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Original article

The role of candidate genetic polymorphisms in the interaction between voriconazole and cyclosporine in patients undergoing allogeneic hematopoietic cell transplantation: An explorative study

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ARTICLE INFO

Article history:

Received 7 November 2019

Accepted 12 February 2020

Available online 22 February 2020

Keywords:

Cyclosporine
 Voriconazole
 Allo-HCT
 Pharmacogenetics
 ABCB1

ABSTRACT

Purpose: To evaluate polymorphisms in genes of drug metabolizing enzymes and transporters involved in cyclosporine and/or voriconazole disposition among patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT).

Methods: DNA from forty patients was genotyped using the DMETPlus array. The average ratio of cyclosporine concentration/dose (C/D in (ng/mL)/(mg/kg)) per participant's weight was computed using available trough levels and daily doses.

Results: The C/D cyclosporine ratio was significantly higher when it was administered with voriconazole as compared to when it was administered alone: median: 116.75 vs. 25.40 (ng/mL)/(mg/kg) with and without voriconazole respectively, ($P < 0.001$). There was also a significant association between the C/D cyclosporine ratio combined with voriconazole and the *ABCB1* 2677 G > T > A (*rs2032582*) genetic polymorphism ($P = 0.05$). In parallel, *ABCB1* variant allele carriers had higher creatinine in combination therapy with a median creatinine (mg/dL) of 0.74 vs. 0.56 for variant allele carriers vs. reference; $P = 0.003$. Interestingly, CYP2C9, CYP2C19, and CYP3A5 extensive metabolizers tended to be associated with lower cyclosporine C/D ratio when combined with voriconazole, but the results were not statistically significant.

Conclusion: To the best of our knowledge, this is the first pharmacogenetic study on the interaction between voriconazole and cyclosporine in patients undergoing allo-HCT. Results suggest that the *ABCB1* 2677 G > T > A genetic polymorphism plays a role in this interaction with cyclosporine related nephrotoxicity. Pre-emptive genotyping for this genetic variant may be warranted for cyclosporine dose optimization. Larger studies are needed to potentially show significant associations with more candidate genes such as *CYP3A4/5*, *CYP2C9*, and *CYP2C19*, among others.

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Introduction

The introduction of standardized treatment guidelines and protocols for patients needing allogeneic hematopoietic cell transplantation (allo-HCT) have led to enhanced quality of life and prolonged survival. Nevertheless, efforts are still needed to optimize safety, toxicity, and efficacy of the therapeutic strategy. Invasive fungal infection (IFI) is a major life-threatening

complication in patients undergoing allo-HCT. In recent years, and mainly due to the institution of prophylaxis with new antifungal agents such as voriconazole, there has been a significant decrease in IFI-related mortality following allo-HCT [1–4]. The combination of calcineurin inhibitors and voriconazole may result in clinically relevant interactions due to competitive inhibition of cytochrome (CYP) P450 enzymes by voriconazole [5]. This resulting elevation in plasma concentrations of cyclosporine implies a reduction in cyclosporine dose to avoid associated risks of toxicity [6–10]. Interestingly, it has been observed that the pattern of interaction is distinct among different individuals, with some sustaining different plasma concentrations of cyclosporine when compared to others receiving the same treatment regimen [11].

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Because of observed inter-individual variabilities in drug therapy, pharmacogenetic studies are performed to investigate the association between polymorphisms of candidate genes and drug response and toxicity [12]. The majority of the cyclosporine-related pharmacogenetic studies have evaluated polymorphisms in the *ABCB1*, *CYP3A4*, and *CYP3A5* genes because of their role in cyclosporine disposition [13]. As for voriconazole, it is now established that *CYP2C19* is the major determinant of its pharmacokinetic variability [14,15]; yet *CYP2C9*, *CYP3A4*, and *ABCB1* were also shown to play a role, and hence the potential inhibition of cyclosporine elimination by voriconazole [16].

To our knowledge, despite a large number of publications in subjects who underwent solid organ transplants [13,14,16–25], there is a paucity of literature evaluating the role of genetic polymorphisms of candidate genes with the variability of the pharmacokinetics of cyclosporine in hematopoietic cell transplant patients [26–28]. It appears that variants in *CYP3A45* and *ABCB1* may be associated with variability in cyclosporine pharmacokinetics; nevertheless, data have been inconclusive and sometimes inconsistent probably due to the variability in patient populations, sample size, dosing strategies, times of plasma collection, and pharmacokinetic modeling [20]. More importantly, none of these studies addressed the concomitant administration of antifungals. As such, no researchers have yet evaluated the potential role of genetic polymorphisms in the interaction between voriconazole and cyclosporine. Furthermore, little is known about the effect of voriconazole/cyclosporine interaction in allogeneic HCT in terms of drug efficacy or toxicity.

