Clinical Pharmacokinetics and Population Pharmacokinetic Analysis of Voriconazole in Transplant Patients

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Transplant patients at high risk of invasive mold infections receive voriconazole for prophylaxis. Low exposure of voriconazole predisposes patients for infection. High concentrations are associated with toxicity. Large variability in voriconazole exposure with a fixed dosing regimen has been observed in transplant patients. The objectives are to characterize the pharmacokinetics of voriconazole in transplant patients, to identify factors associated with the variability in the pharmacokinetics, and to develop adequate dosing guidelines for transplant patients.

Liver, lung and pediatric bone marrow transplant (BMT) patients were enrolled. Multiple blood samples were collected within one dosing interval (totally 75 full pharmacokinetic profiles). Voriconazole plasma concentrations were measured using HPLC. Non-compartmental analysis was performed using WinNonlin. Population pharmacokinetic models were developed using NONMEM. Covariate models were built using a forward addition and reverse removal approach. Precision of parameter estimation was evaluated by bootstrapping. Adequate dosing regimens were developed using Monte Carlo simulations.

There was good correlation between AUCo-∞ and trough voriconazole plasma concentrations in all patient groups. In liver transplant patients, CL/F and V/F of voriconazole significantly decreased with postoperative time, CL/F of voriconazole significantly increased with liver function, and *CYP2C19*2* allele carriers exhibited significantly higher exposure. Donor characteristics had no significant association with pharmacokinetics of voriconazole in liver transplant patients. In lung transplant patients the bioavailability of voriconazole was substantially lower, but significantly increased with postoperative time, and patients with cystic fibrosis (CF) exhibited a significantly lower bioavailability and exposure than non-CF patients. Clearance of voriconazole significantly increased with liver function in BMT patients. BMT patients had significantly higher clearance and significantly lower volume of distribution compared to liver and lung transplant patients, but bioavailability was similar to lung transplant patients.

In conclusion, weight-adjusted or fixed dosing regimens resulted in highly variable exposure of voriconazole in liver transplant, lung transplant and BMT patients. Given that trough

voriconazole concentration is a good measure of drug exposure (AUC), voriconazole dose can be individualized based on trough concentrations. Population analysis demonstrated inadequacy of oral administration of voriconazole and adequacy of intravenous administration during the first few post-operative days, followed by oral doses for optimal drug exposure.

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PREFACE

There are not enough words to acknowledge all the professional, scientific and personal support I have received over the years during my Ph.D. study. Since I moved to the United States, I have been blessed to meet wonderful people who treated me like family.

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Abbreviations

λz	Terminal disposition rate constant
ANOVA	Analysis of variance
AUC	Area under the concentration-vs-time curve
AUMC	Area under moment curve
BID	Twice daily
BMT	Bone marrow transplant
C12	Last plasma concentration at 12 hours
Co	Trough concentrations prior to oral dosing
CF	Cystic fibrosis
CL	Clearance
Clast	Last plasma concentration at 12 hours
Clint	Intrinsic clearance
CLh	Hepatic clearance
CL/F	Apparent systemic clearance over bioavailability
Cmax	Peak plasma concentrations
CV	Coefficient of Variation
CYP450	Cytochrome-P450
F	Bioavailability
FDA	Food and Drug Administration
Fu	Fraction unbound
HPLC	High Performance Liquid Chromatography

IRB	Institutional Review Board
LLOQ	Lowest Limit of Quantification
LSS	Limited sampling strategy
MRT	Mean residence time
POT	Post-operative time
R^2	Coefficient of correlation
SD	Standard deviation
T1/2	Half-life
Tlag	Absorption lag time
Tmax	Time to reach peak concentration
UV	Ultraviolet
V	Volume of Distribution
V1	Volume of Distribution in the central compartment
V2	Volume of Distribution in the peripheral compartment
Vd	Volume of Distribution
Vd/F	Apparent volume of distribution over bioavailability

Chapter I Introduction

1.1 Voriconazole and its use in transplant patients

Due to chronic immunosuppression, infections are common life-threatening complications in organ transplant patients (28). Invasive aspergillosis is one of the most dreaded complications in transplant patients (77) due to its high mortality rate, which can range up to 88.1% (64). Voriconazole (V-Fend®, Pfizer, formerly known as UK-109496), (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1,2,4-triazol-1-yl)butan-2-ol, is a novel broad-spectrum triazole systemic antifungal agent and an ideal drug to prevent invasive aspergillosis. Compared with other azole antifungal agents, it has potent activity against a broader spectrum of clinically significant fungal pathogens, including Aspergillus, Candida, Cryptococcus neoformans, and some unusual organisms such as Fusarium and P. boydii (36, 82, 106, 112, 119). The primary mode of antifungal action of voriconazole is the inhibition of fungal cytochrome P450-dependent ergosterol synthesis (mediated via 14-alpha-sterol demethylase) resulting in a loss of ergosterol in the fungal cell wall.

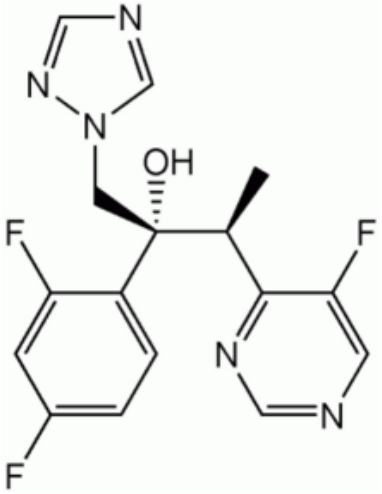


Figure 1. Voriconazole chemical structure

Voriconazole is available in intravenous and oral formulation. It is formulated as lyophilized powder reconstitution into a solution for intravenous infusion, as well as tablets and powder for suspension for oral administration.

1.2 Clinical pharmacokinetic properties of voriconazole

The clinical pharmacokinetic properties of voriconazole have been well characterized in non-transplant patients in clinical trials during its development (25, 88, 89). In addition, Purkins et al. (95-97) investigated the clinical pharmacokinetics of voriconazole in healthy adult volunteers, Robatel et al. (102) studied adult patients with end-stage renal disease (ESRD) undergoing

hemodialysis, Peng et al. (85) studied adult patients with ESRD undergoing peritoneal dialysis, Walsh et al. (130) investigated immunocompromised pediatric patients (2–11 years old), and Lazarus et al. (61) studied non-transplant patients at risk of fungal infections. At the time of our studies, there was no information available on the pharmacokinetics of voriconazole in transplant patients. Population approaches have previously been used to investigate voriconazole pharmacokinetics in non-transplant patients (55, 75, 130), but not in transplant patients. There is a need to evaluate the pharmacokinetics of voriconazole in transplant patients in order to optimize therapy with voriconazole in the patient population.

1.2.1 Absorption

Voriconazole is highly lipophilic and is rapidly absorbed. Its peak concentration after a single oral dose is reached within 2 hours. Its bioavailability after oral administration is estimated to be 96% in non-transplant population based on pooled data from 207 healthy volunteers (88). These results indicate that similar exposure of voriconazole is expected with identical intravenous and oral doses of voriconazole.

1.2.2 Distribution

Voriconazole is extensively distributed into peripheral tissues, and therefore has a large volume of distribution. The steady-state volume of distribution range from approximately 2 L/kg to 4.6 L/kg (88, 89). The unbound fraction of voriconazole in plasma is 42%, and it is independent on

dose and plasma concentration (88, 89). Volume of distribution at steady state is not significantly different between the intravenous and oral administration (95), suggesting that the bioavailability of voriconazole is close to 100%. These observations suggest that changes in plasma protein binding are not expected to markedly alter the pharmacokinetics of voriconazole.

1.2.3 Metabolism and elimination

Voriconazole is extensively metabolized hepatically. Less than 2% of the dose is excreted unchanged in the urine. The major metabolite (N-oxide) accounts for 72% of circulating radiolabelled metabolites in plasma but exhibits no antifungal activity (88). The metabolites of voriconazole are primarily eliminated in the urine, with approximately 80% to 83% of the radioactivity being recovered in the urine (25, 88, 89, 103).

Total body clearance of voriconazole ranged from 13 to 36 L/h in healthy volunteers (102). The mean elimination half-life (t1/2) of voriconazole is about 6 hours following single and multiple oral or intravenous administration (48). Clearance and terminal phase elimination rate constant are not significantly different between the intravenous and oral administration (95).

Voriconazole is metabolized primarily by the Cytochrome P450 (CYP) isoenzymes *CYP2C19*, and to a lesser extent by *CYP2C9* and *CYP3A4* (44, 52, 83, 112) to inactive metabolites. Voriconazole is also an inhibitor of these three enzymes (103). *CYP2C19* demonstrates genetic polymorphism with 3–5% of Caucasians and African Americans populations expected to be poor metabolizers, whereas the prevalence is 15–20% amongst Asians (52, 113). Voriconazole

concentrations (AUCτ) have been reported to be 4 times higher in poor metabolizers than extensive metabolizers (88). This observation indicates that genotype of a patient might significantly alter the pharmacokinetics of voriconazole. However, currently there is no recommendation for dosing adjustment with regard to the genotype of the patients.

1.2.4 Nonlinear pharmacokinetics

Voriconazole exhibits nonlinear pharmacokinetics. The values of Cmax and area under the plasma concentration-vs-time curve during a dosage interval τ (AUCτ) increase disproportionately with the dose following multiple doses of both oral or intravenous administration (88, 89). For oral administration, a 2-fold increase in dose (from 200 to 400mg twice daily) led to a 2.8-fold increase in Cmax (from 1.9 to 5.3 μg/ml) and a 3.9-fold increase in AUCτ (from 9.8 to 37.5 μg*h/ml), respectively. For intravenous administration, a 1.7-fold increase in dose (from 3 to 5 mg/kg twice daily) led to a 2.4-fold increase in Cmax (from 3 to 7.2 μg/ml) and a 3.1-fold increase in AUCτ (from 13.9 to 43.4 μg*h/ml), respectively (95). As a result of nonlinear pharmacokinetics of voriconazole, the t1/2 is dose dependent and is generally greater after multiple oral administrations than after single oral administration due to accumulation of voriconazole after multiple dosing (96). Saturation of metabolism is likely to be the reason for the nonlinear pharmacokinetics of voriconazole since voriconazole is eliminated predominantly by metabolism (103). Therefore changes in dosing should take into account.

1.3 Variability in the pharmacokinetics of voriconazole

Large inter- and intra-individual variability in voriconazole plasma concentrations regardless of the route of administration or the type of patient population has been documented and discussed in the literature (6, 63, 66, 80, 81, 96, 109, 114, 118, 122). Several major factors have been demonstrated to be significantly associated with the variability in the pharmacokinetics of voriconazole. However, nothing is known about various factors that might alter the pharmacokinetics of voriconazole in transplant patients.

1.3.1 Age

Clearance is more rapid in children under the age of 12 years, and therefore higher doses are required to achieve similar voriconazole exposure compared to that in adults (48). Plasma concentrations of voriconazole at steady-state following intravenous administration twice daily have been reported to be similar in children (2–11 years old) receiving 4 mg/kg and in adults receiving 3 mg/kg (88).

1.3.2 Hepatic dysfunction

Patients with cirrhosis demonstrated approximately 50% lower clearance of voriconazole compared to subjects with normal hepatic function (3.6 vs 6.9 L/h) (48). Therefore significantly lower doses of voriconazole should be administered to patients with hepatic dysfunction.

1.3.3 Food effect

Single and multiple oral administration of voriconazole (200mg) with food resulted in reduced bioavailability of voriconazole by approximately 22% and delayed absorption by a mean of 1.1 hours in healthy male volunteers in comparison to fasted state (98). Multiple dose administration of voriconazole with high fat meals resulted in reduced mean Cmax and AUCτ values by 34% and 24%, respectively. This factor must be taken into account when designing and interpreting pharmacokinetic studies of voriconazole.

1.3.4 Drug-drug interactions

Voriconazole serum concentrations are significantly reduced by co-administration of rifampin, rifabutin, phenytoin and are likely to be reduced by carbamazepine and long-acting barbiturates (35, 40, 88). Protease inhibitors (saquinavir, amprenavir and nelfinavir) inhibit the metabolism of voriconazole. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) either inhibit (efavirenz and delavirdine) or induce (efavirenz and nevirapine) the metabolism of voriconazole (20, 88). The potential for interaction of voriconazole with other drugs must be taken into account while using voriconazole in patients who are on multiple drug therapy.

1.3.5 Other factors

Other factors associated with inter-individual variability of voriconazole exposure include alcohol abuse in the past (134), *CYP2C19* genetic polymorphisms including poor as well as ultra-rapid metabolizers (45, 55, 69, 108, 130, 132, 135), gastrointestinal abnormalities (e.g. mucositis or diarrhea) (109) impairing drug absorption, and other factors.

1.4 Variability in the pharmacokinetics of voriconazole in transplant patients

Transplant patients are very unique populations with many unique physiological changes that can potentially alter the pharmacokinetics of voriconazole. In addition to the factors identified in non-transplant population mentioned above, many other factors unique in transplant populations could be significantly associated with the variability in the pharmacokinetics of voriconazole.

1.4.1 Variability in absorption

Variability in absorption with oral voriconazole administration may cause the large inter-individual variability of voriconazole exposure. Firstly, impaired gastrointestinal function after transplant surgery is a common physiological change that is unique to this patient population. The magnitude of decrease in the gastrointestinal function is quite different from patient to patient, and therefore may be a source of between-subject variability and could potentially alter the rate of absorption of voriconazole. Secondly, voriconazole is highly lipophilic and therefore its absorption is likely dependent on secretion of sufficient bile. Variation in bile flow between

patients and variable dissolution of voriconazole in patients may be another source of variability, and could potentially lead to altered bioavailability, especially in liver transplant patients. Finally, administration of voriconazole with food has significant influence on voriconazole absorption, and therefore feeding method is also a source of variability in voriconazole exposure in transplant patients.

1.4.2 Variability in elimination

Variability in elimination may be another factor responsible for the large inter-individual variability of voriconazole exposure, especially in liver transplant patients. First of all, the most relevant physiological factor that can lead to the large variability of voriconazole exposure is differences in liver function caused by physiological characteristics unique to liver transplant patients, because voriconazole is extensively metabolized in the liver with less than 2% of the administered dose being excreted unchanged in urine. There are no clinically relevant effects of renal impairment on the pharmacokinetics of oral or intravenous voriconazole (94). Secondly, voriconazole has demonstrated nonlinear pharmacokinetics due to saturation of metabolism (89, 94), and may be an important contributor in patients with decreased liver function. Voriconazole metabolism may be saturated in some liver transplant patients. Finally, genetic polymorphism of *CYP2C19* (major metabolizing enzyme for voriconazole) among patients can result in inter-individual variability in metabolism (45, 55, 69, 89, 94, 108, 130, 132, 135).

1.4.3 Drug-drug interactions

Potential drug-drug interactions may also contribute to the large inter-individual variability of voriconazole exposure. The transplant patients simultaneously receive many therapeutic agents for treatment and prophylaxis. In vitro studies have shown that voriconazole has the greatest affinity for *CYP2C19*, lower affinity for *CYP2C9* and limited affinity for *CYP3A4* (94). Inhibitors and/or inducers of these enzymes may change the pharmacokinetics of voriconazole (88, 89, 94).

1.5 Therapeutic drug monitoring of voriconazole

1.5.1 Adverse events

Pooled analyses of 289 healthy volunteers and 1657 patients with invasive fungal infections (a total of 1946 subjects) who received voriconazole and participated in clinical trials or compassionate use programs demonstrated that approximately 50% of all voriconazole recipients experienced at least one treatment-related adverse event (25).

Transient visual disturbances are the most commonly reported adverse event. Approximately 30% of patients in the clinical trials experienced altered or enhanced visual perception, blurred vision, color vision change and photophobia (88). Enhanced brightness of light and blurred vision was also reported more frequently with voriconazole (1, 131). The site of action involved

in visual disturbances is thought to be the retina with the exact mechanism remaining to be determined (88, 89).

Approximately 13% of patients treated with voriconazole showed abnormalities in liver function test (88). Serious adverse events including hepatitis and fulminant hepatic failure have also been reported. Approximately 12.4% of the patient (206 of 1655 patients) receiving voriconazole showed clinically significant abnormalities in transaminase levels (i.e. >3 × ULN) according to a pooled analysis of therapeutic trials (88, 89). Furthermore, pharmacokinetic/pharmacodynamic analyses have demonstrated that the incidence of clinically relevant abnormalities in liver function laboratory tests is correlated with voriconazole plasma levels (25).

Dermatological reactions (mainly skin rashes) were observed in 6% of subjects receiving voriconazole in clinical trials (25, 88, 89). Severe skin reactions including erythema multiforme (Steven-Johnson syndrome) (16, 25, 88, 89), toxic epidermal necrolysis (15, 42), photosensitivity reactions (105), pseudoporphyria (19, 111, 120) and phototoxic reactions (99, 126) have also been reported. It is therefore important to maintain the concentration of voriconazole below a threshold to minimize adverse events.

1.5.2 Correlation between voriconazole exposure and efficacy/toxicity

It has been reported that low voriconazole exposure is associated with a poor outcome in patients with aspergillosis (17, 33, 80, 93, 109, 114, 123, 125) and ultimately death of the patients, while high voriconazole plasma concentrations are correlated with an increased risk for toxicity (6, 46,

67, 109, 117). For example, based on a longitudinal linear logistic regression analysis of pooled data from ten clinical trials (1053 patients), there is a significant correlation between voriconazole plasma levels and the incidence of abnormal levels of AST, ALT and bilirubin (67). Every 1 μg/ml increase in voriconazole plasma concentration was predicted to result in an increase in the odds of an AST, ALT, bilirubin and ALP abnormality of 13%, 7%, 17% and 16%, respectively.

1.5.3 Application of correlation between voriconazole exposure and efficacy/toxicity to therapeutic drug monitoring

Simple efficacy measure for the treatment molds are not quite available yet, to which patient dose can be titrated. So far there have only been data in animals for Candida showing a predictive pharmacodynamic parameter (AUC/MIC) and a potential target value (2) with no equivalent data for molds. However, there is a simple HPLC/UV assay available to monitor voriconazole levels and exposure in patients.

Therapeutic monitoring may be important in optimizing therapy with this drug, and has been proposed by several investigators (6, 17, 26, 121). Area under the concentration-vs-time curve (AUC) is commonly used to characterize total drug exposure. However, multiple blood samples throughout the dosing interval are required to estimate AUC, which is inconvenient, costly and not practical in clinical settings. Therefore surrogate marker and limited sampling strategies should be developed to estimate AUC accurately and precisely while minimizing the number of blood samples required.

Despite lack of proof that the trough voriconazole plasma concentration is a good surrogate marker for exposure (AUC) of voriconazole, target trough voriconazole plasma concentrations have been proposed for therapeutic drug monitoring of voriconazole, such as 2.05ug/ml (114), 2ug/ml (123), 1ug/ml (80) and 2~6ug/ml (125). Therapeutic drug monitoring of voriconazole is currently performed in the routine clinical monitoring program at our institution with an intention to keep the trough concentration above 1ug/ml.

1.6 Preliminary data

Based on the 2951 samples collected from the routine therapeutic monitoring program at University of Pittsburgh Medical Center, nearly 16% of the patients on recommended doses did not have any measurable trough plasma concentration, nearly 29% of the patients had trough plasma concentration of less than 0.5ug/ml, and nearly 45% of the patients had trough plasma concentration of less than 1ug/ml (Figure 2-4, Table 1). These patients are at higher risk of fungal infection. This demonstrated that underexposure is a serious problem in voriconazole use in transplant patients. Nearly 5% of the patients have trough plasma concentration of more than 6ug/ml. These patients are at higher risk of toxicity. This suggests that the current dosing regimens developed for non-transplant population may not be adequate for transplant patients, and therefore rational dosing regimens need to be developed by a better understanding of the pharmacokinetics of voriconazole in transplant patients.

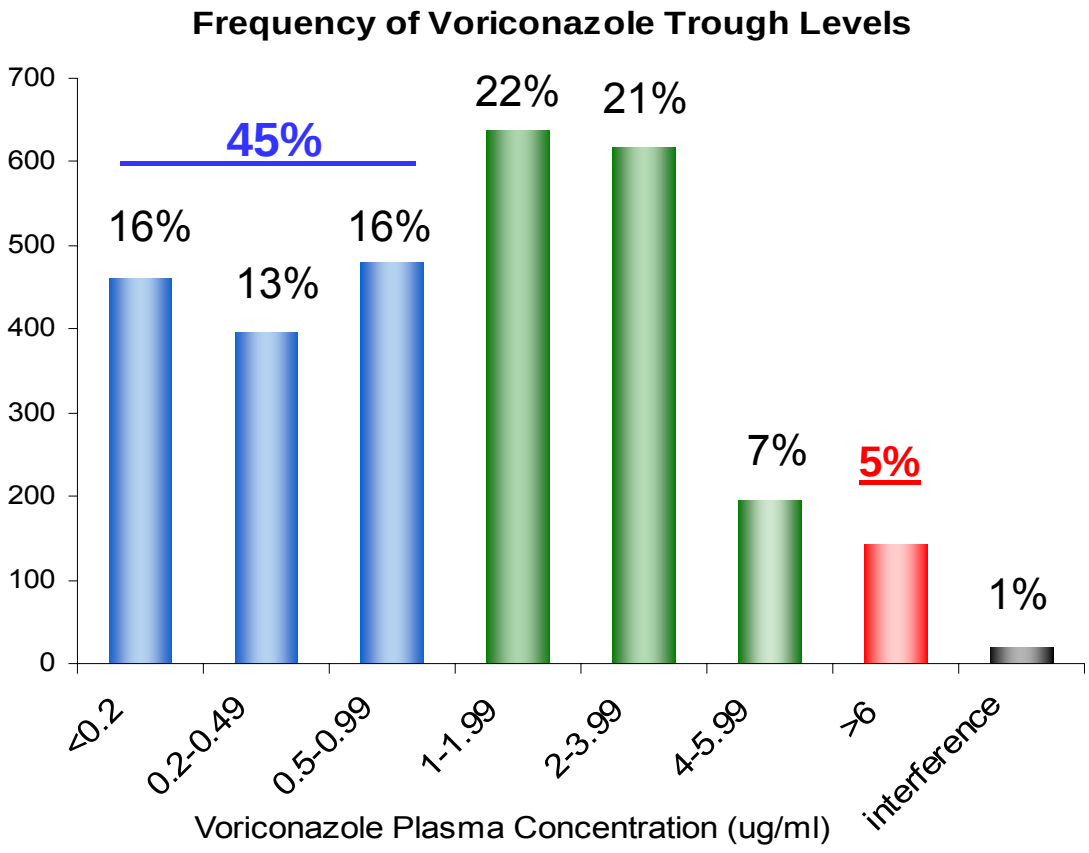


Figure 2. Priliminary data (08/2007): frequency distribution of voriconazole exposure in the routine therapeutic drug monitoring program. Y-axis: number of samples.

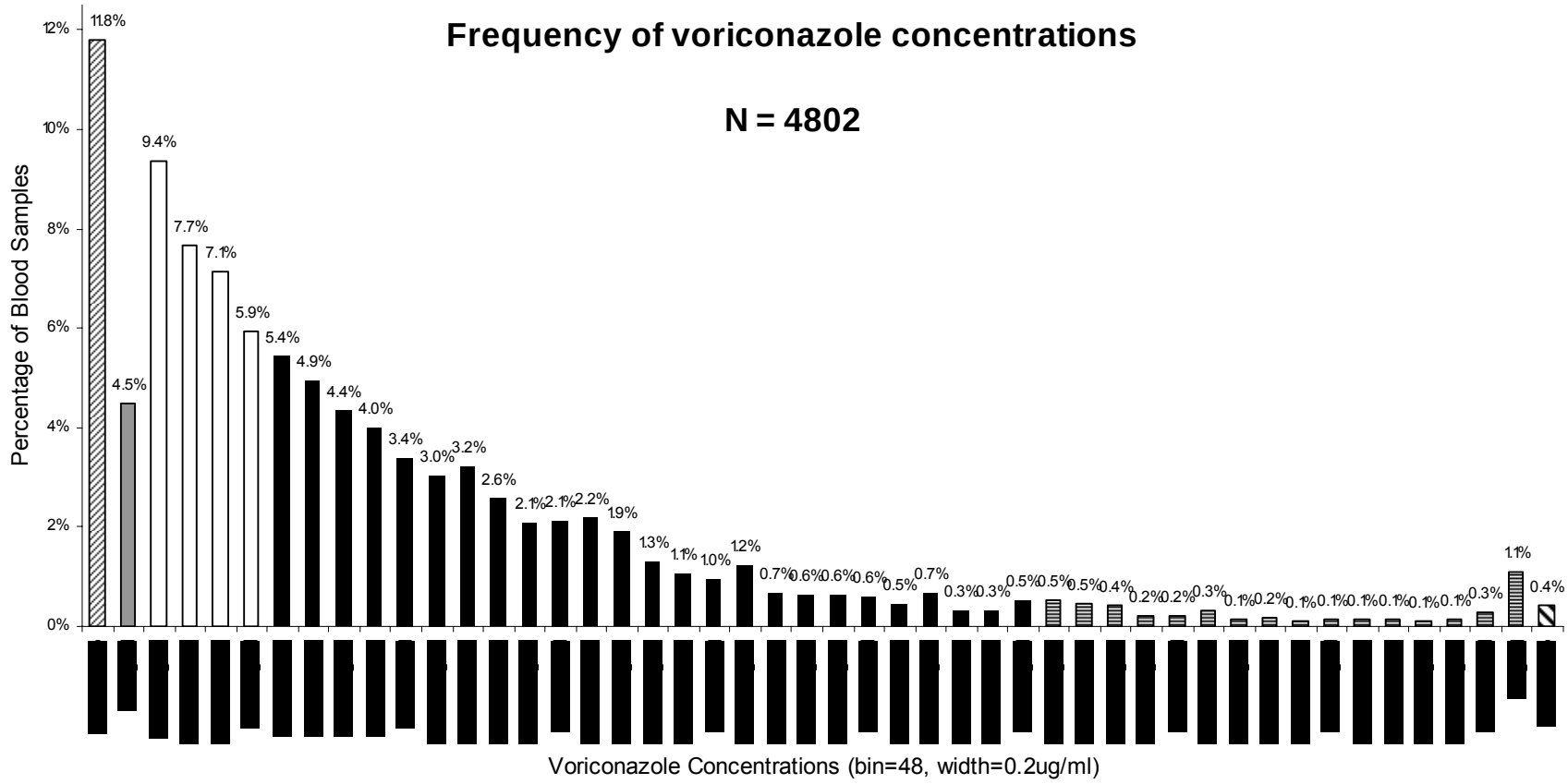


Figure 3. Update of priliminary data (06/2010): frequency distribution of voriconazole exposure in the routine therapeutic drug monitoring program

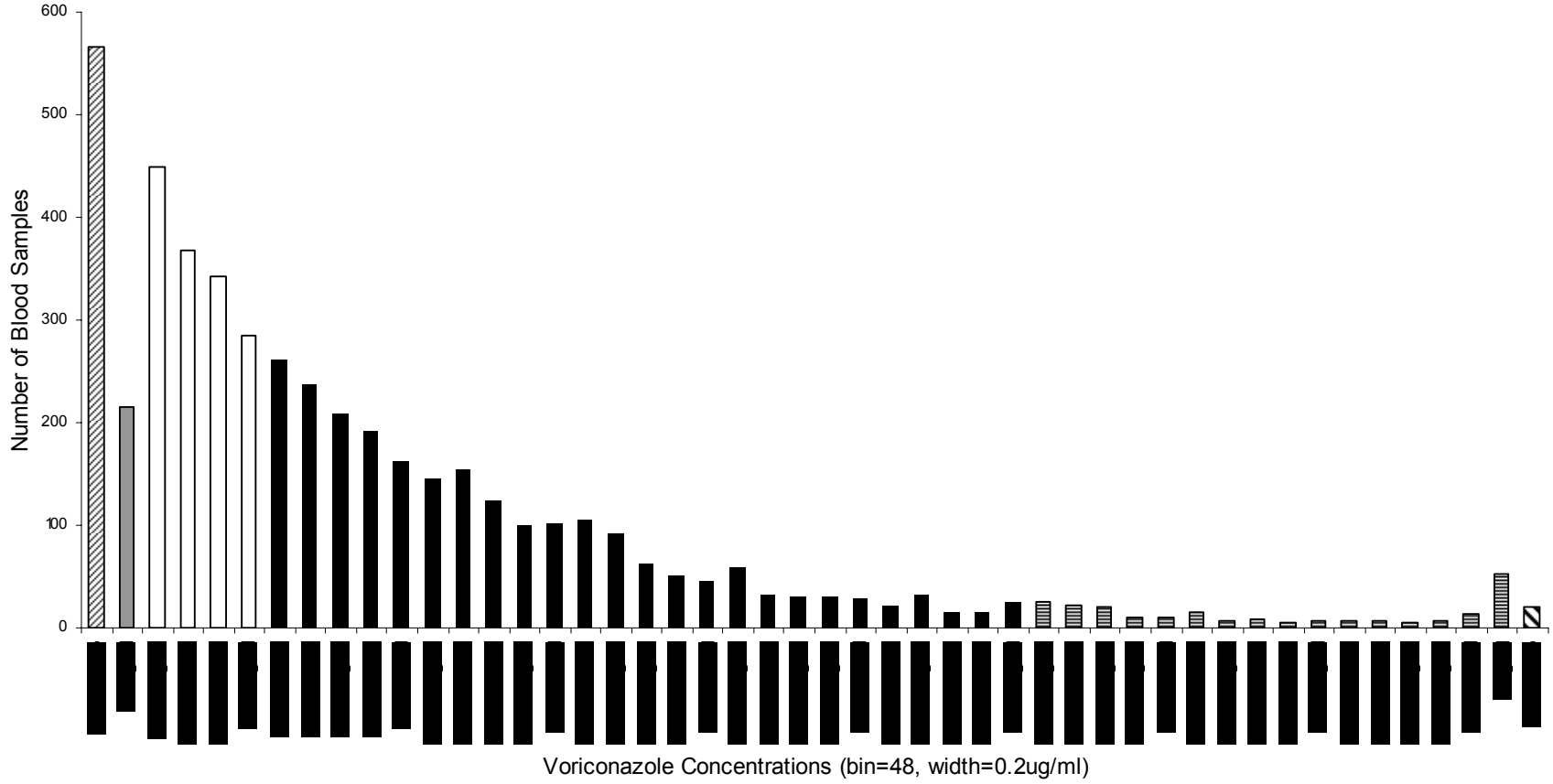


Figure 4. Update of priliminary data (06/2010): frequency distribution of voriconazole exposure in the routine therapeutic drug monitoring program

Table 1. Update of priliminary data (06/2010): frequency distribution of voriconazole exposure in the routine therapeutic drug monitoring program

Therapeutic Range	Voriconazole level	Percentage	Label in Figure 3-4
Below Therapeutic Range (46.4%)	Undetectable	11.8%	
	Below assay LLOQ	4.5%	
	< 1ug/ml	30.1%	
Within Therapeutic Range (48.6%)	1 ~ 6 ug/ml	48.6%	
Above Therapeutic Range (4.6%)	> 6 ug/ml	4.6%	
Interference (0.4%)		0.4%	

1.7 Study populations

Voriconazole prophylactic regimen is typically administrated in liver transplant, lung transplant, small intestine transplant and pediatric bone marrow transplant patients at our institution. Liver, lung and bone marrow transplant patients were studied in this dissertation.

The physiological conditions in transplant patients that may alter the pharmacokinetics of voriconazole may be different in different transplant populations. In liver transplant patients, liver function and gastrointestinal function are likely to play a major role in the pharmacokinetics of voriconazole because liver function and gastrointestinal function may be impaired during the transplant surgery but recovered with time. In contrast, liver function may not be a major factor in lung transplant patients because the majority of the lung transplant patients have normal liver function. Gastrointestinal function is likely to play a major role in the pharmacokinetics of voriconazole in lung transplant patients because gastrointestinal function may be impaired during the transplant surgery but recovered with time. Pediatric bone marrow transplant patients are a unique population, and variable gastrointestinal and hepatic function is likely to be the major factor contributing to the large variability in the pharmacokinetics of voriconazole.

1.8 Hypotheses

A complete understanding of the pharmacokinetics of voriconazole in transplant patients will help in optimizing the use of this drug in transplant patients. We hypothesize that

1. use of a fixed dosing regimen will lead to a large degree of variability in the exposure of voriconazole in transplant patients due to variability in liver function since liver dysfunction would alter the pharmacokinetics of voriconazole.
2. Polymorphism in *CYP* especially *CYP2C19*2* will contribute to the observed variability since *CYP2C19* is the primary enzyme that metabolizes voriconazole.
3. The bioavailability of voriconazole will be lower in liver, lung and bone marrow transplant patients than that reported in non-transplant population due to decreased/variable GI function
4. Trough voriconazole concentration will be a good measure of drug exposure (AUC), and voriconazole dose can be individualized based on trough concentrations measurements. This relationship will hold good in liver, lung and bone marrow transplant patients.

We have evaluated the above hypotheses in liver transplant (Chapter III), lung transplant (Chapter IV) and pediatric bone marrow transplant (Chapter VI) patients using conventional and population pharmacokinetic approaches (Chapter II).

Chapter II Methods

2.1 Pharmacokinetic modeling

Pharmacokinetics evaluates the time course of the processes of absorption, distribution, metabolism and excretion of drugs. Pharmacokinetic modeling employs various pharmacokinetic parameters as descriptors of these processes and mathematically relates the drug concentration in biological fluids, typically in blood or plasma to time. Pharmacokinetics and pharmacokinetic parameters are useful for understanding response/toxicity over time, and important for determination of dose and formulation of the drug to be administrated. Important pharmacokinetic parameters include clearance (CL), volume of distribution (Vd), absorption rate constant (ka), elimination rate constant (k), half life (T1/2), terminal disposition rate constant (λz), area under the curve (AUC), mean residence time (MRT), peak plasma concentrations (Cmax), time to reach peak concentration (Tmax), and area under moment curve (AUMC). Pharmacokinetic parameters have to be determined by modeling drug concentration versus time profiles using either classical or population pharmacokinetic modeling techniques.

In this dissertation, pharmacokinetic modeling was extensively applied to estimate the pharmacokinetic parameters and evaluate the association between patient variables and pharmacokinetic parameters. Classical and population pharmacokinetic modeling techniques were both extensively applied.

2.2 Classical pharmacokinetic modeling

Classical modeling approaches normally employ linear and nonlinear regression to estimate individual pharmacokinetic parameters for each subject. Non-compartment analysis is the most commonly used classical modeling approach. Parameters are often summarized as a mean value and standard deviation as a reflection of inter-individual variability. The industry standard program to implement the analysis is WinNonlin (Pharsight Corp., Mountain View, CA). Non-compartment analysis was applied to all the studies in this dissertation using WinNonlin.

2.3 Population pharmacokinetic modeling

2.3.1 General approaches

Nonlinear mixed-effects (a combination of fixed and random effects, or constant and varying effects) modeling approaches are also normally employed in the analysis of data. Fixed effects include typical population values of pharmacokinetic parameters and covariate parameters. Random effects include both intra- and inter-individual variability. The industry standard program to implement the analysis is NONMEM (GloboMax, Ellicott City, MD). Nonlinear mixed-effects modeling was applied to all the studies in this dissertation using NONMEM.

2.3.2 Model building

The first step in the model building process was to identify the structural model or base model (model without any covariates): $TV(Pj)=\theta j$, where $TV(Pj)$ is the typical value of the jth population parameter.

Inter-individual variability was described using various models, including:

1. exponential model: $P_{ij} = TV(Pj) * EXP(\eta_{ij})$
2. proportional model: $P_{ij} = TV(Pj) * (1 + \eta_{ij})$
3. additive model: $P_{ij} = TV(Pj) + \eta_{ij}$
4. other models such as $Log(P_{ij}) = Log(TV(Pj)) + \eta_{ij}$

where P_{ij} is the ith individual's estimate of the jth basic pharmacokinetic parameter, $TV(Pj)$ is the typical value of the jth population parameter, and η_{ij} is a random variable for the ith individual and the jth basic pharmacokinetic parameter distributed with mean zero and variance of ωj^2.

The residual variability (ε) between the observed and predicted concentrations is all the variability that remains unexplained. It could be due to intra-individual pharmacokinetic variability, model misspecification, variation in concentration measurement, errors in dosing history and sampling time, and other variations. It was also described using various models, for example:

1. additive error model: $Cobs = Cpred + \varepsilon$
2. proportional error model: $Cobs = Cpred * (1 + \varepsilon)$
3. combined error model: $Cobs = Cpred * (1 + \varepsilon) + \varepsilon'$
4. exponential error model: $Cobs = Cpred * EXP(\varepsilon)$

5. other error models such as $C_{obs} = C_{pred} + \varepsilon * C_{pred}^{\theta}$

where Cobs and Cpred are the observed and predicted concentrations, respectively, and ε and ε' are normal random variables with means of zero and variances of δ^2 and δ'^2, respectively.

2.3.3 Covariate evaluation

One of the major goals of population pharmacokinetic modeling as well as this dissertation is to model sources of inter- and intra-individual variability, which is a key issue not readily addressed by classical pharmacokinetic methods. Various covariate screening methods are available to select the covariates to be evaluated, such as nonlinear least-squares based method, Empirical Bayes Estimates based method, likelihood ratio test, direct covariate screening by inclusion of the covariate in the model (most reliable but time-consuming), and others. In this dissertation, covariate relationships were initially explored using Empirical Bayes Estimates based method, and then confirmed by directly incorporating the covariate into the model.

Then a forward and backward stepwise model building process was used to evaluate the association of selected covariates with pharmacokinetic parameters and to build the final covariate submodel. In the forward inclusion step, each covariate was included into the base model and tested one at a time using various approaches to associate the covariate with the parameter, such as linear association, exponential association, and other associations. All the covariates that were considered significant (see below for criteria) were then included to obtain an intermediate multivariate model (full model). Then in the backwards exclusion step, the covariates were removed from the full model one at a time. The tested covariate stayed in the

final model if the model without the tested covariate was significantly "worse" than the full model. The cut-off range of each covariate for data transformation was selected based on clinical considerations.

The significance of a covariate effect and the improvement in the model were assessed under five criteria: (1) a significant decrease in the minimum objective function value (OFV) of the covariate model compared to base model (the decrease in OFV was referred to the chi-squared distribution to assess significance), (2) assessment of the log likelihood ratio test, 3) improved Goodness-of-Fit, (4) increased precision of parameter estimation, and (5) reduced inter-individual and residual variability.

Conclusions of significant or insignificant covariate effects were made cautiously. Several reasons could lead to an exclusion of a significant covariate (false negative), for example, the covariate submodel may be mis-specified, this covariate may not be variable enough in the population studied, or the correct model specification of this covariate is a cut-off model while the values of this covariate in the population studied happen to be all below or above that cut-off value even though this covariate is very variable.

2.3.4 Model evaluation and validation

Bootstrapping was performed to evaluate the precision of the parameter estimation, stability of the model and normality of the distribution of the parameter estimates (78). A series of datasets were generated by repeated random sampling with replacement (resampling), which had the

same size as the original dataset with a different combination of subjects and their data. Parameters were estimated in this series of datasets. As the number of resampling approachesd infinity, the standard deviations of the parameters obtained from bootstrapping should approach to the 'true' standard deviations. Statistics of parameter estimates obtained from bootstrapping were compared with those obtained from the original dataset. An appreciable discrepancy reduces confidence in the model.

