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Variability of Voriconazole Plasma Concentrations after Allogeneic Hematopoietic Stem Cell Transplantation: Impact of Cytochrome P450 Polymorphisms and Comedications on Initial and Subsequent Trough Levels

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Voriconazole (VRC) plasma trough concentrations (C_{min}) are highly variable, and this could affect treatment efficacy and safety in patients undergoing allogeneic hematopoietic stem cell transplantation (AHSCT). We aimed to describe the intra- and interindividual variation of VRC C_{min} throughout the course of VRC therapy and to identify the determinants of this variation. Clinical data, medications, and VRC C_{min} ($n = 308$) of 33 AHSCT patients were retrospectively collected. Cytochrome P450 (CYP450) genotypes of CYP2C19, CYP3A4, and CYP3A5 patients were retrospectively determined before allografting, and a combined genetic score was calculated for each patient. The higher the genetic score, the faster the metabolism of the patient. The VRC C_{min} inter- and intraindividual coefficients of variation were 84% and 68%, respectively. The VRC dose (D) was correlated to VRC C_{min} ($r = 0.412$, $P < 0.0001$) only for oral administration. The administration route and the genetic score significantly affected the initial VRC C_{min}. Considering oral therapy, patients with a genetic score of <2 had higher initial VRC C_{min}/D than patients with a genetic score of >2 ($P = 0.009$). Subsequent VRC C_{min} remained influenced by the genetic score ($P = 0.004$) but were also affected by pump proton inhibitor comedication ($P < 0.0001$). The high variability of VRC C_{min} in AHSCT patients is partially explained by the route of administration, treatment with pump proton inhibitors, and the combined genetic score. This study suggests the interest in combined genetic score determination to individualize *a priori* the VRC dose and underlines the need for longitudinal therapeutic drug monitoring to adapt subsequent doses to maintain the VRC C_{min} within the therapeutic range.

Recipients of allogeneic hematopoietic stem cell transplants (AHSCT) are at high risk of developing invasive fungal infections (IFIs), in particular invasive aspergillosis (IA). Voriconazole (VRC) is a broad-spectrum triazole antifungal used as the first-line treatment of IA. Despite adequate care, mortality due to IA remains very high, reaching 56% in AHSCT patients (1). One possible explanation for this high rate of treatment failure could be insufficient exposure to treatment. Indeed, several studies performed in heterogeneous cohorts of patients with underlying hematological malignancy, solid organ transplantation, surgery, or various chronic diseases have suggested an association between low plasma trough concentrations (C_{min}) and treatment failure. For example, the lack of response to VRC therapy was more frequent in patients with a VRC C_{min} of ≤1 mg/liter (46%) than in those with a VRC C_{min} of >1 mg/liter (12%) (2). Similarly, VRC C_{min} in patients failing to respond to VRC therapy were lower than those in successfully treated patients (3). Finally, a median VRC C_{min} of >2.2 mg/liter was found to be a strong predictor of microbiological or clinical success (4). Thus, a therapeutic target of between 1 and 4 to 6 mg/liter for VRC C_{min} has recently been proposed by the British Society for Medical Mycology (5). However, the VRC C_{min} is frequently below this efficacy threshold in patients suffering from hematologic malignancies (6, 7), notably in AHSCT patients (8).

The VRC C_{min} exhibits large inter- and intraindividual variabilities (4, 9, 10) that could be related in part to nonlinear pharmacokinetics, metabolization via cytochrome P450 (CYP450), and drug-drug interactions (3, 11). Younger age (3, 6, 12), oral administration of VRC (3), and concomitant medication with enzyme inducers like phenytoin, rifampin, or glucocorticoids (3, 13) are associated with decreased VRC C_{min}. In this context, therapeutic drug monitoring (TDM) of VRC is of particular interest. A prospective randomized study has recently demonstrated the interest in VRC TDM, since an improvement of treatment response and a decrease of drug discontinuation due to adverse effects was obtained in patients benefiting from TDM (14). Nevertheless, TDM allows dose adjustment *a posteriori*, whereas it seems crucial to obtain an adequate VRC C_{min} as soon as possible after the start

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of treatment (4, 15). Indeed, an initial VRC C_{min} of ≤0.35 mg/liter appeared to be a strong predictor of mortality (4). Since VRC is extensively metabolized by CYP450 (16), it could be hypothesized that genetics affecting CYP450 have also a strong influence on initial VRC C_{min}, in addition to drug-drug interactions (3, 11, 13) and route of administration (3). Thus, identification of relevant genetic polymorphisms could be useful when proposing *a priori* dose adjustment and to reach an adequate VRC C_{min} from the first determination. The CYPs 2C19, 3A4, 3A5, and to a lesser extent, 2C9, are involved in VRC metabolism (16–18); all of these CYPs exhibit a number of clinically relevant polymorphisms. Genetic studies focusing on the CYP2C19 genotype have already demonstrated a link between the gain-of-function allele *17 and an insufficient VRC C_{min} (19) or between alleles *2 and *3 and an increased VRC C_{min} (20). The impact of the CYP2C19 genotype on VRC pharmacokinetics was later confirmed by several population pharmacokinetic studies (13, 21). Moreover, the presence of a CYP2C19 polymorphism has been associated with a higher frequency of out-of-range VRC C_{min} in lung transplant cystic fibrosis patients (22). Conversely, the variability of CYP3A activity on VRC pharmacokinetics has been less studied. In healthy subjects, cotreatment with a CYP3A inhibitor increased VRC exposure, particularly in poor metabolizers of CYP2C19 (23, 24), and CYP3A5 variants did not influence VRC single-dose pharmacokinetics (25). More recently, the T allele of the rs4646437 polymorphism located in an intronic area of the CYP3A4 was associated with higher VRC levels in Chinese patients (26). However, the impact of the new variant CYP3A4*22 (conferring decreased CYP3A4 activity) (27) on VRC pharmacokinetics has never been investigated.

The intraindividual variability of VRC C_{min} is also considerable (4, 9, 10), requiring regular VRC C_{min} determinations throughout the duration of VRC treatment (5). Some authors reported no correlation between the first and subsequent VRC C_{min} (9), whereas others showed that initial and subsequent VRC C_{min} were correlated only when the initial VRC C_{min} was greater than 2 mg/liter (10).