The aims of this pilot explorative project were to evaluate polymorphisms in genes of drug metabolizing enzymes (DMEs) and transporters involved in cyclosporine and/or voriconazole disposition among patients undergoing allo-HCT and to assess the role of these polymorphisms in the interaction between voriconazole and cyclosporine on cyclosporine pharmacokinetics, toxicity, and efficacy.

Methods

Patients and data collection

We identified forty adult patients for whom DNA samples were available and stored in the Pathology department, who underwent allo-HCT between 2009 and 2016, and for whom informed consent to use their DNA samples and clinical information was obtained. The study was approved by the Institutional Review Board (IRB) of AUB. All procedures performed were in accordance with the ethical standards of the 1964 Helsinki declaration.

Clinical data

Baseline demographics to include age, sex, diagnosis, and concomitant diseases, and drug therapies were collected in addition to details on transplant protocol and antifungal prophylaxis. The incidences of fungal infections and graft versus host disease (GVHD) by day 100 post-transplant were collected [29]. As per protocol, serum creatinine levels were obtained on daily basis, and the following were recorded or computed as potential markers of nephrotoxicity: the highest recorded serum creatinine value during cyclosporine combined with voriconazole therapy, and the mean change in creatinine during cyclosporine combined with voriconazole therapy when compared to baseline, with baseline being the creatinine value just before initiation of cyclosporine.

Genotyping

DNA was extracted from peripheral whole blood or bone marrow aspirate with the QIAmp Blood MINI kit (Qiagen,

Germantown, MD). These were first normalized to 60 ng/ μ l concentration, then genotyped on the DMETPlus arrays from Affymetrix as per protocol. The DMET console software was used for the generation of base calls (<https://www.thermofisher.com/order/catalog/product/901268>).

All samples were collected pre-transplant. In order to validate the array data, 3 genomic DNA controls were run with the samples. In addition, genotyping for 3 *CYP2C19* single nucleotide polymorphisms (SNPs) was performed using Taqman[®] allele discrimination assays on a CFX384 real-time PCR from Biorad. Furthermore, genotyping for *ABCB1* rs2032582 was performed with Sanger sequencing. The primers used were as follows: forward: 5'-GCAGCTATAGGTTCCAGGC-3' and reverse 5'-GAGCATAGTAAG-CAGTAGGGAGT-3'.

Among all variants covered by the DMETPlus array, only those of genes established to be involved in the metabolism or transport of voriconazole or cyclosporine were evaluated [13,16]. Genotype frequencies of each individual variant were computed and tested for Hardy Weinberg Equilibrium (HWE). Then, association analyses were performed with the genotype and phenotype calls that were generated by the software of the array.

Cyclosporine pharmacokinetics

Whole blood was collected in the morning 12h after administration of the evening dose of cyclosporine. Serum was collected, and cyclosporine levels were quantitatively determined by the ARCHITECT Cyclosporine chemiluminescent microparticle immunoassay. The ratio of cyclosporine concentration/dose (C/D: (ng/mL)/(mg/kg)) was computed for each participant by dividing the cyclosporine trough level measured in one day over the total dose per kilogram of cyclosporine administered on the day before. In the case of oral cyclosporine administration, the C/D ratios were corrected for bioavailability (F). Two values were generated per participant: one when cyclosporine was given in combination with voriconazole and one before the start of voriconazole. Since cyclosporine steady state is reached within a day (mean half-life of 6.35 h) [30], for cyclosporine therapy provided before initiation of voriconazole prophylaxis, the C/D ratio of cyclosporine was based on the cyclosporine level that was measured just before voriconazole therapy 24–48 hours after cyclosporine initiation. As for the C/D ratio during voriconazole therapy, an average value was calculated based on all of the available levels during that period (Supplementary Table 1).

Statistical analysis

This is a pilot explorative study based on available data and DNA samples; hence no power analysis was performed. Data were entered and analyzed using SPSS version 24.0 (IBM, USA). A two-sided statistical significance was set at a *P*-value of ≤ 0.05 . The univariate associations were computed using Fisher's exact test, bivariate Pearson correlation, and Kruskal Wallis or Mann Whitney U tests as appropriate.