Visual predictive check was used to evaluate the predictive performance of the model. The 95% prediction intervals (2.5^{th} and 97.5^{th} percentiles of the simulated concentrations) were computed by simulating at least 1000 subjects, and should contain approximately 95% of the observed concentrations to conclude a good predictive performance of the model.

The most rigorous validation method is external validation, and it was applied in this dissertation whenever possible. Concentrations in the validation dataset that was not used for modeling building were predicted using the parameters obtained from the model building dataset (also called index dataset) and then compared to the observed concentrations. Bias (mean prediction error, MPE) and precision (mean absolute prediction error, MAPE, and root mean square prediction error, RMSE) were calculated:

$$MPE = \frac{\sum(Cpred - Cobs)}{N},\ MAPE = \frac{\sum|Cpred - Cobs|}{N},\ RMSE = \sqrt{\frac{\sum(Cpred - Cobs)^2}{N}}$$

where Cpred and Cobs denote the predicted and observed concentrations, respectively. Ideally the mean absolute prediction error should be comparable with the residual variability.

Chapter III Pharmacokinetics of Voriconazole in Liver Transplant Patients

3.1 Abstract

Objectives: To characterize the pharmacokinetics of voriconazole in liver transplant patients, evaluate the potential correlations between pharmacokinetic parameters and patient variables, externally validate the model, and explore limited sampling strategies (LSS) using Bayesian approaches.

Methods: Multiple blood samples were collected within one dosing interval from 15 patients who were initiated on a prophylactic regimen of voriconazole 200 mg enterally (tablets) twice daily starting immediately post transplant. Voriconazole plasma concentrations were measured using high performance liquid chromatography. Non-compartmental pharmacokinetic analysis was performed using WinNonlin. Nonlinear mixed-effects pharmacokinetic models were developed using NONMEM. The final model was internally evaluated using bootstrapping and visual predictive check (VPC), and externally validated by predicting additional samples from different patients that were not used for model-building. Maximum *a posteriori* Bayesian estimators were developed to predict AUC with limited samples (LSS). Mean prediction error (MPE) and mean absolute prediction error (MAPE) were calculated for external validation and LSS.

Results: In non-compartmental analysis, the mean CL/F, Vd/F and half life were 5.8 ± 5.5 L/hr, 94.5 ± 54.9 L and 15.7 ± 7.0 hr, respectively. T1/2, Cmax, trough level, AUCo-∞, AUMCo-∞ and MRTo-∞ were significantly correlated with postoperative time. T1/2, λ, AUCo-∞ and CL/F were significantly correlated with indices of liver function (AST, total bilirubin and INR). Cmax, Clast, AUMCo-∞ and MRTo-∞ were significantly higher in the presence of deficient *CYP2C19*2* alleles. There was a good correlation between AUCo-∞ and trough voriconazole plasma concentrations. In the population analysis, a one-compartment model with an absorption lag time (Tlag) adequately described the data. Population estimates of CL/F and Vd/F were 7.92L/hr and 248L. Levels of CL/F, Vd/F and Tlag decreased with post-operative time and converged to stable levels in about 7 post-operative days. CL/F significantly decreased with increased INR. Co-administration of pantoprazole, race and ALT were also significantly associated with variability in pharmacokinetic parameters but ultimately excluded in the final model. VPC showed that most of the data fell within the 90% prediction interval and were symmetrically distributed around the median. Additional 52 samples from 19 patients were collected for external validation. MPE was 0.206ug/ml (not significantly different from zero) and MAPE was 0.99ug/ml. Trough levels adequately predicted voriconazole exposure in liver transplant patients. Compared to trough levels, LSS using two samples or one sample at a different time provided better MPE, MAPE and correlation (R^2) between the real and LSS-predicted AUC.

Conclusions: A fixed dosing regimen of voriconazole results in a highly variable exposure of voriconazole in liver transplant patients. Given that trough voriconazole concentration is a good measure of drug exposure (AUC), voriconazole dose can be individualized based on trough

concentrations measurements in liver transplant patients. There is a significant association of voriconazole pharmacokinetics with post-operative time and liver function. Donor characteristics had no significant correlation with the pharmacokinetics of voriconazole. Our observations suggested a need for intravenous administration of voriconazole in the immediate post-operative period before an oral dose can be administrated in order to maintain adequate exposure of liver transplant patients to voriconazole.

3.2 Introduction

Voriconazole is typically given orally for a few weeks after liver transplantation for prophylaxis of fungal infections at our institution. Due to hepatic surgical damage and reperfusion injury, liver function in liver transplant patients will be impaired and will be variable immediately after the transplant surgery, and will gradually improve with time.

We hypothesize that use of a fixed dosing regimen will lead to a large degree of variability in the exposure of voriconazole in liver transplant patients due to variability in liver function after liver transplant surgery and due to polymorphism in *CYP* especially *CYP2C19*2*. We also hypothesize that voriconazole trough plasma concentration of voriconazole is a good surrogate marker for drug exposure (AUC). In order to test our hypothesis, we propose four specific aims:

Specific aim 1 will characterize the pharmacokinetics of voriconazole, and evaluate the variability in the pharmacokinetics of voriconazole in adult liver transplant patients. Full pharmacokinetic profiles of voriconazole have been collected in thirteen liver transplant patients within one oral dosing interval (200mg, BID) after transplantation. Non-compartmental pharmacokinetic analysis and nonlinear mixed effects modeling analysis will be performed to estimate the pharmacokinetic parameters and to capture both inter-patient and intra-patient variability in the pharmacokinetic parameter estimates. We predict that the pharmacokinetic parameters estimated in liver transplant patients will be different from non-transplant subjects, there will be a large variability in the pharmacokinetic parameter estimates, and there will be a good correlation between voriconazole trough plasma concentration and AUC.

Specific aim 2 will evaluate the association of patient-specific and donor-specific variables with the pharmacokinetics of voriconazole in liver transplant patients. Patient-specific and donor-specific demographic variables, liver function tests, renal function tests and *CYP* genotypes have been collected from each patient. Their association with the pharmacokinetic parameters of voriconazole will be evaluated using simple linear regression in non-compartmental analysis and evaluated as a covariate in population pharmacokinetic analysis. We predict that low CL/F will be associated with presence of *CYP2C19*2*, poor liver function. Pharmacokinetic parameters will change significantly with time after transplantation.

Specific aim 3 will develop better doing guidelines by validating the population pharmacokinetic model using Bayesian forecasting. Random samples will be collected in liver transplant patients along with the patient-specific and donor-specific variables from the routine therapeutic monitoring program. Voriconazole plasma concentrations will be measured using the same analytical assay as used in specific aim 1. The final model built in specific aim 2 will be used to predict voriconazole plasma concentrations that were not used for model-building by Bayesian forecasting. The predictions will be compared to the actual measurements by calculating the bias and precision. Once the model is validated, voriconazole plasma concentrations and drug exposure will be simulated at different dose levels adjusted by the factors selected in the final model to determine the optimal dosing regimens. We predict that the bias will not be significantly different from zero, and new dosing regimens will depend on presence of *CYP2C19*2* allele and liver function tests.

Specific aim 4 will optimize therapeutic drug monitoring by developing limited sampling strategies (LSS) using Bayesian approaches to predict voriconazole exposure. Maximum *a posteriori* Bayesian estimators will be used to predict voriconazole plasma concentrations (full profiles) using the validated final model as the *a priori* model, actual dosing record and covariate values from the patients in the model-building group without any concentrations as the input, and a few concentrations (limited sampling) as feedback information in the Bayesian estimation. The predictive performance of LSS will be evaluated by comparing true AUC0–12h and LSS-predicted AUC0–12h. Bias and precision will be calculated. We predict that bias, precision and correlation between the True and LSS-predicted AUC (R^2) will be improved by using a two-sample LSS.

3.3 Methods

3.3.1 Patients

Between January 2007 and March 2007, liver transplant recipients who were initiated on a voriconazole prophylactic regimen (200 mg tablets twice daily orally or via a nasogastric tube) immediately post transplant as part of their standard clinical care and who signed informed consent were enrolled in this prospective study. Children under age 18, patients who were receiving any medications known to influence the pharmacokinetics of voriconazole and patients receiving voriconazole to treat an active fungal infection were excluded from this study. Demographic data including age, gender, height, weight, race, laboratory results and current medication use were recorded. All patients received tacrolimus as their primary immunosuppressive agent. The protocol was approved by IRB at the University of Pittsburgh.

3.3.2 Blood Sample Collection

Serial blood samples (3ml) were collected from each patient just prior to (0 hr) and at 0.5, 1, 1.5, 2, 4, 6, 8 and 12 hours following administration of a minimum of 5 oral doses (range from the 5^{th} to 15^{th} dose; mean 7^{th} dose). Blood samples were collected into heparinized Vacutainer® tubes and centrifuged at 3000 rpm for 10 minutes, and plasma was separated and stored at -70°C until analysis.

3.3.3 Genotyping

One ml sample of whole blood was collected and immediately stored at - 80°C for genetic analysis. Additionally, whenever available, allograft biopsy tissue was also collected and stored at - 80°C for future genetic analysis. Genetic analysis was conducted through isolation of genomic DNA using the PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN). Determination of a panel of *CYP2C9*, *CYP2C19* and *CYP3A4 and CYP3A5* allelic variants was performed by TaqMan allele discrimination analyses. The genotyping of *CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP3A4*1B, CYP3A5*3* and *CYP3A5*6* was performed in all 15 patients (blood) and 7 donors (liver) using the Applied Biosystems Drug Metabolism Genotyping Assay kits to genotype for C3608T, A42614C, G19154A, G17948A, A-392G, A6986G and G14690A, respectively. Positive and negative PCR controls were included with each amplification reaction. Blinded duplicate sample analyses were also performed for all genotyping assays. An additional 10% of samples are repeated to avoid further misclassification and verify the reproducibility of the assay. All results are interpreted independently by two laboratory personnel and no discordant results were obtained.

3.3.4 Analytical Assay

Plasma voriconazole concentrations were measured using validated HPLC method that was modified based on previously published assays (34, 86, 87). Sixty µl of 6% perchloric acid (Fisher Scientific, Fair Lawn, NJ) was added into 120 µl of plasma, vortexed, and centrifuged (13,000 rpm) for 4 minutes at room temperature. 50ul of supernatants were injected onto a HPLC

system consisting of a Waters model 510 HPLC pump, a Waters model 717 plus automatic sampler, and a Waters model 2487 UV tunable absorbance detector set to 255 nm. Separation was performed at ambient temperature on 5 μm, 4.6 × 250 mm Waters C18 Symmetry analytical column. The mobile phase consisted of HPLC-grade acetonitrile and water (68:32, v/v, Fisher Scientific, Fair Lawn, NJ). The total run time was 10 minutes at a flow rate of 0.8 ml/min. Chromatographic data were collected and analyzed using Empower Chromatography software (Waters, version 5.0). The assay precision (intraday variability) was 1.3% to 9.0% (0.2 – 9 ug/ml), and the assay bias (interday variability) was 0.7% to 3.1% (0.5 – 9 ug/ml). The linearity range was 0.2 – 9 ug/ml ($R^2 = 0.9998$).

3.3.5 Non-compartmental Pharmacokinetic Analysis

Various pharmacokinetic parameters were calculated using non-compartmental analysis with WinNonlin software (version 4.1; Pharsight Corporation, Mountainview, CA). The parameters calculated after enteral administration of voriconazole included terminal disposition rate constant (λz), terminal disposition halflife (t1/2), area under the curve (AUC), apparent systemic clearance over bioavailability (CL/F), apparent steady state volume of distribution over bioavailability (Vd/F), mean residence time (MRT), peak plasma concentrations (Cmax), time to reach peak concentration (Tmax), last plasma concentration at 12 hours (Clast), and area under moment curve (AUMC). λz and t1/2 were derived from data points during the terminal disposition phase only when at least three data points were available, and the AUCo-∞ and AUMCo-∞ specific for the dose evaluated was calculated using reverse superposition principle. Projected trough voriconazole plasma concentrations (Clast) was used in three patients (#11, #15

and #16) since Clast was missing in these three patients. Each of these parameters is presented as mean and standard deviation. Statistical comparison of different parameters was made using paired two-tailed Student *t*-test (SPSS software, Windows-based version 14.0, Chicago, IL). A *P* value of <0.05 was considered statistically significant.

3.3.6 Statistical Analysis

The relationship between various pharmacokinetic parameters and patient variables and biochemical indices was examined by simple linear regression analysis. A relationship was considered to be statistically significant at $P < 0.05$ for the deviation of the coefficient from zero in the linear regression analysis. The difference between trough concentrations (C0 and C12) was tested using paired two-tailed Student t-test. The effect of dichotomous variables (such as gender, race and concomitant medication) on various pharmacokinetic parameters of voriconazole was tested using unpaired two-tailed Student t-test except for the effect of *CYP2C19* genotype, which was tested using unpaired one-tailed Student t-test since carriers of *CYP2C19*2* and **3* alleles have been identified as poor metabolizers [25]. The effect of feeding methods on various pharmacokinetic parameters of voriconazole was tested using one-way ANOVA. A P value of < 0.05 was considered to be statistically significant in all the statistical tests. The relationship between CL/F, Vd/F and body weight were evaluated using both simple linear model and allometrical model

$$Parameter = A \times (WT / \overline{WT})^{B},$$

where parameter includes CL/F and Vd/F, WT denotes actual body weight, and A and B are co-effeicients and exponents to be estimated using nonlinear regression.

95% confidence bands and 95% prediction bands were calculated and plotted using GraphPad Prism (Version 4.03, GraphPad Software, Inc.) to evaluate the precision of parameter estimation and predictive performance.

3.3.7 Population Pharmacokinetic Analysis

A nonlinear mixed-effects pharmacokinetic model (base model) was developed using NONMEM 6.2.0 (GloboMax, Hanover, MD) using first order conditional estimation method with interaction. Correlations between pharmacokinetic parameters were always incorporated and estimated. One- and two-compartment models were tested with first/zero-order elimination and Michaelis-Menten elimination process since nonlinear pharmacokinetics of voriconazole has been reported (89). Different approaches to describe the absorption phase were tested including zero-/first-order process, an absorption lag time, Erlang distribution and Weibull distribution. Various inter-individual variability structures were tested including:

1. exponential model: $P_{ij} = TV(P_j) \times e^{\eta ij}$
2. proportional model: $P_{ij} = TV(P_j) \times (1 + \eta_{ij})$
3. additive model: $P_{ij} = TV(P_j) + \eta_{ij}$
4. log-additive model: $Log(P_{ij}) = Log(TV(P_j)) + \eta_{ij}$

where Pij is the ith individual's estimate of the jth pharmacokinetic parameter, TV(Pj) is the typical value of the jth pharmacokinetic parameter, and ηij is a random variable for the ith individual and the jth pharmacokinetic parameter distributed with mean zero and variance of ωj^2.

Various residual variability models were tested including:

1. additive error model: $Cobs = Cpred + \varepsilon$
2. proportional error model: $Cobs = Cpred \times (1 + \varepsilon)$
3. combined error model: $Cobs = Cpred \times (1 + \varepsilon) + \varepsilon'$
4. exponential error model: $Cobs = Cpred \times e^{\varepsilon}$
5. other error model: $Cobs = Cpred + \varepsilon \times Cpred^{\theta}$

where Cobs and Cpred are the observed and predicted concentrations, respectively, and ε and ε' are normal random variables with means of zero and variances of δ^2 and δ'^2, respectively.

Covariate relationships were first visually evaluated by plotting Empirical Bayes Estimate against covariates. Covariate effects were then tested by incorporating covariates into the base model (without covariate) one at a time using at least 13 approaches to associate the covariate with the parameter. Different cut-off values for the covariates were also tested. A covariate was considered as significant and a cut-off value was considered optimal if all the following criteria were met: (1) a decrease in objective function value (OFV) of 6.63 for 1 degree of freedom ($p<0.01$), (2) no significant trend in Empirical Bayes Estimates vs covariate plots, (3) improved Goodness-of-Fit, (4) reduced inter-individual variability and (5) clinical plausibility for incorporating the covariate.

Then using the same criteria, an intermediate model (full model) containing all selected covariates was built using a forward addition approach (covariate added one at a time). Then the

final model was obtained using a reverse removal approach (covariate removed one at a time from the full model) using the same criteria.

3.3.8 Model Evaluation

The adequacy of fitting was examined by plotting predicted versus observed concentrations (Goodness-of-Fit), concentrations versus time profiles and weighted residuals versus predicted concentrations.

Precision of parameter estimation, stability of the final model and normality of the distribution of the parameter estimates was evaluated using bootstrapping (resampling repeated 2300 times) using Wings for NONMEM (http://wfn_sourceforge.net). Non-parametric statistics (median, 95% confidence interval) of parameter estimates obtained from bootstrapping were compared with the point parameter estimates obtained from the final model. The distribution of the parameter estimates obtained from bootstrapping was visually inspected for normality, based on which standard error was calculated for each parameter estimate in the final model.

To evaluate the predictive performance of the final model using visual predictive check, 1500 data sets were simulated using the parameter estimates in the final model. The 50th percentile concentration (estimator of the population-predicted concentration) and the 5^{th} and 95^{th} percentile concentrations (90% prediction interval) were plotted and compared to the observed concentrations.

3.3.9 External Validation

The established final model was then externally validated. Additional 52 samples were retrospectively collected from a separate group of 19 adult liver transplant patients in the therapeutic drug monitoring program at our institution who met the same criteria as patients included in the model-building group. Voriconazole plasma concentration in these patients was measured using the same assay as the model-building group. Complete dosing records and the same patient and donor specific factors as the model-building group were obtained. The protocol was approved by IRB at the University of Pittsburgh.

Voriconazole plasma concentrations were predicted by fixing the parameters in the structural and variance model to the parameter estimates in the final model using posthoc Bayesian forecasting with NONMEM 6.2.0. The predicted values were compared with the corresponding observed values. Bias (mean prediction error, MPE) and precision (mean absolute prediction error, MAPE) were calculated with 95% confidence intervals using the following equations:

$$MPE = \frac{\sum (Cpred - Cobs)}{N}$$

$$MAPE = \frac{\sum |Cpred - Cobs|}{N}$$

where Cpred and Cobs denote the predicted and observed concentrations, respectively.

3.3.10 Limited Sampling Strategy (LSS)

The aim of developing LSS using Bayesian approaches was to explore the clinical use of the final model, where dosage regimens, sampling times and covariate values could change frequently, and to predict AUC accurately and precisely using limited number of samples. The validated final model and all the parameter estimates were used as the *a priori* model. Actual dosing record and covariate values from the 13 patients in the model-building group without any concentrations were used the input. A few concentrations (limited sampling) were input as feedback information in the Bayesian estimation with different combinations of 1, 2 or 3 concentrations at 0, 0.5, 1, 1.5, 2 and 4 hours according to clinical constraints. Maximum *a posteriori* Bayesian estimators were used to predict voriconazole plasma concentrations (full profiles) using NONMEM. True AUC0–12h (AUCobs) and LSS-predicted AUC0–12h (AUCpred) was calculated using trapezoidal rules with all actual and predicted concentrations, respectively. The predictive performance of LSS was evaluated by comparing AUCobs and AUCpred. Bias ($MPE\% = \frac{\sum\left(\frac{AUCpred - AUCobs}{AUCobs} \times 100\%\right)}{N}$) and precision ($MAPE\% = \frac{\sum\left|\frac{AUCpred - AUCobs}{AUCobs} \times 100\%\right|}{N}$) were calculated with 95% confidence interval.

3.4 Results

3.4.1 Patients and Data Collection

A total of 15 patients were enrolled in this study. The characteristics of the patients, including the primary diagnosis, days post transplantation on the day of study, methods of feeding at time of study, concomitant medications, MELD (Model for End-Stage Liver Disease) Score, age, gender and race, the characteristics of the donors, including the cold ischemic time, warm ischemic time, the age distribution and the type of liver donation, the laboratory biochemical and hematological profile of the study patients before transplantation and on the day of pharmacokinetics study, and the pharmacogenomic profiles of patients and donors are shown in Table 2.

Table 2. Characteristics of liver transplant patients and donors

Gender (male/female)	11/4
Diagnoses	
Viral Hepatitis (HBV/HCV)	1/5
HCV + Alcohol	2
Non-Alcoholic Steatohepatitis	3
Primary Sclerosing Cholangitis	2
Autoimmune Hepatitis	1
Wilson's Disease	1
MELD Score	20.6 ± 11.3 (8-43)
Patient Age (yr)	56.3 ± 10.3 (41-76)
Weight (kg)	84.1 ± 17.7 (56-121)
Race (Caucasian/Asian)	13/2
Days post transplantation on the day of study	3.7 ± 1.4 (2-7)
Feeding at time of study (tube/clear liquid/regular food)	3/11/1
Anastomosis (T-Tube present§/T-Tube absent§/Roux-en-Y)	6/8/1
Concomitant drug (pantoprazole)/famotidine)	10/5
Cold Ischemic Time (min)	538.9 ± 266.6 (86-935)
Warm Ischemic Time (min)	27.2 ± 5.1 (16.8-37.8)
Donor Age (yr)	47.9 ± 21.6 (14-84)
Cadaveric/Living (n)	13/2
Total bilirubin (mg/dL) †	6.4 ± 5.9 (1.9-25.4) / 6.1 ± 6.4 (0.5-22.9)
AST (aspartate aminotransferase) (U/L) †	1088.9 ±726.2 (180-2405) / 294.8 ± 204.2 (33-620)

Table 2. (continued)

ALT (alanine aminotransferase) (U/L) †	682.6 ± 444.8 (144-1569) / 346.1 ± 263.4 (53-792)
INR (International normalized ratio) †	1.76 ± 0.35 (1.1-2.3) / 1.3 ± 0.16 (1-1.6)
SCr (Serum Creatinine) (mg/dL) †	1.6 ± 0.8 (0.6-2.9) / 1.7 ± 1.3 (0.5-5.2)
Baseline plasma albumin (g/L)	3.1 ± 0.5 (2.3-4.1)
CYP2C9*2 (C3608T) ‡	15 : 0 : 0 (6 : 1 : 0)
CYP2C9*3 (A42614C) ‡	12 : 3 : 0 (5 : 2 : 0)
CYP2C19*2 (G19154A) ‡	12 : 3 : 0 (6 : 1 : 0)
CYP2C19*3 (G17948A) ‡	15 : 0 : 0 (6 : 0 : 0)
CYP3A4*1B (A-392G) ‡	15 : 0 : 0 (6 : 0 : 0)
CYP3A5*3 (A6986G) ‡	0 : 0 : 15 (7 : 0 : 0)
CYP3A5*6 (G14690A) ‡	15 : 0 : 0 (7 : 0 : 0)

Values are all expressed as mean ± standard deviation (range) and measured in patients except specified as measurements in donors.

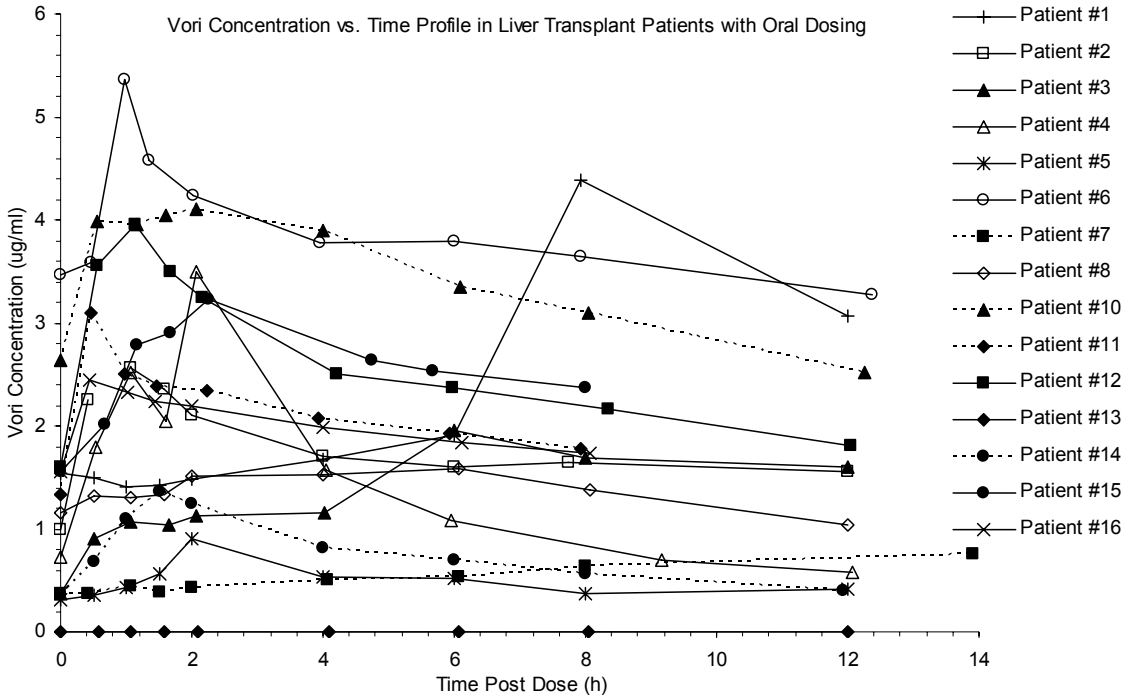
MELD, Model for End-Stage Liver Disease, calculated using the equation MELD score = 3.78[Ln serum bilirubin (mg/dL)] + 11.2[Ln INR] + 9.57[Ln serum creatinine (mg/dL)] + 6.43

§: they were both Duct-to-Duct Anastomosis, but the t-tube had been taken out in some of the patients at the time of study.

† Values are displayed as baseline measurements / measurements on the day of study.

‡ Donor genotype (liver) was displayed in parenthesis. The three values displayed represent wild type homozygous extensive metabolizers (-/-) : heterozygous extensive metabolizers (-/+) : poor metabolizers (+/+).

The mean and individual plasma concentrations of voriconazole over time after enteral voriconazole are shown in Figure 5. Thirty-three percent of the patients had a trough level lower than 1ug/ml, and the rest of the patients had a trough level between 1ug/ml and 6ug/ml. Among all the 15 patients that completed the study, one patient had an undetectable concentration of voriconazole in all of the samples and could not be evaluated (#13, no particular reason was identified), and three patients (#1, #3 and #7) had extremely atypical profiles with fewer than three data points during the terminal disposition phase, and non-compartmental pharmacokinetic analysis could therefore not be readily performed.



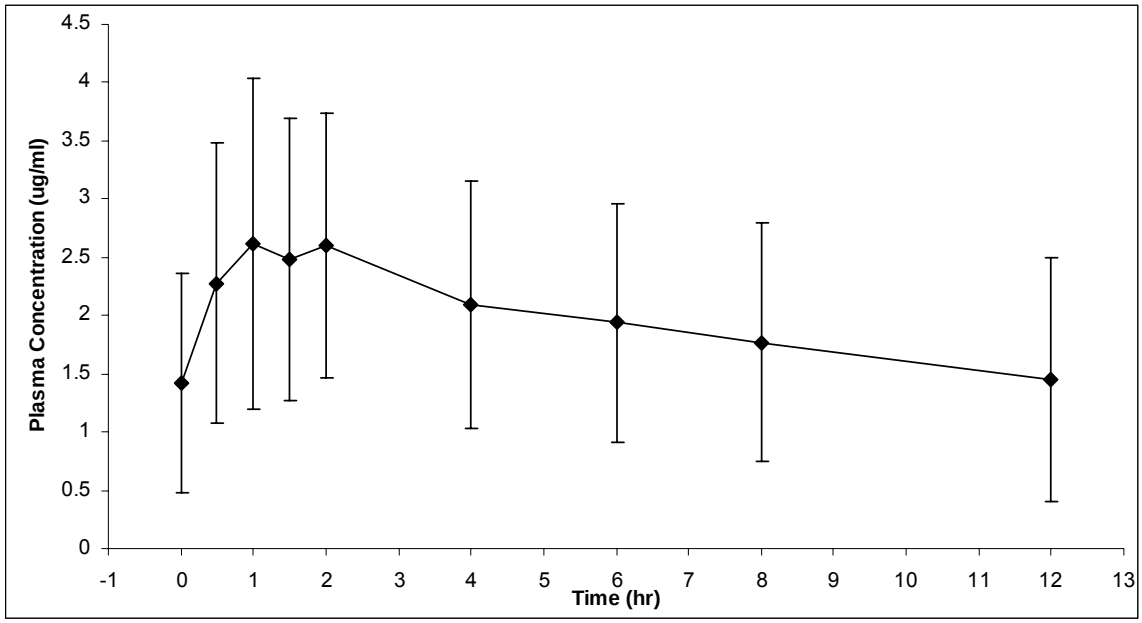


Figure 5. Plasma concentrations profiles of voriconazole in liver transplant patients

Large inter-individual variability can be observed. Patient #1, #3, #7 and #13 had extremely atypical profiles.

Upper figure: plasma concentrations of voriconazole over time during one dosing interval (all patients).

Lower figure: Mean plasma concentrations of voriconazole over time during one dosing interval (mean with SD error bars, patient #1, #3, #7 and #13 excluded, see text).

3.4.2 Non-compartmental Pharmacokinetic Analysis

Complete pharmacokinetic data could be calculated in 11 patients. The pharmacokinetic parameters of voriconazole after enteral administration of voriconazole are shown in Table 3. There was a wide variation in various pharmacokinetic parameters of voriconazole in liver transplant patients after enteral voriconazole administration.

Table 3. Pharmacokinetic parameters of voriconazole (non-compartmental analysis) in liver transplant patients

Patient ID	λz (hr^{-})	HL_λz (hr)	Tmax (hr)	Cmax (ug/ml)	Clast* (ug/ml)	AUCo-∞ (hr* ug/ml)	AUMCo-∞ (hr*hr *ug/ml)	Vz/F_obs (L)	CL/F_obs (L/hr)	MRTo-∞ (hr)
2	0.04	16.1	1.1	2.6	1.6	34.3	1662.8	81.1	3.5	48.5
4	0.12	5.6	2.1	3.5	0.6	15.2	145.7	76.8	9.5	9.6
5	0.07	9.5	2.0	0.9	0.4	7.2	182.9	239.1	17.5	25.4
6	0.02	30.0	1.0	5.4	3.3	37.7	12945.6	46.0	1.1	343.5
8	0.07	9.4	6.1	1.6	1.0	14.7	480.2	89.7	6.6	32.7
10	0.05	14.9	2.1	4.1	2.5	38.1	2610.8	45.3	2.1	68.6
11	0.04	17.8	0.5	3.1	1.52	27.9	2089.1	82.4	3.2	74.9
12	0.04	15.5	1.2	4.0	1.8	34.4	1940.9	63.6	2.8	56.3
14	0.09	7.4	1.5	1.4	0.4	9.2	135.5	165.2	15.5	14.8
15	0.03	21.3	2.3	3.2	2.08	45.7	3826.1	65.8	2.1	83.7
16	0.04	17.7	0.5	2.4	1.49	20.6	2113.1	84.8	3.3	102.4
Mean	0.06	15.0	1.8	2.9	1.6	25.9	2557.5	94.5	6.1	78.2
SD	0.03	7.0	1.5	1.3	0.9	13.1	3643.6	57.6	5.7	92.7
CV (%)	52.69	46.6	84.8	45.4	58.3	50.7	142.5	61.0	92.6	118.6
Median	0.04	15.5	1.5	3.1	1.7	27.9	1940.9	81.1	3.3	56.3
95% CI	0.04-0.08	10.9-19.1	0.9-2.74	2.1-3.7	1.0-2.1	18.1-33.7	404.3-4710.7	60.5-128.6	2.8-9.5	23.4-133.0

Abbreviations: Cmax, maximum concentration; Tmax, time to reach maximum concentration; Clast, concentration at 12 hours; λz, disposition rate constant; HL_λz, apperant half-life; AUCo-∞, area under the curve concentration; CL/F_obs, clearance/bioavailability; Vz/F_obs, volume of distribution/bioavailability; MRTo-∞, mean residence time; AUMCo-∞, area under the first moment curve

* Clast of patient #1, #3, #7 and #13 are 3.07 ug/ml, 1.6 ug/ml, 0.76 ug/ml and 0 ug/ml (unmeasurable).

Projected Clast of patient #11, #15 and #16 was used (see text).

The trough concentrations prior to dosing (Co) and at 12 hours after dosing (C12) are not significantly different (p=0.2794), and the difference between the trough concentrations (C12-Co)/C12 averaged 6.4%, indicating that steady state had been reached in most of the patients at the time of study (Figure 6).

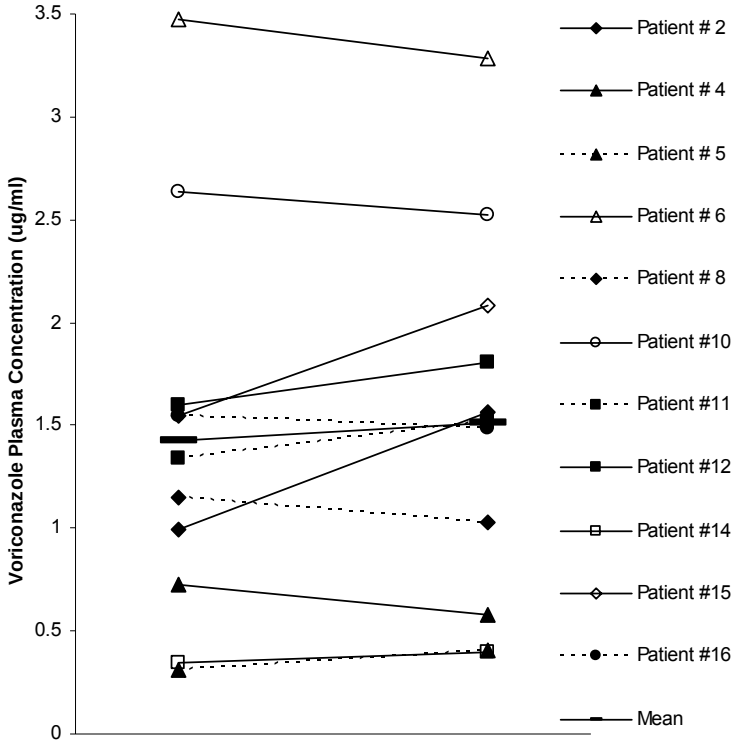


Figure 6. Comparison of trough concentrations in liver transplant patients

Co (left) vs C12 (right)

There was a good correlation (R^2=0.75) between the trough voriconazole plasma concentrations and the corresponding AUCo-∞ (Figure 7, n=11).

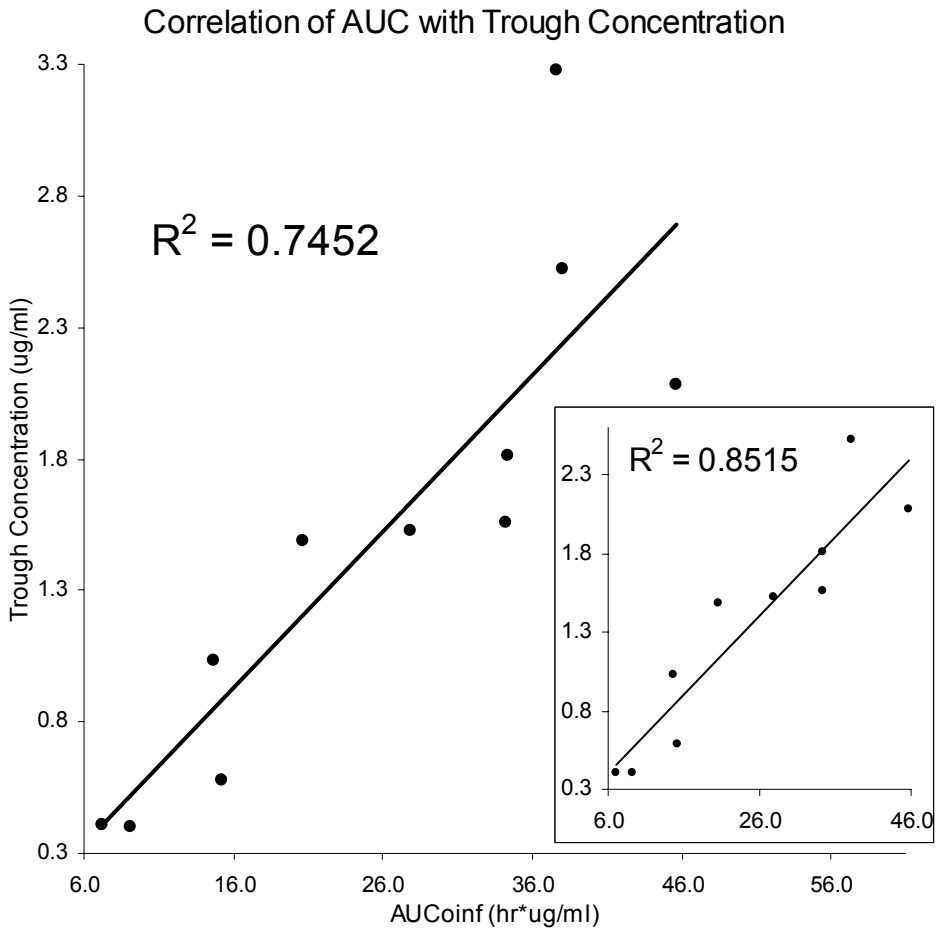


Figure 7. Correlation of AUCo-∞ and trough concentration in liver tranpslant patients

Big figure: R^2=0.745 when AUCo-∞ and trough concentrations (C12) was correlated in all the 11 patients that had typical profiles.

Small figure: R^2=0.852 when AUCo-∞ and trough concentrations (C12) was correlated in 10 patients that had typical profiles with patient #6 omitted.

There were significant correlations between various estimated pharmacokinetic parameters and patient variables and various biochemical indices (linear regression coefficient differs significantly from zero). All the correlations are summarized in Table 4 and Figure 8. The 95% confidence bands and 95% prediction bands were calculated and plotted (Figure 8). The correlation between body weight and the two independent pharmacokinetic parameters CL/F and Vd/F was very poor using both simple linear regression (R^2=0.1345 for CL/F and 0.0308 for Vd/F) and the principle of allometry (R^2=0.0990 for CL/F and 0.1350 for Vd/F).

Table 4. Correlations between patient variables and pharmacokinetic parameter estimates obtained using non-compartmental analysis in liver transplant patients

	Λ	T1/2	Tmax	Cmax	Clast	AUC o-∞	Vd/F	CL/F	AUMC o-∞	MRT o-∞
POT *		0.7415 (+)		0.6132 (+)	0.6256 (+)	0.4564 (+)			0.7444 (+)	0.7004 (+)
ASTo *										0.4395 (+)
Bild *		0.4746 (+)								
INRo *	0.6510 (-)	0.4214 (+)						0.4490 (-)		
INRd *	0.5639 (-)					0.4555 (+)				
RACE †			0.016	0.0402		0.0711	0.0513	0.1023		
PAN †				0.0066	0.0112	0.0939			0.0629	0.0868
T-Tube †						0.0841				
CYP2C19 †				0.0136	0.0352				0.0131	0.0154

Abbreviation: POT, post-operative time; ASTo: baseline AST; Bild: total bilirubin on the day of study; INRo, baseline international normalized ratio; INRd international normalized ratio on the day of study; PAN, pantoprazole; T-Tube, t-tube present or absent at the time of study. CYP2C19, heterozygous extensive metabolizers (*CYP2C19*1/*2*).