This retrospective study aimed to describe the variations of VRC C_{min} throughout the course of VRC therapy in a cohort of AHSCT patients and to identify the determinants of the variability in the first and subsequent VRC C_{min}.

MATERIALS AND METHODS

Patients. This study was conducted in Grenoble University Hospital Center, France. Adult (>18 years old) patients who had received an AHSCT, were treated with VRC, and underwent TDM of VRC between January 2011 and July 2013 were eligible. VRC C_{min} determined before the allograft or before VRC pharmacokinetics reached a steady state and patients with less than three VRC C_{min} determinations were excluded. After treatment initiation (without loading dose) or dose adjustment, a 3-day period was considered necessary to obtain a VRC C_{min} steady state (23). Demographic, biological (transaminase levels), clinical data, records concerning VRC therapy (C_{min}, daily dose, and route of administration), and concomitant medications were retrospectively collected. All patients gave written informed consent for genetic analysis, sample collection, and use of their data. This retrospective study was performed on residual samples stored in a biological sample collection (DRC-2013-1983) and was approved by the regional Ethics Committee.

Classification of invasive aspergillosis. IFIs were classified as possible, probable, or proven according to guidelines from the European Organization for Research and Treatment of Cancer/European Invasive Infections Cooperative Group and the criteria of the National Institute of Allergy and Infectious Diseases-Mycoses Study Group (EORTC/MSG) (28).

Measurement of plasma VRC trough concentration. The plasma VRC trough concentration was determined on samples handled just before the subsequent VRC administration. VRC C_{min} were measured by a validated liquid chromatography-tandem mass spectrometry method (29). Briefly, after protein precipitation, samples were injected into a 2-dimensional chromatographic system. In the first step, samples were cleaned in a perfusion chromatography column before being eluted and transferred to an analytical column. Finally, compounds were detected by tandem mass spectrometry. The plasma drug standard curve ranged from 0.1 to 20 mg/liter. The therapeutic range was between 1 and 5 mg/liter.

Genotyping. Genotyping was performed retrospectively on residual samples from routine biological analyses collected before the allograft. DNA was extracted from white blood cells using Macherey-Nagel NucleoSpin Blood L kit (Macherey-Nagel, Hoerd, France). The quality and quantity of DNA were checked with the NanoDrop 2000 spectrophotometer (Thermo Scientific, Illkirch, France).

CYP2C19 genotyping. CYP2C19 genotyping was performed by direct sequencing after DNA amplification using specific primers for the *2, *3 (associated with low CYP2C19 activity), and *17 (associated with increased CYP2C19 activity) alleles (see Table S1 in the supplemental material). The presence of the wild-type allele CYP2C19*1 was inferred in the absence of CYP2C19*2, CYP2C19*3, and CYP2C19*17. Patients were classified as ultrarapid (URM), extensive (EM), or intermediate (IM) metabolizers according to the established genotype-phenotype relationships proposed by Mega and colleagues (see Table S2 in the supplemental material) (30).

CYP3A genotyping. The CYP3A4*22 allele (associated with low CYP3A4 activity) and the CYP3A5*1 allele (associated with CYP3A5 expression) were studied using the TaqMan allelic discrimination assay (Life Technologies, Illkirch, France). Patients were classified as intermediate metabolizers (IM) if they had the *22 allele and extensive metabolizers (EM) if they did not.

Determination of genetic score. To evaluate the impact of both CYP2C19 and CYP3A genotypes, a combined genetic score was calculated for each patient.

First, a specific genetic score was separately attributed to each CYP2C19 and CYP3A genotype cluster, as previously proposed by Goutelle et al. (31) and Moes et al. (32), and both specific scores were then added to obtain the combined genetic score (see Table 2). Briefly, the specific genetic score for each cytochrome was determined as follows: the absence of any polymorphism (genotype *1/*1 for CYP2C19, absence of CYP3A4*22 and presence of CYP3A5*3/*3 for CYP3A) conferred a score equal to 1, whereas the presence of a gain- or a loss-of-function allele inferred, respectively, an increase or a decrease in the genetic score. The fluctuation of the genetic score was ±0.5 when the gain- or the loss-of-function allele was heterozygous or ±1 when the gain- or the loss-of-function allele was homozygous.

The combined genetic score was calculated to reflect the hepatic clearance (Cl_H) of VRC expressed as follows: Cl_H = Cl VRC N-oxidation + Cl VRC hydroxylation (33). VRC N-oxidation depends on CYP2C19 and CYP3A activities, which exhibited similar K_m values in an *in vitro* study (17), while VRC hydroxylation depends on only the activity of CYP3A isoforms (17). Thus, the same weight was attributed to CYP2C19 and to CYP3A4.

The combined genetic score was expressed in arbitrary units. Patients were classified into three categories of combined genetic score: <2, 2, and >2. A combined genetic score equal to 2 corresponds to normal CYP2C19 and CYP3A activities (or the association of one gain-of-function allele and one loss-of-function allele), whereas a combined genetic score of <2 or >2 reflects diminished or increased CYP2C19 and CYP3A activities, respectively.

TABLE 1 Demographic and clinical characteristics of 33 allogeneic hematopoietic stem cell transplantation patients who experienced therapeutic drug monitoring of voriconazole

Characteristic	Value for characteristic
Demographics	
Age, yr[a]	52.2 (35.1–60.1)
Male, no. (%)	20 (61)
BMI, kg/m^{2}[a]	22.5 (16.6–31.4)
Classification of IFI, no. (%)[b]	
Proven	2 (6)
Probable	15 (46)
Possible	14 (42)
ND[c]	2 (6)
Hematological diagnoses, no. (%)	
Acute myeloid leukemia	22 (67)
Acute lymphoblastic leukemia	5 (15)
Non-Hodgkin lymphoma	3 (9)
Chronic lymphocytic leukemia	2 (6)
Myelodysplastic syndrome	1 (3)
Conditioning regimens, no. (%)	
Myeloablative	8 (24)
Non-myeloablative	25 (76)
Matching, no. (%)	
Yes	22 (67)
No	11 (33)

[a] These data are expressed as median (10th to 90th percentiles).
[b] IFI, invasive fungal infection. All patients were treated for invasive pulmonary aspergillosis, except one patient who had invasive *Candida* infection.
[c] ND, not determined.