Results

Sample characteristics

This study included 40 subjects [median age 43 years (20–65)] with 15 (37.5%) females and 25 (62.5%) males). Thirty-nine (98%) were transplanted from a matched related donor, and one patient was transplanted from a matched unrelated donor. Twenty-one (54%) were transplanted for acute myelogenous leukemia, 12 (30%) for acute lymphoblastic leukemia, 4 (10%) for a myelodysplastic syndrome, 2 for Hodgkin lymphoma, and one for diffuse large

B-cell lymphoma, and these frequencies were representative of the trends of allo-HCT activities in Lebanon [31]. Thirty-one (78%) had the transplant while in complete remission, 5 (13%) with progressive disease, and 4 (10%) with stable disease. All received conditioning as per standard protocols [32,33]. Twenty-eight (70%) received fludarabine and busulfan conditioning, 8 (20%) received thiotepa, busulfan, and fludarabine conditioning, 3 (8%) received sequential conditioning, and one patient received busulfan and cyclophosphamide conditioning.

All patients received cyclosporine 1.5 mg/kg twice per day for GVHD prophylaxis as of day 3 before transplantation. Thirty-five (88%) were given rabbit ATG (Thymoglobulin; Genzyme, Lyon, France) intravenously at a dose of 2.5 mg/kg/day on day minus 2 and day minus 1. None of the subjects had a history of IFI prior to transplant, and all received voriconazole 200 mg twice a day as primary prophylaxis for fungal infections starting 1 day before transplantation. The cyclosporine dose was not empirically reduced on that day. None of the subjects was on any potent CYP inhibitors or inducers.

Cyclosporine pharmacokinetics

All except 1 subject (N = 39) were started on IV cyclosporine, of whom the majority (N = 31; 78%) were then shifted to PO cyclosporine as appropriate. When patients were shifted from IV to PO cyclosporine, they received the same daily dose that was then adjusted based on the trough level after 48 h. Voriconazole was initiated as 200 mg PO twice per day for the majority of subjects (N = 31), or started as IV and then shifted to PO (N = 9). Cyclosporine trough levels before the start of voriconazole were available for 29 patients. The median number of measurements of C/D was 10 (Min-Max: 2–21) for cyclosporine administered with voriconazole (Supplementary Table 1).

Interaction between voriconazole and cyclosporine

There certainly was an interaction between voriconazole and cyclosporine since the cyclosporine C/D ratios was significantly higher when it was administered with voriconazole as compared to when it was administered alone: median (Min-Max) C/D of cyclosporine (ng/mL)/(mg/kg) of 116.75 (52.70–385.92) vs. 25.40 (8.20–114.65) with and without voriconazole respectively; $P < 0.001$ (Fig. 1A). The same was true even when excluding subjects for whom cyclosporine concentrations prior to starting

voriconazole were not available, with a median (Min-Max) C/D of cyclosporine of 114.39 (52.70–385.92) (ng/mL)/(mg/kg) with voriconazole (Fig. 1B). Of note, age at transplant significantly positively correlated with cyclosporine C/D when combined with voriconazole (Pearson correlation = 0.319; $P = 0.045$).

Genetic polymorphisms in candidate genes

All samples and the 3 controls were successfully genotyped on the DMETPlus platform with a call rate of > 99%. There was 100% concordance with the *CYP2C19* and *ABCB1* genotyping by real-time PCR and Sanger sequencing, respectively.

The analyzed candidate genes are shown in Table 1. Among 115 variants that are covered by the array, only 12 appeared. *FMO1* and *FMO5* genetic polymorphisms were not available in the DMETPlus array, and variants of the *FMO2* gene are not included in the analysis due to the high “no call” rate (8 out of 40 samples). All included variants were in HWE ($P > 0.05$), and their minor allele frequencies (MAF) were comparable to those of European ancestry (Table 1).

The phenotype frequencies are shown in Table 2. All subjects were *CYP3A4**1/*1, hence the *CYP3A4* gene was not included in the association analysis. In addition, the *ABCB1* 1199 G > A polymorphism was not included in the phenotype call due to the absence of a clinical effect. Similarly, the *CYP2C19**9 and *15 variants were excluded due to the fact that they are not part of the dosing guidelines of voriconazole [14].

Role of genetic polymorphisms in the interaction between voriconazole and cyclosporine

As shown in Table 2, there was a statistically significant association between the C/D ratio of cyclosporine when combined with voriconazole and the 2677 G > T > A *ABCB1* genetic polymorphism ($P = 0.05$) (Fig. 2A). In addition, the statistical significance remained after categorization of the cyclosporine C/D ratio when combined with voriconazole into two categories, one below the median and one above it, whereby a higher percentage of *ABCB1* variant allele carriers were in the high C/D ratio category [16(80%) for variant allele carriers vs. 10 (50%) for the reference allele ($P = 0.045$)]. Interestingly, *CYP2C9*, *CYP2C19* and *CYP3A5* extensive metabolizers tended to be associated with lower cyclosporine C/D ratio when combined with voriconazole, but the results were not statistically significant. Of note that *CYP2C19**2/*2 was an outlier;