*: r-square for linear regression between the two variables is displayed in the table. Signs in the parenthesis indicate positive (+) or negative (-) association.

†: p value is displayed in the table.

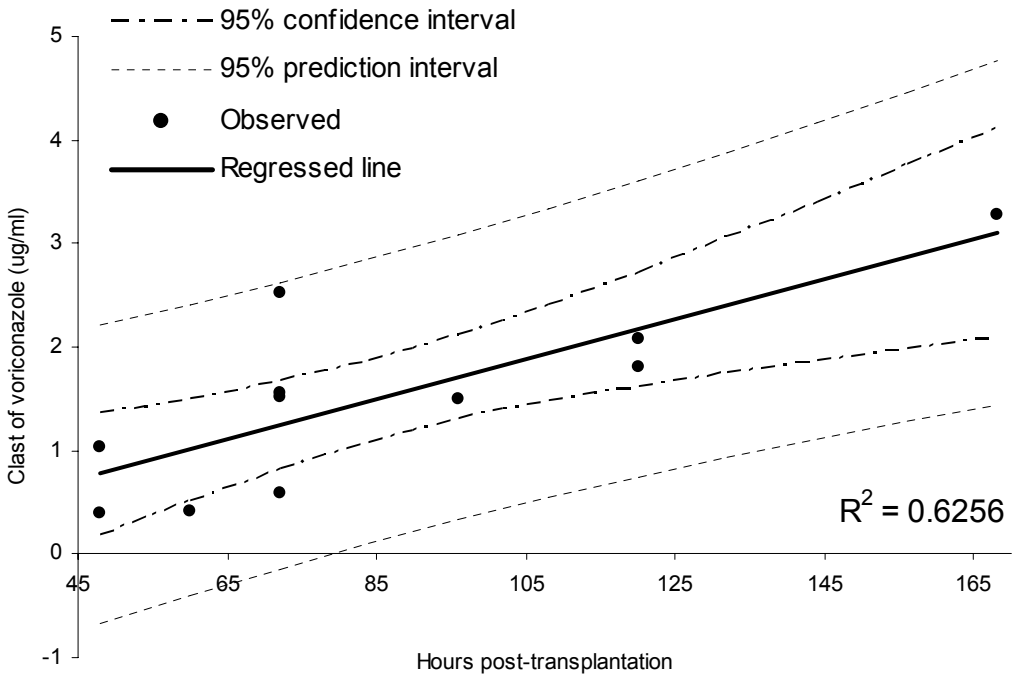


Figure 8. Correlation between post-operative time (hours post-transplantation) and trough plasma concentrations Clast in liver tranpslant patients

Data from all the 11 patients that had typical profiles are displayed. 95% confidence bands and 95% prediction bands are plotted.

Despite the small number of subjects in this study, the presence of deficient *CYP2C19*2* alleles and race were significantly associated with some pharmacokinetic parameters of voriconazole. Compared to homozygous extensive metabolizers (*CYP2C19*1/*1*), Cmax, Clast, AUMCo-∞ and MRTo-∞ were significantly higher in heterozygous extensive metabolizers (CYP2C19*1/*2)

by 1.9-fold, 2.0-fold, 5.1-fold and 3.9-fold, respectively. Compared to Caucasian patients (n=9), Tmax was significantly higher (p=0.016) by 3-fold and Cmax was significantly lower (p=0.0402) by 2.6-fold in Asian patients (n=2) (Table 4). In addition, Vd/F was 2.1-fold higher (p=0.0513), CL/F was 2.5-fold higher (p=0.1023), and AUCo-∞ was 2.7-fold lower (p=0.0711) in Asian patients compared to Caucasian patients, although this did not reach to statistical significance.

Interestingly, concomitant pantoprazole treatment with oral voriconazole was associated with a statistically significant decrease in voriconazole exposure. Voriconazole half-life, Cmax, Clast, AUCo-∞, MRTo-∞ and AUMCo-∞ were significantly lower by 37%-70% in patients receiving concomitant pantoprazole treatment compared to those not on pantoprazole (Table 4). CL/F was 3.5-fold higher in patients on concomitant pantoprazole treatment compared to those not on pantoprazole, although this did not reach to statistical significance (p=0.0533). Feeding methods (regular diet, clear liquids, tube feedings) have no effect on the pharmacokinetic parameters of voriconazole.

It is also worth mentioning that all of the donor variables including CIT (Cold Ischemic Time), WIT (Warm Ischemic Time), donor age and type of liver donation poorly correlated with all the estimated pharmacokinetic parameters (R^2<0.4).

3.4.3 Population Pharmacokinetic Analysis

A one-compartment model with first-order absorption and elimination with an absorption lag time (Tlag) adequately described the data. Other elimination and absorption models tested did

not result in significant decrease in OFV and thus did not significantly improve the model fit. Inter-individual and residual variability was best described by an exponential model and a combined proportional and additive error model, respectively. The population estimates of CL/F, Vd/F, ka and Tlag, inter-individual and residual variability were summarized in Table 5. The additive error estimate was comparable with the lowest limit of quantification (LLOQ) of the assay 0.2ug/ml. Although individual predictions agreed well with observations, population predictions were strongly biased in the base model (Figure 9a).

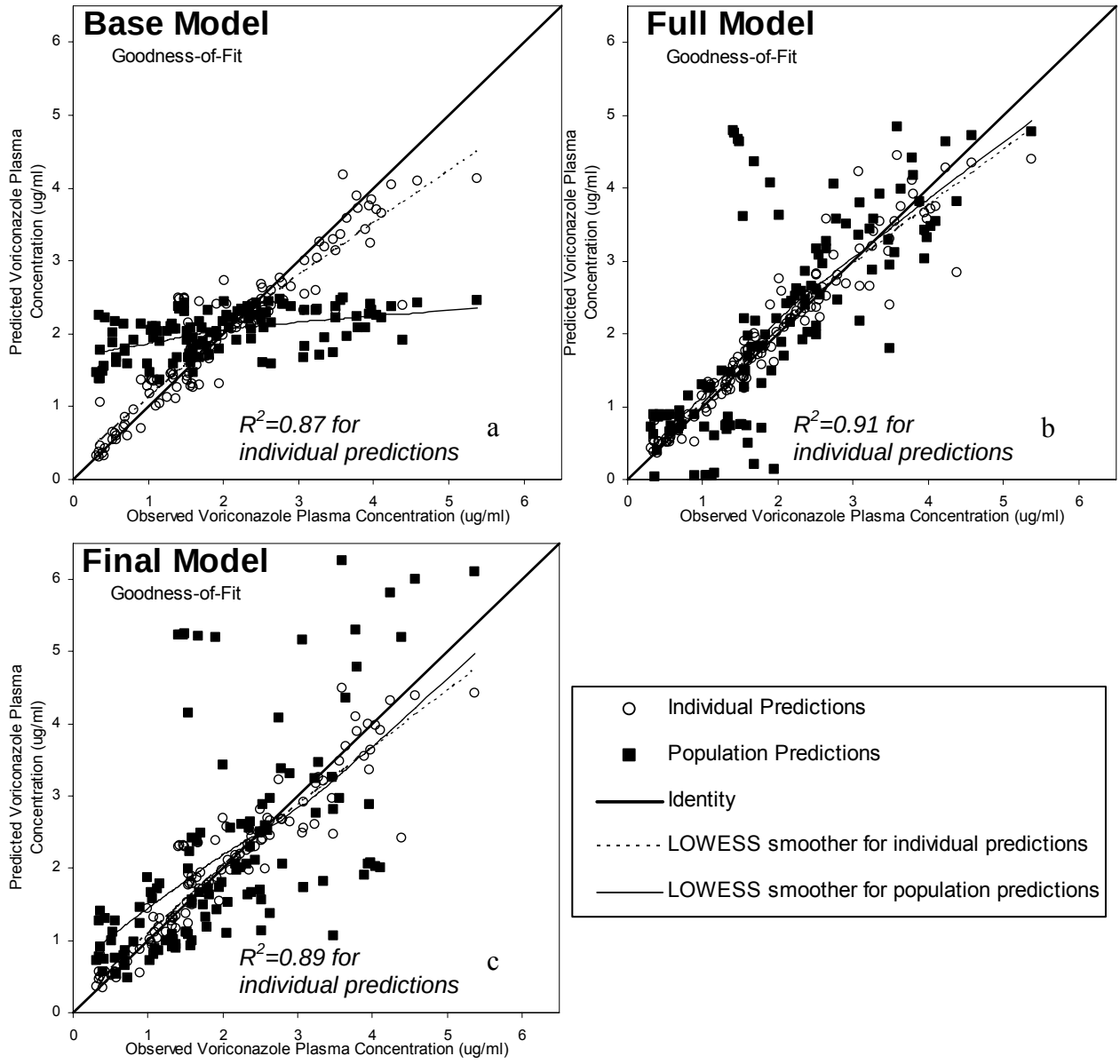


Figure 9. Goodness-of-Fit of base model, full model and final model in liver tranpslant patients

a: Goodness-of-Fit of base model. Individual predictions (hollow circle) agreed well with observations (R^2=0.87). Population predictions (solid square) were strongly biased (over-prediction at low concentrations and under-prediction at high concentrations), indicated by the LOWESS smoother of population predictions (thin solid line) which appears to be almost horizontal.

b: Goodness-of-Fit of full model. Individual predictions (hollow circle) agreed well with observations (R^2=0.91). Population predictions (solid square) were substantially improved compared to the base model. The strong bias in population predictions observed in the base model was basically corrected.

c: Goodness-of-Fit of final model. Individual predictions (hollow circle) agreed well with observations (R^2=0.89). Population predictions (solid square) were substantially improved compared to the base model. The LOWESS smoother of the population predictions (thin solid line) was close to the identity line (thick solid line), indicating that population predictions in the final model was not significantly biased.

To explore covariate relationships, all the covariates were tested one at a time. The best cut-off value for post-operative time (POT) was found to be 168 hours (1 week) using the criteria listed in the METHODS section, meaning that POT was set to 168 hours for any POT greater than 168 hours. CL/F and Vd/F significantly decreased with POT. High CL/F was significantly associated with low international normalized ratio (INR) or alanine aminotransferase (ALT) and with pantoprazole co-administration. Caucasian patients seemed to have significantly lower CL/F and Vd/F than Asian patients. Short Tlag was significantly associated with high INR and with pantoprazole co-administration.

Table 5. Population pharmacokinetic modeling process in liver tranpslant patients

Parameter / Model	Significant Covariate	ΔOFV *	P value †	Equation
CL/F ‡	POT	-22.10	<0.00001	$CL/F = 6.58 \times \left(\frac{POT}{86.77}\right)^{-1.12}$
	INR	-24.40	<0.00001	$CL/F = 39.7 - 28.9 \times \frac{INR}{1.29}$
	RACE	-26.51	<0.00001	$CL/F = 53 - 46 \times CAU$
	PANT	-29.58	<0.00001	$CL/F = 4.68 \times 2.3^{PANT}$
	ALT	-7.21	<0.01	$CL/F = 6.9 - 1.57 \times \left(\frac{ALT - 338.15}{246.29}\right)$
Vd/F ‡	POT	-12.87	<0.001	$Vd/F = 106 \times \exp\left(1 - 0.461 \times \frac{POT - 86.77}{36.71}\right)$
	RACE	-18.11	<0.0001	$Vd/F = 1440 \times (1 - 0.788 \times CAU)$
KA ‡	POT	-12.29	<0.001	$KA = 839 \times \left(\frac{POT}{86.77}\right)^{-8.74}$
	PANT	-11.13	<0.001	$KA = 0.293 \times 4320^{PANT}$
Tlag ‡	PANT	-11.11	<0.001	$Tlag = 0.5 \times 0.005^{PANT}$
	INR	-6.78	<0.01	$Tlag = 0.00118 \times \left(1 + \left(\frac{INR}{1.29}\right)^{-12.2}\right)$
	POT	-9.42	<0.01	$Tlag = 0.01 \times 10.5^{\left(\frac{POT}{86.77}\right)}$
Full model	POT, INR, RACE, PANT	-76.54	<0.00001	Not shown

Table 5. (continued)

Final model	POT, INR	-32.78	<0.00001	$\mathrm{CL/F} = (10.6 - 3.92 \times \frac{INR - 1.29}{0.17}) \times \left(\frac{POT}{86.77}\right)^{-1.51}$ $\mathrm{Vd/F} = 776 \times \exp\left(-1.3 \times \frac{POT}{86.77}\right)$ $\mathrm{KA} = 316 \times \left(\frac{POT}{86.77}\right)^{10.9}$ $\mathrm{Tlag} = 0.817 \times 0.0838^{\frac{POT}{86.77}}$

* ΔOFV: change in the objective function value compared to the base model

† A decrease in OFV was referred to the chi-squared distribution to assess significance.

‡ These are the results in the covariate relationship exploration step. All the covariates were incorporated one at a time into these parameters to explore the covariate relationship.

POT: post-operative time. POT was set to a constant of 168 hours if the post-operative time was greater than 168 hours.

ALT: alanine aminotransferase

INR: International normalized ratio

CAU: 1 for Caucasian patients and 0 for Asian patients

PANT: 1 for co-administered pantoprazole and 0 for no co-administered pantoprazole

Tlag: absorption lag time

The forward addition model building step resulted in the full model containing all the covariates selected in the exploration process mentioned above except for ALT (Table 5). The reverse removal model building step resulted in the final model containing POT as a significant covariate on all the pharmacokinetic parameters, and INR as a significant covariate on CL/F (Table 5).

Base model, full model and final model all showed adequacy of fitting. Figure 9 showed a good correlation (R^2>0.87) between individual predictions and observations. Weighted residuals were approximately normally distributed and were mostly within about 2 units of the null ordinate in all three models.

The full model and final model seemed to be superior over the base model and the covariates selected in the full model and final model explained a large portion of the variability in the population predictions in the base model. Population predictions were substantially improved in the full model and final model compared to the base model (Figure 9). LOWESS smoothers showed that the strong bias in population predictions observed in the base model was reduced in the full model and final model. Inter-individual variability decreased by 38.1% in CL/F and by 64.8% in Tlag in the final model compared to the base model (Table 5). The OFV decreased by 32.78 for 5 degree of freedom ($p<0.00001$) in the final model compared to the base model. Finally, Concentration-vs-Time plots also confirmed the superiority of the final model.

According to the equations and the individual parameter estimates in the final model, CL/F, Vd/F and Tlag decreased rapidly and dramatically over time after the surgery and eventually reached stable levels at some point within 7 post-operative days. The individual CL/F, Vd/F and Tlag

estimates covered a wide range of values at the early period of time after the surgery. CL/F ranged from 102 L/hr to 383 L/hr, Vd/F ranged from 191 L to 1944 L, Tlag ranged from 0.32 hours to 1.04 hours. However, the individual parameter estimates eventually converged to stable levels at some point within 7 post-operative days (Figure 10).

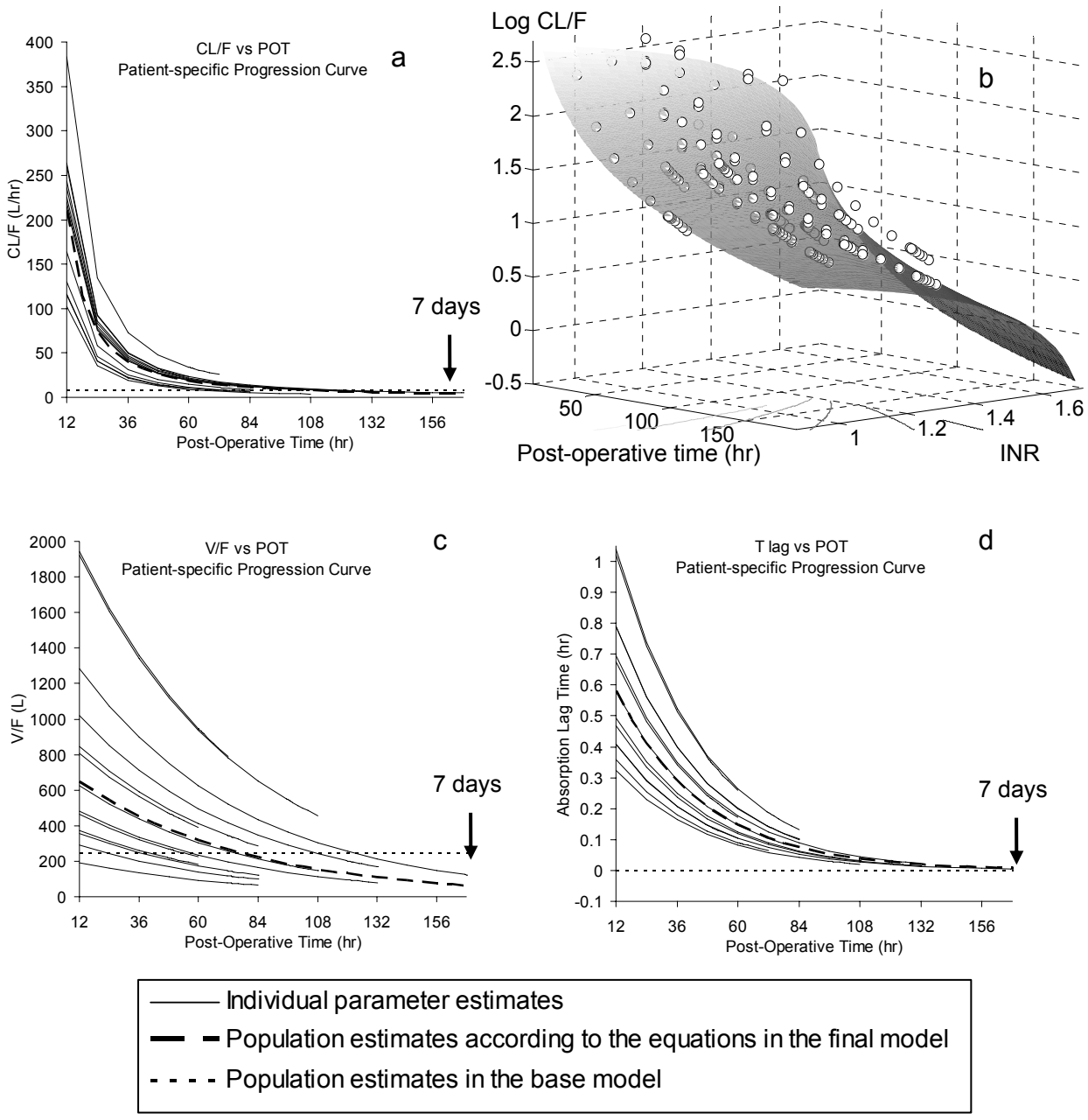


Figure 10. Covariate relationships in the final model in liver tranpslant patients

a, c and d: both the population estimates of CL/F, Vd/F and Tlag according to the equations (thick dash line) and the individual estimates of CL/F, Vd/F and Tlag (thin solid lines) decreased rapidly and dramatically over time after the

surgery and eventually reached the population levels (thin horizontal dash line) at some point within 7 post-operative days. The individual CL/F, Vd/F and Tlag estimates covered a wide range (high variability) at the early period of time after the surgery. CL/F ranged from 102 L/hr to 383 L/hr, Vd/F ranged from 191 L to 1944 L, Tlag ranged from 0.32 hours to 1.04 hours. This variability decreased over time and the individual parameter estimates eventually converged to the population levels at some point within 7 post-operative days.

b: 3D plot of the covariate relationship between CL/F and post-operative time and INR. Individual estimates of CL/F (hollow circles) are symmetrically distributed around the population estimates according to the equation (surface). CL/F decreased with post-operative time and increased INR.

3.4.4 Model Evaluation

In the bootstrapping analysis for the final model, 1281 out of 2300 runs successfully converged and were incorporated into the non-parametric analysis. The point population estimates of all parameters were similar to the median values obtained from bootstrapping and fell within the 95% confident intervals (Table 6), indicating precise and stable parameter estimation in the final model. Most of the parameter estimates seemed to be normally distributed, confirming the normality assumptions for model building.

Table 6. Comparison between population estimates in the base model and final model and comparison of population estimates in the final model and non-parametric statistics obtained from bootstrapping analysis of the final model in liver tranpslant patients

	θ1	Θ2	θ3	θ4	θ5	θ6	θ7	θ8	θ9	Inter-individual variability (%)				Residual	
										CL/F	Vd/F	KA	Tlag	Proportional	Additive (ug/ml)
Population estimates (base model) *	7.9	248	52.4	0.001						82.7	80.5	162.8	182.5	0.46	0.1
Population estimates (final model) †	10.6	776	316	0.817	-1.3	0.084	-3.92	-1.51	10.9	51.2	84	151.7	64.31	0.43	0.3
Lower boundary of 95% CI ‡	5.24	56	5.88	0.001	-3.37	0.001	-5.5	-3.29	3.26	5.5	42.1	51.2	30.2	0.22	0.1
Median ‡	9.7	327	99	0.423	-0.62	0.21	-2.41	-1.48	10.4	48.4	71.3	121.2	87.3	0.37	0.28
Upper boundary of 95% CI ‡	13	5580	105	8.152	0.94	8.03	0.02	-0.91	18.6	72.3	109	209.6	165.6	0.49	0.51

CI: confidence interval

*: In this row (base model), θ1 = CL/F, θ2 = Vd/F, θ3 = KA and θ4 = absorption lag time (Tlag)

‡: non-parametric statistics obtained from bootstrapping analysis of the final model

† and ‡: In these rows (final model), θ's are numbered according to the following equations in the final model:

$$\text{CL/F} = (\theta 1 - \theta 7 \times \frac{INR - 1.29}{0.17}) \times \left(\frac{POT}{86.77}\right)^{\theta 8},\ \text{Vd/F} = \theta 2 \times \exp\left(\theta 5 \times \frac{POT}{86.77}\right),\ \text{KA} = \theta 3 \times \left(\frac{POT}{86.77}\right)^{\theta 9},\ \text{Tlag} = \theta 4 \times \theta 6^{\frac{POT}{86.77}}$$

In the visual predictive check, most of the data fell within the 90% prediction interval and were symmetrically distributed around the median (Figure 11), indicating good predictive performance of the final model. Figure 11 (hollow circle, observed concentrations) also illustrated the distribution of samples during post-operative time.

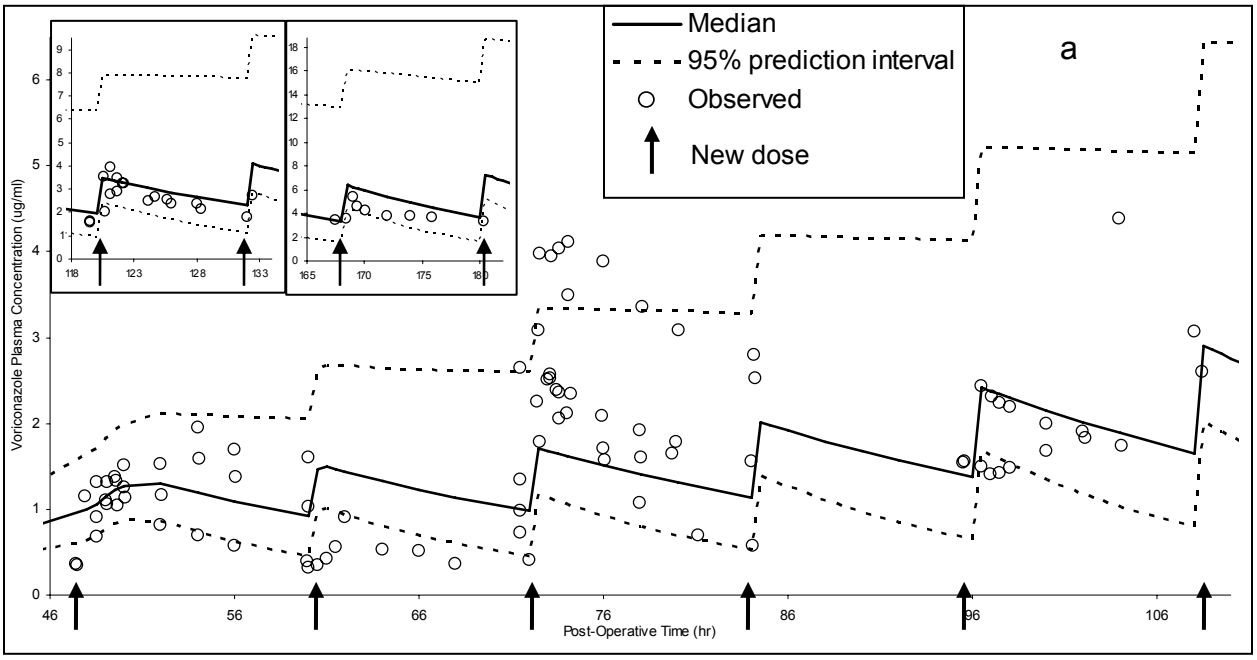


Figure 11. Visual predictive check of the final model in liver tranpslant patients

Most of the observations (hollow circles) fell within the 90% prediction interval (dash lines) and were symmetrically distributed around the median (solid line).

3.4.5 External Validation

Patient characteristics did not significantly differ between the model-building and validation groups (Table 7).

Table 7. Comparison of characteristics of liver transplant patients and donors between index group and validation group

	Index group	Validation group
Number of samples/patients	117/13	52/19
Voriconazole plasma concentration (ug/ml)	2.04 ± 1.12 (0.31-5.37)	1.30 ± 1.68 (0-6)
Gender (male/female)	10/3	16/3
MELD Score	20.5 ± 11.7 (8-43)	NA
Patient Age (yr)	55.8 ± 10.9 (41-76)	51.5 ± 12.3 (29-66)
Weight (kg)	83.5 ± 18.9 (56-121)	83.8 ± 24.5 (50-135)
Height (cm)	173.1 ± 6.7 (157.5-182.9)	173.6 ± 10.5 (149.9-188)
Race (Caucasian / Asian / African American)	11/2/0	16/0/2
Feeding at time of study (tube/clear liquid/regular food)	2/10/1	NA

Table 7. (continued)

Anastomosis *	6/7	NA
Concomitant drug (pantoprazole/famotidine)	8/5	NA
Cold Ischemic Time (min)	530.8 ± 273.2 (86-935)	586.6 ± 158.5 (354-875)
Warm Ischemic Time (min)	27.5 ± 5.4 (16.8-37.8)	30.1 ± 12.0 (12-57)
Donor Age (yr)	51.4 ± 20.8 (14-84)	49.2 ± 19.0 (16-82)
Cadaveric/Living (n)	11/2	18/1
Total bilirubin (mg/dL) †	6.4 ± 6.4 (1.9-25.4) / 6.4 ± 6.9 (0.5-22.9)	6.7 ± 4.5 (2-17.6) / 6.6 ± 7.7 (0.6-26.5)
AST (U/L) †	1053.3 ± 720.7 (180-2405) / 307.8 ± 210.8 (33-620)	1220.9 ± 732.7 (225-3007) / 51.6 ± 58.4 (14-379)
ALT (U/L) †	653.7 ± 367.6 (244-1516) / 338.2 ± 246.3 (108-792)	615.1 ± 548.3 (118-2401) / 47.7 ±39.6 (11-207)
INR †	1.78 ± 0.36 (1.1-2.3) / 1.29 ± 0.17 (1-1.6)	1.97 ± 0.48 (1.4-3.3) / 1.35 ± 0.16 (1-1.6)
SCr (mg/dL) †	1.6 ± 0.9 (0.6-2.9) / 1.8 ± 1.4 (0.5-5.2)	1.7 ±0.8 (0.7-3.1) / 1.9 ± 1.1 (0.5-7.1)
Plasma albumin (g/L) †	NA / 3.2 ± 0.5 (2.3-4.1)	2.5 ± 0.6 (1.2-3.1) / 2.8 ± 0.6 (1.4-3.9)

Values are all expressed as mean ± standard deviation (range) except specified otherwise. Values were measured in patients except specified as measurements in donors.

NA: not available

MELD, Model for End-Stage Liver Disease, calculated using the equation MELD score = 3.78[Ln serum bilirubin (mg/dL)] + 11.2[Ln INR] + 9.57[Ln serum creatinine (mg/dL)] + 6.43

AST: aspartate aminotransferase

ALT: alanine aminotransferase

INR: International normalized ratio

SCr: Serum Creatinine

* Values are displayed as the number of patients with T-Tube present/T-Tube absent at the time of study. All the patients had Duct-to-Duct anastomosis, but the t-tube had been taken out in some of the patients at the time of study.

† Values are displayed as baseline measurements / measurements on the day of study.

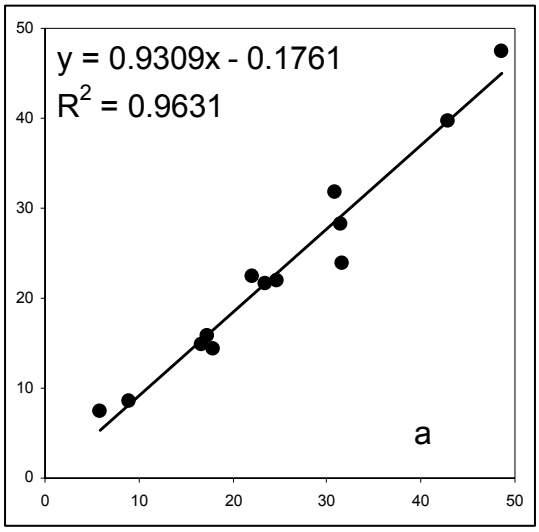
‡ Donor genotype (liver) was displayed in parenthesis. The three values displayed represent wild type homozygous extensive metabolizers (-/-) : heterozygous extensive metabolizers (-/+) : poor metabolizers (+/+).

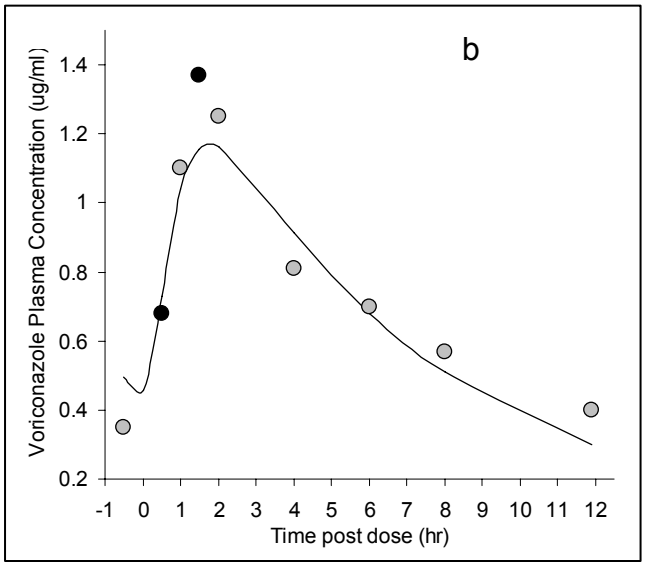
In the external validation, the POT in the validation group was longer than the model-building group. Despite this, the bias (MPE) was only 0.206ug/ml (95% confidence interval: -1.4–0.55ug/ml) and was not significantly different from zero ($p>0.23$). MPE was comparable to the LLOQ of the assay 0.2ug/ml and additive residual error in the final model 0.3ug/ml. The precision (MAPE) was 0.99ug/ml. Predicted concentrations agreed well with observed concentrations without significant bias. Prediction errors were symmetrically distributed around zero without significant patterns.

3.4.6 Limited Sampling Strategy (LSS)

A one-sample limited sampling strategy (LSS) using only the trough level (0 hour) did not have the best predictive performance. Bias, precision and correlation between True and LSS-predicted AUC (R^2) were improved by using a sample at a different sampling time or by using an additional sample. R^2 increased to 0.963 by using one sample at 4 hour (Figure 12a). MPE% of

AUC prediction (bias) decreased to -0.47% (not significant different from zero, p>0.94) by using one sample at 1 hour. MAPE% (precision) decreased to 8.91% by using two samples at 0.5 and 1.5 hours. Three-sample LSS did not further improve the prediction. Examples of posterior individual fitting of typical and atypical pharmacokinetic profiles using two samples at 0.5 and 1.5 hours are shown in Figure 12b and 12c, respectively.





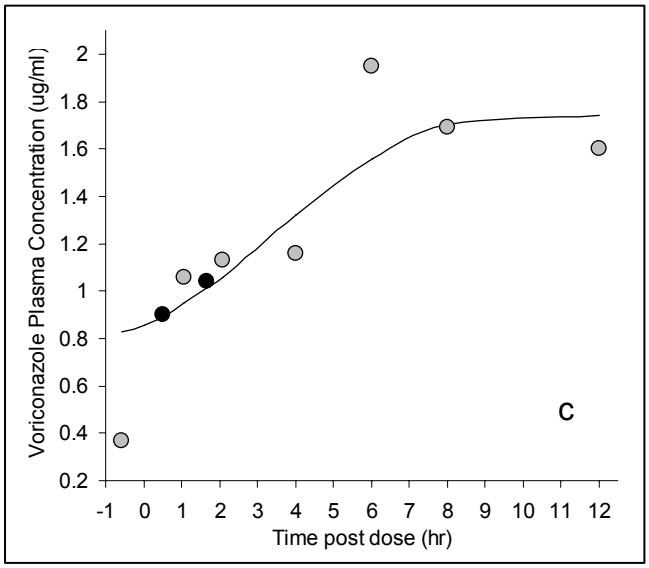


Figure 12. Predictive performance of the limited sampling strategies in liver tranpslant patients

a: Predictive performance of the one-sample limited sampling strategy using one sample at 4 hours. Predicted AUC was well correlated with observed AUC.

b and c: Predictive performance of the two-sample limited sampling strategy using samples at 0.5 and 1.5 hours (examples of posterior individual fitting of a typical pharmacokinetic profile (b) and an atypical pharmacokinetic profile (c)). Black circles are samples used as feedback information (limited sampling). Grey circles are other observed concentrations.

3.5 Discussion

Limited pharmacokinetic data on voriconazole in transplant patients exists in the literature. To date this is the first study to evaluate the pharmacokinetics of voriconazole in liver transplant patients.

This study involved intense blood sampling (nine data points from each patient in a single dosing interval) in the immediate post-transplant period (within seven days) in a small group of relatively homogenous liver transplant patients (n = 15), which allowed accurate and precise parameter estimation. The pharmacokinetic profiles of voriconazole are characterized by an early and sharp increase of voriconazole concentration, with the peak concentration being reached around 1 to 2 hours after dosing. These profiles were consistent with rapid absorption of voriconazole. This observation is similar to what has been reported in non-transplant patients (88, 89, 94).

The additive residual was close to the LLOQ of the assay used. CL/F and V/F estimated in this study were similar to those in non-transplant population (89). Nonlinear pharmacokinetics was not observed in this study (Michaelis-Menten elimination process did not improve the fitting). Evaluated internally in various ways, the final model showed adequacy and good stability and predictive performance.

Despite of the relative homogeneity of the population studied, a large inter-individual variability in voriconazole pharmacokinetics was demonstrated. This is in accordance with the large

variability in voriconazole pharmacokinetics previously reported (6, 10, 50, 63, 66, 80, 81, 96, 109, 114, 118, 122) and the unpublished preliminary data from our research group. The large variability observed in liver transplant patients may be explained by variations in absorption (gastrointestinal function, bile flow and food effect), elimination (liver function, saturated metabolism and *CYP2C19* polymorphism) and drug-drug interaction (CYP450 inhibitors/inducers).

It is important to identify patient factors that significantly contribute to this large inter- and intra-individual variability by exploring the correlations between pharmacokinetic parameters (especially drug exposure) and patient variables. The covariates tested in this study covered a wide range of values within each of the categories tested. In the non-compartmental analysis, we have observed that patients with higher total bilirubin, international normalized ratio (INR) and AST, indicative of hepatic dysfunction and hepatocellulary injury, had higher voriconazole exposure characterized by lower λz (elimination rate constant), higher half-life, higher AUCo-∞ and lower CL/F. We have also identified a positive association between the post-operative time (POT) and voriconazole exposure characterized by increased half-life, Cmax, Clast, AUCo-∞, AUMCo-∞ and MRTo-∞. This suggested an increase in voriconazole exposure with increased time post-transplantation.

In the population analysis, we have also demonstrated that the most important factor associated with voriconazole pharmacokinetics was POT, which is consistent with non-compartmental analysis. CL/F, Vd/F and Tlag decreased rapidly and dramatically over POT and eventually converged to stable levels at some point around 7 days.

The primary reason for the increase in voriconazole exposure characterized by increased Cmax, Clast and AUCo-∞ and the decrease of CL/F and Vd/F with POT is likely to be increased bioavailability (F) with POT due to improved gastrointestinal function. Gastrointestinal complications have been observed after transplant surgery (5, 12, 56, 141) and gastrointestinal function recovers gradually with POT. Results in the study of lung transplant patients showed that voriconazole bioavailability was as low as 10% in the early period of time after lung transplantation, but increased over POT. Although metabolism and clearance may be increased after transplantation due to recovery in improved hepatic function, recovery in gastrointestinal function is likely to contribute to a greater extent, resulting in decreased CL/F. Furthermore, improved gastrointestinal function is also likely to be the reason for the decrease of Tlag with POT. In addition, increased bile production and secretion may also contribute to an increased bioavailability and decreased Tlag over POT.

The secondary reason for a decreased CL/F with POT may be a decreased unbound fraction in the blood (fu) caused by increased plasma protein synthesis with recovered hepatic function. Voriconazole is a low- to intermediate-clearance drug. Voriconazole clearance is highly variable in different studies from 15 to 35.25L/hr (48, 63, 88, 96, 102, 118), and oral clearance varies from 8.1 to 23.4L/hr (61, 95, 101). Therefore the hepatic extraction ratio should range from 0.09 to 0.39. For a low clearance drug $Cl_{apparent} \approx \mathrm{fu} \times \mathrm{Clint}$ (fu denotes fraction unbound. Clint denotes intrinsic clearance). Therefore, a decreased fu over POT could lead to a decreased clearance. Considering the great extent of the change in CL/F, a decreased fu alone may not contribute to the change in CL/F with POT.

An increased plasma protein synthesis is unlikely to be responsible for a decreased Vd/F over POT. Voriconazole is highly lipophilic and extensively distributed into tissues (Vd=4.6L/kg), but is not very extensively bound to plasma proteins (fu>0.42) (94). Therefore the drug binding in tissues should be predominant in determining Vd rather than plasma protein binding, and therefore change in fu in the blood may contribute very little to a change in Vd/F. Resolution of ascites after the transplant surgery may be another possible explanation for decreased Vd/F over time.

Individual parameter estimates of CL/F, Vd/F and Tlag eventually converged to stable levels at some point around day 7. The reason is likely that the physiological factors that determine voriconazole pharmacokinetics (e.g. liver function, gastrointestinal function) were highly variable at the early period of time after the transplant surgery, but recovered and improved with POT towards normal population values.

Higher total bilirubin, INR, AST and ALT, indicative of hepatic dysfunction and hepatocellulary injury is associated with low elimination rate constant, long half life, high AUCo-∞ and low CL/F of voriconazole. The reason is very likely that voriconazole is extensively metabolized in the liver with less than 2% of the administered dose excreted unchanged in urine and faeces (89, 94, 103).