Data analysis. Statistical analyses were performed using Statview software, version 5.0 (SAS Institute, Cary, NC). Quantitative data are expressed as median and 10th and 90th percentiles. Statistical analyses were performed separately on the initial VRC C_{min} and on the subsequent VRC C_{min}. Comparisons between groups (male versus female, intravenous [i.v.] therapy versus oral therapy, CYP2C19 and CYP3A phenotypes, and categories of genetic score) were performed with nonparametric tests (Mann-Whitney test to compare 2 groups or Kruskal-Wallis test followed by Bonferroni adjusted *t* test to compare at least 3 groups). A Wilcoxon rank test was used for the comparison of pairwise series. As the numbers of VRC C_{min} determinations varied among patients, the frequency of a VRC C_{min} of ≤1 mg/liter was calculated for each patient. The percentages of VRC C_{min} of ≤1 mg/liter according to the combined genetic score groups were compared using Fisher's test at the first VRC C_{min} determination and using the Mann-Whitney test for subsequent VRC C_{min}. A Spearman test was used to study the correlation between two quantitative variables. The Hardy-Weinberg equilibrium was tested for each polymorphism by the online method of Rodriguez et al. (34). Univariate and multivariate linear regression analyses were used to identify the determinants contributing to the variability of VRC C_{min}. A *P* value of <0.05 was considered statistically significant.

RESULTS

Population characteristics. Thirty-three AHSCT patients were included in our study. Table 1 summarizes their demographic characteristics, hematological diagnoses, and conditioning regimens. All patients received VRC therapy for secondary prophylaxis of IFI. The classification of IFI is detailed in Table 1, but for 2 patients, IFI could not be retrospectively classified.

CYP450 polymorphisms. Genotyping was performed for 88% of patients ($n = 29$) (blood samples before the allograft were not available for the other patients). The proportions of the CYP2C19 and -3A genotypes are described in Tables S2 and S3 in the supplemental material. For CYP2C19, 37% ($n = 11$), 34% ($n = 10$), and 28% ($n = 8$) of patients were classified as URM, EM, and IM, respectively. All patients classified as IM for CYP2C19 expressed the *2 allele, while no *3 allele was detected in our cohort. The CYP3A4*22 polymorphism was detected in 24% ($n = 7$) of patients, and a single patient expressed CYP3A5. The Hardy-Weinberg equilibrium was respected for each allele (CYP2C19*2, $\chi^2 = 0.74$, $P = 0.39$; CYP2C19*17, $\chi^2 = 0.34$, $P = 0.56$; CYP3A4*22, $\chi^2 = 0.55$, $P = 0.46$), and no linkage disequilibrium was detected between the CYP3A4 and CYP2C19 genotypes ($P = 0.46$). Concerning the combined genetic score, 34% of patients ($n = 10$) had a genetic score of <2, 34% ($n = 10$) had a genetic score equal to 2, and 31% ($n = 9$) had a genetic score of >2 (Table 2).

VRC therapeutic drug monitoring. A total of 308 VRC C_{min} were analyzed (Table 3), representing a median of 9 dosages per patient (range, 3 to 25 dosages) for a median follow-up duration of VRC C_{min} TDM of 94 days (range, 14 to 269 days). The median delay between two VRC C_{min} determinations was 7 days (range, 4 to 21 days). The descriptions of VRC C_{min}, proportions of oral VRC therapy, and comedications are detailed in Table 3. The interindividual coefficient of variation (CV) was 84%, whereas the median intraindividual CV was 68% (range, 14 to 185%). Forty-two percent of VRC C_{min} were out of the therapeutic range, and 85% ($n = 28$) of patients had at least one inadequate VRC C_{min} during their course of treatment. Figure 1a illustrates this high intra- and interindividual variability.

TABLE 2 Calculated combined genetic scores according to CYP2C19 and CYP3A genotypes

CYP3A genotype	CYP specific genetic score	Combined score for CYP2C19 (no. of patients)[a]			
		*2/*1, score of 0.5	*1/*1, score of 1	*17/*1, score of 1.5	*17/*17, score of 2
3A4*22/*1 + 3A5*3/*3	0.5	1 (3)	1.5 (2)	2 (2)	2.5 (0)
3A4*1/*1 + 3A5*3/*3	1	1.5 (5)	2 (8)	2.5 (7)	3 (1)
3A4*1/*1 + 3A5*1/*3	1.5	2 (0)	2.5 (0)	3 (0)	3.5 (1)

[a] The 3 genetic score categories are indicated as follows: a genetic score of <2 is indicated by light gray shading, a genetic score equal to 2 is indicated by medium gray shading, and a genetic score of >2 is indicated by dark gray shading.

TABLE 3 Voriconazole plasma trough concentrations and concomitant treatments

Parameter[a]	Result for VRC C_{min} determinations		
	All ($n = 308$)	Initial ($n = 33$)	Subsequent ($n = 275$)
VRC C_{min}, mg/liter			
Mean	1.6	1.9	1.6
Median	1.3	1.4	1.3
Range	<0.1–9.7	<0.1–9.7	<0.1–8.1
VRC C_{min} level, %			
≤1 mg/liter	40	33	36
>5 mg/liter	2	6	1
Frequency of VRC oral therapy, %[b]	82	58	85
Determination during hospitalization, %	29	64	25
VRC dosing, mg/day[c]	400 (400–600)	400 (400–617)	400 (400–600)
Concomitant treatment, %			
Cyclosporine/tacrolimus/other[d]	47/13/40	73/9/18	44/14/42
Enzyme inhibitor(s)[e]	41	15	44
Glucocorticoid(s)[f]	29	18	30
Esomeprazole/pantoprazole/rabeprazole/no PPI	40/55/3/2	42/49/3/6	40/55/3/2

[a] VRC, voriconazole; C_{min}, trough concentration; PPI, pump proton inhibitor.
[b] Only the tablet form was used for oral VRC therapy.
[c] These data are expressed as median (10th to 90th percentiles).
[d] "Other" included immunosuppressive therapy with mycophenolate or everolimus or the absence of immunosuppressive therapy.
[e] Enzyme inhibitors included dihydropyridines, macrolides, verapamil, amiodarone, amitriptyline, citalopram, and metronidazole (all of these drugs are CYP2C19 and/or CYP3A inhibitors).
[f] The glucocorticoids used were prednisone, prednisolone, and methylprednisolone.