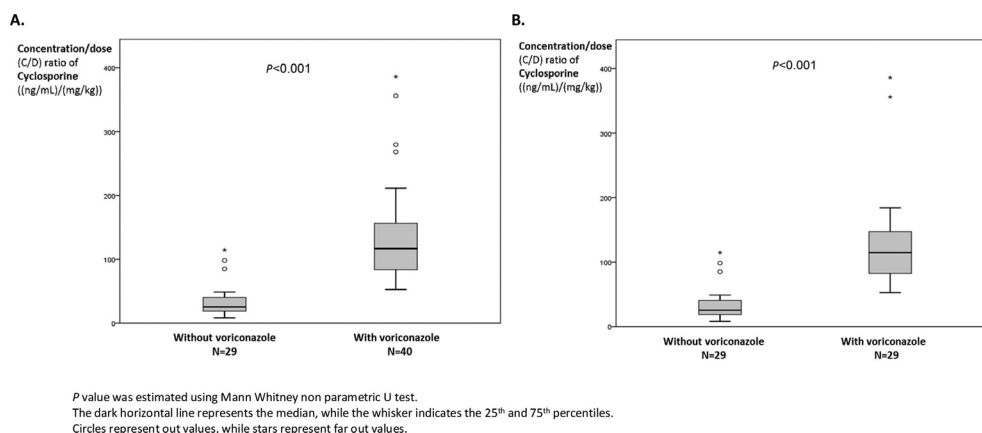


Fig. 1. Box plots of cyclosporine C/D ratio with and without voriconazole **A.** for all 40 subjects and **B.** excluding those for whom cyclosporine concentrations were not available prior to voriconazole therapy.

P value was estimated using Mann Whitney non parametric U test. The dark horizontal line represents the median, while the whisker indicates the 25th and 75th percentiles. Circles represent out values, while stars represent far out values

Table 1
Description and frequencies of the 12 variants and related phenotypes in the candidate genes that were revealed in this study out of the 115 candidate gene variants that are covered by the DMETPlus array.

Candidate gene	Role	Number of variants evaluated by the DMETPlus array	Unique identifiers of variants revealed in this study sample	Description of variants revealed in this study sample	HAPMAP CEU ¹ MAF	Study sample MAF	Phenotype
ABCB1	Export of cyclosporine out of the cell	40	rs2032582	2677 G > T > A (A893SorT) ²	0.47 (T) ³	0.41(T); 0.03 (A)	Increased export
CYP2C9	Metabolism of voriconazole	18	rs2229109	1199 G > A (S400 N)	0.03	0.03	No effect
			rs1799853	3608C > T (R144C) (*2)	0.10	0.21	Reduced metabolism
			rs1057910	42614A > C (I359 L) (*3)	0.06	0.08	Reduced metabolism
			rs9332131	10601delA(K273X) (*6)	N/A	0.01	No enzyme activity
CYP2C19	Metabolism of voriconazole	18	rs4244285	19154G > A (P227 P) (*2A)	0.16	0.10	No enzyme activity
			rs17878459	12460G > C (E92D) (*2B)	N/A	0.03	No enzyme activity
			rs17884712	12784G > A (R144 H) (*9)	N/A	0.05	Reduced metabolism
			rs17882687	55A > C (I19 L) (*15)	N/A	0.04	Unknown
			rs12248560	-806C > T (*17)	0.22	0.24	Increased metabolism
CYP3A4	Metabolism of voriconazole and cyclosporine	25	None	None	N/A	N/A	Normal metabolism
CYP3A5	Metabolism of cyclosporine	14	rs776746	6986A > G (Splice defect) (*3C)	0.96	0.88	No enzyme activity
			rs10264272	14690G > A (Splice defect) (*6)	0	0.01	No enzyme activity

MAF = Minor allele frequency; HWE = Hardy Weinberg Equilibrium; N/A = Not applicable or Not available.

¹Data from Utah Residents of Northern and Western European ancestry (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_viewTable.cgi?pop=1409).

²The genotype distribution of allele carriers was as such: GT = 16; TT = 8; GA = 1 and AT = 1.

³For the *ABCB1* rs2032582, the A minor allele did not figure in the HAPMAP CEU database. Its frequency was however 0.0249 in Europeans of the Genome Aggregation Database (https://www.ncbi.nlm.nih.gov/snp/rs2032582#frequency_tab).

Table 2
Association between phenotypes of the candidate genes and cyclosporine C/D with voriconazole.