For a low clearance drug $Cl_{apparent} \approx \mathrm{fu} \times \mathrm{Clint}$ (fu denotes fraction unbound. Clint denotes intrinsic clearance). Clint depends on liver function of the patient. Therefore patients with higher

total bilirubin, INR, AST and ALT, indicative of hepatic dysfunction and hepatocellulary injury, had higher voriconazole exposure and lower CL/F.

The presence of *CYP2C19*2* alleles resulted in higher Cmax, Clast, AUMCo-∞ and MRTo-∞. This observation in this study is in accordance with recently published data in healthy volunteers (45, 69, 132). It has been reported that voriconazole exposure (AUC) is increased by 4-fold in poor metabolizers compared to homozygous extensive metabolizers. Nearly 15–20% of Asians and 3–5% of Caucasians are poor metabolizers (88, 89, 94). There is also an average 2-fold increase in exposure to voriconazole in heterozygous versus homozygous extensive metabolizers (88, 89, 94). The presence of deficient activity *CYP2C19*2* alleles resulted in higher Cmax, AUMCo-∞ and MRTo-∞. However, *CYP2C19* genetic analysis in this study did not include the newly identified excessive allele *17 (ultra-rapid metabolizer) (132), and only included the deficient alleles *2 and *3, which account for more than 85% of defective CYP2C19 alleles in Caucasians (18). Therefore the existence of excessive alleles and other defective alleles and thus misclassification of patients can not be ruled out.

The possible effect of race on voriconazole pharmacokinetics observed in this study has never been reported before. Asian patients seemed to have a higher CL/F and Vd/F and a slower absorption process than Caucasian patients characterized by higher Tmax and lower Cmax, but this remains to be further investigated.

In addition, the possible effect of co-administered pantoprazole on the exposure of voriconazole might be due to decreased absorption of voriconazole caused by proton pump inhibition since it

has been reported that pantoprazole causes no apparent induction or inhibition of cytochrome P450 enzyme systems (139). Pantoprazole sodium is a proton pump inhibitor (PPI) that covalently binds to the (H(+), K(+))-ATPase enzyme system at the secretory surface of the gastric parietal cell. This action suppresses the final step in gastric acid production and leads to inhibition of both basal and stimulated acid secretion. Pantoprazole produces extensive and long lasting inhibition of gastric acid secretion. PPI agents may reduce absorption of azoles by increasing gastric pH. However, this explanation is also questionable because significant decrease in voriconazole exposure due to decreased absorption caused by proton pump inhibition has never been reported. By contrast, a PPI agent omeprazole has been reported to cause an increase in voriconazole exposure due to inhibition of metabolizing enzyme (89, 137). Therefore further investigation is required to make any conclusion on the effect of co-administered pantoprazole on the exposure of voriconazole.

Donor characteristics have been shown to have no effect on voriconazole pharmacokinetics in this study. If this observation is unbiased, current voriconazole dosing regimen in liver transplant patients without consideration of donor characteristics should be an adequate dosing strategy. However, it is important to point out that an exclusion of a factor does not necessarily mean that this covariate has no significant influence on the pharmacokinetic parameters, especially in this study with a small homogeneous group of patients in the immediate post-transplant period. Many reasons can lead to an exclusion of donor characteristics as a significant factor for voriconazole pharmacokinetics in this study. Firstly, some of the donor characteristics are not variable in the population studied. Secondly, simple linear regression is not the adequate model to assess the correlation between donor characteristics and voriconazole pharmacokinetics. Thirdly, some of

the donor characteristics may only have significant effects on voriconazole pharmacokinetics when their values are above (or below) a certain threshold value. If the values of these donor characteristics in this study were all below (or above) this threshold value, these donor characteristics would be excluded as a significant factor influencing voriconazole pharmacokinetics, no matter how variable this covariate is. Finally, some of the donor characteristics may only have significant effects on voriconazole pharmacokinetics when evaluated with interaction and co-effects with other patient/donor factors together. When evaluated alone without interaction with other factors, a significant factor could be identified as insignificant, which is a limitation of this study that will be discussed in the next sections. Therefore, further investigation on the effects of donor characteristics on voriconazole pharmacokinetics is required to make a conclusion.

The final model was externally validated using retrospectively collected random samples that were not used for model-building, which is considered to be the most rigorous validation method because the established model has to be able to predict completely new data. Although the patients in the validation group had measurements taken at longer POT, the final model was still able to provide accurate and precise concentration predictions, suggesting that the pharmacokinetic parameters remain relatively stable after 7 days post-transplant.

A large variability in voriconazole exposure following a fixed doing regimen necessitates individualizing voriconazole dosing to maximize therapeutic efficacy and minimize toxicity in liver transplant patients, especially considering that 33.3% of the patients in this study had a trough level below 1ug/ml. As mentioned previously, there is no simple efficacy measure to

which patient dose can be titrated, but there is a simple HPLC/UV assay available to monitor voriconazole levels. Therapeutic monitoring is currently performed in the routine clinical monitoring program at our institution with an intention to keep the trough concentration above 1ug/ml. However, trough plasma concentrations have never been documented as surrogate markers of voriconazole exposure in liver transplant recipients.

The good correlation (R^2=0.85) observed in this study between the trough voriconazole plasma concentrations and the corresponding AUCo-∞ indicates that trough voriconazole concentration is a good measure of voriconazole exposure (AUC) in patients.

A maximum a posteriori (MAP) Bayesian estimator was developed and evaluated using the model-building group in this study. Interestingly, the predictive performance of two-sample LSS was not always superior over one-sample LSS. This suggested that one-sample LSS, which are clinically more applicable, efficient, convenient and economical, might be sufficient for reasonable AUC estimation.

There are two main approaches to develop LSS: multi-covariate linear regression (MLR) and MAP Bayesian method that was used in this study. Compared to MLR, the LSS developed in this study has a number of advantages: (1) sampling times and dosage regimens are flexible as long as they are well recorded, which accommodates clinical constraints that dosage regimens are frequently changed and precisely timed sampling is difficult. (2) Covariates that significantly affect pharmacokinetics are included in the analysis, which is particularly important to transplant patients since the covariates could change dramatically from time to time. (3) The LSS can be

continuously updated by incorporating new data to the population parameter estimation, thus improving performance. (4) Bayesian forecasting allows prediction of several pharmacokinetic parameters simultaneously (eg. AUC, clearance, volume of distribution). (5) Full pharmacokinetic profiles and response can be readily simulated, which allows visual comparison, and doses can be calculated. (6) Atypical and typical pharmacokinetic profiles could both be relatively accurately and precisely predicted using the same model developed using MAP (Figure 12b and 12c), while LSS developed using MLR may not be useful for atypical profiles.

These findings are likely to be clinically relevant because it suggests that voriconazole dose should be relatively high immediately after transplantation, especially in patients with good liver function as measured by low total bilirubin, INR, ALT and AST, in order to avoid ineffectiveness of the prophylaxis/treatment and its consequences (fungal infections, especially invasive aspergillosis). Voriconazole dose should be then gradually reduced, especially in patients with poor liver function as defined by high AST, total bilirubin or INR, in order to avoid toxicity caused by high voriconazole exposure. Intravenous administration of voriconazole appears to provide adequate drug exposure in the study of lung transplant patients. Based on the simulations and observations in lung transplant patients, we recommend administration of an intravenous dose of 200mg during the first two days after transplant to avoid low exposure. On day 3, patients should receive either a high oral dose of 400mg or be continued on an intravenous dose of 200mg. Starting from day 4, patients should receive an oral dose of 200mg that appeared to be sufficient to maintain the voriconazole plasma concentrations between 1ug/ml and 6ug/ml due to the change of pharmacokinetic parameters with POT in order to avoid toxicity caused by high voriconazole exposure. However, since voriconazole is currently only given orally to liver

transplant patients at our institution and bioavailability was not able to be characterized in this study, further investigations are warranted in order to make detailed recommendation of optimal voriconazole dose regimen in liver transplant patients, and therapeutic drug monitoring is still necessary in liver transplant patients.

In conclusion, this study has demonstrated that there is a large inter-individual variability in the pharmacokinetics of voriconazole in liver transplant patients. A fixed dosing regimen leads to widely variable exposure of voriconazole in liver transplant patients and therefore is not optimal for voriconazole therapy for prophylaxis and treatment in liver transplant patients. Donor characteristics seem to have no significant influence on voriconazole pharmacokinetics, but further investigation is required due to the small number of subjects evaluated in this study. Voriconazole CL/F, Vd/F and Tlag decreased rapidly and dramatically with postoperative time and eventually converged to stable levels at some point within 7 days. Postoperative time and poor liver function are positively associated with voriconazole exposure and half-life, which may be useful for dosage adjustment. Poor liver function is associated with low CL/F. CL/F and Vd/F are not correlated with body weight, which does not support weight-based dosing strategy. Trough concentrations (target 1ug/ml – 6ug/ml) are good measure of voriconazole exposure (AUCo-∞), and should be used in practice to individualize voriconazole dosage. Limited sampling strategies developed using Bayesian approaches in this study have shown potential to accurately and precisely estimate voriconazole exposure with one or two blood samples and no rigid sampling time or dosage regimens required, but definitely required external validation before used in practice to individualize voriconazole dosage. Routine therapeutic drug monitoring for voriconazole is warranted. This evaluation will allow for an assessment of the

adequacy of the prophylactic regimen in achieving therapeutic drug concentrations in all subjects, and could potentially help identify patients at risk for extremes in voriconazole exposure.

Chapter IV Pharmacokinetics of Voriconazole in Lung Transplant Patients

4.1 Abstract

Objectives: To characterize the pharmacokinetics and bioavailability of voriconazole in adult lung transplant patients during early post-operative period, identify factors significantly associated with various pharmacokinetic parameters, and make recommendations for adequate dosing regimens.

Methods: Thirteen lung transplant patients received two intravenous infusions (6mg/kg, bid) immediately post-transplant followed by oral doses (200mg, bid) of voriconazole for prophylaxis. Blood samples (n=9/interval) were collected during one intravenous and one oral dosing interval from each patient. Voriconazole plasma concentrations were measured by HPLC. NONMEM was used to develop pharmacokinetic models, evaluate covariate relationships and perform Monte Carlo simulations.

Results: There was a good correlation (R^2=0.98) between AUCo-∞ and trough concentrations. A two-compartment model adequately described the data. Population estimates of bioavailability, clearance, Vc and Vp were 45.9%, 3.45L/hr, 54.7L and 143L. Cystic fibrosis (CF) patients exhibited a significantly lower bioavailability (23.7%, n=3) than non-CF patients (63.3%, n=10). Bioavailability increased with post-operative time and reached steady levels in about one week. Vp increased with body weight.

Conclusions: Bioavailability of voriconazole is substantially lower in lung transplant patients than non-transplant subjects, but significantly increases with post-operative time. CF patients exhibit significantly lower bioavailability and exposure of voriconazole, and therefore need higher doses. Weight-adjusted or fixed dosing regimens resulted in highly variable exposure of voriconazole. Voriconazole dose can be individualized based on trough concentrations as a good measure of drug exposure. Simulations demonstrated inadequacy of oral administration of voriconazole and adequacy of intravenous administration during the first post-operative day followed by oral doses.

4.2 Introduction

The bioavailability of voriconazole after oral administration is 96% in non-transplant population (89). However, gastrointestinal complications observed after transplant surgery (5, 12, 56, 141) may cause clinically significant lower bioavailability of voriconazole. Our observation of a large portion of the samples with no measurable plasma concentration or with a plasma concentration of less than 1ug/ml suggested that the absorption and bioavailability may be altered in transplant patients. Therefore it is important to understand bioavailability of voriconazole in transplant patients. However, to date, the bioavailability of voriconazole in transplant patients and the pharmacokinetics of voriconazole in lung transplant patients have not been reported.

Voriconazole is typically given as an intravenous infusion for the first day after lung transplantation and then given orally for prophylaxis at our institution, which provides an opportunity for studying the bioavailability of voriconazole in solid organ transplant patients.

We hypothesize that bioavailability of voriconazole in lung transplant patients is lower than that reported in non-transplant population due to decreased/variable GI function during the early post-operative time period and will improve with time. In order to test our hypothesis, we propose two specific aims:

Specific aim 1 will characterize bioavailability of voriconazole and evaluate the variability in bioavailability of voriconazole in lung transplant patients. Voriconazole plasma concentrations have been measured in 13 lung transplant patients within one intravenous infusion dosing

interval and one oral dosing interval after transplantation. Non-compartmental pharmacokinetic analysis and nonlinear mixed effects modeling analysis will be performed to estimate the bioavailability, and to capture both inter-patient and intra-patient variability in the bioavailability estimate. We predict that the bioavailability estimated in lung transplant patients will be lower than that in non-transplant subjects, and there will be a large variability in the bioavailability estimate.

Specific aim 2 will evaluate the association of patient variables with bioavailability of voriconazole in lung transplant patients. Patient demographic variables have been collected for each patient, and their association with the bioavailability of voriconazole will be evaluated using simple linear regression in non-compartmental analysis and evaluated as a covariate in population pharmacokinetic analysis. We predict that bioavailability will be associated with post-operative time due to gradually recovered GI function after the transplantation surgery.

4.3 Methods

4.3.1 Patients

The protocol was approved by IRB at the University of Pittsburgh. Lung transplant recipients who were initiated on a voriconazole prophylactic regimen immediately post transplant as part of their standard clinical care and who signed informed consent were enrolled in this prospective study. Two intravenous doses were administered first as a 2-hour intravenous infusion (6mg/kg, bid) followed by oral doses (200mg, bid) for a duration of 3 months post transplant. The exclusion criteria were: children under age 18; co-administration of medications known to influence voriconazole pharmacokinetics; administration of voriconazole to treat an active fungal infection; pre-transplant voriconazole administration; or voriconazole dosing regimens other than that associated with fixed oral dosage. Complete dosing history, demographic data, laboratory tests and current medication use were recorded. All patients received tacrolimus as their primary immunosuppressive agent.

4.3.2 Blood Sampling and Analytical Assay

Serial blood samples (7ml) were collected within one intravenous and one oral dosing interval from each patient. The sampling time was just prior to (0 hr) and at 0.5, 1, 1.5, 2, 4, 6, 8 and 12 hours following the 2^{nd} intravenous dose and following administration of a minimum of 5 oral doses (range from the 5^{th} to 37^{th} dose; mean 15^{th} dose). Blood samples were processed and

analyzed for voriconazole plasma concentration using a validated HPLC method previously described.

4.3.3 Non-compartmental Pharmacokinetic Analysis

The difference between trough concentrations prior to oral dosing (Co) and at 12 hours after oral dosing (C12) was tested using paired two-tailed Student *t*-test to confirm attainment of steady state. Area under the plasma concentration-vs-time curve specific for the dose evaluated (AUCo-∞) was calculated using trapezoid rule and reverse superposition principle. Time to peak concentration (Tmax) and peak plasma concentrations (Cmax) were directly read off the concentration-vs-time profiles.

4.3.4 Population Pharmacokinetic Analysis

A nonlinear mixed-effects pharmacokinetic model (base model) was developed using NONMEM 6.2.0 (GloboMax, Hanover, MD) using first order conditional estimation method with interaction. Correlations between pharmacokinetic parameters were always incorporated and estimated. One- and two-compartment models were tested with first/zero-order elimination and Michaelis-Menten elimination process since nonlinear pharmacokinetics of voriconazole has been reported (89). Inter-individual variability was described using exponential model $P_{ij} = TV(P_j) \times e^{\eta ij}$, where Pij is the ith individual's estimate of the jth pharmacokinetic parameter, TV(Pj) is the typical value of the jth pharmacokinetic parameter, and ηij is a random variable for the ith

individual and the jth pharmacokinetic parameter distributed with mean zero and variance of ωj^2. Residual variability was described using combined error model $Cobs = Cpred \times (1+\varepsilon) + \varepsilon'$, where Cobs and Cpred are the observed and predicted concentrations, respectively, and ε and ε' are normal random variables with means of zero and variances of σ^2 and σ'^2, respectively. The adequacy of fitting was examined by plotting predicted versus observed concentrations (Goodness-of-Fit), concentrations versus time profiles and weighted residuals versus predicted concentrations.

4.3.5 Covariate Relationship Exploration

Association between patient variables and pharmacokinetic parameters were first visually evaluated by plotting Empirical Bayes Estimates (EBE) against patient variables. Patient variables were then incorporated into the base model one at a time using at least 13 approaches to associate the patient variable with the parameter. A patient variable was considered as significant if all the following criteria were met: (1) a decrease in objective function value (OFV) of 6.63 for 1 degree of freedom ($p<0.01$), (2) no significant trend in EBE-vs-patient variables plots, (3) improved Goodness-of-Fit, (4) reduced inter-individual variability and (5) clinical plausibility for incorporating the patient variable.

4.3.6 Monte Carlo Simulations

Voriconazole concentration-vs-time profiles in patients with and without CF (200mg, oral, BID) were simulated using NONMEM to illustrate that CF patients may exhibit significantly lower exposure of voriconazole than non-CF patients, and that CF patients may experience underexposure of voriconazole with trough concentrations of <1ug/ml. The simulation procedure is based on drawing random samples for each of the pharmacokinetic parameters from their statistical distributions reflecting inter-individual variability. Every random draw generates a parameter set that characterizes the pharmacokinetics of a "virtual" subject and is subsequently used to generate the concentration-vs-time profile of this "virtual" subject. A total of 1500 "virtual" CF subjects and 1500 "virtual" non-CF subjects were simulated using this procedure. This simulation ensemble closely matches the original population statistics. Concentration-vs-time profiles of the "virtual" populations were summarized and compared by their median and 5% and 95% percentiles (90% prediction interval). The width of the 90% prediction interval reflects the degree of inter-individual variability in the original population.

In order to illustrate voriconazole exposure under different clinical scenarios and thus make clinical recommendation of adequate dosing regimens, voriconazole concentration-vs-time profiles were simulated for five hypothetical dosing regimens (BID): oral administration only (200mg, 400mg, 600mg) or combined administration of two doses of a 2-hour intravenous infusion (6mg/kg) followed by oral administration (200mg, 400mg). 1500 "virtual" subjects were simulated for each regimen using the same procedure mentioned above. In addition, simulation of individual profiles was also performed using a fixed dose of 200mg or a body weight-adjusted dose of 3mg/kg, and compared with each other, in order to evaluate whether the

variability among the pharmacokinetic profiles was reduced by using a body weight-adjusted dose as compared to a fixed dose.

4.4 Results

4.4.1 Patients

A total of 13 patients were enrolled in this study. Table 8 summarizes the characteristics of the patients, including the primary diagnosis, age, body weight, race, gender, days post-transplant on the day of the oral study and laboratory biochemical profiles prior to transplant, immediately after transplant and on the day of the oral study. One patient did not complete the oral study.

Table 8. Characteristics of lung transplant patients

Diagnoses	
Cystic fibrosis	3
Emphysema	5
Idiopathic pulmonary fibrosis	4
Scleroderma	1
Patient Age (yr)	50.9 ± 16.1 (19-70)
Weight (kg)	68.0 ± 15.2 (46-91)
Ideal body weight (kg)	59.6 ± 8.2 (45.5-75.3)
Race (Caucasian/Other)	12/0
Gender (male/female)	7/6
Days post-transplant on the day of oral study	8.5 ± 4.4 (3-19)
Alkaline phosphatase (U/L) *	82.4 ±31.8 (54-169)
Alanine aminotransferase (U/L) *	30.3 ± 8.3 (22-52)
Aaspartate aminotransferase (U/L) *	28.1 ± 14.5 (20-75)
Gamma-glutamyl transpeptidase (U/L) *	35.1 ± 19.0 (15-71)
Serum Creatinine (mg/dL) †	0.78 ± 0.16 (0.5-1) / 0.85 ± 0.22 (0.5-1.1)
Creatinine Clearance (ml/min) †	85.6 ± 36.9 (55.6-177.8) / 85.7 ± 40.4 (40.5-177.8)

Values are all expressed as mean ± standard deviation (range) except specified otherwise.

* Values measured before the transplantation.

† Values are displayed as measurements within one day after the transplantation / measurements on the day of the oral study.

There was a wide variation in voriconazole plasma concentrations (Figure 13). Most voriconazole plasma concentrations (72.5%) were maintained within 1-6ug/ml, while 17.9% and 9.7% of voriconazole plasma concentrations were below 1ug/ml or above 6ug/ml, respectively.

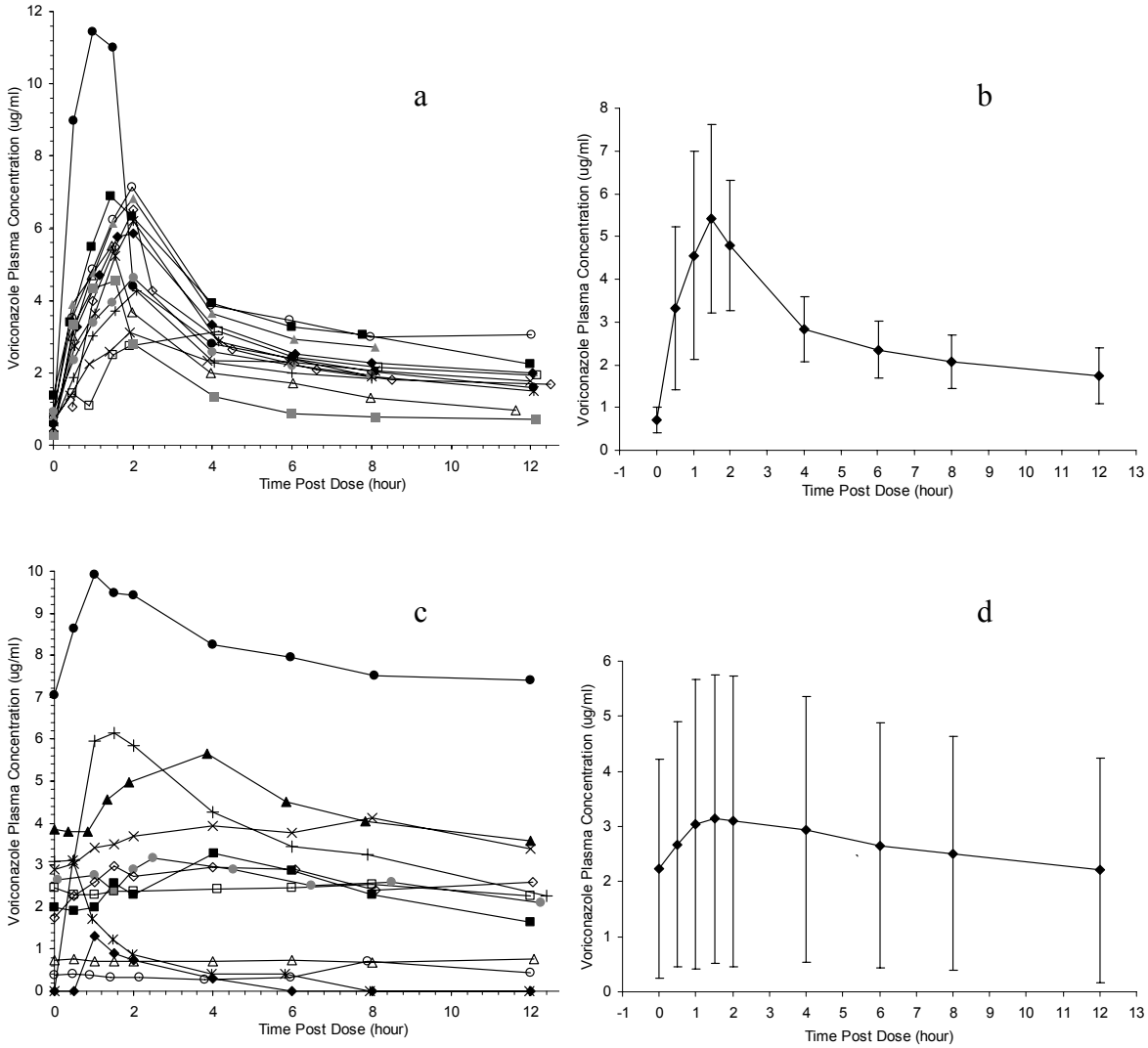


Figure 13. Plasma concentrations-vs-time profiles of voriconazole in lung transplant patients

a: individual plasma concentrations-vs-time profiles of voriconazole collected during an intravenous infusion dosing interval

b: mean plasma concentrations-vs-time profiles of voriconazole with standard deviation error bars collected during an intravenous infusion dosing interval

c: individual plasma concentrations-vs-time profiles of voriconazole collected during an oral dosing interval (one patient did not complete oral study)

d: mean plasma concentrations-vs-time profiles of voriconazole with standard deviation error bars collected during an oral dosing interval

4.4.2 Non-compartmental analysis

Trough concentrations Co and C12 were not significantly different (p=0.82), and the difference between the trough concentrations (C12-Co)/C12 averaged -2.7%, indicating that steady state had been reached in most of the patients at the time of the oral study (Figure 14).

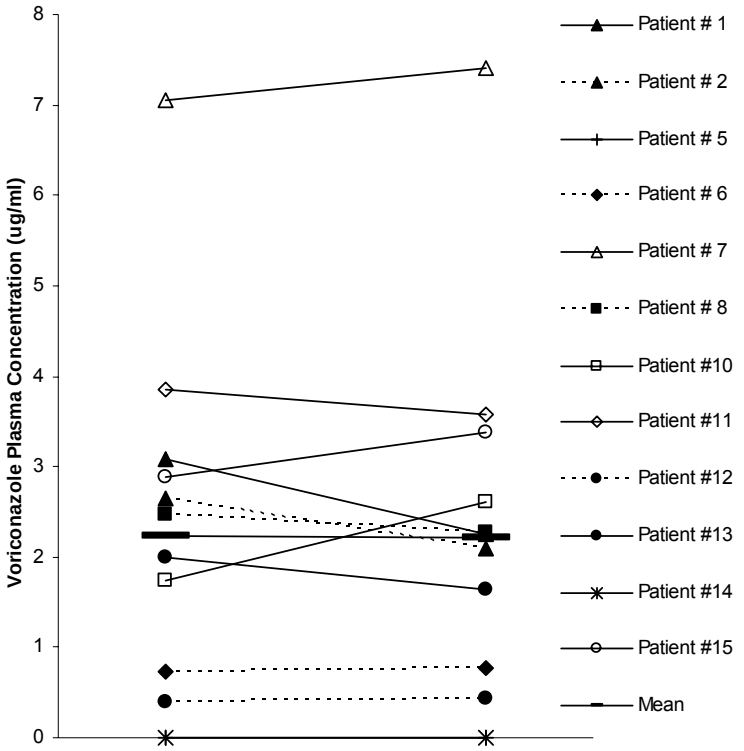


Figure 14. Comparison of trough concentrations in the oral study in lung transplant patients

Co (left) vs C12 (right)

Figure 15 illustrates a good correlation between voriconazole trough plasma concentrations and the corresponding AUCo-∞ both for intravenous infusion (non-steady state, R^2=0.86) and oral dose (steady state, R^2=0.98). Tmax (±SD) for oral dose was 1.9±1.3 hours. Cmax (±SD) for intravenous infusion and oral dose was 5.9±2.2ug/ml and 3.6±2.6ug/ml, respectively.

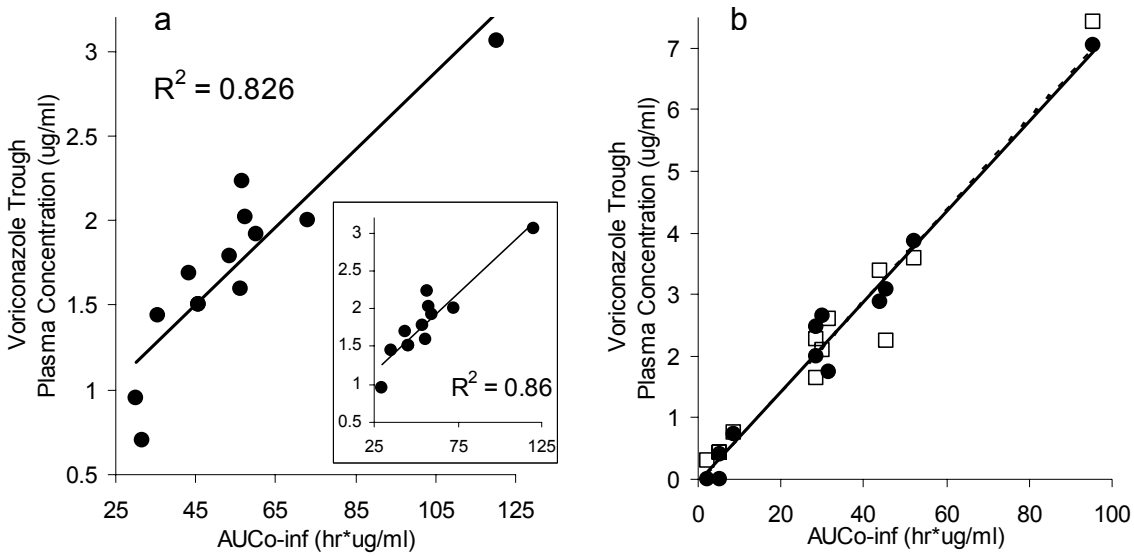


Figure 15. Correlation of AUCo-∞ with voriconazole trough plasma concentrations in lung transplant patients

a: R^2=0.83 when AUCo-∞ and trough concentrations (C12) were correlated (main figure) during an intravenous infusion dosing interval (non-steady state). R^2=0.86 when a potential outlier is omitted (inset figure). Two patients have very similar C12 and AUC and therefore can not be separated in the figure.

b: R^2=0.98 (dash line) and R^2=0.96 (solid line) when AUCo-∞ was correlated with trough concentrations Co (●) and C12 (□), respectively, during an oral dosing interval (steady state, one patient did not complete oral study).

4.4.3 Population Pharmacokinetic Analysis

A two-compartment model with first-order absorption and elimination adequately described the data. The population estimates (inter-individual) of bioavailability, clearance, volume of distribution of central compartment (Vc) and peripheral compartment (Vp), inter-compartment clearance (Q), and absorption rate constant (ka) were 45.9% (82.9%), 3.45L/hr (107%), 54.7L (78.4%), 143L (88.3%), 22.6L/hr (50.1%) and 0.591hr^{-} (115.2%). The proportional and additive residual variability was 0.31 and 0.49ug/ml, respectively. Individual predictions agreed well with observations (Figure 16). Weighted residuals were approximately normally distributed and were mostly within about 2 units of the null ordinate.

CL/F in lung transplant (7.52L/hr) patients was similar to that in liver transplant patients (7.92 L/hr). Vd/F in lung transplant patients (430.7 L) was significantly higher than that in liver transplant patients (248 L).

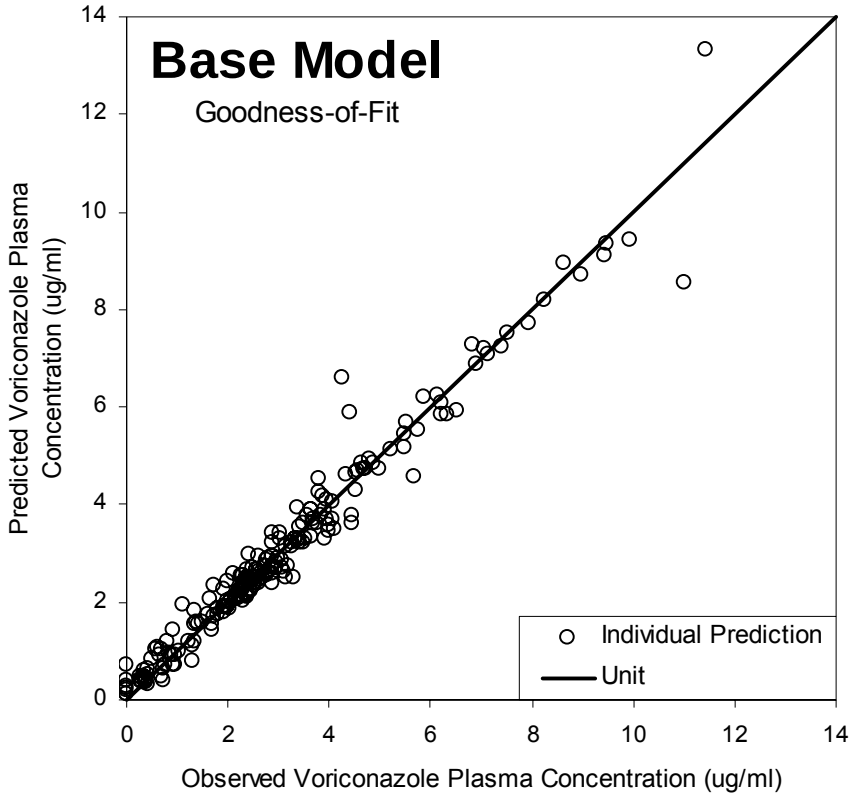


Figure 16. Goodness-of-Fit of base model in lung transplant patients

Individual predictions agreed well with observations (R^2=0.96).

Based on the individual estimates obtained from the base model, mean bioavailability (±SD) was 23.7% (±19.4%, n=3) and 63.3% (±15.2%, n=10) in CF and non-CF patients, respectively. Bioavailability was significantly lower in CF patients than non-CF patients (p=0.0032, two-tailed Student *t*-test).

4.4.4 Covariate Relationship Exploration

Model 1: Cystic Fibrosis (CF)

The most important patient variable associated with bioavailability was CF. OFV decreased by 11.65 from -47.55 (base model) to -59.20 when CF was incorporated in bioavailability, indicating substantial model improvement (p=0.0006). Inter-individual variability in bioavailability decreased by 30.7% from 82.9% (base model) to 57.5%, while inter-individual variability in other pharmacokinetic parameters did not change significantly.

The association between CF and bioavailability (F) was best described using the equation $F = F_{CF} + F' \times K_{non-CF}$ (Model 1), where F_{CF} denotes bioavailability of CF patients, F' denotes the difference in bioavailability between CF and non-CF patients, and $K_{non\text{-}CF}$=1 for non-CF patients and 0 for CF patients. Population estimates of F_{CF} and F' were 10.7% and 72%, respectively. Based on the model, bioavailability of voriconazole was significantly lower in CF patients (10.7%) than non-CF patients (82.7%) by 87%.

Model 2: Post-operative Time (POT)

Another important factor associated with bioavailability was POT. OFV decreased by 10.94 from -47.55 (base model) to -58.49 when POT was incorporated in bioavailability, indicating substantial model improvement (p=0.0009). The association between POT and bioavailability (F) was best described using the equation $F = \frac{F_{\max} \times POT}{POT + F_c}$ (Model 2), where Fmax denotes the

maximal bioavailability that can be reached in the patients in this study, and Fc is a constant. Inter-individual variability was incorporated both in Fmax and Fc and estimated. Population estimates (inter-individual variability) of Fmax and Fc were 61.9% (61.5%) and 1.97 hours (217.3%), respectively. Even the maximal bioavailability (61.9%) in lung transplant patient population was still much lower than that in non-transplant subjects (96%). The small value of Fc indicates that bioavailability would increase rapidly with POT.

According to the equation and individual parameter estimates obtained from Model 2, bioavailability of voriconazole significantly and rapidly increased with POT in most of the patients, and eventually reached maximal levels within one week post-transplant (Figure 17). Figure 17 also illustrates that bioavailability was significantly lower in CF patients than non-CF patients. The large variability demonstrated in Figure 17 is consistent with the large inter-individual variability in Fmax and Fc.

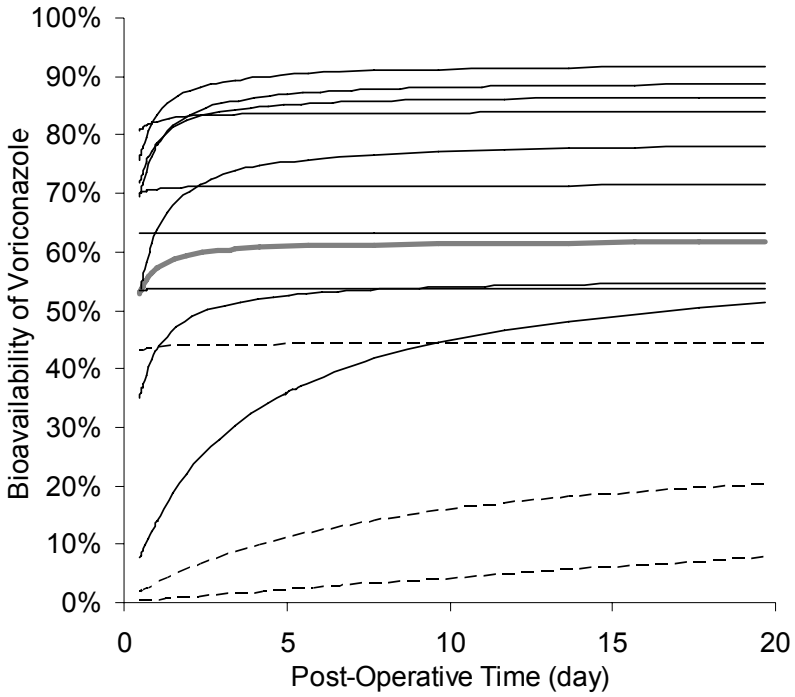


Figure 17. Change of bioavailability of voriconazole over post-operative time in lung transplant patients with and without cystic fibrosis

Individual parameter estimates of bioavailability obtained from Model 2 were plotted against post-operative time (POT). Bioavailability significantly and rapidly increased with POT in most of the patients, and eventually reached the maximal level within one week after transplant. Bioavailability was significantly lower in cystic fibrosis (CF) patients (dash line) than non-CF patients (solid line). Grey solid line: population estimates from Model 2.

Model 3: Body Weight (WT)

Vp significantly increased with WT. OFV decreased by 7.29 from -47.55 (base model) to -54.84 when WT was incorporated in Vp, indicating substantial model improvement (p=0.0069). Inter-

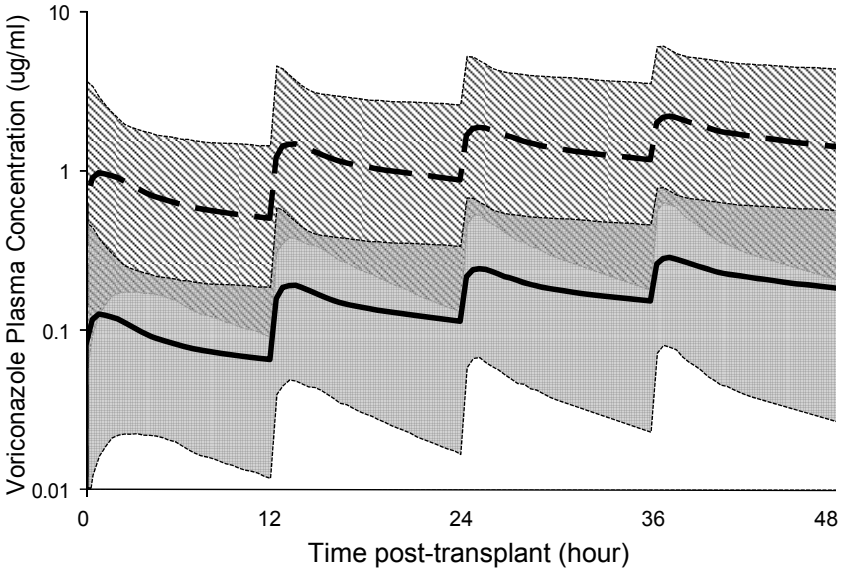
individual variability in bioavailability decreased by 31.9% from 88.3% (base model) to 61.2%, while inter-individual variability in other pharmacokinetic parameters did not change significantly. The association between Vp and WT was best described using the equation $Vp = TV(Vp) \times \left(WT \div \overline{WT}\right)^a$ (Model 3), where TV(Vp) denotes typical value of Vp in the patients in this study, i.e. the Vp in a patient with average body weight (68kg), and a is a constant to be estimated. Population estimates of TV(Vp) and a were 148L and 3.56, respectively.