During the course of treatment of the 33 patients, 30 VRC dose adjustments for 16 patients were performed, representing 11% of 275 follow-up VRC C_{min} for 48% of patients. Seventy percent ($n = 21$) of these dose modifications were realized without changing the VRC route of administration, while 27% ($n = 8$) resulted from an i.v.-to-oral switch, and 3% ($n = 1$) were discontinuations of VRC therapy due to an overdose.

Figure 1b shows the relationships between VRC daily dose (expressed in mg per day) and VRC C_{min} for both routes of administration. Considering oral administration, the VRC C_{min} presented a CV of 72% and was weakly correlated to the VRC daily dose ($r = 0.412$, $P < 0.0001$), while i.v. administration led to a VRC C_{min} CV of 101%, and no correlation was found with the daily dose ($r = 0.096$, $P = 0.5$).

Initial VRC trough concentrations. The description of the initial VRC C_{min} is detailed in Table 3. Thirty-nine percent of initial VRC C_{min} were out of the therapeutic range, with the majority under the lower therapeutic limit: 33% were ≤1 mg/liter, and 6% were >5 mg/liter. VRC i.v. administration was associated with a higher initial VRC C_{min} compared to oral treatment (i.v., 1.7 mg/liter [range, 0.3 to 5.9 mg/liter], versus oral, 1.0 mg/liter [range, 0.1 to 2.9 mg/liter]; $P = 0.03$). Sex, age, body mass index (BMI), and proton pump inhibitor (PPI) treatment or enzymatic inhibitor treatment as well as transaminase levels had no impact on the initial VRC C_{min} (data not shown).

Influence of CYP450 polymorphisms on initial VRC trough concentrations. The influence of CYP450 polymorphisms was studied on the initial VRC C_{min} adjusted on VRC dose (VRC C_{min}/D) to overcome the influence of VRC dose. After oral administration, the CYP2C19 phenotype affected the initial VRC C_{min}/D (Kruskal Wallis test, $P = 0.04$), with a higher initial VRC C_{min}/D in IM compared to URM patients (*post hoc* test, $P = 0.007$) (Fig. 2a). Similarly, the presence of the *22 allele for CYP3A4 was associated with a statistically significant increased VRC C_{min}/D (Fig. 2c). When patients were stratified on CYP2C19 phenotype, expression of CYP3A4*22 was associated with increased initial VRC C_{min}/D whatever the CYP2C19 phenotype (see Fig. S1 in the supplemental material). In univariate analysis, the combined genetic score was significantly correlated to VRC C_{min}/D ($r = -0.748$; $P = 0.002$). As shown in Fig. 2, a genetic score of >2 was associated with a lower VRC C_{min}/D compared to a genetic score of <2 (*post hoc* test, $P = 0.009$) (Fig. 2e).

Studying all initial VRC C_{min} independently of the VRC route of administration provided similar results: a trend toward an influence of the CYP2C19 phenotype (Kruskal-Wallis test, $P = 0.08$) (Fig. 2b) and the *22 allele (Mann-Whitney test, $P = 0.05$) (Fig. 2d) as well as the combined genetic score (Kruskal-Wallis test, $P = 0.05$) (Fig. 2f) on the first VRC C_{min}/D.

None of the patients having a combined genetic score of <2 presented an initial VRC C_{min} of ≤1 mg/liter, while 47% of the patients having a combined genetic score of ≥2 had an initial VRC C_{min} of ≤1 mg/liter (Fisher's test, $P = 0.01$).

In a multiple linear regression model integrating the VRC route of administration, initial VRC dose, and combined genetic score, the combined genetic score remained an independent predictor of initial VRC C_{min} ($r^2 = 0.33$; $P = 0.04$).

Subsequent VRC trough concentrations. Longitudinal TDM of VRC C_{min} is detailed in Table 3. Thirty-seven percent of the subsequent VRC C_{min} were outside the therapeutic range, with 36% at ≤1 mg/liter and 1% at >5 mg/liter. The route of administration had no impact on follow-up VRC C_{min} (i.v., 0.9 mg/liter