Candidate gene	Genotype	Phenotype	Participants N(%)	Cyclosporine C/D with voriconazole (ng/mL)/(mg/kg)				
				Median (range)	P value ¹	<116.75 N(%)	≥116.75 N(%)	P value ²
ABCB1	Ref/Ref	Normal	14 (35)	89.99 (86.54)		10 (71.43)	4 (28.57)	
	Variant allele carriers (rs2032582): G/T; T/T; A/T	Increased export	26 (65) ³	67.84 (152.46)	0.05	10 (38.46)	16 (61.54)	0.045
CYP2C9	*1/*6 ; *2/*3	IM	4 (10)	137.85 (155.41)		1 (25.0)	3 (75.0)	
	*1/*2 ; *1/*3	EM or IM	17 (42.5)	118.26 (332.59)		8 (47.06)	9 (52.94)	
	*1/*1	EM	19 (47.5)	115.49 (302.30)	0.934	11 (57.89)	8 (42.11)	0.546
CYP2C19	*2/*2	Slow	1 (2.5)	115.49 (0)		1 (100)	0 (0)	
	*1/*2 ; *2/*17	Intermediate	6 (15)	137.99 (150.75)		2 (33.33)	4 (66.67)	
	*1/*1	Normal	15 (37.5)	118.26 (333.23)		6 (40.0)	9 (60.0)	
	*1/*17	Rapid	16 (40)	108.23 (300.68)		9 (56.25)	7 (43.75)	
CYP3A5	*17/*17	Ultra-Rapid	2 (5)	82.61 (58.05)	0.694	2 (100)	0 (0)	0.403
	*3C/*3C ; *3C/*6	PM	32 (80)	117.44 (332.33)		16 (50.0)	16 (50.0)	
	*1A/*3C	IM	7 (17.5)	116.88 (131.58)		3 (42.86)	4 (57.14)	
	*1A/*1A	EM	1 (2.5)	68.83 (0)	0.353	1 (100)	0 (0)	1.000

All subjects were CYP3A4*1/*1, hence the CYP3A4 gene was not included in the association analysis. In addition, the *ABCB1* 1199 G > A polymorphism was not included in the phenotype call due to the absence of clinical effect. Similarly, the CYP2C19*9 and *15 variants were excluded due to the fact that they are not part of the dosing guidelines of voriconazole (14).

IM = Intermediate metabolizer; EM = Extensive (normal) metabolizer; PM = poor metabolizer.

¹Mann Whitney U test or Kruskal Wallis test as applicable.

²Fisher's Exact test.

³The genotype distribution of allele carriers was as such: GT = 16; TT = 8; GA = 1 and AT = 1.

nevertheless excluding it from the analysis did not lead to any statistical significance.

Role of genetic polymorphisms in the cyclosporine related toxicity and outcome

As shown in Table 3, only the *ABCB1* 2677 G > T > A variant allele carriers had statistically significantly higher creatinine during treatment with median creatinine (mg/dL) of 0.74 vs. 0.56 for allele carriers compared to reference; $P = 0.003$ (Fig. 2B). No significant results appeared in association with the change in creatinine during combination therapy from baseline. Of note that *ABCB1* 2677 G > T > A variant allele carriers had significantly higher baseline creatinine when compared to wild type [median (min-

max) (mg/dL): 0.8 (0.4–1.7) and 0.6 (0.4–1.0) respectively, $P = 0.041$].

Cyclosporine outcome and toxicity

One subject (2.5%) developed proven fungal infection according to the (EORTC/MSG) consensus group despite prophylaxis [34]. Nine (22.5%) developed GVHD by day 100 post-transplant, 8 of which were grade 2 and 1 was grade 3. The highest median (Min-Max) serum creatinine (mg/dL) during cyclosporine therapy was 0.73 (0.41–1.50) with 9 (22.5%) subjects having had values of more than 1.3 mg/dL at least once during therapy (Table 3). The only statistically significant association with baseline characteristics (age and gender) was with nephrotoxicity whereby males had