4.4.5 Monte Carlo Simulations

Statistical distribution of pharmacokinetic parameters and inter-individual variability obtained from Model 1 (see above) was used to simulate CF and non-CF "virtual" subjects (Figure 18a). Median voriconazole plasma concentration and median AUC were 6.7 times higher in non-CF patients than CF patients. Furthermore, 90% prediction interval of CF patients did not include the median concentration-vs-time profiles of non-CF patients, and vice versa. This indicates significantly lower exposure of voriconazole in CF patients than non-CF patients.

Ninety percent prediction interval of the entire concentration-vs-time profiles (including peak levels) in CF patients remain below 1ug/ml for the first three days post-transplant. 90% prediction interval of trough concentration remains below 1ug/ml for the first four days post-transplant. Median concentration-vs-time profile in CF patients remains below 0.5ug/ml for the entire duration of study. This indicates underexposure of voriconazole in CF patients with trough concentration of <1ug/ml in 90% of the patients during the first four days post-transplant. In addition, the large inter-individual variability is confirmed by wide 90% prediction intervals.

Statistical distributions of pharmacokinetic parameters and inter-individual variability obtained from Model 2 (see above) were used to simulate different dosing regimens. Median trough concentrations stay above 1ug/ml since the first loading dose and are maintained between 2 and 3ug/ml at steady state when patients receive two 2-hour intravenous infusions followed by oral doses (Figure 18b). In contrast, simulation with mere oral administration (BID) at 200mg, 400mg and 600mg results in median trough concentration below 1ug/ml for the first 3.5 days, 1.5 days and 1 day post-transplant, respectively. In addition, simulated individual profiles using a fixed dose of 200mg or a body weight-adjusted dose of 3mg/kg were compared with each other, and the variability among the pharmacokinetic profiles was not reduced by using a body weight-adjusted dose as compared to a fixed dose, which confirmed the adequacy of fixed oral dosing regimens.



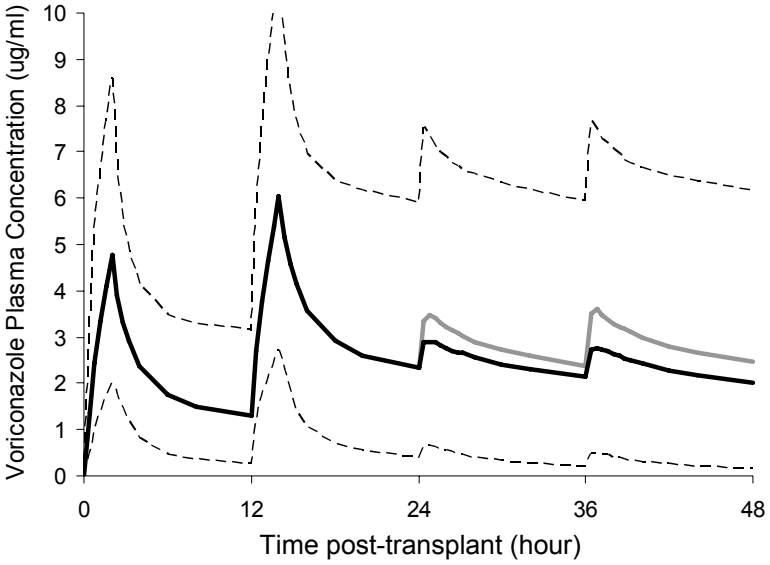


Figure 18. Monte Carlo simulation in lung transplant patients

a: simulated voriconazole concentration-vs-time profiles during first two days post-transplant in lung transplant patients with and without cystic fibrosis (CF). Median of simulated voriconazole concentration in CF patients (dash

line) and non-CF patients (solid line) with 90% prediction intervals of CF patients () and non-CF patients () is displayed. Extension of the profiles beyond two days post-transplant is not shown.

b: simulated voriconazole concentration-vs-time profiles (extended until steady state is reached) in lung transplant patients receiving two doses of 2-hour intravenous infusion (6mg/kg) followed by oral doses (BID). Median of simulated voriconazole concentration with intravenous infusion followed by oral dose of 200mg (black solid line) and 400mg (grey solid line) are compared. Only 90% prediction interval for intravenous infusion followed by oral dose of 200mg (dash line) is displayed.

4.5 Discussion

To date this is the first evaluation of bioavailability of voriconazole in transplant patients, and the first pharmacokinetic study of voriconazole in lung transplant patients.

Prospective intense sampling (nine samples per dosing interval) in early post-transplant period in a small group of relatively homogenous patients (n=13) was used in this study to provide accurate and precise parameter estimation. Oral pharmacokinetic profiles of voriconazole are characterized by an early and sharp increase of voriconazole concentration, with the peak concentration being reached around 2 hours after dosing. This observation is consistent with rapid absorption of voriconazole and similar to what has been reported in non-transplant patients (89). Despite the relative homogeneity of the population studied, a large inter-individual variability in voriconazole pharmacokinetics was demonstrated. This is consistent with previous reports (49, 118). Nonlinear pharmacokinetics was not observed in this study (Michaelis-Menten elimination process did not improve the fitting).

The large inter-individual variability in voriconazole exposure has given rise to concerns about voriconazole dose management in transplant patients, especially when it results in underexposure. Preliminary data showed that nearly 15.2% of transplant patients on recommended doses have undetectable trough concentrations, and nearly 45% of the patients have trough concentrations of <1ug/ml. Drug underexposure may be caused by decreased absorption or increased elimination. Elimination of voriconazole is determined by liver function and Cytochrome P450 polymorphism. Therefore elimination is unlikely to increase in lung transplant patients.

Therefore we hypothesized that decreased bioavailability is responsible for underexposure of voriconazole in transplant patients. Bioavailability of voriconazole is substantially lower in lung transplant patients during the early post-operative period (45.9%) in this study than that in non-transplant subjects (96%), likely due to gastrointestinal complications observed after transplant surgery (5, 12, 56, 141).

Furthermore, we demonstrated that bioavailability of voriconazole was significantly lower in CF patients than non-CF patients by 87%. It is typical that the mean bioavailability calculated using individual estimates of bioavailability obtained from base model (23.7% for CF patients and 63.3% for non-CF patients) were different from the population estimates in Model 2 (10.7% for CF patients and 82.7% for non-CF patients). Unlike the mean, the population estimate is the posterior mode of the marginal likelihood distribution for that parameter value versus the objective function (i.e. the maximum likelihood point in the distribution). If the distributions are not strictly normal (log normal is enough to skew this), the mean will not equal the mode.

Low voriconazole exposure observed in patients with CF in this study agree with the observations reported by Berge et al. (4) that voriconazole plasma concentrations were <0.5ug/ml in over 30% of CF lung transplant patients, and <1.5ug/ml in nearly 70% of the patients. However, the authors did not perform pharmacokinetic analysis to reveal the cause of underexposure since only trough and peak concentrations from therapeutic drug monitoring were obtained. Population pharmacokinetic analysis and Monte Carlo simulation in our study demonstrated that the reduced bioavailability in CF patients is the potential cause of underexposure.

CF is well known to cause malabsorption and reduced bioavailability of several highly lipophilic compounds, such as vitamin A, D, E and K (27), cyclosporine (124) and ibuprofen (38). Due to its high lipophilicity and low water-solubility, absorption of voriconazole highly depends on digestion of fat and the subsequent formation of micelle. However, this process is severely impaired in CF patients for many reasons. First of all, pancreatic insufficiency in CF patients causes impaired digestion of fat. Obstruction in the small pancreatic ducts in CF patients leads to decreased secretion of pancreatic enzymes (lipase) into the intestine, resulting in impaired lipolysis of dietary triacylglycerols. Furthermore, decreased secretion of pancreatic bicarbonate causes low duodenal pH, which considerably reduces pancreatic lipase activity. Secondly, a diminished bile salt pool causes impaired formation and absorption of micelle (31). Bile salts readily precipitate at low duodenal pH, and thus the duodenal bile salt concentration may fall below the critical micellar concentration. Furthermore, precipitated bile salts are not reabsorbed for the enterohepatic circulation, and therefore lost in great amount. Finally, intestinal mucosal dysfunction, alterations in the intestinal mucus layer (dehydration of the luminal surface and altered mucus secretion with distended crypts along the mucosal surface) and accelerated intestinal transit time in CF patients may also contribute to malabsorption of fat and highly lipophilic drugs such as voriconazole (24, 124). In addition, gastric acid hypersecretion in CF patients (14) may further lower duodenal pH, and thus further reduce pancreatic lipase activity and increase precipitation and loss of bile salts.

It is important to identify factors that significantly contribute to the large inter- and intra-individual variability of voriconazole in this population by exploring associations between

patient variables and pharmacokinetic parameters. The eleven patient variables tested in this study covered a wide range of values within each of the categories tested. Bioavailability increased rapidly over POT and reached maximal levels within one week in most of the patients, likely to be due to improved gastrointestinal function over POT. CL/F and Vd/F of voriconazole rapidly and dramatically decrease with POT in liver transplant patients. We propose increased bioavailability with POT as the primary reason, which is partly supported by this study.

A final model was also built as described previously (39). However, despite the statistically significant improvement of the final model and the covariate models (Model 1, 2 and 3) compared to base model, visual inspection of the Goodness-of-Fit plots of the final model and covariate models only showed a corrected bias of population predictions at low concentrations. This suggested that the patient variables tested and selected in this study (CF, POT and body weight) only explain part of the variability in the pharmacokinetics of voriconazole in lung transplant patients, while some other variables that were not collected in this study are still needed to account for the remaining variability. Future studies should collect more variables and further explore factors that are significantly associated with pharmacokinetics of voriconazole in lung transplant patients.

Vd/F in lung transplant patients (430.7 L) was significantly higher than that in liver transplant patients (248 L), likely due to different study design. Voriconazole was administrated intravenously in this study in lung transplant patients but not in the study of liver transplant patients. A two-compartment model is more likely to be observed following intravenous

administration, resulting in larger volume of distribution as compared to a one-compartment model, due to the addition of the peripheral compartment.

As discussed previously, the large variability in voriconazole exposure following weight-adjusted or fixed doing regimens necessitates individualizing voriconazole dosing to maximize therapeutic efficacy and minimize toxicity in lung transplant patients. Therapeutic monitoring is currently performed in the routine clinical monitoring program at our institution with an intention to keep the trough concentration above 1ug/ml. However, trough concentrations have never been documented as surrogate markers of voriconazole exposure in lung transplant patients.

The good correlation observed in this study between the voriconazole trough plasma concentrations and the corresponding AUCo-∞ both for intravenous infusion (non-steady state, R^2=0.86) and oral dose (steady state, R^2=0.98) indicates that trough concentration is a good measure of voriconazole exposure in this population.

These findings are likely to be clinically relevant. Based on Monte Carlo simulations, CF patients are very likely to experience underexposure of voriconazole and therefore need higher doses. Mere oral administration of voriconazole is likely to cause underexposure of voriconazole in lung transplant patients in early post-transplant period, while intravenous administration during the first post-operative day followed by oral doses is likely to result in appropriate drug exposure. However, therapeutic drug monitoring of voriconazole is still necessary in lung transplant patients due to the large inter-individual variability.

In conclusion, a population pharmacokinetic model was developed for voriconazole in lung transplant patients in early post-operative period. Large inter-individual variability in voriconazole pharmacokinetics was demonstrated. Bioavailability of voriconazole is substantially lower in lung transplant patients (45.9%) than non-transplant subjects (96%), but significantly increased with post-operative time, likely due to recovery of gastrointestinal functions. Exposure and bioavailability of voriconazole is significantly lower in CF patients, likely due to impaired absorption of voriconazole caused by physiological changes associated with CF. We recommend intravenous infusion (6mg/kg) during the first post-operative day followed by oral doses (200mg or 400mg) as an adequate dosing regimen in lung transplant patients. Given the large variability in the pharmacokinetics and the good correlation between AUC and trough concentrations, trough concentrations should be used to individualize voriconazole dose.

Chapter V Double-peak profiles of voriconazole in transplant patients

5.1 Abstract

Objectives: To apply the two-portion absorption model to describe atypical voriconazole profiles.

Methods: NONMEM (ADVAN5) was used to develop the simplified two-portion absorption model assuming discontinuous absorption of the available dose in two portions: F1 and F2 (F1+F2=1). Delayed transfer of each portion from the stomach to the gut and the sequential absorption was described by first-order processes with lag-times (Tlag1 and Tlag2) and transfer/absorption rate constants (ktra1 and ktra2). Precision of parameter estimation was evaluated using bootstrapping.

Results: Full pharmacokinetic profiles (8–9 samples/profile) with a single delayed wide peak or two peaks were observed in 23 transplant patients. A one-compartment model with first-order elimination in association with the simplified two-portion absorption model adequately described the data and showed superiority over one- and two-compartment models with an absorption lag time. The population estimates of F1, Tlag1, Tlag2, ktra1 and ktra2 were 0.27, 0.24 hours, 2.03 hours, 0.15 hr^{-1} and 0.004 hr^{-1}, respectively. Tlag1 was significantly smaller than Tlag2.

Conclusions: Atypical voriconazole pharmacokinetic profiles were probably caused by impaired gastrointestinal functions that are common in the early post-transplant period, and could be

reliably described by a simplified two-portion absorption model. Twenty-seven percent of the available dose seemed to be rapidly absorbed immediately, with the remainder being slowly absorbed. This model was useful to understand the mechanisms of atypical profiles of voriconazole and to improve estimation of voriconazole exposure in liver, lung and small intestine transplant patients.

5.2 Introduction

Double-peak phenomena of concentration-vs-time curves have been observed with a number of orally administered drugs, and some of them have been extensively investigated (7-9, 11, 22, 32, 37, 41, 58-60, 65, 71-73, 76, 90-92, 104, 107, 110, 115, 128, 133, 136, 140, 142). However, to date there have been no reports about such observations with voriconazole.

Five major physiological mechanisms have been hypothesized for double-peak profiles following oral administration. These include: (1) enterohepatic recirculation (84), (2) site-specific absorption of the drug from two distinct absorption sites along the gastrointestinal tract that are separated by a region of relatively low absorption (53), (3) active intestinal secretion of the drug from the systemic circulation into the gut lumen followed by a reabsorption of the secreted drug (exsorption) (62), (4) progressive solubilization of the drug along the gastrointestinal tract and its subsequent absorption (79), and (5) gastric retention of a portion of the drug dose due to delayed gastric emptying and/or variable gastrointestinal motility (76).

Enterohepatic recirculation and exsorption can be easily excluded as the cause of the secondary peak in oral voriconazole pharmacokinetic profiles since no secondary peak was observed following intravenous administration in lung transplant patients (10) or following oral administration in non-transplant populations (63, 109, 118). Furthermore, there is no evidence that a significant portion of an oral voriconazole dose is recovered in the bile in human subjects (63, 100, 109, 118). Site-specific absorption and progressive solubilization are also unlikely since the underlying physiological mechanisms of these two hypotheses are unlikely to differ

between transplant and non-transplant populations, and no secondary peak has been observed with the same formulation of voriconazole in non-transplant populations (63, 109, 118).

In contrast, gastric retention of a portion of the drug dose is the most likely reason for the double-peak phenomenon with voriconazole in solid organ transplant patients since delayed gastric emptying and decreased/variable gastrointestinal motility has been frequently observed after transplant surgery (5, 12, 56, 141). One of the earliest ideas of two-portion absorption was proposed by Suverkrup et al. (116), and further developed by Zimmerman et al. (143), Kaniwa et al. (54) and Oberle et al. (76). However, these models have a large number of parameters and could be overparameterized if the pharmacokinetic profiles are characterized by a limited number of samples. Instead of applying the general two-portion absorption model, a simplified version may be justifiable to model voriconazole double-peak profiles.

We hypothesize that the complex pharmacokinetic profiles of voriconazole with a single delayed wide peak or two peaks in solid organ transplant patients can be described and interpreted by discontinuous absorption caused by gastric retention a portion of the drug dose due to delayed gastric emptying and/or variable gastrointestinal motility. In order to test our hypothesis, we propose two specific aims:

Specific aim 1 will develop a two-portion absorption model. Complex pharmacokinetic profiles of voriconazole will be collected from our in-house database. Each individual profile will be fitted using the two-portion absorption model. A population model that simultaneously fits all the data will also be developed. Estimated parameters will be compared with corresponding

physiological parameters measured in healthy population. We predict that the two-portion absorption model will be adequate and versatile for describing both types of complex voriconazole pharmacokinetic profiles with either a wide delayed peak or two peaks. There will be a large variability in all the parameter estimates. The lag times of the two portions will be significantly different. The difference between the lag times for the two portions will be similar as or bigger than the normal time of the gastric emptying cycle. The transfer rate constants for the two portions will be smaller than the normal values of the gastric emptying rate.

Specific aim 2 will evaluate the superiority of the two-portion absorption model over conventional one- or two-compartment models with an absorption lag time. Conventional one- or two-compartment models with an absorption lag time will be developed to fit each individual profile and to simultaneously fit all the data, and their Goodness-of-Fit and adequacy will be compared with the two-portion absorption model. We predict that the two-portion absorption model will lead to a statistically significant lower objective function value (OFV) and values of akaike information criterion (AIC) and substantially improved Goodness-of-Fit and Concentration-vs-Time plots.

5.3 Methods

5.3.1 Patients and Data Collection

Atypical full pharmacokinetic profiles with a single delayed wide peak or two peaks were selected from our in-house database containing 48 patients. All patients with atypical profiles were chosen. All patients received voriconazole orally post transplant as part of their standard clinical care. All patients received no medications known to influence voriconazole pharmacokinetics and received tacrolimus as their primary immunosuppressive agent. Complete dosing record was obtained. Plasma voriconazole concentrations were measured using a validated HPLC method as previously described (50).

5.3.2 Simplified Two-Portion Absorption Model

The two-portion absorption model assumes negligible absorption from the stomach and discontinuous sequential absorption of the drug in two portions with the same absorption rate constant from the gut compartment. A fraction (F1) of the dose is transferred from the stomach to the gut first while the transfer of the remaining fraction (F2=1–F1) is delayed (Figure 19a). This delayed transfer of each portion was described by first-order processes with lag-times (Tlag1 and Tlag2 for each portion with Tlag1<Tlag2) and transfer rate constants (ktr1 and ktr2 for each portion). Once transferred into the small intestine, the dose was absorbed immediately

without any delay, which was described by first-order processes with an absorption rate constant (ka).

Given that voriconazole is highly lipophlic and rapidly absorbed (high ka) (63, 109, 118) while the gastrointestinal motility is low after transplant surgery (low ktr1 and ktr2), we assumed that the transfer of each portion of the drug dose from the stomach to the gut lumen is the rate limiting step. Under this assumption, ktr1 and ka were combined as ktra1, and ktr2 and ka were combined as ktra2 (Figure 19b). This simplification of the two-portion absorption model reduced the number of parameters to be estimated especially considering the limited number of samples available in this study.

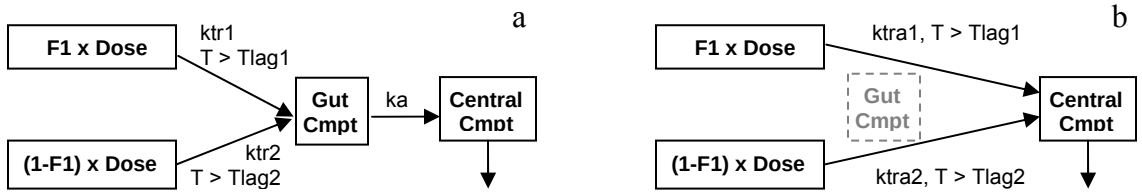


Figure 19. Scheme of the simplified two-portion absorption model

a: General two-portion absorption model. A first fraction (F1) of the dose is transferred from the stomach to the gut first while the transfer of the remaining fraction (F2=1–F1) is more delayed. This delayed transfer of each portion was described by first-order processes with transfer rate constants (ktr1 and ktr2 for each portion) and lag-times (Tlag1 and Tlag2 for each portion). Once transferred into the gut, the dose was absorbed immediately, which was described by first-order processes with an absorption rate constant (ka).

b: Simplified two-portion absorption model. ktr1 and ka were combined as ktra1 and ktr2 and ka were combined as ktra2 (see text). Otherwise the model is exactly the same as the general two-portion absorption model.

5.3.3 Individual Modeling

One- and two-compartment models associated with the simplified two-portion absorption model were developed to fit each individual profile using nonlinear regression as implemented in NONMEM 6.2.0 (GloboMax, Hanover, MD) using ADVAN5 subroutine with the following

seven basic pharmacokinetic parameters: the fraction of the first portion F1, the two transfer/absorption rate constants ktra1 and ktra2, and the two lag-times associated with the transfer of each portion Tlag1 and Tlag2 and the characteristic parameters of a one-compartment model. The individual nonlinear regression analysis was done to serve as a comparator to the nonlinear mixed effects population approach as well as provide initial estimates for the models. This sequential process facilitated the identification of the underlying candidate structural models.

5.3.4 Population Modeling

The population approach was then applied to develop a combined population model that simultaneously fitted all the double-peak profiles using First-Order Conditional Estimation with interaction methods in NONMEM. Correlations between pharmacokinetic parameters were always incorporated and estimated. Various inter-individual variability structures were tested including:

1. exponential model: $P_{ij} = TV(P_j) \times e^{\eta ij}$
2. proportional model: $P_{ij} = TV(P_j) \times (1 + \eta_{ij})$
3. additive model: $P_{ij} = TV(P_j) + \eta_{ij}$
4. log-additive model: $Log(P_{ij}) = Log(TV(P_j)) + \eta_{ij}$

where Pij is the ith individual's estimate of the jth pharmacokinetic parameter, TV(Pj) is the typical value of the jth pharmacokinetic parameter, and ηij is a random variable for the ith individual and the jth pharmacokinetic parameter distributed with mean zero and variance of ωj^2.

Various residual variability models were tested including:

1. additive error model: $Cobs = Cpred + \varepsilon$
2. proportional error model: $Cobs = Cpred \times (1 + \varepsilon)$
3. combined error model: $Cobs = Cpred \times (1 + \varepsilon) + \varepsilon'$
4. exponential error model: $Cobs = Cpred \times e^{\varepsilon}$
5. other error model: $Cobs = Cpred + \varepsilon \times Cpred^{\theta}$

where Cobs and Cpred are the observed and predicted concentrations, respectively, and ε and ε' are normal random variables with means of zero and variances of δ^2 and δ'^2, respectively.

5.3.5 Model Evaluation

The adequacy of fitting was examined by plotting predicted versus observed concentrations (Goodness-of-Fit), concentrations versus time and weighted residuals versus predicted concentrations. The simplified two-portion absorption model was compared to one- and two-compartment model with first-order absorption and an absorption lag time to examine its superiority in describing the atypical profiles. The decrease in objective function values (OFV) was referred to the chi-squared distribution to assess significance of improvement. Akaike

information criterion (AIC) was calculated as OFV plus two times the number of parameters and compared between the models.

In the population modeling process, precision of parameter estimation, stability of the final model and normality of the distribution of the parameter estimates was evaluated using bootstrapping (resampling repeated 2000 times) using Wings for NONMEM (http://wfn_sourceforge.net). Non-parametric statistics (median, 95% confidence interval) of parameter estimates obtained from bootstrapping were compared with the point parameter estimates obtained from the population model. The distribution of the parameter estimates obtained from bootstrapping was visually inspected for normality.

5.4 Results

5.4.1 Patients and Data Collection

Nine liver transplant, nine lung transplant and five small intestine transplant patients out of 48 patients manifested atypical pharmacokinetic profiles. In each profile, nine blood samples were taken just prior to (0 hr) and at 0.5, 1, 1.5, 2, 4, 6, 8 and 12 hours after dosing except for 3 small intestine transplant patients, whose samples at 1 hour were not scheduled. Profiles with a single delayed wide peak were manifested in one liver transplant and one lung transplant patient (numbered as Liver1 and Lung1 in Table 10), while all the other patients seemed to have double-peak profiles.

5.4.2 Individual Modeling

A one-compartment model associated with the simplified two-portion absorption model adequately described the individual profiles. In the two profiles with a single delayed wide peak, the estimated F1, Tlag1, Tlag2, ktra1 and ktra2 were 0, 0 hours, 5.99 hours, 1.63×10^{15} hr^{-1} and 0.18 hr^{-1} for the one liver transplant patient, and 0.496, 0.0001 hours, 6.06 hours, 0.004 hr^{-1} and 0.18 hr^{-1} for the one lung transplant patient.

Table 9 summarizes individual parameter estimates of the double-peak profiles. There was large inter-individual variability in all the parameter estimates. The estimated Tlag1 was significantly

smaller than Tlag2 in all the three types of transplant populations, which confirmed the discontinuous absorption and suggested that the transfer of the bigger portion (F2) from the stomach to the gut lumen is more delayed than the smaller portion (F1). The estimated ktra1 tended to be larger than ktra2 in all the three types of transplant, suggesting that the transfer of the bigger portion (F2) from the stomach to the gut lumen is slower than the smaller portion. All the parameter estimates were not significantly different between the three types of transplant populations.

Table 9. Summary of individual parameter estimates in patients representing voriconazole double-peak profiles using the simplified two-portion absorption model

	Liver (n=8)	Lung (n=8)	Small Intestine (n=5)	p †
F1	0.24 ± 0.20 (0.04 ~ 0.496)	0.23 ± 0.22 (0.01 ~ 0.499)	0.26 ± 0.18 (0.11 ~ 0.46)	0.9601
Tlag1 (hr)	0.31 ± 0.33 (0 ~ 0.93)	0.42 ± 0.40 (0 ~ 0.98)	0.74 ± 0.63 (0.001 ~ 1.43)	0.2564
Tlag2 (hr)	1.91 ± 1.33 (0.53 ~ 4)	3.78 ± 2.33 (0.85 ~ 7.7)	4.18 ± 3.35 (0.94 ~ 8.29)	0.1716
ktra1 (hr^{-1})	2.50 ± 5.32 (0.03 ~ 15.6)	2.36 ± 3.29 (0.14 ~ 8.25)	0.41 ± 0.5 (0.05 ~ 1.19)	0.5284
ktra2 (hr^{-1})	0.08 ± 0.06 (0.002 ~ 0.17)	0.26 ± 0.64 (0.004 ~ 1.83)	0.05 ± 0.04 (0.002 ~ 0.11)	0.5780
ΔTlag (hr)	1.29 (0.53 ~ 3.44)	3.41 (0.85 ~ 7.23)	1.36 (0.50 ~ 8.29)	0.24
p ‡	0.0024	0.0021	0.045	

F1: the fraction of the first portion. Therefore the other portion F2=1–F1

ktra1 and **ktra2**: the two transfer/absorption rate constants for each portion.

Tlag1 and **Tlag2**: the two lag-times associated with the transfer of each portion.

Δ**Tlag**: the two-portion interval (difference between the lag times for the two portions of the oral dose). ΔTlag=Tlag2–Tlag1. Values are displayed as median (range).

†: comparison of the parameter estimates between the three types of transplant populations. P value was obtained using ANOVA.

‡: p value that indicates whether Tlag1 is significantly larger than Tlag2 (one-tail Student t-test).

Values are all expressed as mean ± standard deviation (range) except for ΔTlag expressed as median (range).

5.4.3 Population Modeling

Based on the results obtained in the individual modeling process that all the parameter estimates were not significantly different between the three types of transplant populations, a combined population model that simultaneously fitted all the double-peak profiles was developed. A one-compartment model associated with the simplified two-portion absorption model adequately described all the data simultaneously. Inter-individual and residual variability was best described by an exponential model and a combined proportional and additive error model, respectively. The population estimates of F1, Tlag1, Tlag2, ktra1 and ktra2 were 0.27, 0.24 hours, 2.03 hours, 0.15 hr^{-1} and 0.004 hr^{-1}, respectively. These values were similar to that obtained during the individual modeling process. The large inter-individual variability agreed with that observed during individual modeling process. The population estimates of proportional and additive residual errors were 0.25 and 0.48ug/ml, respectively.

5.4.4 Model Evaluation

In the individual modeling process, most of the double-peak profiles were better described by the one-compartment model associated with the simplified two-portion absorption model compared to one- or two-compartment models associated with first-order absorption and an absorption lag time. The profiles with a single delayed wide peak were equally well described by the simplified two-portion absorption model and one- or two-compartment models. Compared to one- or two-

compartment model, the simplified two-portion model led to a statistically significant lower objective function value (OFV, Table 10) and akaike information criterion (AIC) values in most of the patients (not shown) and substantially improved Goodness-of-Fit and Concentration-vs-Time plots. Figure 20 represented typical profiles with a single delayed wide peak and two peaks in each type of transplant population, and showed successful prediction of the single delayed wide peak or the double peaks.

Table 10. Comparison of a one-compartment model associated with the simplified two-portion absorption model and one- and two-compartment model associated with an absorption lag time

	OFV			Change in OFV	
Patient *	Two-portion	1-CMPT	2-CMPT	Two-potion vs 1-CMPT †	Two-portion vs 2-CMPT ‡
Liver 1 **	-3.394	-3.394	-3.394	0 (p=1)	0 (p=1)
Liver 2	-44.904	-11.177	-11.177	33.727 (p<0.000001)	33.727 (p<0.000001)
Liver 3	-31.437	-0.747	-7.513	30.69 (p<0.000001)	23.924 (p<0.000001)
Liver 4	-52.169	-35.527	-35.527	16.642 (p<0.0009)	16.642 (p<0.00005)
Liver 5	-39.554	-26.001	-31.537	13.553 (p<0.0036)	8.017 (p=0.0047)
Liver 6	-47.172	-5.778	-6.047	41.394 (p<0.000001)	41.125 (p<0.000001)
Liver 7	-41.165	-20.563	-36.565	20.602 (p<0.0002)	4.6 (p<0.0320)
Liver 8	-59.929	-28.415	-38.748	31.514 (p<0.000001)	21.181 (p<0.000005)
Liver 9	-26.479	-11.746	-17.042	14.733 (p<0.0021)	9.437 (p<0.0022)
Lung 1 **	-66.82	-62.332	-62.332	4.488 (p<0.2134)	4.488 (p<0.0342)
Lung 2	-28.844	-9.311	-16.583	19.533 (p<0.0003)	12.261 (p<0.0005)

Table 10. (continued)

Lung 3	-23.065	-12.565	-18.705	10.5 (p<0.0148)	4.36 (p=0.0368)
Lung 4	-41.583	-22.454	-37.376	19.129 (p<0.0003)	4.207 (p<0.0403)
Lung 5	-34.369	19.489	11.97	53.858 (p<0.000001)	46.339 (p<0.000001)
Lung 6	-21.634	-17.143	-17.483	4.491 (p<0.2131)	4.151 (p<0.0417)
Lung 7	-32.414	-14.413	-16.292	18.001 (p<0.0005)	16.122 (p<0.0001)
Lung 8	-30.859	-13.508	-13.508	17.351 (p<0.0006)	17.351 (p<0.00005)
Lung 9	-26.048	-15.655	-22.388	10.393 (p<0.0156)	3.66 (p<0.0558)
SI 1	-35.472	-9.394	-14.696	26.078 (p<0.00001)	20.776 (p<0.00001)
SI 2	-22.612	-8.384	-14.727	14.228 (p<0.0027)	7.885 (p<0.005)
SI 3	-20.841	-11.113	-15.197	9.728 (p<0.0211)	5.644 (p<0.0176)
SI 4	-16.939	-9.353	-5.314	7.586 (p<0.0554)	11.625 (p<0.0007)
SI 5	-19.049	-8.844	-8.844	10.205 (p<0.0170)	10.205 (p<0.0014)
POP §	-68.792	-41.427	-55.019	27.365 (p<0.000005)	13.773 (p<0.0002)

OFV: minimum objective function value

Two-portion: one-compartment model associated with the simplified two-portion absorption model

1-CMPT: one-compartment model associated with first-order absorption and an absorption lag time

2-CMPT: two-compartment model associated with first-order absorption and an absorption lag time

*: patient was numbered with the type of transplant. Liver: liver transplant. Lung: lung transplant. SI: small intestine transplant.

**: these patients manifested profiles with a single delayed wide peak. All the other patients had double-peak profiles.

†: $OFV_{1-cmpt} - OFV_{two-portion}$. The change in OFV was referred to chi-square distribution for 3 degrees of freedom to calculate p value since the simplified two-portion absorption model had seven parameters while the one-compartment model had four.

‡: $OFV_{2-cmpt} - OFV_{two-portion}$. The change in OFV was referred to chi-square distribution for 1 degree of freedom to calculate p value since the simplified two-portion absorption model had seven parameters while the two-compartment model had six.

§: population model that simultaneously fits all the data

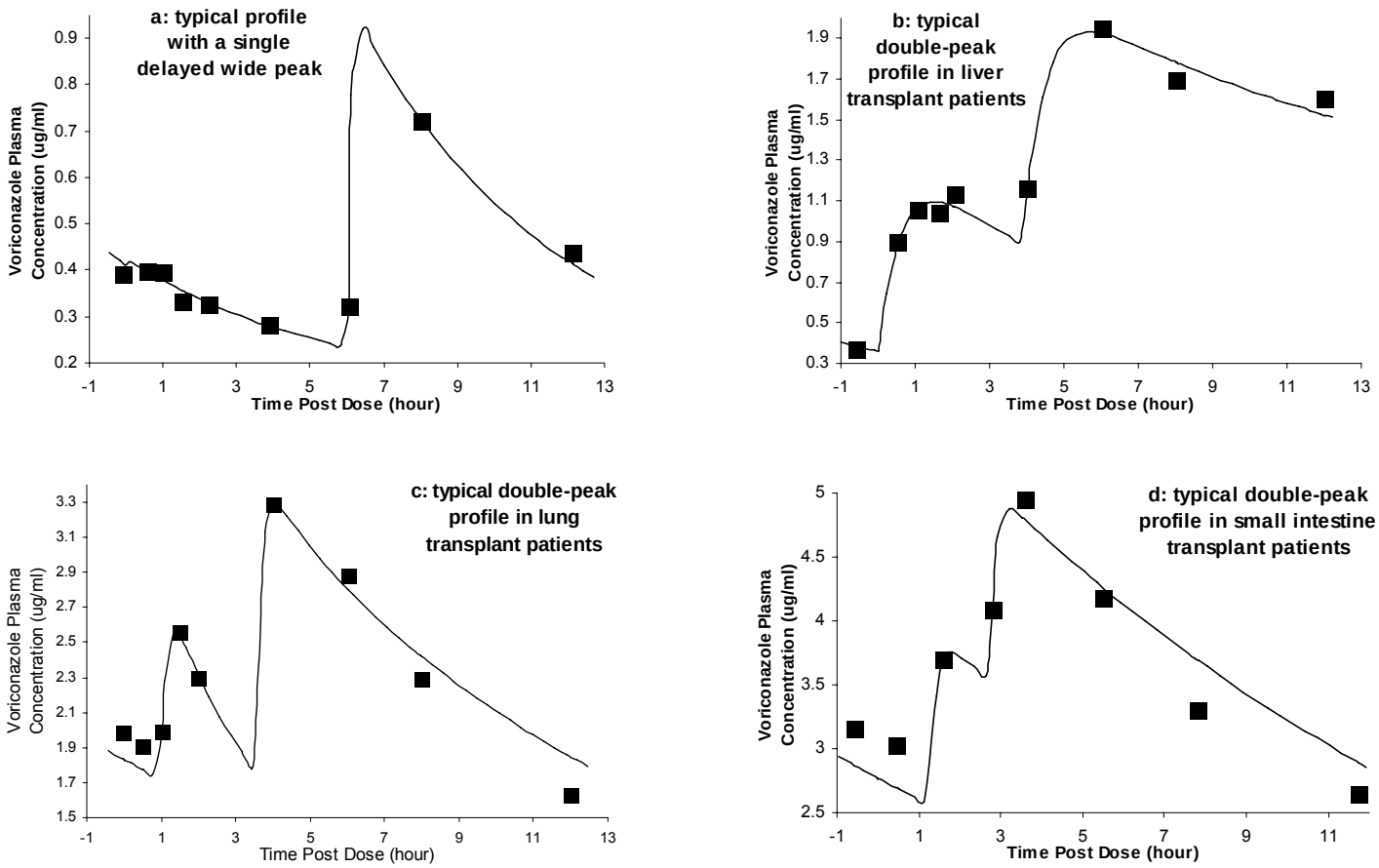


Figure 20. Typical posterior individual fittings

The curves represent the individual predictions in the individual modeling process (see text). The points represent observed concentrations.

a: Typical posterior individual fitting in a patient representing a profile with a single delayed wide peak

b: Typical posterior individual fitting in a liver transplant patient with a double-peak profile

c: Typical posterior individual fitting in a lung transplant patient with a double-peak profile

d: Typical posterior individual fitting in a small intestine transplant patient with a double-peak profile

In the population modeling process, a good correlation was observed between the individual predictions and the observations (R^2=0.98) using the simplified two-portion absorption model (Figure 21). LOWESS smoother showed no apparent bias. Weighted residuals were approximately normally distributed and were mostly within about 3 units of the null ordinate. The simplified two-portion absorption model yielded an OFV of -68.792, which was significantly lower than the OFV yielded using a one-compartment model (-41.427, $p<0.000005$) or two-compartment model (-55.019, $p<0.0002$) associated with first-order absorption and an absorption lag time (Table 10). The AIC obtained using the simplified two-portion absorption model (-52.792) was substantially lower than those obtained using a one-compartment model (-31.427) or two-compartment model (-31.019) associated with first-order absorption and an absorption lag time.