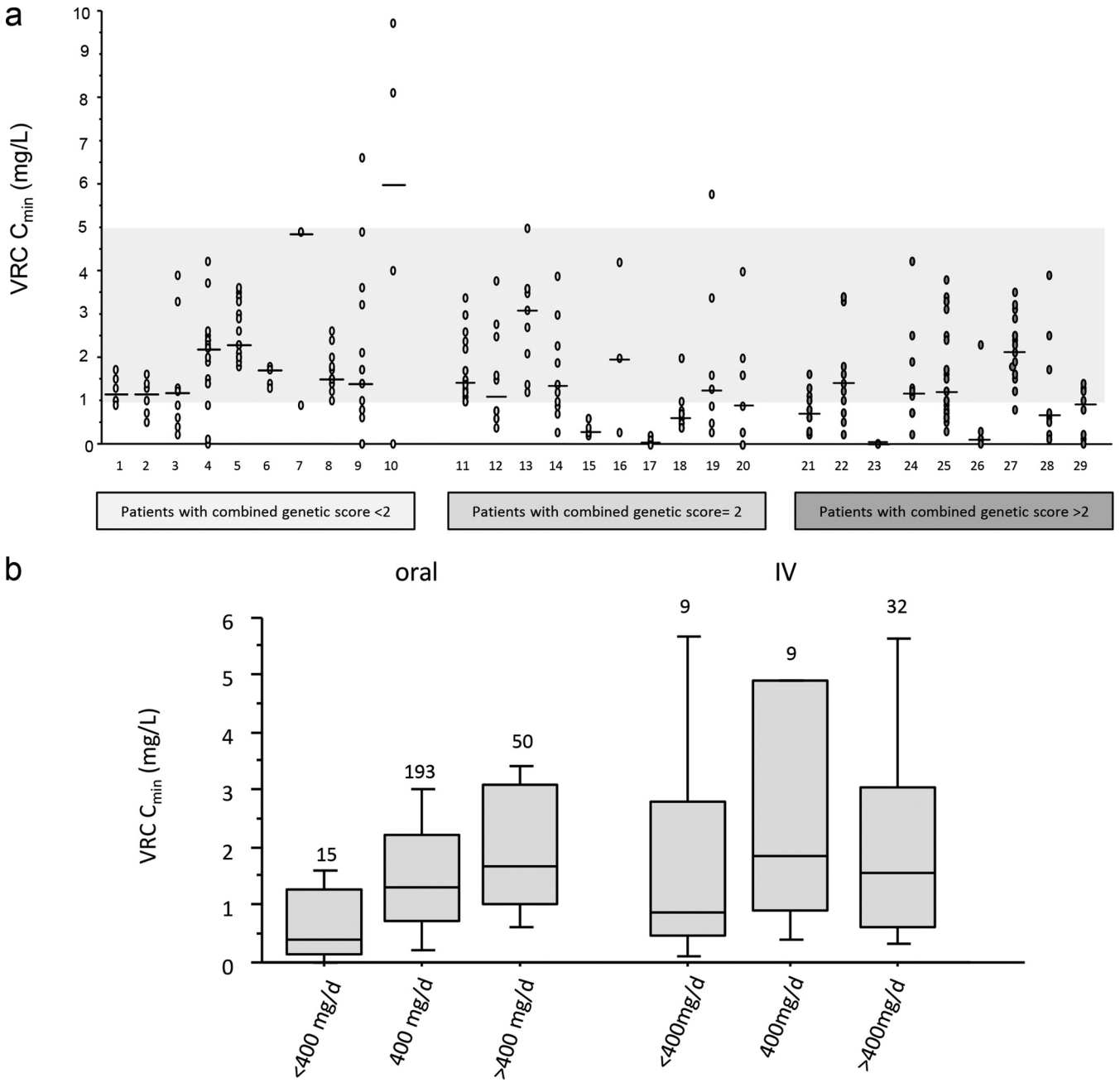

FIG 1 Variability of voriconazole plasma trough concentrations. (a) Serial voriconazole plasma trough concentrations determined in 33 post-AHSCT patients. All patients had at least 3 voriconazole concentration determinations, including several similar voriconazole concentrations for patients 7 and 23. The area shaded in gray represents the therapeutic range. (b) Relationships between voriconazole dose (expressed as mg per day) and plasma trough concentration for both routes of administration. Data are presented as interquartile range (boxes), data range (whiskers), and median (horizontal line). Numbers are indicated above boxes.

[range, 0.3 to 4.8 mg/liter], versus oral, 1.4 mg/liter [range, 0.2 to 3.3 mg/liter]; $P = 0.9$). As observed for initial VRC C_{min}, age, BMI, and transaminase levels were not related to subsequent VRC C_{min} (data not shown).

Influence of CYP450 polymorphisms on subsequent VRC trough concentrations. The combined genetic score influenced the subsequent VRC C_{min} or subsequent VRC C_{min}/D (Fig. 3a and b) (Kruskal-Wallis test, $P = 0.004$ and $P < 0.0001$, respectively). The subsequent VRC C_{min} of patients having a combined genetic score of >2 were lower than those of patients with a combined genetic score of <2 (*post hoc* test, $P = 0.002$).

Figure 3c shows the evolution of VRC C_{min} during repeated TDM in patients stratified according to the combined genetic score. As previously shown in Fig. 2c, the initial VRC C_{min} was significantly affected by the combined genetic score (Kruskal-Wallis test, $P = 0.03$), while at each subsequent TDM VRC C_{min} measurement, the VRC C_{min} was not significantly different between the combined genetic score categories.

