Age (years)	62 (43–68)
Weight (kg)	75 (67–95)
Height (cm)	170 (168–185)
BMI (kg/m ²)	24.5 (22.1–28.2)
Underlying conditions	
Haematological malignancy	15 (75)
Solid organ transplantation	1 (5)
Other ^a	4 (20)
Voriconazole treatment	
Route of administration	
intravenous	14 (70.0)
oral	6 (30.0)
Daily dose (mg/kg)	5.9 (4.4–8.1)
Co-administered medication	
Proton pump inhibitor	12 (60)

^aOther diagnosed diseases included cystic fibrosis, chronic obstructive pulmonary disease and vasculitis.

changes result in down-regulation of various CYP iso-enzymes at the level of gene transcription, resulting in a loss of mRNA of the corresponding CYP-isoenzymes and subsequently a decrease in protein and enzyme activity. As a result, the metabolism of drugs that are metabolized by CYP-iso enzymes decreases and hence the serum concentration increases.^{21–24} Therefore, drugs with a narrow therapeutic window can accumulate to toxic serum concentrations during severe inflammation. This phenomenon was previously seen for among others theophylline, a CYP1A2 substrate and midazolam, a CYP3A4 substrate.^{24,25}

As mentioned before, voriconazole is extensively metabolized by CYP iso-enzymes, primarily by CYP2C19, and to a lesser extent by CYP2C9 and CYP3A4. Several *in vitro* studies using cell lines representative for expression of many CYP450 genes *in vivo* have shown that CYP2C19 activity is reduced during inflammation.^{26,27} Furthermore, voriconazole has a narrow therapeutic window and voriconazole serum concentrations are associated with efficacy and safety. Therefore, reduced metabolism of voriconazole can result in high serum concentrations, which are associated with an increased risk of neurotoxicity and liver toxicity.^{6,8,28} In addition, the probability and severity of drug–drug interactions between different CYP450 substrates can be increased due to the decreased enzyme activity.

An *in vitro* study with human hepatocytes showed that the expression of CYP2C19 mRNA was decreased by ~30%–50% in human hepatocytes and appears to depend on the pro-inflammatory cytokine IL-6.²⁹ In this study we measured the acute phase protein CRP, instead of IL-6. In contrast to the pro-inflammatory cytokine IL-6, CRP concentrations were routinely measured for the included patients. However, the synthesis of CRP is stimulated by pro-inflammatory cytokines (including IL-6). Therefore, CRP concentrations could be used instead of IL-6 concentrations for reflection of the degree of inflammation.³⁰ Additionally, since CRP expression in the liver is induced by IL-6, which is released by macrophages and T cells, CRP concentrations will show a delayed response at the beginning of an infection and will therefore be relatively normal during the initial phase of an infection and increase in a later phase.³¹ The same applies for voriconazole metabolism, which will probably be normal at the beginning of an infection and will decrease in a later phase. In previous studies this was already observed and voriconazole concentrations showed a similar trend as CRP concentrations over time.^{15,16} A future study investigating IL-6 concentration over time would therefore be very interesting as it may be an early predictor of decreased metabolism of voriconazole.

With the longitudinal data analysis, we showed that both the metabolic ratio and the voriconazole trough concentration were significantly influenced by the CRP concentration (both $P < 0.001$). These results show that inflammation indeed influences the metabolic ratio of voriconazole and the trough concentration, after correction for other factors that could influence these parameters. The increase in voriconazole serum concentration observed in this prospective observational study confirmed the results from earlier studies. Unfortunately, the study was not designed to determine the individual influence of other factors that significantly influenced the metabolic ratio, voriconazole concentration or voriconazole-N-oxide concentration.

During voriconazole treatment, seven patients were admitted to an ICU. In general, patients admitted to an ICU are critically ill. The pharmacokinetics of drugs in critically ill patients can differ from those who are less ill.³² However, by including multiple measurements in time for all patients, factors such as underlying disease and the general condition of the patient are taken into account with the longitudinal data analysis.

Furthermore, voriconazole trough concentrations were measured at steady state, because the metabolic ratio could be different during the loading phase of voriconazole due to non-linear pharmacokinetics of voriconazole.

Since therapeutic drug monitoring of voriconazole is routinely performed in our hospital, the occurrence of sub-therapeutic or

toxic voriconazole serum concentrations was prevented by voriconazole dose adjustments by the attending physician. This may have influenced the extent of the effect. However, the longitudinal data analysis showed that the degree of inflammation, as reflected by CRP, had a significant influence ($P < 0.001$) on the metabolism of voriconazole and voriconazole trough concentration, despite dose adjustments after performing therapeutic drug monitoring.