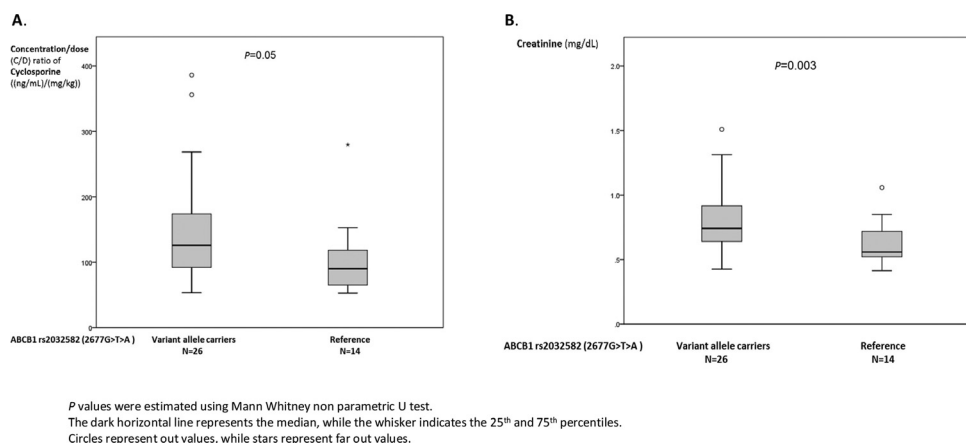


Fig. 2. Box plots of the association between *ABCB1* genotypes and **A.** cyclosporine C/D ratio with voriconazole and **B.** highest serum creatinine values during combination therapy. P values were estimated using Mann Whitney non parametric U test. The dark horizontal line represents the median, while the whisker indicates the 25th and 75th percentiles. Circles represent out values, while stars represent far out values.

higher serum creatinine values when compared to females; for instance, all 9 subjects who had values of more than 1.3 mg/dL at least once during therapy were males ($P = 0.007$), and the median (Min-Max) creatinine (mg/dL) were 0.75 (0.46–1.51) for males compared to 0.58 (0.41–1.03) for females ($P = 0.001$). Of note that these results are to be expected considering the difference in muscle mass between males and females.

Higher cyclosporine C/D ratio, when combined with voriconazole, was associated with a lower incidence of GVHD by day 100 post-transplant [median C/D of cyclosporine of 124.4 vs. 65.2 (ng/mL)/(mg/kg) for those who did not develop GVHD vs. those who did respectively; $P = 0.034$] (Fig. 3A). In addition, there was a statistically significant positive correlation between the C/D ratio of cyclosporine combined with voriconazole and nephrotoxicity whereby higher cyclosporine C/D ratios were correlated with the highest creatinine levels during combined therapy (Pearson correlation = 0.432; $P = 0.005$) (Fig. 3B). There was no significant correlation between these ratios and the change in creatinine during combination therapy from baseline (Pearson correlation = 0.005; $P = 0.975$). Of note that, when analyzing outcome and toxicity with cyclosporine trough levels when combined with voriconazole, there was also a significant association whereby

higher cyclosporine levels were associated with lower incidence of GVHD by day 100 post-transplant [median cyclosporine (ng/mL) of 229.00 vs. 184.75 for those who did not develop GVHD vs. those who did respectively; $P = 0.015$]. No significant correlations were revealed with nephrotoxicity.

Discussion

To the best of our knowledge, this is the first pharmacogenetic study on the interaction between voriconazole and cyclosporine in patients undergoing allo-HCT evaluating a number of variants in candidate genes involved in metabolism and transport of voriconazole and cyclosporine. Results suggest that the 2677G > T > A genetic polymorphism in the *ABCB1* drug transporter plays a role in the interaction between both drugs and cyclosporine related nephrotoxicity in patients undergoing allo-HCT. In addition, and similar to previous studies in patients undergoing renal transplant [8,9] and allo-HCT [6,7,10,11], there certainly is an interaction between voriconazole and cyclosporine depicted as higher cyclosporine C/D ratios when combined with voriconazole. This interaction is associated with a lower incidence of GVHD but at the expense of a higher risk of nephrotoxicity. To

Table 3
Association between phenotypes or genotypes of the candidate genes and cyclosporine related outcome and toxicity.