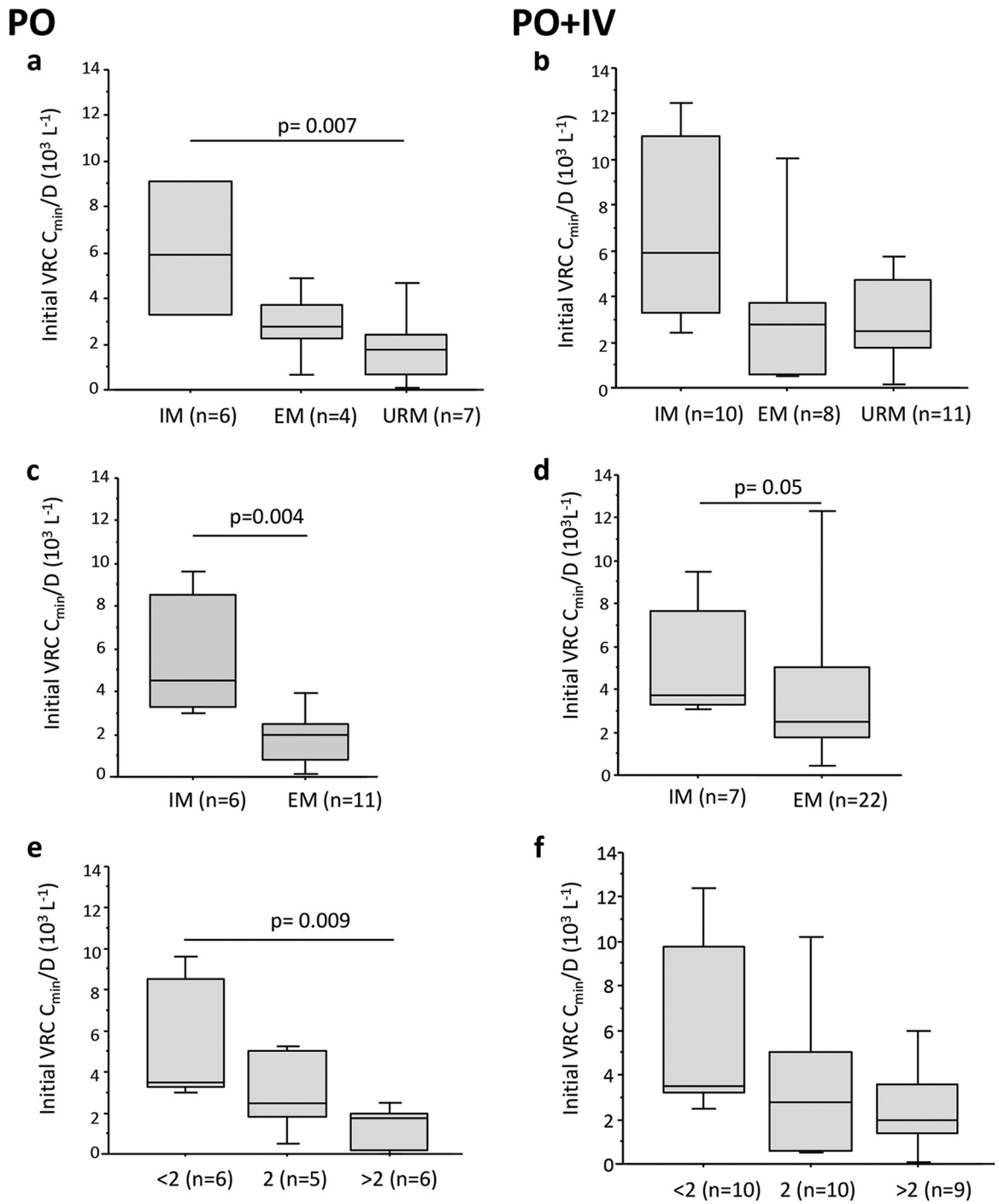

FIG 2 Influence of CYP450 polymorphisms on initial voriconazole plasma trough concentrations adjusted on the dose (VRC C_{min}/D). (a and b) Influence of CYP2C19 phenotypes on initial VRC C_{min}/D ratio obtained during VRC oral treatment (PO) (a) and on all initial VRC C_{min}/D ratios (oral and i.v. [PO + IV]) (b). (c and d) Influence of CYP3A phenotypes on the initial VRC C_{min}/D ratio obtained during VRC oral treatment (c) and on all initial VRC C_{min}/D ratios (d). (e and f) Influence of the combined genetic score on the initial VRC C_{min}/D ratio obtained during VRC oral treatment (e) and on all initial VRC C_{min}/D ratios (f). Data are presented as interquartile range (boxes), data range (whiskers), and median (horizontal line). Only the significant *P* value obtained for the *post hoc* Bonferroni test (<0.0167) is shown. Abbreviations: VRC, voriconazole; CYP, cytochrome; PO, *per os*; IV, intravenous; URM, ultrarapid metabolizer; EM, extensive metabolizer; IM, intermediate metabolizer.

The frequency of VRC C_{min} of ≤1 mg/liter was higher ($P = 0.05$) in patients having a combined genetic score of ≥2 (50% [range, 0 to 100%]) than in those with a combined genetic score of <2 (21% [range, 0 to 56%]).

Influence of diarrhea on subsequent VRC trough concentrations. Clinical events contemporary with VRC C_{min} measurements were available for 77% of subsequent VRC C_{min} ($n = 212$). Since diarrhea could impact VRC C_{min} by reducing VRC absorp-