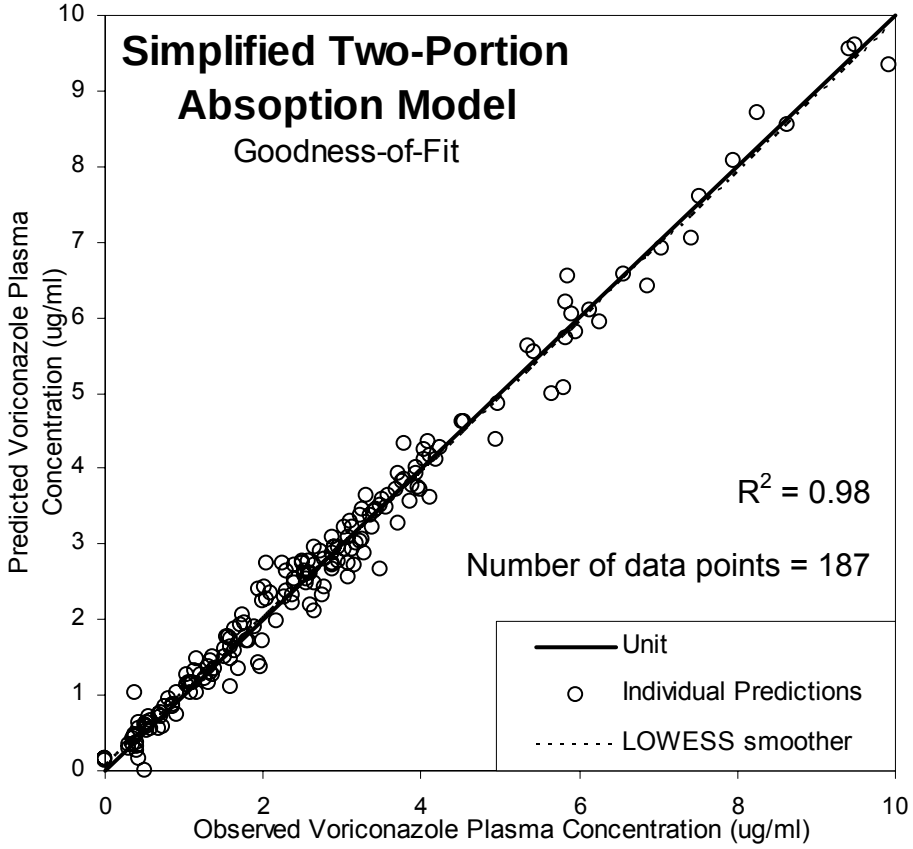


Figure 21. Goodness-of-Fit of individual predictions in population modeling process using the simplified two-portion absorption model

Individual predictions (hollow circle) agreed well with observations (R^2=0.98) without obvious bias indicated by the LOWESS smoother.

All these evaluations in the individual and population modeling process showed adequacy of the simplified two-portion absorption model in fitting voriconazole atypical profiles and confirmed the superiority of the two-portion absorption model over the other two models in describing voriconazole double-peak profiles.

In the bootstrapping analysis for the simplified two-portion absorption model, 916 runs successfully converged and were incorporated into the non-parametric analysis. The point population estimates of all parameters were similar to the median values obtained from bootstrapping and fell within the 95% confident intervals. The distribution of the parameter estimates obtained from the bootstrapping appeared to be normally distributed.

5.5 Discussion

This study is the first report on atypical profiles with a single delayed wide peak or a secondary peak following oral voriconazole administration in solid organ transplant patients (liver, lung and small intestine), although double-peak phenomenon has been reported and investigated with a number of orally administered drugs, such as cimetidine (60, 71-73, 76), ranitidine (72, 73, 107, 115, 140), cyclosporine (32, 104), alprazolam (133), talinolol (136), phenazopyridine (110), veralipride (91), valproate (7), celiprolol (65), furosemide (58), piroxicam (92, 128, 142), flurbiprofen (22), famotidine (59), furosemide (37), piretanide (8), danazol (11), acebutolol (90) and cephalosporins (9, 41).

The simplified two-portion absorption model developed in this study seemed to be versatile for describing both types of atypical voriconazole pharmacokinetic profiles with either a wide delayed peak or a secondary peak, confirming the usefulness of this model to describe atypical profiles of orally administered drugs especially double-peak profiles.

The underlying physiological mechanism for the two-portion absorption model is the cyclical and periodical nature of gastric emptying and gastrointestinal motility in the fasted state (76). During fasting, the upper gastrointestinal tract is cleared periodically following a cyclic pattern called migrating motility complex (MMC) (127, 138). The pattern is characterized by four phases. Phase I is a quiescent period (absence of motor activity) and lasts for 20–90 minutes. Phase II is an intermittent and irregular contraction process and lasts for 10–135 minutes. Then the strength of the contractions gradually increases, resulting in a short period of intense

contractions called Phase III, which is the activity phase and lasts for about 15 minutes in the stomach and 6 minutes in the duodenum. Then the intensity and frequency of contractions decreased (i.e. Phase IV), which is a transitional period from Phase III to the next Phase I, but Phase IV was only found occasionally. Gastric emptying is mainly associated with the active phase of MMC (Phase III).

The situation described by the two-portion absorption model happens when the drug dose is partially emptied in one MMC with the remainder being emptied in another MMC. In this case, the two-portion interval (difference between the lag times for the two portions of the oral dose, ΔTlag=Tlag2–Tlag1) should be similar to MMC periodicity (interval between Phase III activity fronts in two successive MMC cycles ranging from 1.3–2.5 hours (21, 29, 30, 47, 57, 127)).

In the double-peak profiles in this study, the two-portion interval (ΔTlag=Tlag2–Tlag1) ranged from 0.5–8.29 hours. The small intestine transplant patients had the largest range (0.5–8.29 hours). This range was substantially larger than the range of MMC periodicity in healthy subjects (1.3–2.5 hours), suggesting more variable gastric emptying and gastrointestinal motility in solid organ transplant patients, especially in small intestine transplant patients. In the patients with moderate ΔTlag within the range of healthy subjects (1.3–2.5 hours) in this study, the two portions of the dose may be transferred during two consecutive MMC cycles. In the patients with small ΔTlag of less than 1.3 hours in this study, the two portions of the dose may be transferred within one MMC cycle instead of over two consecutive MMC cycles in these subjects. One portion might be emptied in phase I or phase II with the remainder being emptied in phase III. There may be two explanations for the patients with large ΔTlag of greater than 2.5 hours. The

two portions of the dose could be transferred either during two inconsecutive MMC cycles, or during two consecutive MMC cycles that could be substantially longer in solid organ transplant patients than healthy subjects.

In healthy subjects, the transfer rate constants from the stomach to the gut lumen (ktr1 and ktr2) should be similar to the gastric emptying rate even in the situation of discontinuous absorption (140). Gastric emptying of non-nutrient liquids is approximately a first-order process (29, 57) although it is variable (13) and may depend on volume. The half-life of liquids during gastric emptying is 9–40 minutes (3, 43) depending on volume (43, 70). Therefore the gastric emptying rate should be approximately 1.04–4.62 hr^{-1} in healthy subjects.

In this study, the transfer/absorption rate constants ktra1 and ktra2 should be approximately equal to the gastric emptying rate in those patients since the gastric emptying is the rate limiting step as previously discussed. The values of ktra1 and ktra2 estimated in this study were basically smaller than the gastric emptying rate in healthy subjects (1.04–4.62 hr^{-}). ktra1 was within this range only in 1 subject while ktra2 was within this range only in 4 subjects. This suggested decreased/variable gastric emptying and gastrointestinal motility in solid organ transplant patients as the cause the double-peak profiles.

The five parameters in the two-portion absorption model (F1, Tlag1, Tlag2, ktra1 and ktra2) have different impact on the maximum concentration of each peak, appearance of the secondary peak and the degree of separation between the two peaks, which has been discussed in details by Yin et al. (140).

Based on the underlying physiological mechanism of the two-portion absorption model and the discussion above, atypical profiles of voriconazole are more likely to be observed in the early post-transplant period after transplant surgery since recovery of gastrointestinal function takes place gradually with time after transplant. In this study, the post-operative time (POT) ranged from 1.5 to 5 days with a median POT of 2 days in the liver transplant patients, ranged from 3 to 18.75 days with a median POT of 7 days in lung transplant patients, and ranged from 12 to 1279 days with a median POT of 91 days in small intestine transplant patients. This suggested that the frequency of atypical profiles is high in the first two weeks after transplant surgery in liver and lung transplant patients, but they can be observed in both early and late post-transplant periods in small intestine transplant patients. However, profiles from the late post-transplant period greater than 19 days were not available in liver and lung transplant patients in this study. Therefore the association between POT and the frequency of voriconazole atypical profiles should be further investigated.

In addition to the physiological changes of gastrointestinal function in transplant patients, the physical properties of voriconazole could also contribute to the discontinuous absorption. The active substance, voriconazole, (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1,2,4-triazol-1-yl)butan-2-ol (formerly known as UK-109496), is a weak base and classified as a low solubility, high permeability compound (25) (Class II in the Biopharmaceutical Classification System) with a predicted water solubility of only 98ug/ml (23). All the patients in this study took voriconazole tablets, which could be incompletely dissolved. The discontinuous

dissolution of the ingested dose could also contribute to the discontinuous absorption as liquids and solids have different kinetics through the gastrointestinal tract.

In conclusion, the atypical profiles with a single delayed wide peak or two peaks and abnormal absorption of orally administered voriconazole in liver, lung and small intestine transplant patients were probably caused by impaired and variable gastrointestinal motility that is common in the early post-transplant period. These profiles were reliably described using a simplified two-portion absorption model. Approximately 27% of the available dose seemed to be rapidly absorbed shortly after dosing with the remainder being slowly absorbed. Since appropriate pharmacokinetic models are necessary to provide physicians with convenient limited sampling strategies to estimate voriconazole exposure, the model developed in this study for voriconazole could be useful to improve estimation of voriconazole exposure and therefore contribute to development of limited sampling strategies in liver, lung and small intestine transplant patients. Future studies with a large sample size are warranted to validate the model and make the model clinically applicable.

Chapter VI Pharmacokinetics of Voriconazole in Pediatric Bone Marrow Transplant Patients

6.1 Abstract

Objectives: To characterize the pharmacokinetics of voriconazole in pediatric bone marrow transplant patients during pre-operative and early post-operative period and identify patient factors significantly associated with various pharmacokinetic parameters.

Methods: Pediatric bone marrow transplant patients who received voriconazole before and immediately after transplant for prophylaxis were recruited. The pre-transplant dosing regimen consisted of oral dose only. The post-transplant dosing regimen consisted of intravenous infusions followed by oral dose. The initial dose was 6mg/kg twice daily and was adjusted based on therapeutic drug monitoring. Blood samples (n=8/interval) were collected during one pre-transplant oral dosing interval, one post-transplant intravenous dosing interval and one post-transplant oral dosing interval from each patient. Voriconazole plasma concentrations were measured by HPLC. NONMEM was used to develop pharmacokinetic models.

Results: Eleven pediatric bone marrow transplant patients were recruited. A two-compartment model adequately described the data. There was a good correlation (R^2=0.94) between AUCo-∞ and trough concentrations. Population estimates of bioavailability, clearance, Vc and Vp were

46.5%, 5.76L/hr, 19.4L and 58.8L. Clearance significantly decreased with increased indices of liver function tests. Volume of distribution significantly increased with body weight. Bioavailability significantly decreased with decreased indices of liver function tests. Bioavailability of voriconazole is similar between lung transplant and pediatric bone marrow transplant patients. Compared to liver transplant and lung transplant patients, pediatric bone marrow transplant patients had significantly higher apparent oral clearance and significantly lower volume of distribution.

Conclusions: Bioavailability of voriconazole is significantly lower in pediatric bone marrow transplant patients than non-transplant adult subjects. Incorporation of patient variables associated with the pharmacokinetics of voriconazole may assist in optimizing the dosage regimen of voriconazole in pediatric bone marrow transplant patients. Voriconazole levels should be monitored and the dose can be individualized based on trough concentrations as a good measure of drug exposure.

6.2 Introduction

Due to neutropenia, graft-versus host disease and chronic immunosuppression, infections are common life-threatening complications in bone marrow transplant (BMT) patients [14]. In particular invasive fungal infections [15-17], such as invasive aspergillosis can be life threatening [18]. As part of standard clinical care, voriconazole is administered to BMT patients orally for several weeks prior to the transplantation, intravenously for several weeks after the transplantation, and then orally for several months for prophylactic purpose at our institution. This provides an opportunity for studying the bioavailability of voriconazole and the impact of transplantation on the pharmacokinetics of voriconazole in BMT patients.

We hypothesize that use of a weight-adjusted or fixed dosing regimen of voriconazole will lead to a large degree of variability in drug exposure among BMT patients due to variability in absorption and elimination caused by physiological characteristics unique to BMT patients. In order to test our hypothesis, we propose three specific aims:

Specific aim 1 will characterize the pharmacokinetics of voriconazole, evaluate the variability in the pharmacokinetics of voriconazole and compare the pharmacokinetics of voriconazole of pediatric BMT patients with non-transplant adult subjects. Voriconazole plasma concentrations was measured in 11 BMT patients following administration of one oral dose prior to the transplantation, and one intravenous dose and one oral dose after transplantation. Non-compartmental pharmacokinetic analysis and nonlinear mixed-effects modeling analysis was performed to estimate the pharmacokinetic parameters, and to capture both inter-patient and

intra-patient variability in the pharmacokinetic parameter estimates. We predict that the pharmacokinetic parameters estimated in BMT patients will be different from non-transplant adult subjects, especially the bioavailability will be lower and there will be a large variability in the pharmacokinetic parameter estimates.

Specific aim 2 will evaluate the association of patient variables with the pharmacokinetics of voriconazole in BMT patients. Patient-specific demographic variables, liver and renal function tests and blood counts will be collected from each patient, and their association with the pharmacokinetic parameters of voriconazole will be evaluated as a covariate in population pharmacokinetic analysis. We predict that low clearance or CL/F will be associated with poor liver function, and volume of distribution will be associated with body weight.

6.3 Methods

6.3.1 Patients

The protocol was approved by IRB at the University of Pittsburgh. Pediatric bone marrow transplant recipients who were initiated on a voriconazole prophylactic regimen several weeks prior to the transplantation and immediately post transplant as part of their standard clinical care and who signed informed consent were enrolled in this prospective study. The pre-transplant dosing regimen consisted of oral dose only. The post-transplant dosing regimen consisted of intravenous infusions followed by oral dose for several months. The initial dose was 6mg/kg twice daily and adjusted based on therapeutic drug monitoring. The exclusion criteria were co-administration of medications known to influence voriconazole pharmacokinetics or administration of voriconazole to treat an active fungal infection. Complete dosing history, demographic data, laboratory tests and current medication use were recorded. All patients received tacrolimus or cyclosporine as their primary immunosuppressive agent.

6.3.2 Blood Sampling and Analytical Assay

Serial blood samples (1.2ml) were collected from each patient just prior to (0 hr) and at 0.5, 1, 1.5, 2, 4, 6 and 12 hours after the dose of voriconazole on three separate phases (Figure 22 and Table 11). The first phase was performed approximately within a month pre transplant. The second phase was anticipated within the first few days after transplant when the subject appears

to be clinically stable and is on intravenous voriconazole. The third phase was anticipated to be within a few days after the second phase, when the patient is on oral therapy. Blood samples were processed and analyzed for voriconazole plasma concentration using a validated HPLC method previously described (51). The assay precision (intraday variability) was 1.3% to 9.0% (0.2 – 9 ug/ml), and the assay bias (interday variability) was 0.7% to 3.1% (0.5 – 9 ug/ml). The linearity range was 0.2 – 9 ug/ml ($R^2 = 0.9998$).

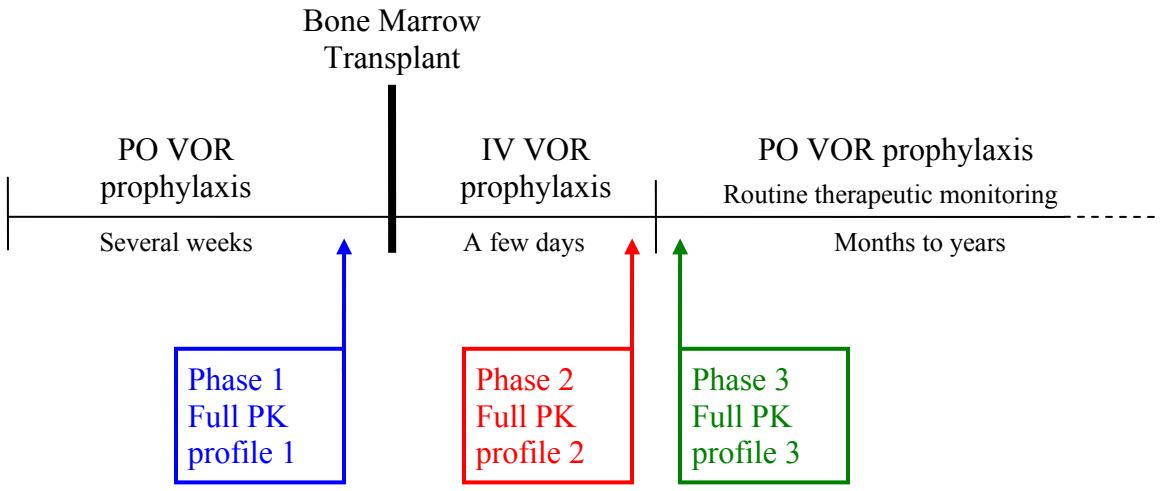


Figure 22. Study design and sampling scheme of the pharmacokinetic study of voriconazole in pediatric bone marrow transplant patients

VOR: voriconazole, IV: intravenous, PO: oral, PK: pharmacokinetic

Table 11. Study design and sampling scheme of the pharmacokinetic study of voriconazole in pediatric bone marrow transplant patients

	Phase 1	Phase 2	Phase 3
Route	Intravenous	Intravenous	Oral
Before/after transplant	Before transplant	After transplant	
Ideal time	After several doses, or as late as possible	As late as possible during the intravenous dosing period	As soon as possible after change from intravenous dosing to oral dosing
Justification of the ideal time	To make sure the steady state is reached	To minimize confounding between occasion 2 and 3 so that bioavailability can be adequately calculated	
Blood sampling	8 samples (total 9.6 ml blood)	8 samples (total 9.6 ml blood)	8 samples (total 9.6 ml blood)

6.3.3 Non-compartmental Pharmacokinetic Analysis

The difference between trough concentrations prior to dosing (Co) and at 12 hours after dosing (C12) was tested using paired two-tailed Student t-test to confirm attainment of steady state in

each phase. Time to peak concentration (Tmax) and peak plasma concentrations (Cmax) were directly read off the concentration-vs-time profiles. Various pharmacokinetic parameters were calculated using non-compartmental analysis with WinNonlin software (version 4.1; Pharsight Corporation, Mountainview, CA). The parameters calculated included terminal disposition rate constant (λz), terminal disposition halflife (t1/2), area under the plasma concentration-vs-time curve (AUC), apparent systemic clearance over bioavailability (CL/F), apparent steady state volume of distribution over bioavailability (Vd/F), and time to reach peak concentration (Tmax). λ z and t1/2 were derived from data points during the terminal disposition phase only when at least three data points were available, and the AUCo-∞ specific for the dose evaluated was calculated using trapezoid rule and reverse superposition principle. Projected trough voriconazole plasma concentrations (Clast) was used if the first plasma concentration at 0 hours (Co) were not taken within 5 minutes before dosing or the last plasma concentration at 12 hours (Clast) were not taken at exactly 12 hours. Each of these parameters is presented as mean and standard deviation. Statistical comparison of different parameters was made using paired two-tailed Student t-test (SPSS software, Windows-based version 14.0, Chicago, IL). A P value of <0.05 was considered statistically significant.

6.3.4 Population Pharmacokinetic Analysis

A nonlinear mixed-effects pharmacokinetic model (base model) was developed using NONMEM 7.1.0 (GloboMax, Hanover, MD) using first order conditional estimation method with interaction. Correlations between pharmacokinetic parameters were always incorporated and estimated. One- and two-compartment models were tested with first/zero-order elimination and Michaelis-

Menten elimination process since nonlinear pharmacokinetics of voriconazole has been reported (88, 89). Inter-individual variability was described using exponential model $P_{ij} = TV(P_j) \times e^{\eta ij}$, where Pij is the ith individual's estimate of the jth pharmacokinetic parameter, TV(Pj) is the typical value of the jth pharmacokinetic parameter, and ηij is a random variable for the ith individual and the jth pharmacokinetic parameter distributed with mean zero and variance of ωj2. Residual variability was described using combined error model $Cobs = Cpred \times (1 + \varepsilon) + \varepsilon'$, where Cobs and Cpred are the observed and predicted concentrations, respectively, and ε and ε' are normal random variables with means of zero and variances of σ2 and σ'2, respectively. The adequacy of fitting was examined by plotting predicted versus observed concentrations (Goodness-of-Fit), concentrations versus time profiles and weighted residuals versus predicted concentrations.

6.3.5 Covariate Relationship Exploration

Association between patient variables and pharmacokinetic parameters were first visually evaluated by plotting Empirical Bayes Estimates (EBE) against patient variables. Patient variables were then incorporated into the base model one at a time using at least 13 approaches to associate the patient variable with the parameter. A patient variable was considered as significant if all the following criteria were met: (1) a decrease in objective function value (OFV) of 6.63 for 1 degree of freedom ($p<0.01$), (2) no significant trend in EBE-vs-patient variables plots, (3) improved Goodness-of-Fit, (4) reduced inter-individual variability and (5) clinical plausibility for incorporating the patient variable.

6.4 Results

6.4.1 Patients

A total of 11 patients were enrolled in this study. Table 12 summarizes the characteristics of the patients including age, gender and days post-transplant on the day of the study in each phase. Table 13 summarizes the conditioning regimens of the patients. Three patients did not complete the phase 2 intravenous study, and two patients did not complete the phase 3 oral study. Graft-versus-host was diagnosed in three patients. Figure 23 (a – r) summarizes the characteristics of the patients over the period of time from the initiation of voriconazole administration until the time of discharge. These characteristics included body weight, laboratory biochemical profiles and voriconazole dose. The dash lines in the figures represent the normal range of these indices. A large degree of inter-individual and intra-individual variability was observed in these characteristics of the patients. Most of these characteristics of the patients varied substantially over time and were out of normal range.

Table 12. Characteristics of patients in the pharmacokinetic study of bone marrow transplant patients

Patient ID	Age	Gender	Phase 1 Oral Pre-transplant	Phase 2 Intravenous Post-transplant	Phase 3 Oral Post-transplant
#1	16	Female	-3	10	
#2	5	Female	-4	8	41
#3	22	Male	-5		110
#4	5	Female	-6		43
#5	2	Female	-8	16	104
#6	3	Male	-7 *		68
#7	0.6	Female	-1	22	33
#8	15	Female	-7	7	83
#9	7	Male	-10	10	72
#10	16	Female	-1	6	31
#11	1.9	Male	-7	19	53
Mean	8.5		-5.4	12.3	63.8
SD	7.4		2.9	6.0	28.5
Median	5.0		-6.0	10.0	60.5
CV	86.6%		53.6%	48.7%	44.6%
Min	0.6		-10	6	31
Max	22		-1	22	110

* Voriconazole was administrated intravenously (clinical decision).

Table 13. Conditioning regimens of patients in the pharmacokinetic study of bone marrow transplant patients

Patient ID	Drug / Irradiation	Days before Transplant	Dose	Frequency	Total Dose
1	ATGAM	4	15 mg/kg/dose	q12h x 6 doses	3600 mg
	Cyclophosphamide	5	50 mg/kg/dose	once a day x 4	8000 mg
	Busulfex	9	0.8 mg/kg/dose	q6h x 4 days	572 mg
2	TBI	3	1.8 Gy/fraction	7 fraction over 4 days	12.6 Gy
	Cyclophosphamide	5	60 mg/kg/dose	once a day x 2	2040 mg
3	ATGAM	4	15 mg/kg/dose	q12h x 6 doses	6750 mg
	TBI	4	1.8 Gy/fraction	7 fraction over 4 days	12.6 Gy
	Cyclophosphamide	6	60 mg/kg/dose	once a day x 2	9000 mg
4	Cyclophosphamide	5	50mg/kg/dose	daily for 4 days	3660 mg
	Busulfex	9	1.1mg/kg/dose	q6h x 4 days	280 mg
5	ATGAM	4	15mg/kg/dose	q12h x 6 doses	1140 mg
	Cyclophosphamide	5	50mg/kg/dose	daily for 4 days	2500 mg
	Busulfex	9	1.1mg/kg/dose	q6h x 4 days	210 mg
6	ATGAM	32	15mg/kg/dose	q12h x 6 doses	1650 mg
	Cyclophosphamide	5	50mg/kg/dose	daily for 4 days	2920 mg
	Busulfex	9	0.8mg/kg/dose	q6h x 4 days	207 mg
7	Melphalan	2	140mg/m2	once	50 mg
	Fludarabine	7	1mg/kg/dose	daily for 5 days	35 mg
	Alemtuzumab	8	0.2mg/kg/dose	daily for 5 days	7.5 mg
8	TBI	4	1.8 Gy/fraction	7 fraction over 4 days	12.6 Gy
	Cyclophosphamide	7	60mg/kg/dose	once a day x 2	7200 mg
9	Cyclophosphamide	8	50mg/kg/dose	daily for 4 days	4980 mg

Table 13. (continured)

10	Busulfex	12	1.1mg/kg/dose	q6h x 4 days	420 mg
	TBI	4	1.8 Gy/fraction	7 fraction over 4 days	12.6 Gy
	Cyclophosphamide	27	60mg/kg/dose	once a day x 2	7850 mg
11	ATGAM	4	15mg/kg/dose	q12h x 6 doses	1200 mg
	Cyclophosphamide	5	50 mg/kg/dose	daily for 4 days	2740 mg
	Busulfex	9	1.1mg/kg/dose	q6h x 4 days	194 mg

TBI: total body irradiation

Figure 23. Characteristics of patients in the pharmacokinetic study of bone marrow transplant patients

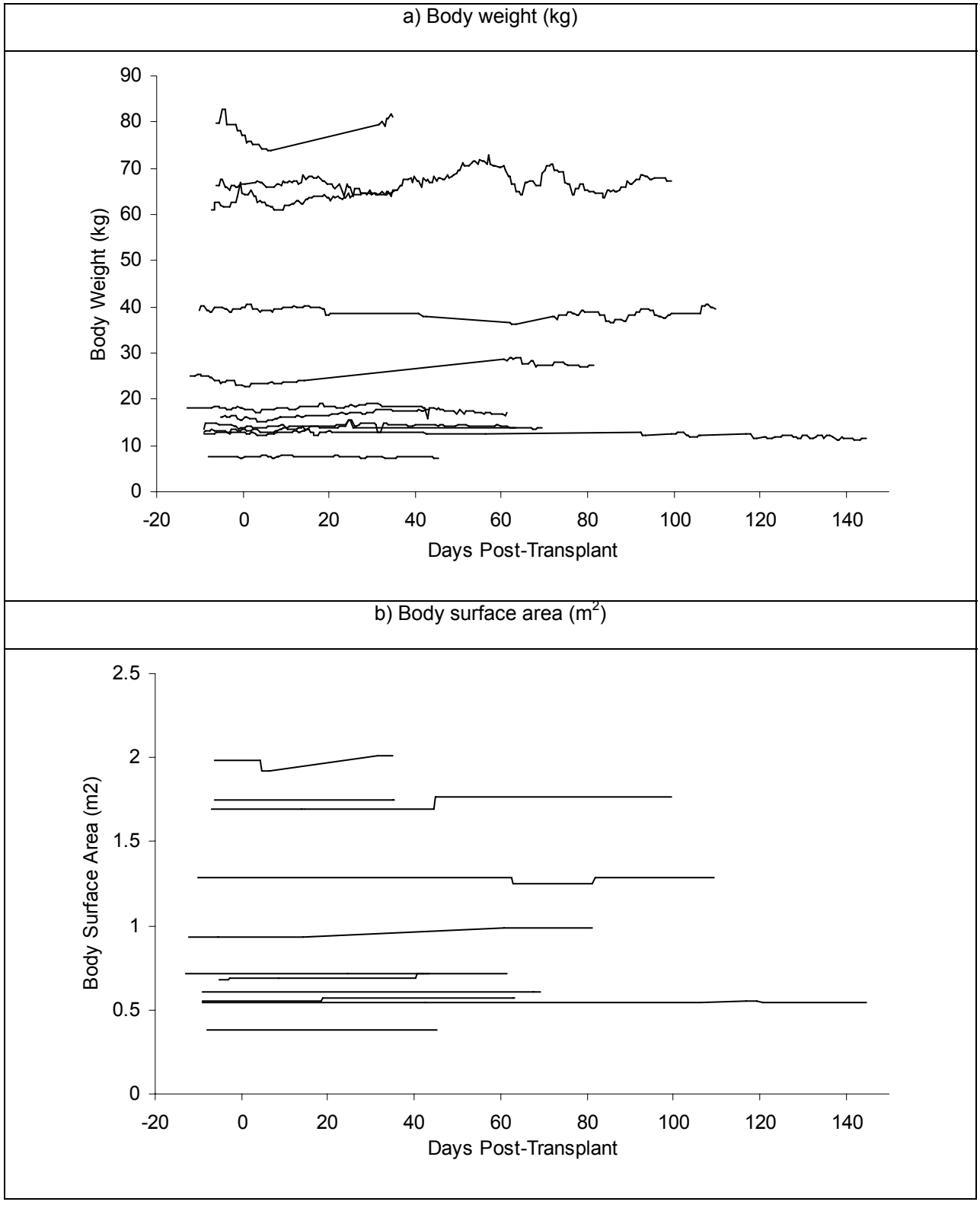
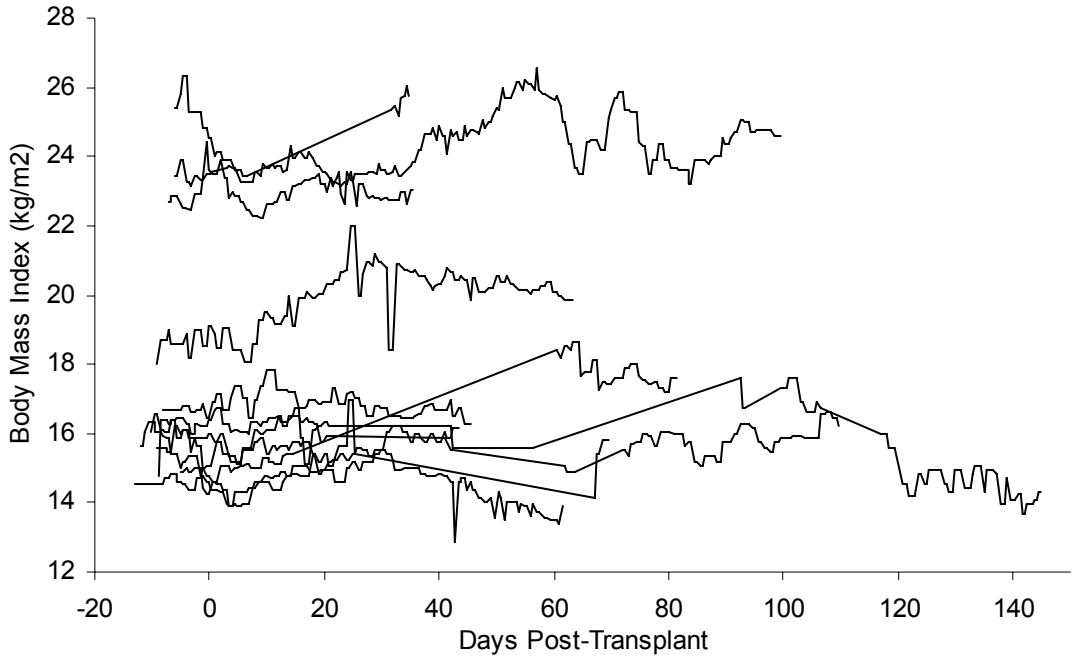


Figure 23. (continued)

c) Body Mass Index (kg/m^2)



d) Serum Creatinine (mg/dL)

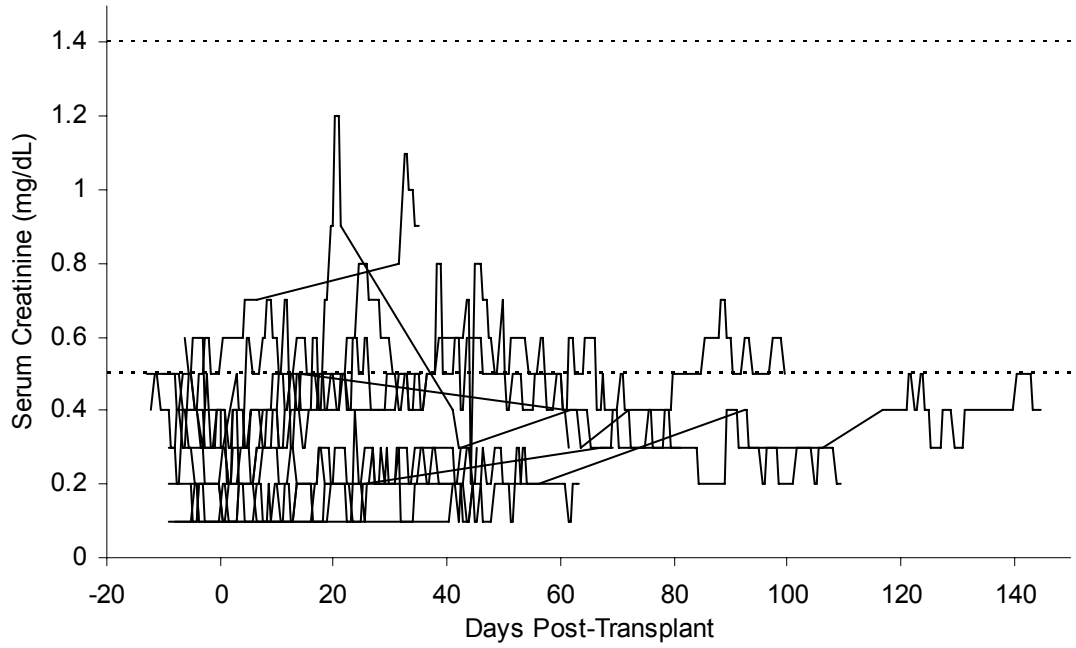
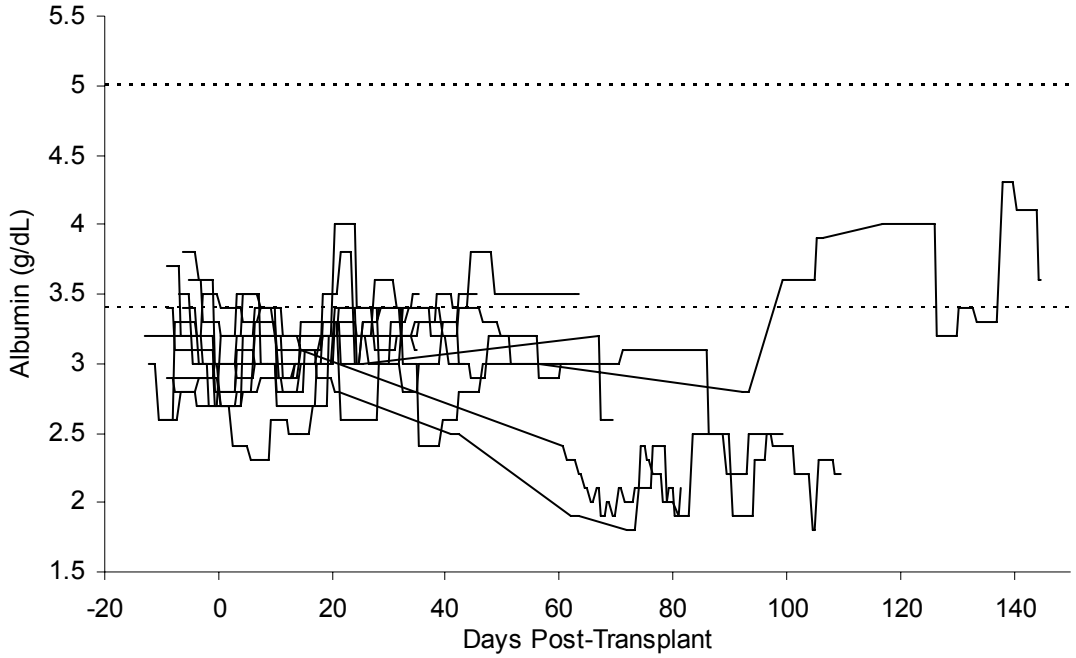


Figure 23. (continued)

e) Albumin (g/dL)



f) Total Bilirubin (mg/dL)

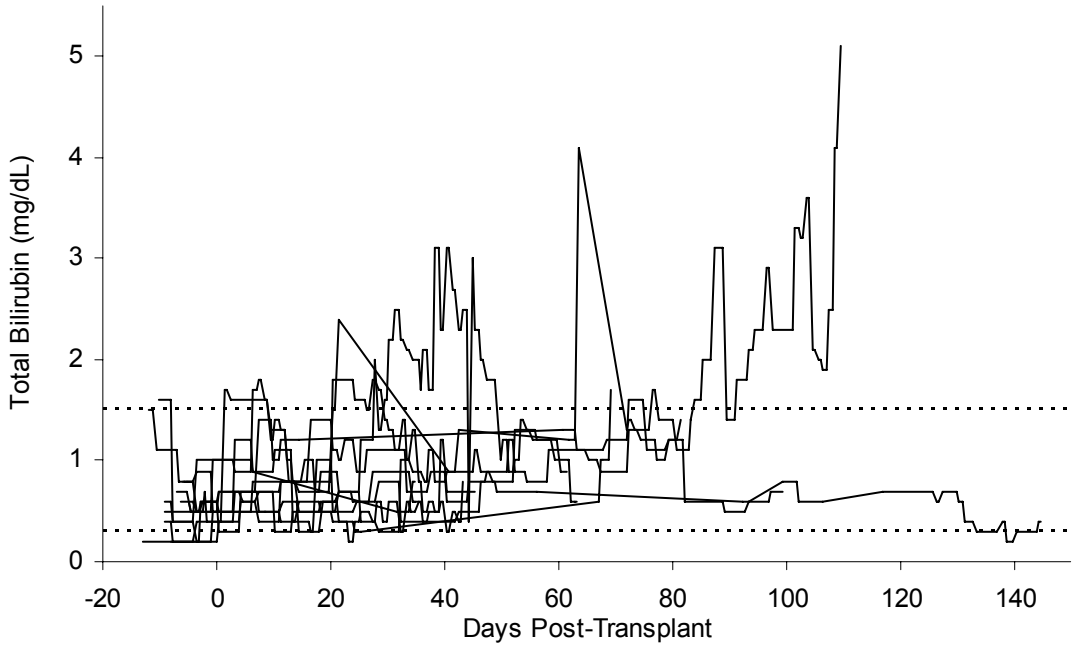
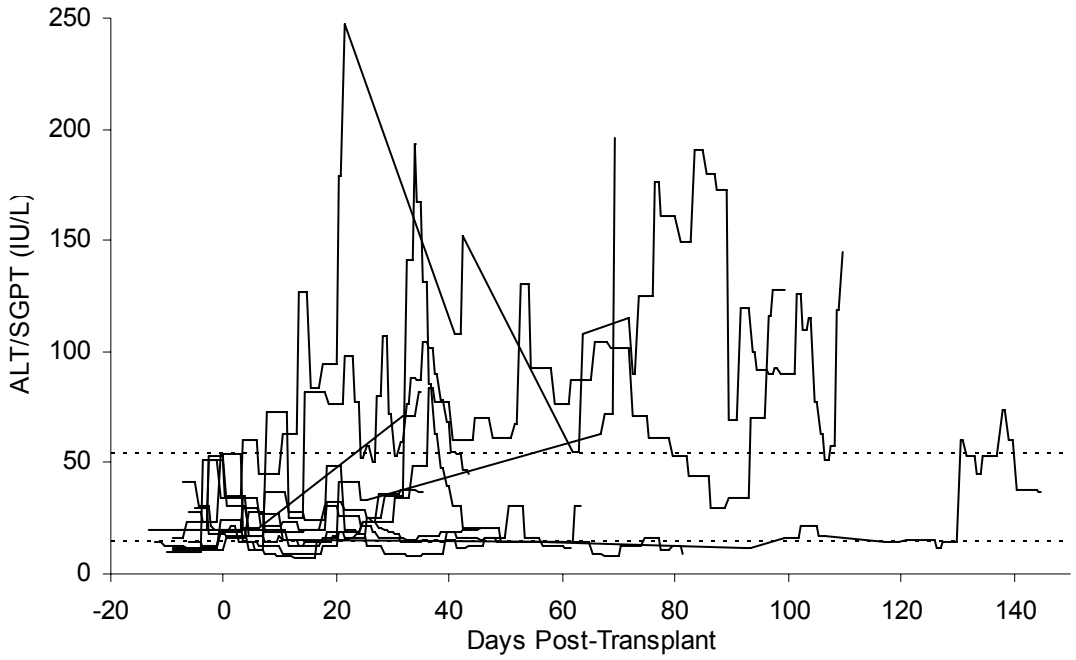