Candidate gene	Phenotype or genotype	Fungal infection			GVHD by day 100 post-transplant			Highest creatinine during combination therapy			Change in creatinine during combination therapy from baseline			
		NO N(%)	YES N(%)	P^1	NO N(%)	YES N(%)	P^1	<1.3 N(%)	≥1.3 N(%)	P^1	Median (range)	P^2	Median (range)	P^2
ABCB1	Reference	13(92.2)	1(7.1)	0.418	12(85.7)	2(14.3)	0.310	12(85.7)	2(17.5)	0.310	0.56 (0.64)	0.003	-0.02 (0.54)	0.922
	Variant allele carriers	22(84.6)	4(15.4)		19(73.1)	7(26.9)		19(73.1)	7(26.9)		0.74 (1.08)		-0.04 (0.67)	
CYP2C9	IM	4(100)	0(0)	0.800	4(100)	0(0)	0.473	3(75.0)	1(25.0)	0.648	0.80 (0.60)	0.458	0.00 (0.24)	0.816
	EM or IM	14(82.4)	3(17.6)		14(82.4)	3(17.6)		12(70.6)	5(29.4)		0.72 (1.09)		-0.03 (0.67)	
CYP2C19	EM	17(89.5)	2(10.5)	0.796	13(68.4)	6(31.6)	0.778	16(84.2)	3(15.8)	0.359	0.72 (0.13)	0.112	-0.02 (0.61)	0.202
	Slow	1(100)	0(0)		1(100)	0(0)		1(100)	0(0)		0.55 (0)		0.06 (0)	
	Intermediate	6(100)	0(0)		5(83.3)	1(16.7)		6(100)	0(0)		0.68 (0.33)		-0.12(0.34)	
	Normal	12(80.0)	3(20.0)		12(80.0)	3(20.0)		10(66.7)	5(33.3)		0.78 (0.78)		0.02 (0.67)	
CYP3A5	Rapid	14(87.5)	2(12.5)	1.000	12(75.0)	4(25.0)	0.472	13(81.3)	3(18.8)	0.731	0.62 (1.08)	0.327	-0.04(0.40)	0.300
	Ultra-Rapid	2(100)	0(0)		1(50.0)	1(50.0)		1(50.0)	1(50.0)		0.72 (0.39)		0.12(0.40)	
	PM	28(87.5)	4(12.5)		26(81.3)	6(18.8)		25(78.1)	7(21.9)		0.72 (1.09)		-0.03(0.61)	
	IM	6(85.7)	1(14.3)		4(57.1)	3(42.9)		5(71.4)	2(28.6)		0.85 (0.75)		0.03 (0.56)	
	EM	1(100)	0(0)		1(100)	0(0)		1(100)	0(0)		0.63 (0)		-0.18 (0)	

IM = Intermediate metabolizer; EM = Extensive (normal) metabolizer; PM = poor metabolizer; GVHD = Graft vs. Host Disease.

¹Fisher's Exact test.

²Mann Whitney U test or Kruskal Wallis test as applicable.

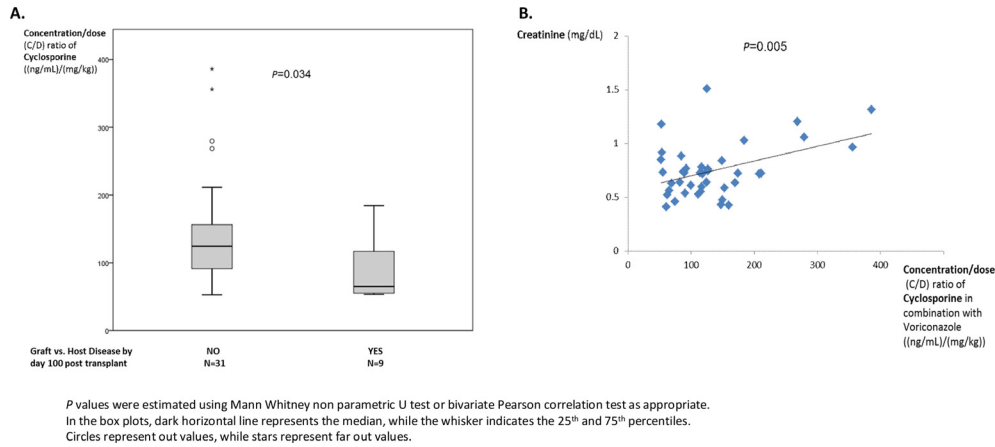


Fig. 3. Cyclosporine related outcome and toxicity: **A.** Box plots of the association between cyclosporine C/D ratio with voriconazole and incidence of graft vs. host disease by day 100 post-transplant and **B.** Correlation of cyclosporine C/D ratio with voriconazole and highest serum creatinine values during combination therapy. *P* values were estimated using Mann Whitney non parametric U test or bivariate Pearson correlation test as appropriate. In the box plots, dark horizontal line represents the median, while the whisker indicates the 25th and 75th percentiles. Circles represent out values, while stars represent far out values.

our knowledge, this pharmacokinetic–pharmacodynamic association has not been previously reported in subjects undergoing allo–HCT, though it has been shown in other settings such as in renal transplant recipients [35]. This interaction is potentially through the competitive inhibition of CYP3A4 by voriconazole when combined with cyclosporine since this enzyme is involved in the metabolism of both drugs [13,16]. No CYP3A4 variants were revealed in the participants of this study to evaluate whether they play any role in the interaction between both drugs.