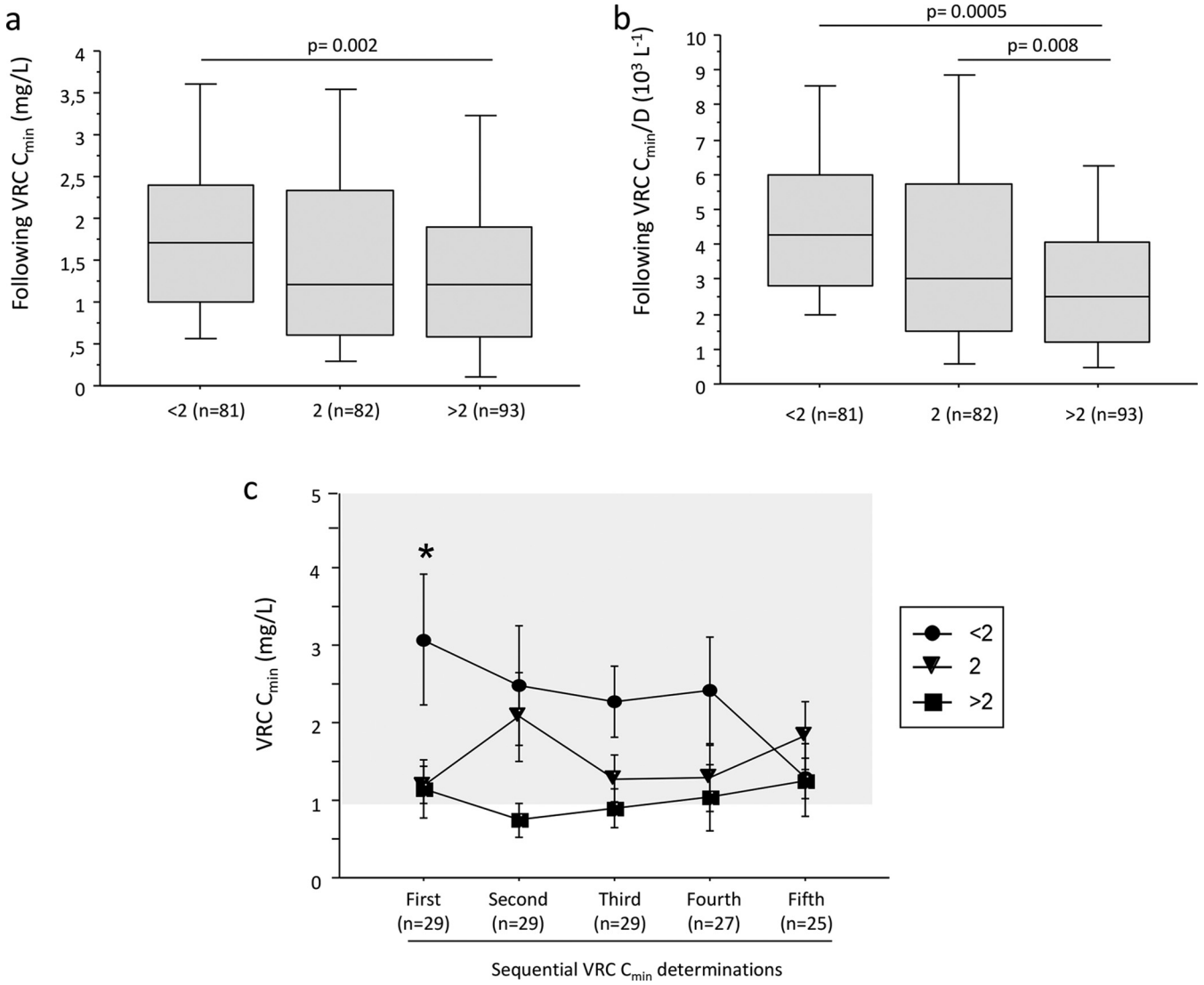

FIG 3 Influence of the genetic score on follow-up voriconazole plasma trough concentrations. Shown are the follow-up VRC C_{min} (a) or C_{min}/D ratio (b) according to the combined genetic score. Data are presented as interquartile range (boxes), data range (whiskers), and median (horizontal line), and only the significant *P* value obtained for the *post hoc* Bonferroni test (<0.0167 for three groups) is shown. (c) Temporal evolution of VRC trough concentrations throughout longitudinal TDM according to the combined genetic score. The area shaded in gray represents the therapeutic range. Solid circles, triangles, and squares represent the mean (± standard deviation) VRC C_{min} for patients having genetic scores of <2, equal to 2, and >2, respectively. The asterisk indicates a *P* value of <0.05 for the Kruskal-Wallis test. Second, third, fourth, and fifth determinations of VRC C_{min} were obtained after median periods of 7 (range, 2.8 to 28), 14 (range, 7.0 to 36), 35 (range, 11 to 59), and 53 (range, 20 to 87) days after the first determination.

tion, the effect of diarrhea was investigated during *per os* VRC therapy. Among the 235 VRC C_{min} obtained during oral VRC treatment, clinical events were available for 193 VRC C_{min}. Eleven episodes of diarrhea were reported by 9 different patients (i.e., a frequency of 6%). VRC C_{min} was not influenced by diarrhea (VRC C_{min} with and without diarrhea, 1.6 mg/liter [range, 0.3 to 3.8 mg/liter] versus 1.3 mg/liter [range, 0.2 to 2.9 mg/liter]; $P = 0.2$). The influence of gastrointestinal graft versus host disease (GVHD) or mucositis could not be tested in our cohort because only two episodes of gastrointestinal GVHD and one of mucositis were reported during the course of VRC oral treatment.

Influence of concomitant medication on subsequent VRC trough concentrations. Data on concomitant treatments were available for 95% of VRC C_{min} ($n = 259$). Ninety-four percent ($n = 244$) of the subsequent VRC C_{min} were determined while the patient was on PPI treatment; esomeprazole and pantoprazole were the most frequently used PPIs (Table 3). Each PPI influenced the VRC C_{min} differently (Kruskal-Wallis test, $P < 0.0001$) (Fig. 4). *Post hoc* analysis revealed a higher VRC C_{min} when the PPI was esomeprazole compared to pantoprazole ($P < 0.0001$), whereas there was no statistically significant difference between rabeprazole and esomeprazole or pantoprazole. This difference was also observed after stratification of patients according to combined genetic scores (data not shown).

Treatment with glucocorticoids had no impact on VRC C_{min} (1.3 mg/liter [range, 0.1 to 3.2 mg/liter] with treatment versus 1.4 mg/liter [range, 0.3 to 3.4 mg/liter] without; $P = 0.2$). Since a multicenter study recently reported an interaction between gluco-

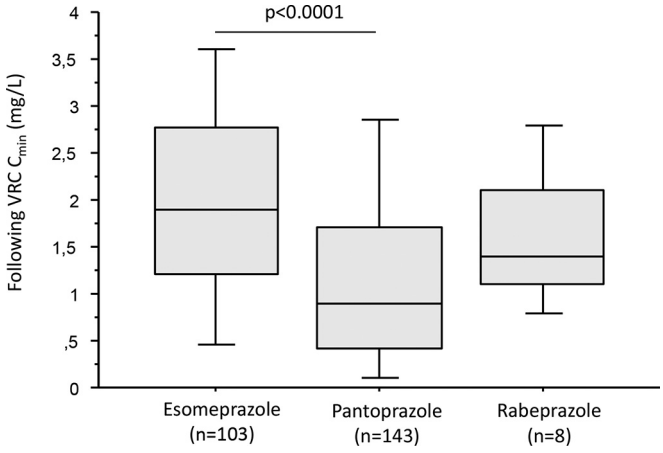

FIG 4 Influence of pump proton inhibitor treatment on voriconazole plasma trough concentrations. Data are presented as interquartile range (boxes), data range (whiskers), and median (horizontal line).

corticoids and VRC (3), a pairwise patient analysis was performed to further explore this drug-drug interaction. Patients who benefited from VRC C_{min} determinations with and without concomitant treatment by corticoids for at least 7 days were selected ($n = 10$). For each patient, the mean VRC C_{min} with or without glucocorticoid treatment was calculated. To overcome the influence of the route of administration, only VRC C_{min} obtained during oral therapy were used. Comparison of VRC C_{min} in these paired series confirmed the absence of any effect of glucocorticoid treatment on VRC C_{min} (see Fig. S2 in the supplemental material).

A possible interaction between PPI and steroid use was excluded since the frequencies of steroid use during esomeprazole and pantoprazole treatment were similar (36% of steroid use during esomeprazole treatment versus 29% for pantoprazole; χ^2 test, $P = 0.4$).

Immunosuppressive therapies, including calcineurin inhibitors, mycophenolate, or everolimus, had no effect on subsequent VRC C_{min}. Similarly, the subsequent VRC C_{min} was not influenced by comedication with various CYP2C19 and/or CYP3A enzyme inhibitors (data not shown).

DISCUSSION

The present study demonstrates that in a homogeneous cohort of AHSCT patients, the VRC C_{min} exhibits high inter- and intraindividual variability, resulting in almost half of the VRC C_{min} being out of the therapeutic range. On the whole, this variability could be partially explained by a combined genetic score that takes into account both CYP2C19 and 3A genotypes, the VRC route of administration, and any comedication with PPI.