Figure 23. (continued)

g) Alanine aminotransferase / ALT / SGPT (IU/L)



h) Aspartate aminotransferase AST / SGOT (IU/L)

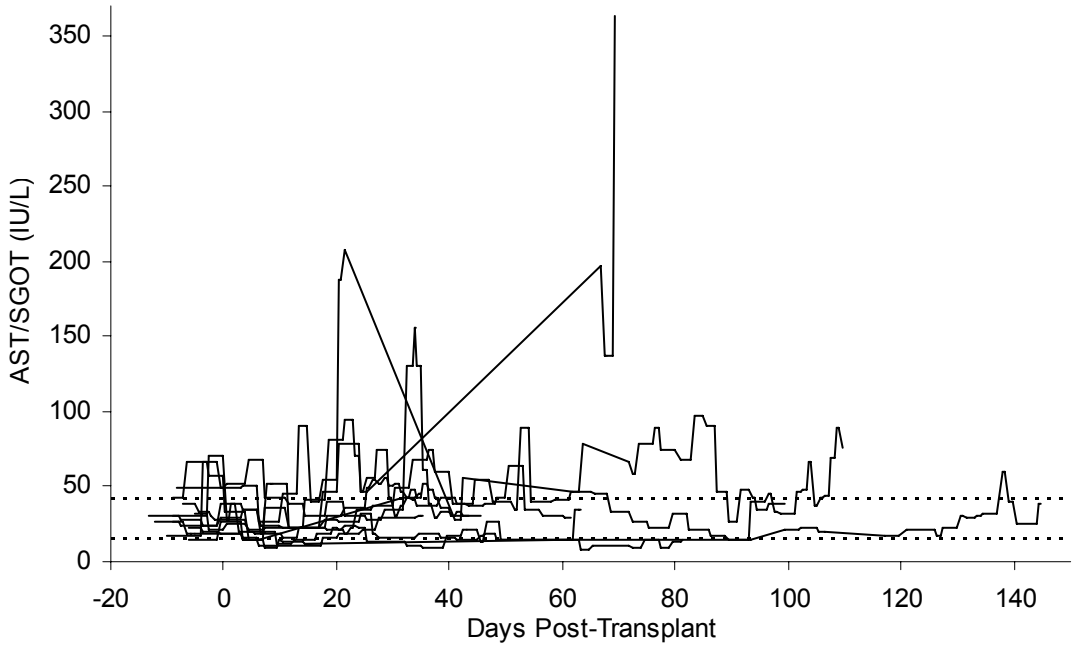
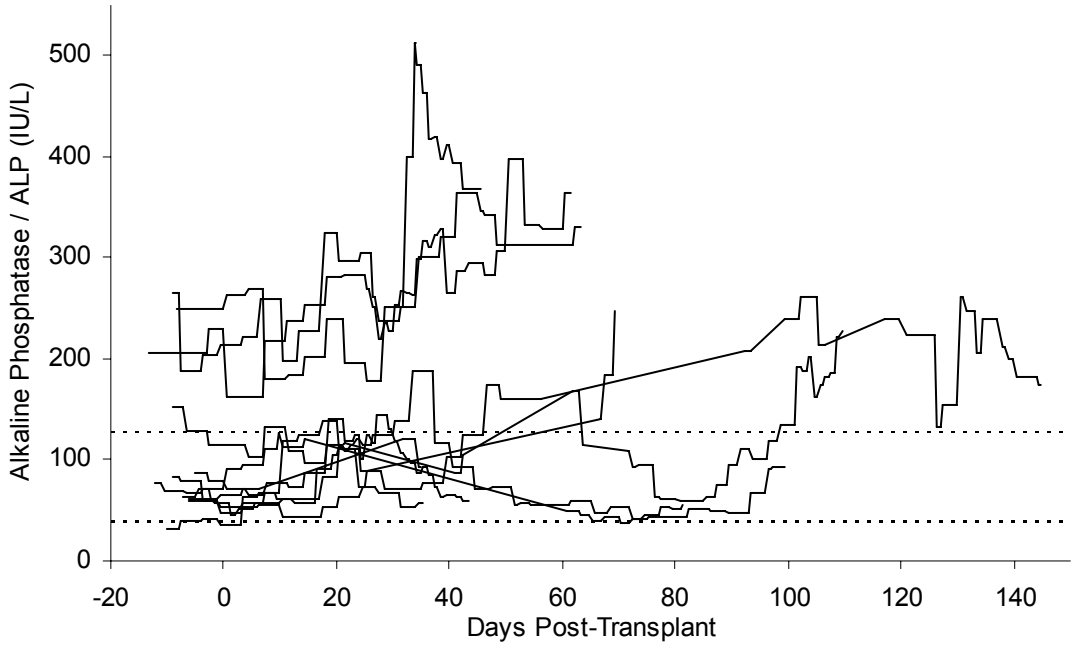


Figure 23. (continued)

i) Alkaline Phosphatase / ALP (IU/L)



j) Gamma-glutamyl transpeptidase / GGTP (IU/L)

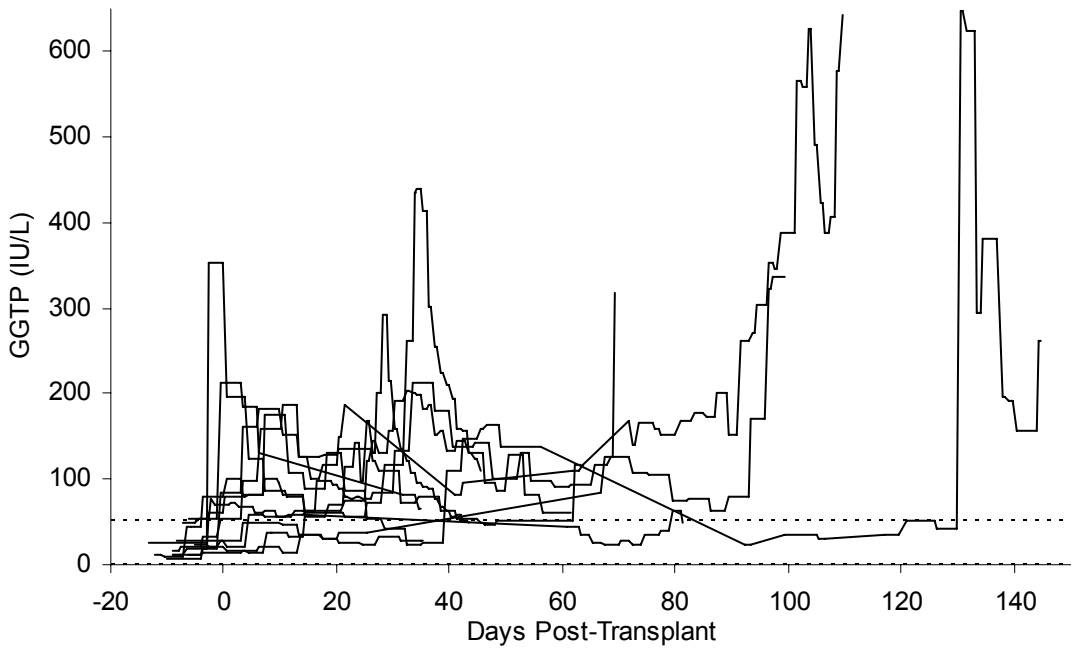
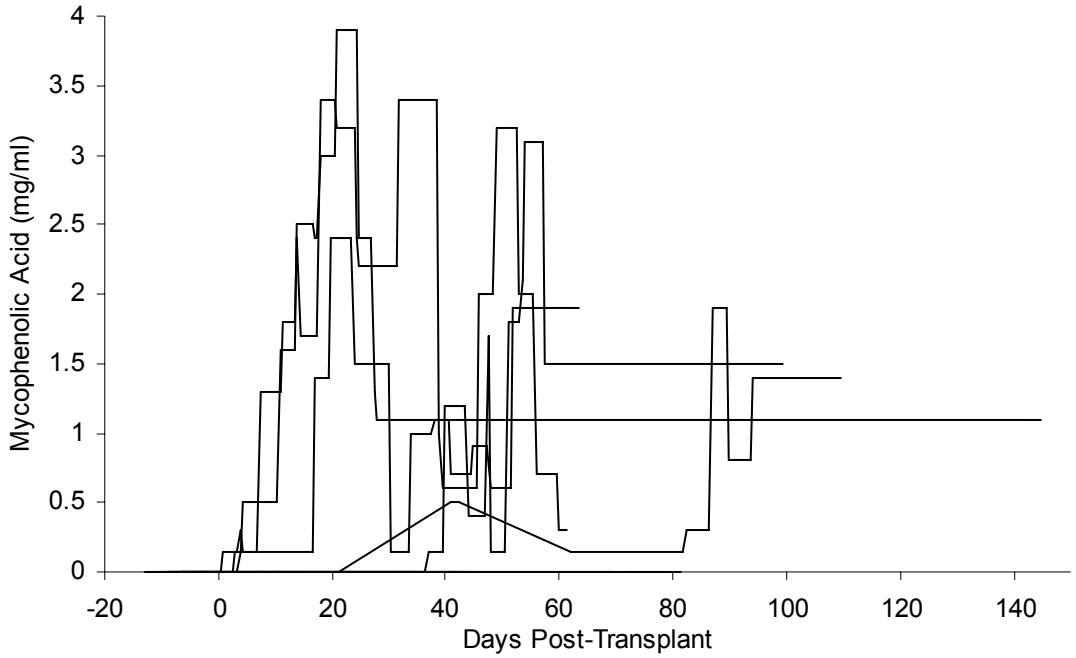


Figure 23. (continued)

k) Mycophenolic acid plasma concentration (mg/ml)



l) White blood cell count / WBC (X10E+09/L)

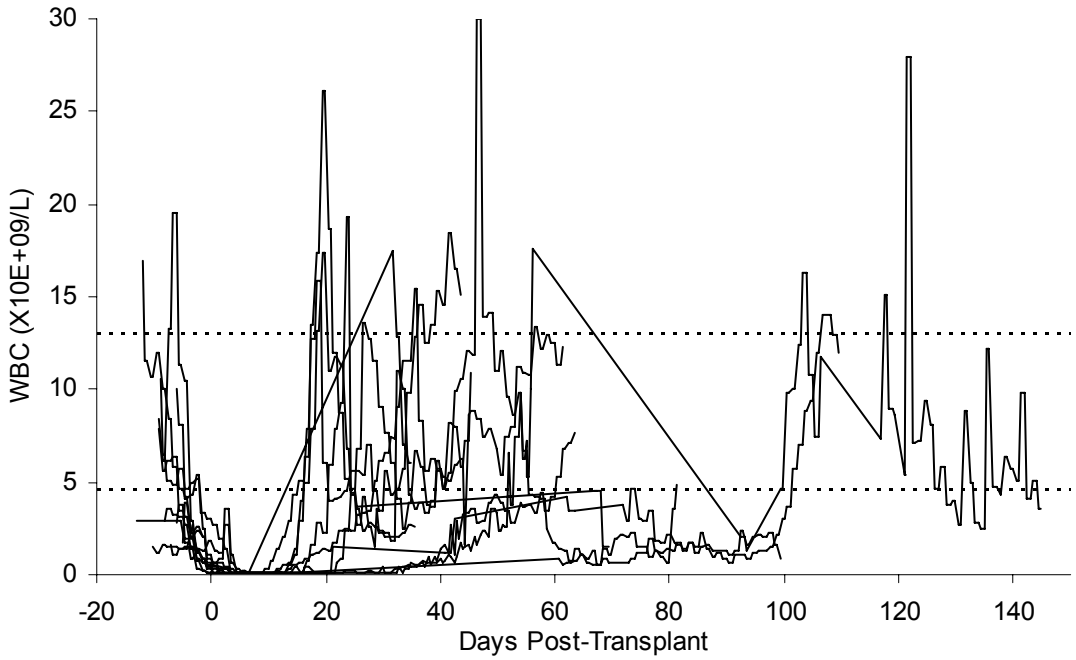
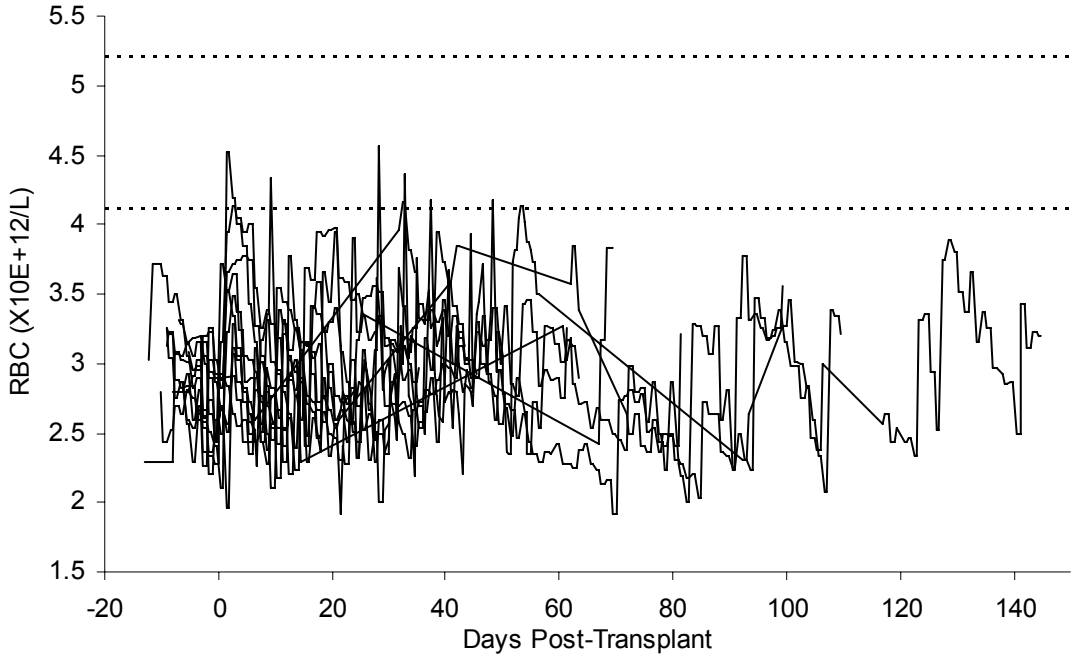


Figure 23. (continued)

m) Red blood cell count / RBC (X10E+12/L)



n) Hemoglobin / HGB (g/dL)

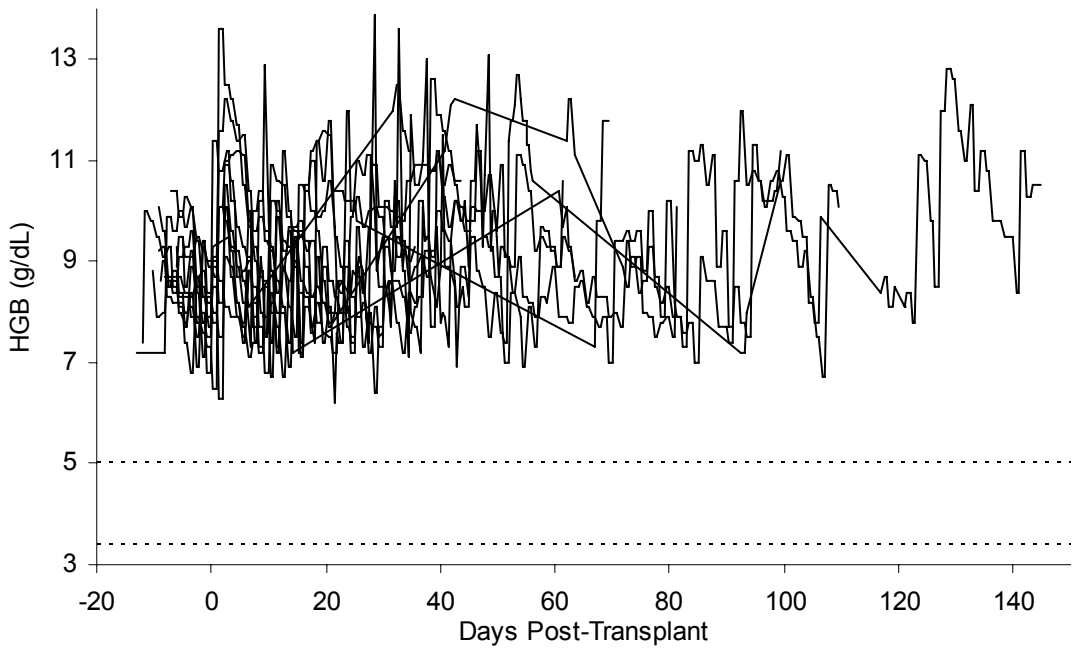
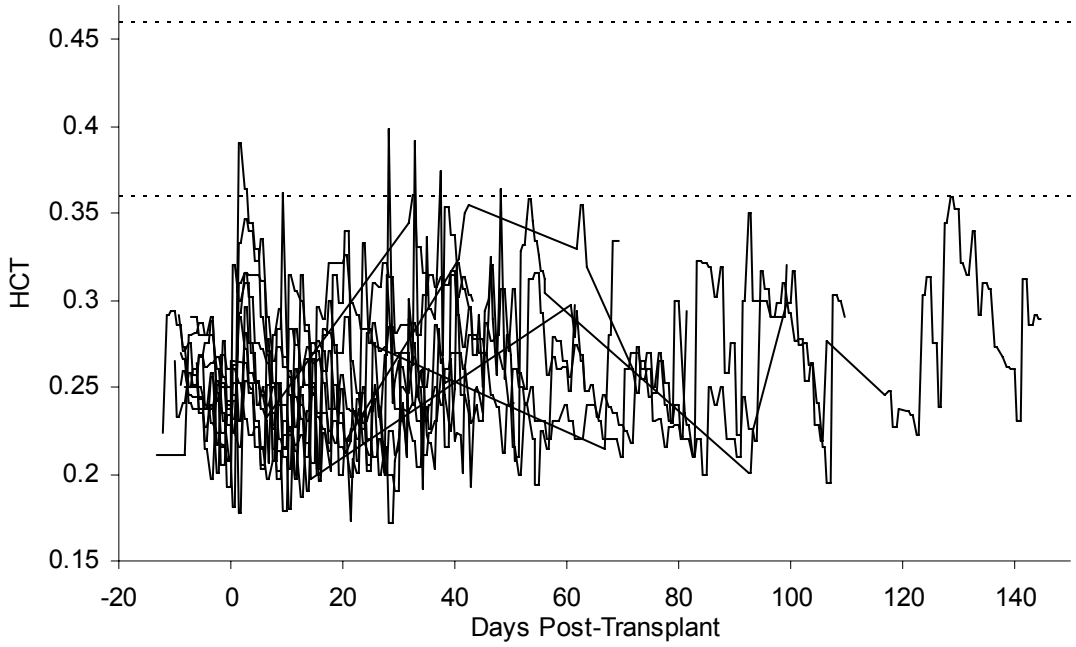


Figure 23. (continued)

o) Hematocrit / HCT



p) Tacrolimus whole blood concentration (ng/ml)

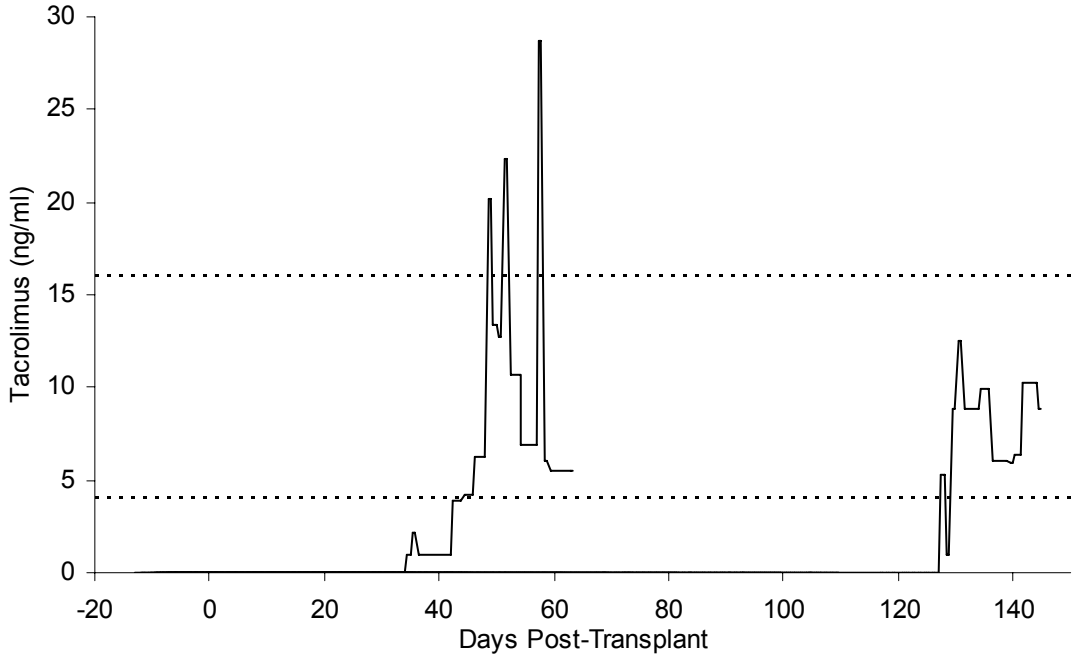
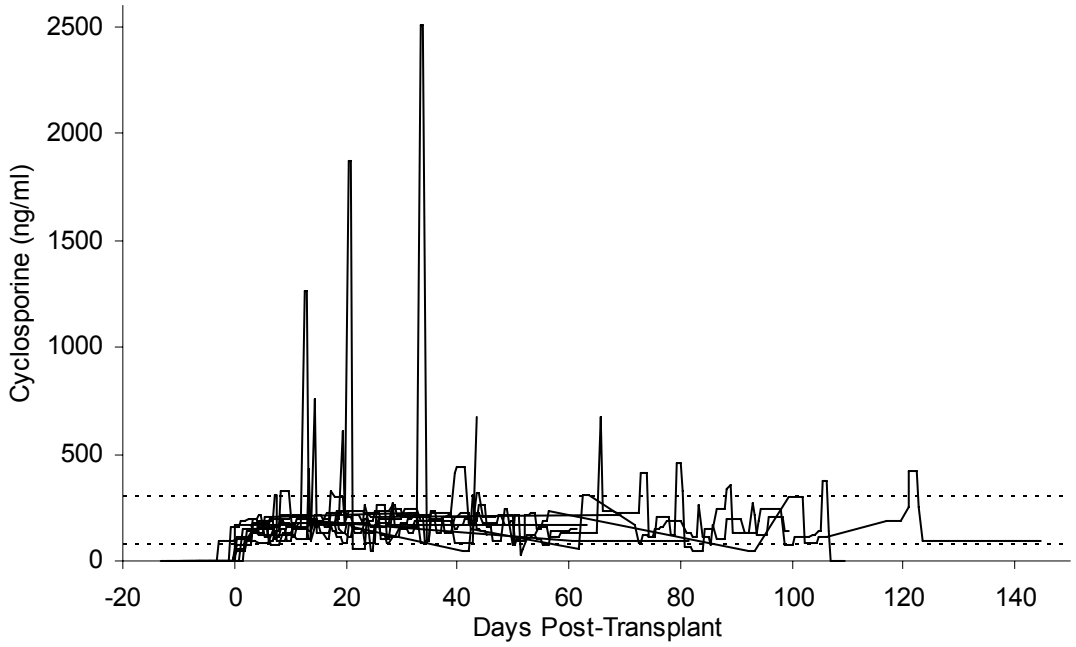


Figure 23. (continued)

q) Cyclosporine whole blood concentration (ng/ml)



r) Voriconazole Dose (mg)

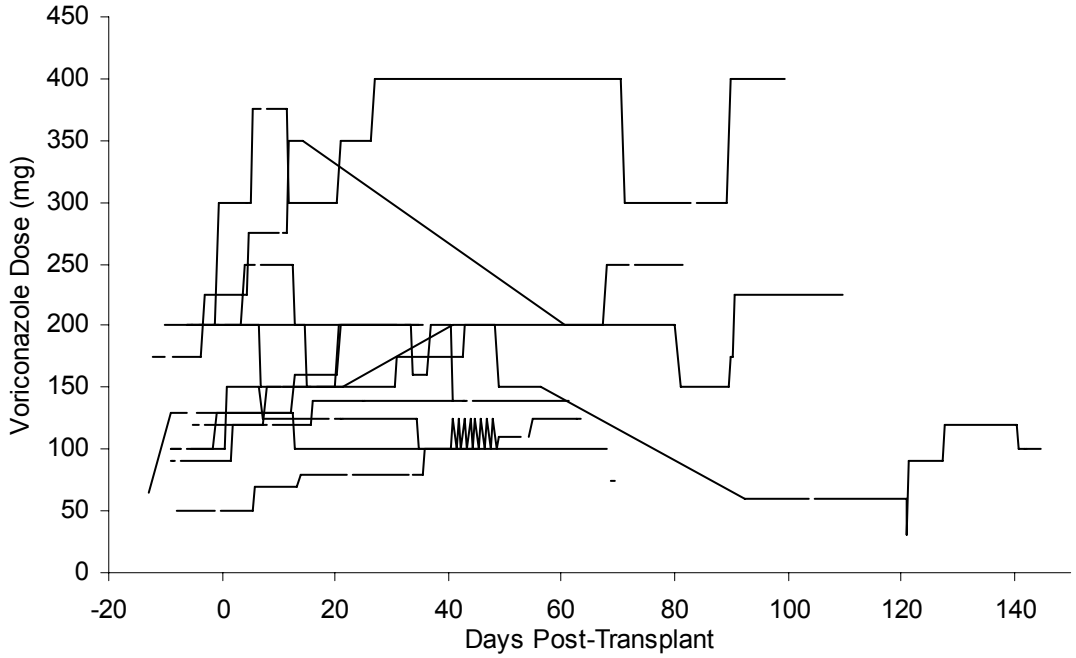


Figure 24 illustrated the time post-transplant when each pharmacokinetic profile was collected and the range of the voriconazole plasma concentrations of each profile. Figure 25 and 26 summarized individual pharmacokinetic profiles in each study phase. There was a wide variation in voriconazole plasma concentrations. Among the total of 228 blood samples collected, less than half of the voriconazole plasma concentrations (45.4%) were maintained within 1-6ug/ml. Underexposure appeared to be predominant with 46.2% and 8.4% of voriconazole plasma concentrations below 1ug/ml or above 6ug/ml, respectively. This percentage was similar to the preliminary data as mentioned previously.

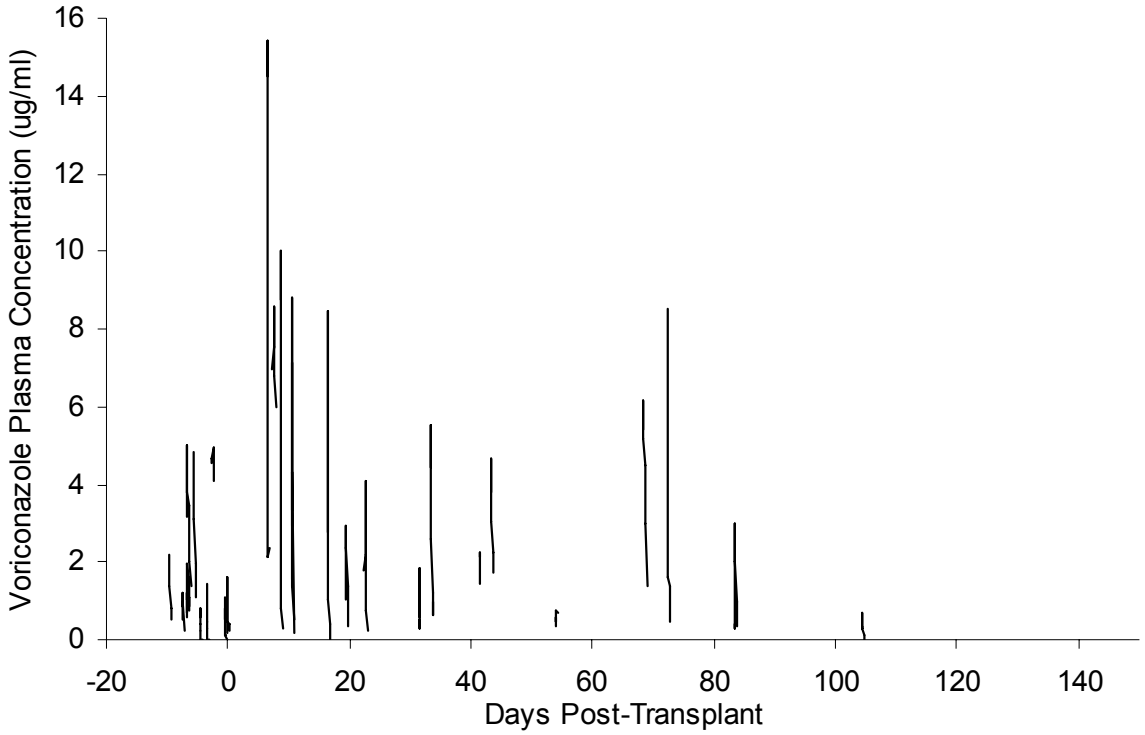


Figure 24. Collection time (post-transplant) and the range of the voriconazole plasma concentrations of each pharmacokinetic profile in the pharmacokinetic study of voriconazole in pediatric bone marrow transplant patients

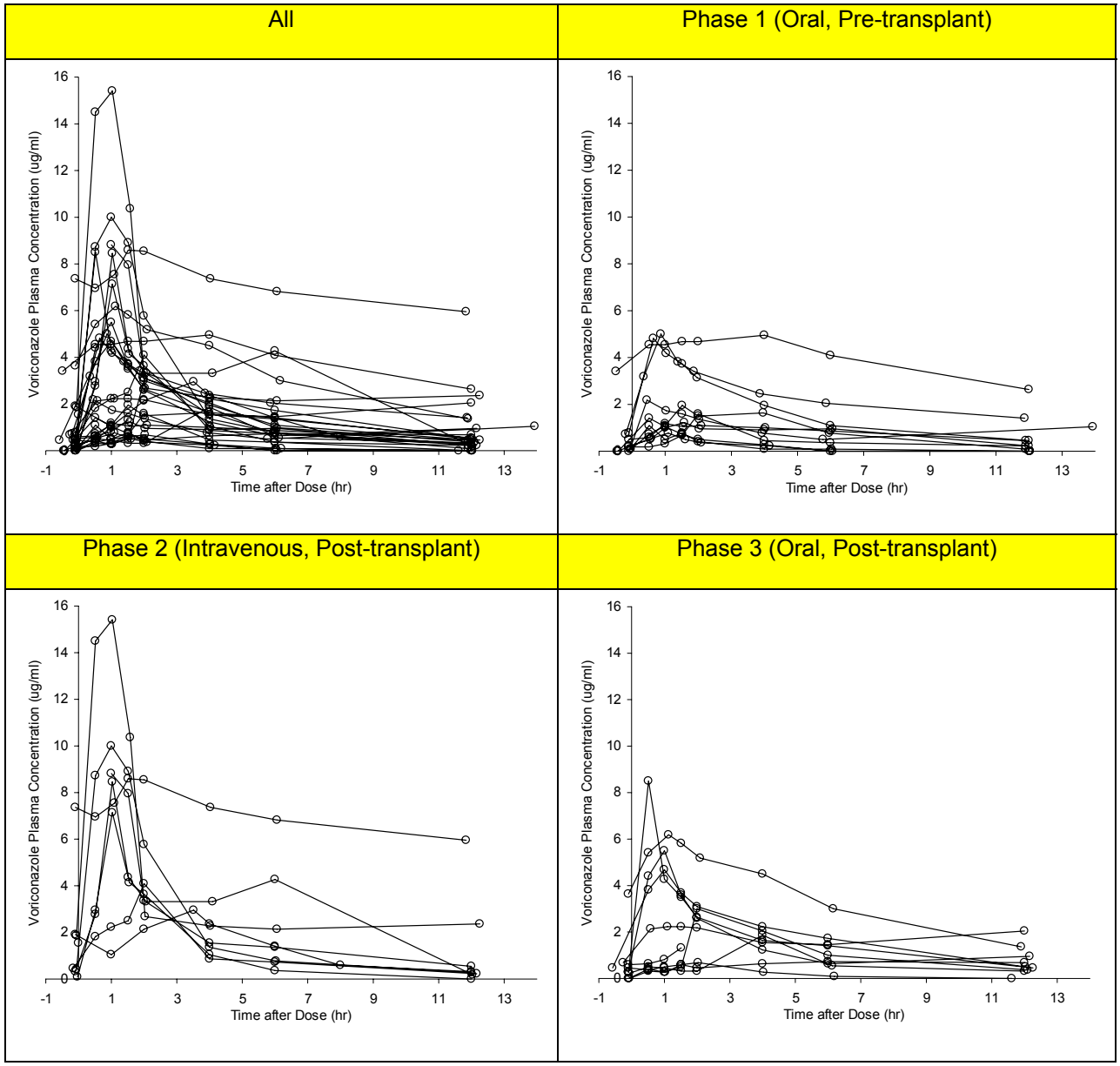


Figure 25. Individual pharmacokinetic profiles of voriconazole in the pharmacokinetic study of voriconazole in pediatric bone marrow transplant patients

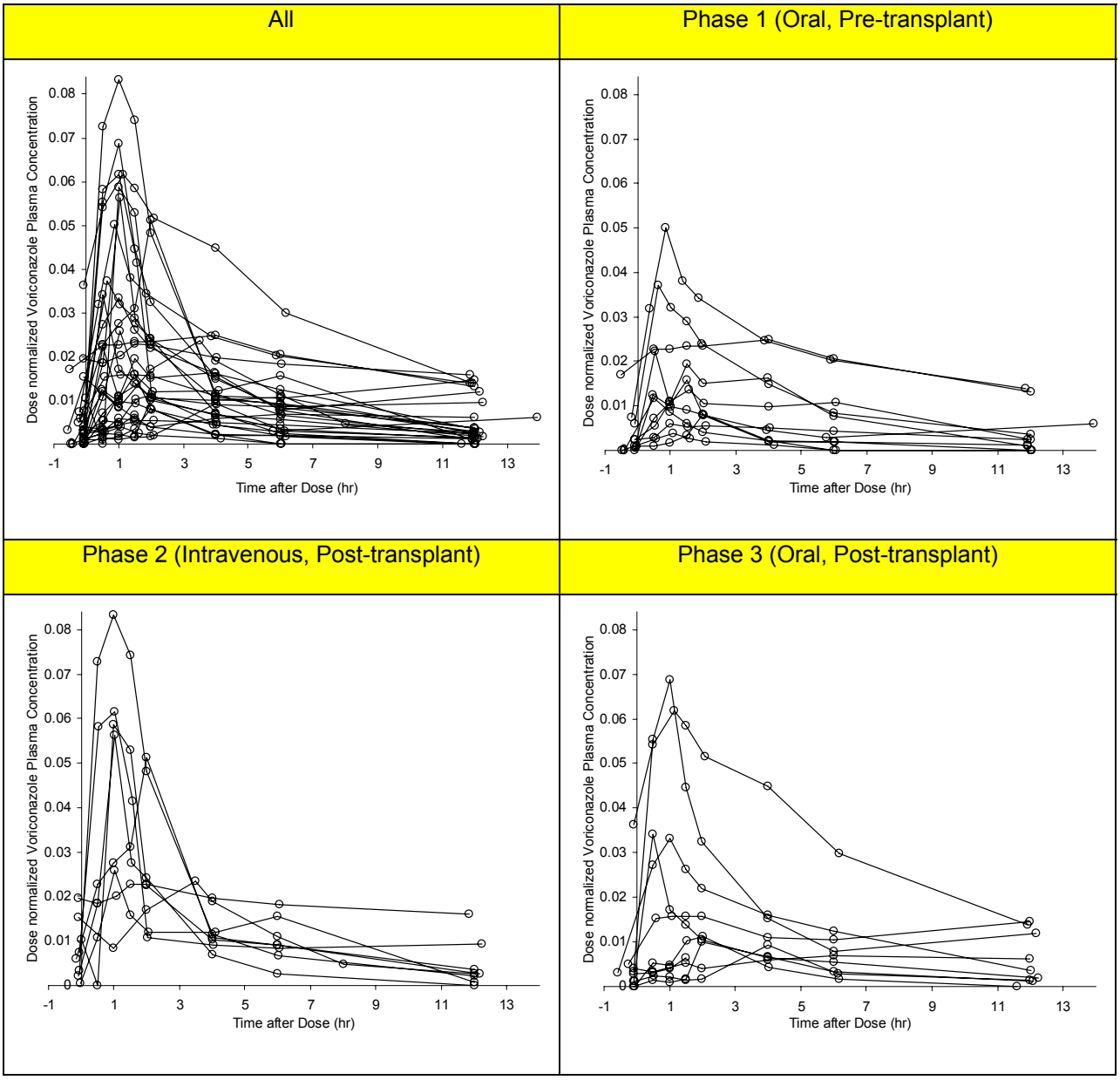


Figure 26. Individual dose-normalized pharmacokinetic profiles of voriconazole in the pharmacokinetic study of voriconazole in pediatric bone marrow transplant patients

6.4.2 Non-compartmental analysis

Figure 27 illustrated that the trough concentrations Co and C12 were not significantly different in phase 1 (p= 0.2161), phase 2 (p=0.0867) or phase 3 (p=0.6087), indicating that steady state had been reached in most of the patients at the time of study in each phase.