Concerning CYP3A5 that is known to be involved in the metabolism of cyclosporine, there was a trend of lower cyclosporine C/D ratios in intermediate and extensive metabolizers, a trend that could have been significant with a higher sample size especially that the polymorphism is relatively rare. In agreement with Kim et al. [17], there was no association between this same CYP3A5 variant and the pharmacokinetics of cyclosporine in a cohort of 34 hematopoietic allo–HCT patients. This is in contrast to data from 2 much larger cohorts by Koh et al. [28] (N = 156) and Qiu et al. [27] (N = 91) whereby the CYP3A5*3 polymorphism was associated with significantly higher cyclosporine concentrations in bone marrow transplant patients. Similarly, in a larger cohort of renal transplant patients (N = 68), expressers of CYP3A5 showed lesser plasma levels of cyclosporine than non-expressers in the first-week post grafting [35]. As for the other enzymes CYP2C9 and CYP2C19 that are involved in the metabolism of voriconazole, although there were no significant associations with the C/D ratio of cyclosporine, there was a trend of finding lower ratios in extensive CYP2C9 and ultra CYP2C19 metabolizers, but the results were not statistically significant probably due to the small sample size. It is possible that the lower C/D ratios are secondary to a lower inhibition of cyclosporine metabolism that results from these phenotypes being associated with lower voriconazole concentrations. This, however, cannot be ascertained, as it is not part of standard clinical practice to measure voriconazole levels during allo–HCT. Nevertheless, it was recently shown by Masoumi et al. [10] on 29 recipients of allo–HCT that there was a significant and positive correlation between voriconazole plasma concentrations and percent change in cyclosporine C/D ratio. Interestingly, in a pharmacogenetic study on Australian patients with hematological malignancies requiring voriconazole treatment for invasive fungal infection, despite the smaller sample size (N = 19), CYP2C19 intermediate metabolizers were associated with higher voriconazole levels and more clinical toxicity when compared to extensive and ultra

CYP2C19 metabolizers. Of note that the frequency of the intermediate metabolizer phenotype was higher than the one we found in our sample: 26% vs. 15% [15]. It remains unknown why one subject with the CYP2C19 slow metabolizer phenotype had a lower C/D ratio than the intermediate phenotype. The subject is also extensive CYP2C9 metabolizer (*1/*1) which could have contributed to the higher C/D cyclosporine ratio.

Concerning *ABCB1* 2677 G > T > A, this variant is known to be a non-synonymous exon 21 variant of the *ABCB1* gene and to be associated with increased drug export [36,37]. Therefore our results are in line with expectations whereby allele carriers were associated with increased cyclosporine C/D ratios and related nephrotoxicity. As a matter of fact, the pharmacodynamic results are in line with Garcia et al. [35] who showed allele carriers of the *ABCB1* 3435C > T polymorphisms, a variant that is in high linkage disequilibrium with the *ABCB1* 2677 G > T > A variant [36,37], to be associated with cyclosporine related gingival hyperplasia and nephrotoxicity in recipients of kidney transplant. This is however in contrast to an evaluation of 121 patients who underwent myeloablative hematopoietic cell transplantation whereby both *ABCB1* variants were not found to be associated with the incidence of kidney disease [38]. Of note that the non-significant results concerning the association of the *ABCB1* polymorphism with the change in creatinine may be due to the significant association of variant allele carriers with higher baseline creatinine values, an association that has been previously reported in the literature [39,40]. Concerning cyclosporine plasma concentrations, our results are congruent with those of Qiu et al. [27] in the 91 bone marrow transplant recipients whereby homozygotes for the *ABCB1* 3435C > T variant alleles had significantly higher dose-adjusted cyclosporine concentrations. This is in contrast to the no associations reported in 2 much smaller cohorts of hematopoietic SCT subjects by Kim et al. [17] (N = 34) and Onizuka et al. [26] (N = 21). The statistical significance that we reached in our study is probably due to the relatively higher frequency of the polymorphism and hence more power, it could also be attributed to the difference in the patient populations.

This study suffers from some limitations. For instance, although the C/D ratios for orally administered cyclosporine were corrected for bioavailability, the F value that we used was 0.33, a value that may not be very accurate for all patients due to major inter- and intra-individual variability in cyclosporine bioavailability secondary to the high expression of CYP3A4 in the intestine [39,40]. In addition, we did not adjust for additional potential influencing

factors such as liver function, weight and albumin levels due to the relatively low sample size.

Conclusions

To the best of our knowledge, this is the first pharmacogenetic study on the interaction between voriconazole and cyclosporine in patients undergoing allo–HCT. Results suggest that 2677 G > T > A genetic polymorphism in the *ABCB1* drug transporter plays a role in this interaction with cyclosporine related nephrotoxicity. Pre-emptive genotyping for this genetic variant may be warranted for cyclosporine dose optimization and potentially closer follow up of renal function. Larger studies are needed to potentially show significant associations with more candidate genes such as *CYP3A4/5*, *CYP2C9*, and *CYP2C19*, among others.

Declaration of Competing Interest

We have no Conflict of Interest.

Acknowledgements

This study was funded by an investigator initiated research grant from Pfizer.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.retram.2020.02.001>.

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