CYP2C19 genotype also plays an important role in the metabolism of voriconazole. The serum concentration of voriconazole is substantially higher in poor metabolizers of the CYP2C19 genotype compared with extensive metabolizers, while the serum concentration of voriconazole is lower in ultra-rapid metabolizers.^{33,34} Therefore, the impact of inflammation on the metabolic ratio and the voriconazole trough concentration could differ for different genotypes. In this study, the CYP2C19 genotype was determined for just over half of the included patients. The largest group of these patients (45%) were extensive metabolizers and no poor metabolizers were found, which is in line with the low prevalence of poor metabolizers among Caucasians (3%–5%).³³ Approximately 25% of the included patients showed ultra-rapid metabolism, which is in the same range as the percentage of ultra-rapid metabolizers in the Caucasian population.³⁵ As expected, patient genotype influences the extent of voriconazole metabolism and hence the voriconazole trough concentration during severe inflammation. However, these data should be interpreted with caution, due to the small number of patients included in this pharmacogenetic analysis.

In our hospital, CYP2C19 genotyping for patients treated with voriconazole is not routinely performed. Instead, we measure both voriconazole and voriconazole-N-oxide concentrations to be able to assess the overall influence of genotype and other relevant factors for instance, drug–drug interactions and drug absorption on voriconazole metabolism.³⁶

PPIs were the only potentially interacting co-medication used by patients in this study. Since omeprazole, esomeprazole and pantoprazole are metabolized by CYP2C19, concomitant use of these PPIs with voriconazole may influence the metabolism of voriconazole. In general, concomitant use of voriconazole and omeprazole does not require adjustment of the voriconazole dose.³⁷ Therefore, the influence of PPIs on the metabolism of voriconazole over time seems minimal. This is supported by the observation that the longitudinal data analysis did not show a significant influence of PPIs on voriconazole trough concentration nor on the metabolic ratio. However, the extent of this drug–drug interaction can be more pronounced in intermediate metabolizers compared with, e.g. extensive metabolizers during severe inflammation, since CYP2C19 enzyme activity is already decreased in intermediate metabolizers. The lack of significant influence of PPIs that was observed could be explained by the small number of intermediate metabolizers in our study. Therefore, further research should be performed to determine the probability and severity of this and other drug–drug interactions during severe inflammation for different genotypes, particularly for intermediate metabolizers.

The findings in our study may help to understand the variability of the voriconazole serum concentration in adults in clinical practice. Since inclusion criteria were limited by voriconazole use, selection bias is not likely and the inflammatory status of patients should therefore be taken into account to guide dosing with voriconazole.

The metabolism of voriconazole is not only very variable in adults, but also in paediatric patients.³⁸ However, the metabolic clearance in children differs from adults. For instance, metabolic clearance of voriconazole via flavin-containing monooxygenase 3 is higher in children compared with adults;³⁹ however, hepatic flavin-containing monooxygenase activity also seems to be decreased during inflammation.⁴ Therefore, further research is required to establish the influence of inflammation on the metabolism of voriconazole in paediatric patients. In addition, CYP2C19 is not the only iso-enzyme that is influenced by inflammation. The metabolic capacity of several CYP iso-enzymes is decreased during severe inflammation, including CYP1A2, CYP2C9 and CYP3A4. In several inflammatory conditions, for instance HIV and cancer, pheno-conversion can occur. This means that a genotypic extensive metabolizer can convert into a phenotypic poor metabolizer, which results in a reduced metabolic capacity.²⁴ Therefore, in general, the influence of inflammation on the metabolism of drugs with a small therapeutic window and primarily metabolized by CYP450 iso-enzymes should be investigated to optimize treatment with these drugs in clinical practice. LC-MS/MS assays, including both parent drug and metabolite, are particularly useful for such studies.⁴⁰

In conclusion, this study clearly demonstrates that the metabolism of voriconazole is decreased during inflammation as reflected by a reduction in the formation of voriconazole-N-oxide. As a result, the voriconazole serum concentration is increased, and following recovery, serum concentrations are expected to drop when inflammation subsides. Therefore, frequent monitoring of the voriconazole serum concentration during voriconazole treatment is recommended during and after severe inflammation, to maintain the voriconazole serum concentration within the therapeutic range.

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Author contributions

Study concept and design: A. V., M. t. A., J. W. C. A. Acquisition of data: A. V., M. t. A., L. F. R. S., J. G. Z. Statistical analysis: A. V., E. R. v. d. H. Analysis and

data interpretation: A. V., M. t. A., E. R. v. d. H., D. J. T., J. W. C. A. Obtained funding: J. W. C. A. All authors revised the manuscript for important intellectual content and approved the final manuscript.

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