Early optimal antifungal therapy improves the clinical outcome in patients with IFI (35, 36), and an initial VRC C_{min} of $\leq$0.35 mg/liter is a strong predictor of mortality (4). These studies suggested the importance of adjusting the VRC dose right from the first administration to reach the VRC C_{min} within the therapeutic range early on. Our study demonstrates for the first time that a genetic score combining both CYP2C19 and -3A4 genotypes is an independent predictor of initial VRC C_{min}. The impact of a CYP2C19 phenotype on the initial VRC C_{min} was already suggested by a study on a Korean cohort of patients with hematological diseases, without reaching statistical significance (37). In our cohort, CYP2C19 IM patients had a statistically significantly higher initial VRC C_{min}/D than CYP2C19 URM patients, confirming the importance of CYP2C19 polymorphisms on the variability of the initial VRC C_{min}. In addition to CYP2C19, VRC is also metabolized through the CYP3A pathway (16), which also exhibits clinically relevant polymorphisms. Our study provides the first demonstration that the CYP3A4*22 polymorphism also significantly influences the VRC C_{min} in AHSCT patients, since it was associated with a higher initial VRC C_{min}/D. Recently, the rs4646437 polymorphism of the CYP3A4 gene was also associated with higher VRC levels in Chinese patients (26). These genetic data, combined with several studies focusing on drug-drug interaction (23, 24), suggest the importance of CYP3A4 activity in VRC metabolism.

Since the frequencies of CYP2C19 and CYP3A single nucleotide polymorphisms were independent of one another, it was therefore of interest to calculate a combined genetic score integrating both CYP genotypes. Interestingly, a combined genetic score of <2 was associated with a higher VRC C_{min}/D compared to a combined genetic score of >2. Moreover, none of the patients having a combined genetic score of <2 presented an initial VRC C_{min} inferior to the efficacy threshold fixed at 1 mg/liter, while 47% of the patients with a combined genetic score of ≥ 2 had a subtherapeutic initial VRC C_{min}. These data suggest that this combined genetic score could help to individualize the VRC dose from the first administration onwards, as recently suggested for CYP2C19 (13, 38), and reduce the risk of out-of-range VRC C_{min} at the first determination.

Throughout VRC treatment, repeated adequate sustained VRC C_{min} measurements, rather than a single VRC C_{min} determination, have been shown to ensure treatment success (4, 12, 39). However, few studies have focused on longitudinal VRC TDM (4, 10, 12, 22), even though it is recommended to repeat VRC C_{min} determinations during therapy (5) in view of the large VRC intraindividual variability (4, 7), especially in AHSCT patients (10).

Follow-up VRC C_{min} remained influenced by the combined genetic score, despite dose adjustment being made during longitudinal TDM. However, this finding could be explained by the low frequency of dose adjustments in our cohort; in fact, VRC dose adjustments were made for only 16% of patients, while 85% of patients had at least one VRC C_{min} outside the therapeutic range and would be likely to need a VRC dose adjustment. Nevertheless, the genetics-related variability of VRC C_{min} was slightly reduced during TDM, notably after 5 VRC C_{min} measurements, although VRC C_{min} variability remained high.

Concomitant treatments are known to affect VRC C_{min}, notably drugs able to influence VRC metabolism by competition at the CYP catalytic site, such as PPIs (3, 7), or by induction of protein expression, such as phenytoin (3). In our cohort, PPIs were used widely, and pantoprazole was associated with lower VRC C_{min} than esomeprazole. Consistent with these findings, an *in vitro* study on human liver microsomes has demonstrated that pantoprazole has a lower inhibitory potency on CYP2C19 activity than esomeprazole and to a lesser extent rabeprazole (40). Conversely, PPIs exhibited no inhibition of CYP3A activity at concentrations in the same order of magnitude as the plasma levels encountered *in vivo* (40). Thus, the reduced VRC C_{min} in patients treated with pantoprazole we observed could be explained by its lower inhibitory potency on CYP2C19 activity. However, the effect of PPIs, especially pantoprazole on the VRC C_{min}, is far from fully under-

stood since clinical studies investigating VRC pharmacokinetics have demonstrated either increased (3) or decreased (6, 41) VRC C_{min} in patients treated with pantoprazole compared to those in untreated PPI patients. Since it is well known that PPIs competitively inhibit CYP2C19 (3, 7, 40), taken together, these results suggest that PPI treatment significantly contributes to the variability of VRC C_{min}.

Another drug-drug interaction, between VRC and glucocorticoids, leading to reduced VRC C_{min} has been suggested by a single study (3) and has been proposed to be the result of CYP induction by glucocorticoids. However, our data and those from other studies (6, 42) did not support such an interaction, and in the present study, steroid use induced no significant change in VRC C_{min} both in the overall analysis performed with all VRC C_{min} and in the pairwise patient analysis. These conflicting results could be explained by the heterogeneity of the studied populations and the type and dose of the glucocorticoid. Indeed, AHSCT patients in our study preferentially received prednisone, prednisolone, and/or methylprednisolone at low and decreasing doses, whereas the effect of glucocorticoids was previously demonstrated in a heterogeneous population, including patients suffering from autoimmune diseases for whom dexamethasone treatment was more frequent and glucocorticoid doses were higher (3).

In our cohort, sex, age, and BMI had no impact on VRC C_{min}, whereas previous studies have identified age (3, 6, 12) and BMI (3, 6) as factors influencing VRC C_{min}. However, the patients in the latter studies were much older and had a higher BMI than those of our cohort, which could contribute to explaining these discrepancies.

There are several limitations of our study. First, this study was retrospective, performed in a single center and on a small number of patients: thus, the influence of the combined genetic score on VRC efficacy and safety could not be evaluated. Second, we have arbitrarily chosen to attribute similar weights to CYP2C19 and CYP3A since (i) VRC N-oxidation results from the simultaneous action of CYP2C19 and CYP3A (17, 18), both CYPs exhibiting similar K_m values (17), and (ii) VRC oxidation depends on only CYP3A isoforms (17). However, we acknowledge that the relative contributions of each CYP and relationships between CYP polymorphisms and activities remain to be further defined.

In conclusion, our data confirm the influence of CYP2C19 and provide strong evidence that CYP3A genotypes also influence VRC exposure in AHSCT patients. In this regard, the determination of a genetic score taking into account both CYP genotypes could be of clinical interest to adjust *a priori* the first VRC doses. Our data provide the groundwork for a multicenter prospective trial to confirm the clinical benefit of VRC first-dose adjustment for the combined genetic score, route of administration, and comedications and adaptation of the following doses according to longitudinal TDM.

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