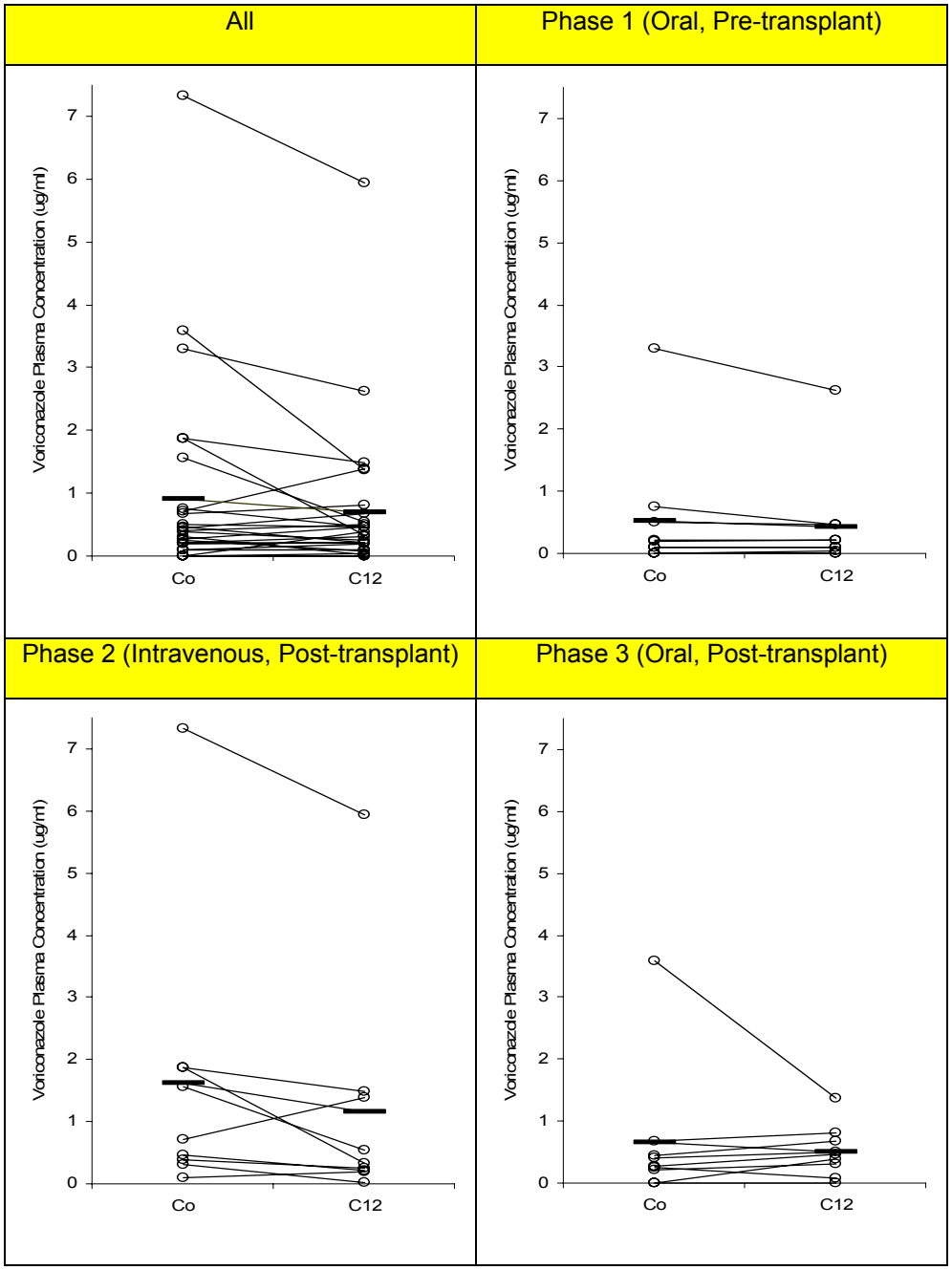


Figure 27. Comparison of trough concentrations in pharmacokinetic study of voriconazole in pediatric bone marrow transplant patients

Hollow circle: individual levels. Bold bar: average levels.

Figure 28 illustrates a good correlation between voriconazole trough plasma concentrations and the corresponding AUCo-∞ at steady state (R^2=0.94).

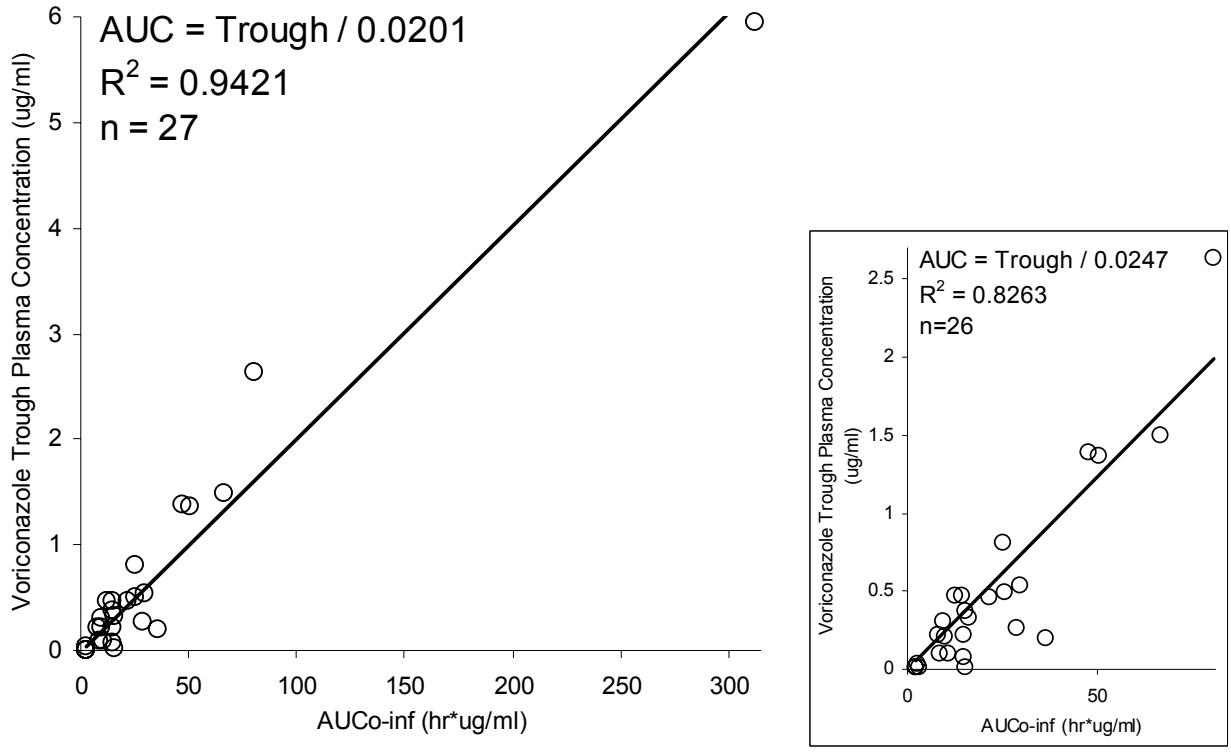


Figure 28. Correlation of AUCo-∞ with voriconazole trough plasma concentrations in pediatric bone marrow transplant patients

Left figure: correlation of AUCo-∞ with voriconazole trough plasma concentrations in pediatric bone marrow transplant patients with all data points.

Right figure: Correlation of AUCo-∞ with voriconazole trough plasma concentrations in pediatric bone marrow transplant patients with the point of the highest concentration omitted.

The pharmacokinetic parameters of voriconazole estimated using non-compartmental analysis are shown in Table 14 – 22. There was a wide variation in various pharmacokinetic parameters of voriconazole in pediatric bone marrow transplant patients after oral and intravenous voriconazole administration.

Table 14. Dose of voriconazole (mg) in pediatric bone marrow transplant patients

Patient ID	Phase 1 Oral, Pre-transplant	Phase 2 Intravenous, Post-transplant	Phase 3 Oral, Post-transplant
#1	200	150	
#2	120	120	140
#3	200		200
#4	130		140
#5	90	150	60
#6		100 *	100
#7	50	80	80
#8	200	375	300
#9	175	275	250
#10	200	250	200
#11	100	125	
Mean	**146.50**	**180.56**	**163.33**
SD	**55.68**	**97.96**	**80.31**
Median	**152.5**	**150**	**140**
CV	**38.0%**	**54.3%**	**49.2%**
Max	**200**	**375**	**300**
Min	**50**	**80**	**60**

* Voriconazole was administrated prior to the transplant (clinical decision).

Table 15. Tmax (hour) of voriconazole in pediatric bone marrow transplant patients

Patient ID	Phase 1 Oral, Pre-transplant	Phase 2 Intravenous, Post-transplant	Phase 3 Oral, Post-transplant
#1	4.0	1.0	
#2	0.5	1.0	1.1
#3	1.1		
#4	0.6		1.0
#5	1.6	1.0	2.0
#6		0.9 *	1.1
#7	0.5	2.0	1.0
#8	1.0	1.5	2
#9	0.5	1.0	0.5
#10	2.0	1.0	4.0
#11	1.5	3.5	
Mean	**1.3**	**1.4**	**1.6**
SD	**1.1**	**0.9**	**1.1**
Median	**1.04**	**1.03**	**1.11**
CV	**81.1%**	**59.1%**	**69.3%**
Max	**4.0**	**3.5**	**4.0**
Min	**0.5**	**0.9**	**0.5**

* Voriconazole was administrated prior to the transplant (clinical decision).

Table 16. Cmax (ug/ml) of voriconazole in pediatric bone marrow transplant patients

Patient ID	Phase 1 Oral, Pre-transplant	Phase 2 Intravenous, Post-transplant	Phase 3 Oral, Post-transplant
#1	4.96	8.81	
#2	1.42	9.99	2.22
#3	0.78		
#4	4.83		4.66
#5	1.23	8.44	0.67
#6		5.01 *	6.17
#7	1.11	4.09	5.5
#8	1.19	8.57	2.99
#9	2.17	7.12	8.52
#10	1.61	15.4	1.87
#11	1.94	2.94	
Mean	**2.12**	**7.82**	**4.08**
SD	**1.52**	**3.71**	**2.61**
Median	**1.52**	**8.44**	**3.83**
CV	**71.3%**	**47.5%**	**64.0%**
Max	**4.96**	**15.40**	**8.52**
Min	**0.78**	**2.94**	**0.67**

* Voriconazole was administrated prior to the transplant (clinical decision).

Table 17. Dose normalized AUC (ug*hr/ml) and bioavailability of voriconazole in pediatric bone marrow transplant patients

Patient ID	Phase 1 Oral, Pre-transplant	Phase 2 Intravenous, Post-transplant	Phase 3 Oral, Post-transplant	Bioavailability
#1	0.184	0.122		
#2	0.025	0.226	0.130	58%
#3	0.013			
#4	0.139		0.165	
#5	0.098	0.099	0.039	39%
#6		0.371 *	0.241	
#7	0.044	0.153	0.176	115%
#8	0.046	0.073	0.051	70%
#9	0.046	0.131	0.043	33%
#10	0.029	0.140	0.042	30%
#11	0.103	0.070		
Mean	**0.073**	**0.154**	**0.111**	**58%**
SD	**0.056**	**0.094**	**0.078**	**32%**
Median	**0.05**	**0.13**	**0.09**	**48%**
CV	**77.0%**	**61.0%**	**70.2%**	**55.9%**
Max	**0.184**	**0.371**	**0.241**	**115%**
Min	**0.013**	**0.070**	**0.039**	**30%**

* Voriconazole was administrated prior to the transplant (clinical decision).

Table 18. Half life (hour) of voriconazole in pediatric bone marrow transplant patients

Patient ID	Phase 1 Oral, Pre-transplant	Phase 2 Intravenous, Post-transplant	Phase 3 Oral, Post-transplant
#1	8.87	5.06	
#2	1.45	3.42	6.99
#3	2.70		
#4	3.32		3.73
#5	3.51	1.26	1.52
#6		5.45 *	5.01
#7	1.53	3.87	1.96
#8	7.17	4.07	3.33
#9	2.58	1.77	4.15
#10	7.63	4.91	3.50
#11	1.99	2.64	
Mean	**4.07**	**3.61**	**3.77**
SD	**2.75**	**1.47**	**1.72**
Median	**3.01**	**3.87**	**3.61**
CV	**67.4%**	**40.9%**	**45.5%**
Max	**8.87**	**5.45**	**6.99**
Min	**1.45**	**1.26**	**1.52**
P **	**0.0539**	**0.0011**	**0.008**

* Voriconazole was administrated prior to the transplant (clinical decision).

** P: the significance of comparison between the half life of voriconazole in pediatric bone marrow transplant patients and that in healthy subjects (6 hours) using two-tailed t-test.

Table 19. Clearance (L/hr) of voriconazole in pediatric bone marrow transplant patients

Patient ID	Phase 1 Oral, Pre-transplant	Phase 2 Intravenous, Post-transplant	Phase 3 Oral, Post-transplant
#1	5.42	8.17	
#2	40.21	4.43	7.68
#3	74.53		
#4	7.19		6.07
#5	10.24	10.10	25.79
#6		2.70 *	4.15
#7	22.50	6.55	5.67
#8	21.57	13.74	19.61
#9	21.96	7.64	23.03
#10	33.92	7.13	23.57
#11	9.70	14.22	
Mean	**24.72**	**8.30**	**14.45**
SD	**20.93**	**3.86**	**9.34**
Median	**21.76**	**7.64**	**13.64**
CV	**84.7%**	**46.5%**	**64.7%**
Max	**74.53**	**14.22**	**25.79**
Min	**5.42**	**2.70**	**4.15**

* Voriconazole was administrated prior to the transplant (clinical decision).

Table 20. Clearance normalized to body weight (L/kg) of voriconazole in pediatric bone marrow transplant patients

Patient ID	Phase 1 Oral, Pre-transplant	Phase 2 Intravenous, Post-transplant	Phase 3 Oral, Post-transplant
#1	0.14	0.20	
#2	2.47	0.28	0.44
#3	0.90		
#4	0.40		0.34
#5	0.83	0.82	2.15
#6		0.18 *	0.30
#7	3.05	0.86	0.77
#8	0.35	0.22	0.30
#9	0.87	0.32	0.82
#10	0.51	0.11	0.37
#11	0.74	1.01	
Mean	**1.03**	**0.45**	**0.69**
SD	**0.96**	**0.35**	**0.63**
Median	**0.78**	**0.28**	**0.40**
CV	**93.4%**	**78.1%**	**91.5%**
Max	**3.05**	**1.01**	**2.15**
Min	**0.14**	**0.11**	**0.30**

* Voriconazole was administrated prior to the transplant (clinical decision).

Table 21. Volume of distribution (L) of voriconazole in pediatric bone marrow transplant patients

Patient ID	Phase 1 Oral, Pre-transplant	Phase 2 Intravenous, Post-transplant	Phase 3 Oral, Post-transplant
#1	69.38	59.73	
#2	84.20	21.84	77.39
#3	290.41		
#4	34.47		32.67
#5	51.81	18.36	56.52
#6		21.22 *	30.01
#7	49.52	36.57	16.06
#8	223.09	80.68	94.23
#9	81.81	19.47	137.83
#10	373.44	50.52	118.90
#11	27.82	54.14	
Mean	**128.60**	**40.28**	**70.45**
SD	**121.97**	**22.19**	**44.27**
Median	**75.60**	**36.57**	**66.96**
CV	**94.8%**	**55.1%**	**62.8%**
Max	**373.44**	**80.68**	**137.83**
Min	**27.82**	**18.36**	**16.06**

* Voriconazole was administrated prior to the transplant (clinical decision).

Table 22. Volume of distribution normalized to body weight (L/kg) of voriconazole in pediatric bone marrow transplant patients

Patient ID	Phase 1 Oral, Pre-transplant	Phase 2 Intravenous, Post-transplant	Phase 3 Oral, Post-transplant
#1	1.77	1.49	
#2	5.17	1.36	4.42
#3	3.51		
#4	1.90		1.80
#5	4.18	1.50	4.71
#6		1.43 *	2.14
#7	6.72	4.83	2.17
#8	3.59	1.32	1.47
#9	3.25	0.82	4.92
#10	5.61	0.77	1.85
#11	2.12	3.83	
Mean	**3.78**	**1.93**	**2.94**
SD	**1.66**	**1.41**	**1.47**
Median	**3.55**	**1.43**	**2.16**
CV	**43.9%**	**73.3%**	**50.1%**
Max	**6.72**	**4.83**	**4.92**
Min	**1.77**	**0.77**	**1.47**

* Voriconazole was administrated prior to the transplant (clinical decision).

The half life of voriconazole appeared to be significantly smaller than that in healthy adult subjects (6 hours) following intravenous and oral voriconazole administration in the post-transplant period (3.61±1.47 hours and 3.77±1.72 hours), and appeared to be smaller than that in healthy subjects (6 hours) following oral voriconazole administration in the pre-transplant period (4.07±2.75 hours). However, the half life of voriconazole appeared to be similar following oral and intravenous voriconazole administration in the pre- and post-transplant period.

The half life of voriconazole in pediatric bone marrow transplant patients (3.61 ~ 4.07 hours) was comparable with the value reported in previously published pediatric studies, such as that in children below age of 12 (3.58 hours) as reported by Karlsson et al. (55), that reported by Neely et al. (5.63 hours for children under 12 years of age and 3.47 hours for children above 12 years of age) (74), and that in immunocompromised children under 11 years of age (4.33 hours) as reported by Walsh et al. (130). However, the half life of voriconazole in pediatric bone marrow patients appeared to be significantly lower than that in immunocompromised children under 11 years of age (7.66 hours) as reported by Michael et al. (68).

The post-transplant bioavailability of voriconazole in pediatric bone marrow patients (58%±32%) was significantly lower than that in healthy adult subjects (96%) with a p value of 0.0167. The post-transplant bioavailability of voriconazole in pediatric bone marrow patients (58%±32%) was comparable with the value reported in previously published pediatric studies, such as that in immunocompromised children (65%) as reported by Walsh et al. (129), that in children below

age of 12 (44.6%) as reported by Karlsson et al. (55), and that reported by Neely et al. (75% for children under 12 years of age and 81% for children above 12 years of age) (74).

The oral clearance of voriconazole in pediatric bone marrow patients was similar before and after transplant. However, the clearance of voriconazole following oral voriconazole administration was significantly lower compared to that following intravenous voriconazole administration in pediatric bone marrow patients in the post-transplant period ($p=0.0288$).

The oral clearance of voriconazole appeared to be similar to the lower range of clearance of voriconazole reported in healthy adult subjects (13 – 36 L/hr) in both pre-transplant (24.72±20.93 L/hr) and post-transplant (14.45±9.34 L/hr) period. However, the clearance of voriconazole following intravenous voriconazole administration in the post-transplant period (8.30±3.86 L/hr) appeared to be significantly lower than the lowest clearance reported in healthy subjects (13 L/hr) with a p value of 0.0137.

The weight-normalized clearance of voriconazole following intravenous administration after transplant in pediatric bone marrow patients (0.45 L/hr/kg) was comparable with the value reported in previously published pediatric studies, such as that in children below age of 12 (0.58 L/hr/kg) as reported by Karlsson et al. (55), that reported by Neely et al. (0.32 L/hr/kg for children under 12 years of age and 0.2 L/hr/kg for children above 12 years of age) (74), that in immunocompromised children under 11 years of age (0.19 L/hr/kg) as reported by Michael et al. (68), and that in immunocompromised children under 11 years of age (0.4 L/hr/kg) as reported by Walsh et al. (130).

The weight-normalized volume of distribution of voriconazole in pediatric bone marrow patients appeared to be similar to the lower range of volume of distribution of voriconazole reported in healthy adult subjects (2 – 4.6 L/kg). The weight-normalized volume of distribution of voriconazole following intravenous administration in pediatric bone marrow patients after transplant (1.93 L/kg) was comparable with the value reported in previously published pediatric studies, such as that in children below age of 12 (3.0 L/kg) as reported by Karlsson et al. (55), that reported by Neely et al. (2.6 L/kg for children under 12 years of age and 1.0 L/kg for children above 12 years of age) (74), that in immunocompromised children under 11 years of age (2.1 L/kg) as reported by Michael et al. (68), and that in immunocompromised children under 11 years of age (2.5 L/kg) as reported by Walsh et al. (130).

There appeared to be no significant difference in pharmacokinetic parameters between phase 1 (pre-transplant oral administration) and phase 3 (post-transplant oral administration). Half life and Tmax of voriconazole was similar between phase 2 (post-transplant intravenous administration) and phase 3 (post-transplant oral administration), while Cmax, dose-normalized AUC, clearance and volume of distribution were significantly lower in phase 3 as compared to phase 1 due to low bioavailability.

Table 23. Comparison of pharmacokinetic parameters estimated in this study with previously reported values

	T1/2	F	CL/WT	Vd/WT
Walsh et al. (129)		65%		
Karlsson et al. (55)	3.58	44.6%	0.58	3
Neely et al. (74), (<12 years old)	5.63	75%	0.32	2.6
Neely et al. (74), (>12 years old)	3.47	81%	0.2	1
Michael et al. (68)	7.66		0.19	2.1
Walsh et al. (130)	4.33		0.4	2.5
This study (non-compartment analysis)	3.61 ~ 4.07	58%	0.45	1.93
This study (population analysis)		46.5%	0.18	2.45

6.4.3 Population Pharmacokinetic Analysis

A two-compartment model with first-order absorption and elimination adequately described the data. The population estimates (inter-individual) of bioavailability, clearance, volume of distribution of central compartment (Vc) and peripheral compartment (Vp), inter-compartment clearance (Q), and absorption rate constant (ka) were 46.5% (104.4%), 5.76L/hr (62.0%), 19.4L (62.0%), 58.8L (122.3%), 6.94L/hr (84.3%) and 0.98hr^{-1} (118.5%). The proportional and additive residual variability was 0.65 and 0.31ug/ml, respectively. Individual predictions agreed well with observations (Figure 29). There is no significant bias in population predictions (Figure 30a). Weighted residuals were approximately normally distributed and were mostly within about 4 units of the null ordinate (Figure 31).

Bioavailability of voriconazole is similar between lung transplant (45.9%) and pediatric bone marrow transplant patients (46.5%). CL/F in pediatric bone marrow transplant patients (12.39L/hr) was significantly higher as compared to liver transplant (7.92 L/hr) and lung transplant (7.52L/hr) patients. Vd/F in pediatric bone marrow transplant patients (168.2 L) was significantly lower as compared to liver transplant (248 L) and lung transplant (430.7 L) patients.

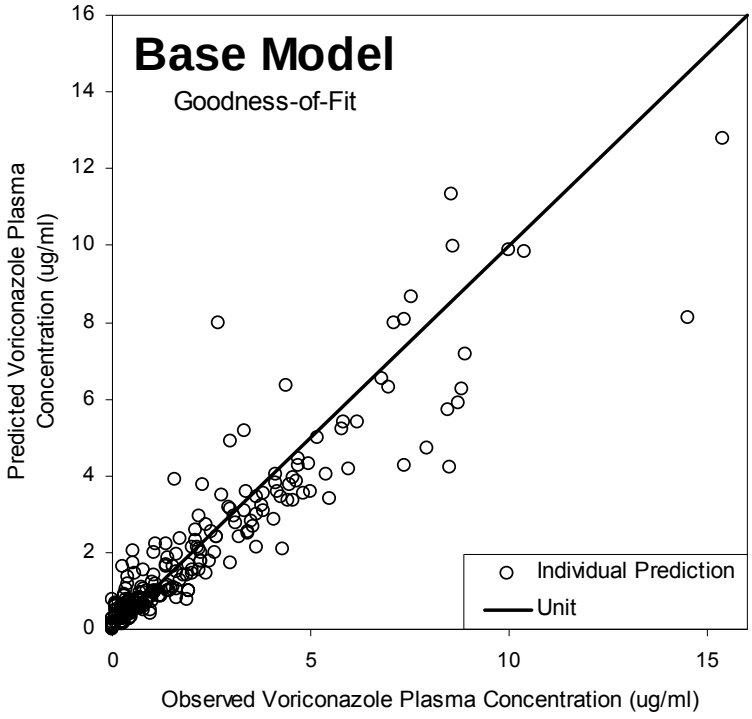


Figure 29. Goodness-of-Fit of base model in pediatric bone marrow transplant patients

Individual predictions agreed well with observations (R^2=0.85).

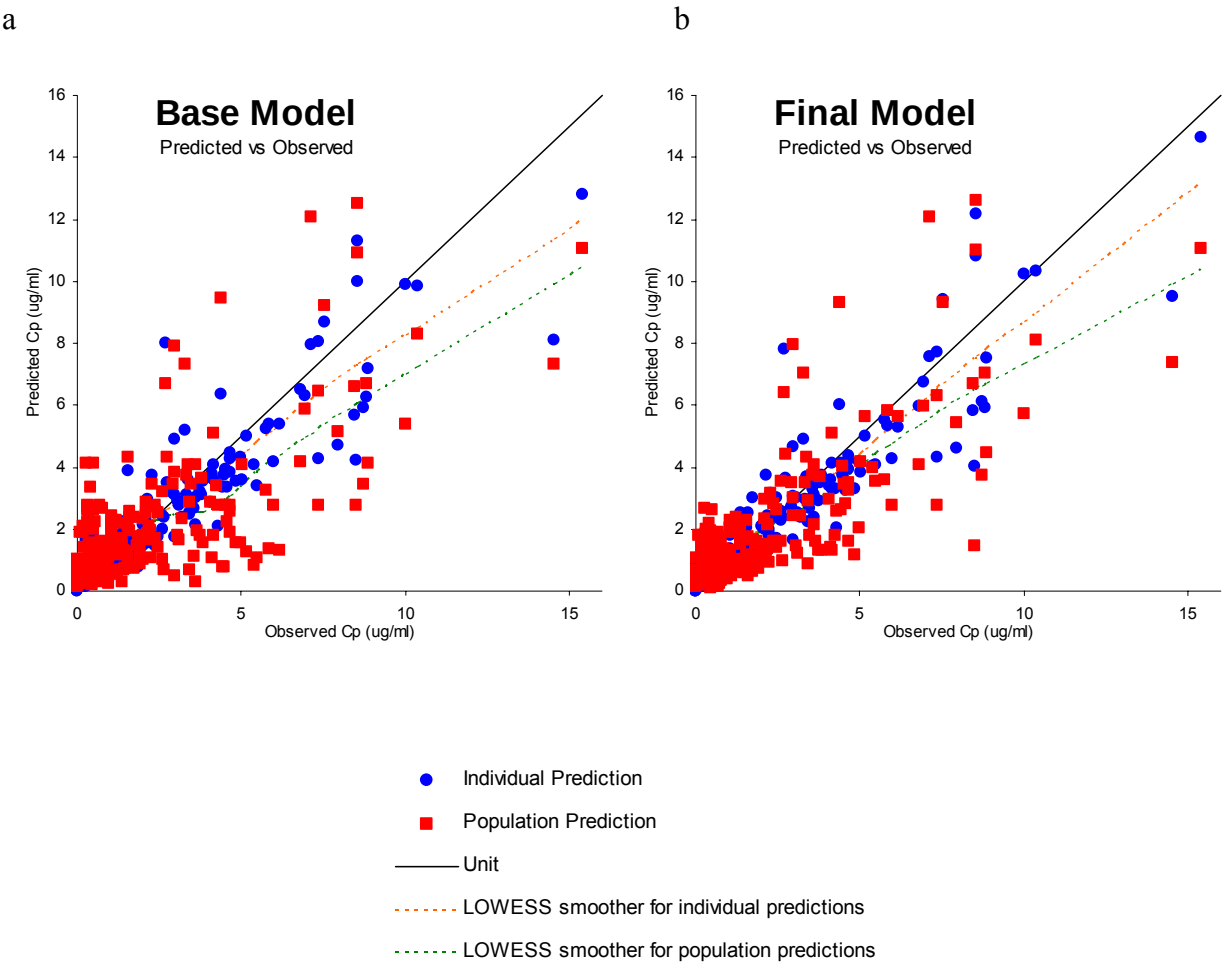


Figure 30. Goodness-of-Fit of base model in pediatric bone marrow transplant patients

a: Goodness-of-Fit of base model. Individual predictions agreed well with observations (R^2=0.85). Population predictions were biased (R^2=0.4969) with over-prediction at low concentrations and under-prediction at high concentrations, indicated by the LOWESS smoother of population predictions.

b: Goodness-of-Fit of final model. Individual predictions agreed well with observations (R^2=0.86). Population predictions were substantially improved compared to the base model (R^2=0.6217), especially at low concentrations, indicated by the LOWESS smoother of population predictions.

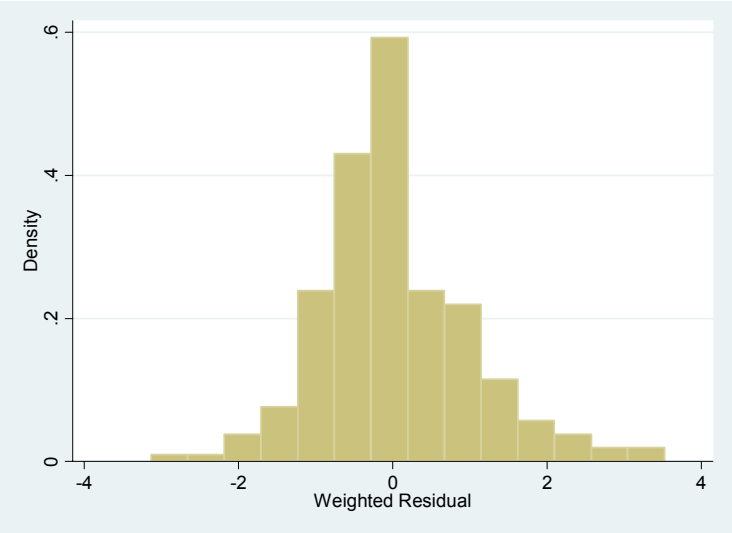


Figure 31. Weighted residual of base model in pediatric bone marrow transplant patients

Weighted residuals were approximately normally distributed and were mostly within about 4 units of the null ordinate.

To explore covariate relationships, all the covariates were tested one at a time (Table 24). CL significantly decreased with body weight as opposite to allometric principles. CL significantly increased with albumin levels. Furthermore, low CL was significantly associated with bad liver function as indicated by high levels of ALT, AST and GGTP. In addition, low CL was significantly associated with high blood count as indicated by high levels of WBC, HGB, HCT and RBC.

Volume of distribution in the central (V2) and peripheral (V3) compartment significantly increased with body weight. V2 significantly increased with albumin levels and blood count as indicated by WBC and RBC. However, V3 significantly decreased with albumin levels and blood count as indicated by WBC, HGB, HCT and RBC.

Bioavailability significantly decreased with body weight, age and albumin levels. Furthermore, high bioavailability was significantly associated with bad liver function as indicated by high levels of total bilirubin, ALT, AST and GGTP. In addition, high bioavailability was significantly associated with high blood count as indicated by high levels of WBC, HGB, HCT and RBC.

A final model was developed using forward addition and reverse removal approach as described previously:

$$CL = 5.53 \times \exp\left(-0.00478 \times \left(AST - 29.9\right)\right)$$

$$F = 32.6\% \times 1.01^{\left(AST - 29.9\right)}$$

Increased AST level was significantly associated with decreased clearance and increased bioavailability of voriconazole in pediatric bone marrow transplant patients. The final model resulted in an OFV of 38.394 with a decrease of 50.534 compared to the base model, indicating that the final model was substantially improved compared to the base model ($p<0.00001$). The Goodness-of-Fit of the final model was demonstrated in Figure 30b. Individual predictions agreed well with observations ($R^2=0.86$). Population predictions were substantially improved ($R^2=0.6217$) compared to the base model ($R^2=4969$), especially at low concentrations.

Table 24. Exploration of covariate relationships in pediatric bone marrow tranpslant patients

Parameter	Significant Covariate	ΔOFV *	P value †	Equation
CL ‡	WT	-19.99	<0.00001	$CL = 4.77 \times \exp(-0.0021 \times (WT - 31.9))$
	ALB	-22.53	<0.00001	$CL = 5.25 \times \exp(0.2 \times (ALB - 3.27))$
	ALT	-30.35	<0.00001	$CL = 5.98 \times \exp(-0.0093 \times (ALT - 19.64))$
	AST	-45.17	<0.00001	$CL = 4.51 \times \exp(-0.0248 \times (AST - 29.9))$
	GGTP	-26.44	<0.00001	$CL = 5.58 \times \exp(-0.0024 \times (GGTP - 22.3))$
	WBC	-21.90	<0.00001	$CL = 4.77 \times 0.986^{(WBC-6.11)}$
	HGB	-25.29	<0.00001	$CL = 5.15 \times \exp(-0.0171 \times (HGB - 8.92))$
	HCT	-25.88	<0.00001	$CL = 4.75 \times 0.0023^{(HCT-0.258)}$
	RBC	-20.21	<0.00001	$CL = 5.22 \times 0.825^{(RBC-2.96)}$
V2 ‡	WT	-21.31	<0.00001	$V2 = 21.6 + 0.0287 \times (WT - 31.9)$
	ALB	-25.12	<0.00001	$V2 = 25.2 + 9.08 \times (ALB - 3.27)$
	WBC	-22.40	<0.00001	$V2 = 22.7 + 0.45 \times (WBC - 6.11)$
	RBC	-21.44	<0.00001	$V2 = 21.7 + 2.34 \times (RBC - 2.96)$
V3 ‡	WT	-21.67	<0.00001	$V3 = 102 + 0.244 \times (WT - 31.9)$
	ALB	-22.78	<0.00001	$V3 = 120 \times \exp(-0.893 \times (ALB - 3.27))$
	WBC	-29.69	<0.00001	$V3 = 30.4 \times 0.786^{(WBC-6.11)}$
	HGB	-26.21	<0.00001	$V3 = 255 \times \exp(-1.98 \times (HGB - 8.92))$
	HCT	-17.80	<0.00005	$V3 = 82.5 \times \exp(-60.9 \times (HCT - 0.258))$
	RBC	-23.48	<0.00001	$V3 = 278 \times \exp(-5.77 \times (RBC - 2.96))$

Table 24. (continued)

	WT	-21.28	<0.00001	$F = 56.7\% \times \exp(-0.0211 \times (WT - 31.9))$
	AGE	-29.72	<0.00001	$F = 45.7\% \times \exp(-0.0809 \times (AGE - 9.3))$
	ALB	-24.35	<0.00001	$F = 42.5\% - 0.148 \times (ALB - 3.27)$
	BIL	-21.63	<0.00001	$F = 42.5\% + 0.0182 \times (BIL - 0.7)$
	ALT	-37.01	<0.00001	$F = 36.3\% \times 1.01^{(ALT - 19.64)}$
F ‡	AST	-46.86	<0.00001	$F = 33.6\% \times 1.02^{(AST - 29.9)}$
	GGTP	-28.62	<0.00001	$F = 39.1\% + 0.0012 \times (GGTP - 22.3)$
	WBC	-21.28	<0.00001	$F = 42.6\% + 0.0019 \times (WBC - 6.11)$
	HGB	-32.01	<0.00001	$F = 46.9\% + 0.122 \times (HGB - 8.92)$
	HCT	-32.65	<0.00001	$F = 48.2\% + 4.04 \times (HCT - 0.258)$
	RBC	-26.94	<0.00001	$F = 47.7\% + 0.197 \times (RBC - 2.96)$

* ΔOFV: change in the objective function value compared to the base model

† A decrease in OFV was referred to the chi-squared distribution to assess significance.

‡ These are the results in the covariate relationship exploration step. All the covariates were incorporated one at a time into these parameters to explore the covariate relationship.

CL: clearance

V2: volume of distribution of central compartment

V3: volume of distribution of peripheral compartment

F: bioavailability

WT: body weight

BIL: total bilirubin

ALT: alanine aminotransferase

AST: aspartate aminotransferase

ALP: Alkaline phosphatase

GGTP: Gamma glutamyl transpeptidase

ALB: albumin

WBC: white blood cell count

HGB: hemoglobin

HCT: hematocrit

RBC: red blood cell count

6.5 Discussion

To date this is the first evaluation of bioavailability and pharmacokinetics of voriconazole in pediatric bone marrow transplant patients.

This study involved intense blood sampling (eight data points from each patient in a single dosing interval) in a small group of relatively homogenous pediatric bone marrow transplant patients (n = 11), which allowed accurate and precise parameter estimation. The pharmacokinetic profiles of voriconazole are characterized by an early and sharp increase of voriconazole concentration, with the peak concentration being reached around 1 to 2 hours after dosing. These profiles were consistent with rapid absorption of voriconazole. This observation is similar to what has been reported in non-transplant adult patients (88, 89, 94).

Pharmacokinetics of voriconazole was studied both in the pre-transplant and post-transplant period. No significant difference in CL/F or Vd/F was observed between these time periods. However, this did not exclude the possibility that CL, Vd and F were all changed after transplant, resulting in unchanged CL/F and Vd/F. Therefore further investigation is warranted to study the change in pharmacokinetics of voriconazole after transplant.

Despite of the relative homogeneity of the population studied, a large inter-individual variability in voriconazole pharmacokinetics was demonstrated. This is in accordance with the large

variability in voriconazole pharmacokinetics previously reported (6, 10, 50, 63, 66, 80, 81, 96, 109, 114, 118, 122) and the unpublished preliminary data from our research group.

It is important to identify patient factors that significantly contribute to this large inter- and intra-individual variability by exploring the correlations between pharmacokinetic parameters (especially drug exposure) and patient variables. The covariates tested in this study covered a wide range of values within each of the categories tested. We have demonstrated that the most important factors associated with voriconazole pharmacokinetics were body weight and indices of liver function tests.

Clearance of voriconazole significantly decreased with increased indices of liver function tests in pediatric bone marrow transplant patients. The reason is very likely that voriconazole is extensively metabolized in the liver with less than 2% of the administered dose excreted unchanged in urine and faeces (89, 94, 103). For a low clearance drug $Cl_{apparent} \approx \text{fu} \times \text{Clint}$ (fu denotes fraction unbound. Clint denotes intrinsic clearance). Clint depends on liver function of the patient. Therefore patients with higher indices of liver function tests, indicative of hepatic dysfunction and hepatocellulary injury, had lower CL/F of voriconazole. Clearance of voriconazole also significantly decreased with body weight in pediatric bone marrow transplant patients, which is opposite to the allometric principles. The reason is very likely that body weight is correlated with age of children, and it is well known that younger children have a higher rate of metabolism.

Bioavailability of voriconazole significantly decreased with decreased indices of liver function tests. The primary reason is likely to be the positive correlation between gastrointestinal function with liver function. The secondary reason could be increased first-pass metabolism due to increased liver function indicated by decreased indices of liver function tests.

CL/F in pediatric bone marrow transplant patients (12.39L/hr) was significantly higher as compared to liver transplant (7.92 L/hr) and lung transplant (7.52L/hr) patients, likely due to higher rate of metabolism in children as compared to adults. Vd/F in pediatric bone marrow transplant patients (168.2 L) was significantly lower as compared to liver transplant (248 L) and lung transplant (430.7 L) patients, likely due to lower body size of children as compared to adults.

Despite the statistically significant improvement of the final model and the covariate models compared to base model, visual inspection of the Goodness-of-Fit plots of the final model and covariate models only showed a corrected bias of population predictions at low concentrations. This suggested that the patient variables tested and selected in this study only explain part of the variability in the pharmacokinetics of voriconazole in pediatric bone marrow transplant patients, while some other variables that were not collected in this study are still needed to account for the remaining variability. Future studies should collect more variables and further explore factors that are significantly associated with pharmacokinetics of voriconazole in pediatric bone marrow transplant patients.

As discussed previously, the large variability in voriconazole exposure following weight-adjusted or fixed doing regimens necessitates individualizing voriconazole dosing to maximize

therapeutic efficacy and minimize toxicity in pediatric bone marrow transplant patients. Therapeutic monitoring is currently performed in the routine clinical monitoring program at our institution with an intention to keep the trough concentration above 1ug/ml. However, trough concentrations have never been documented as surrogate markers of voriconazole exposure in pediatric bone marrow transplant patients.

The good correlation observed in this study between the voriconazole trough plasma concentrations and the corresponding AUCo-∞ at steady state (R^2=0.94) indicates that trough concentration is a good measure of voriconazole exposure in this population.

These findings are likely to be clinically relevant because it suggests that voriconazole dose should be relatively higher in pediatric bone marrow transplant patients due to significantly reduced bioavailability, especially in patients with good liver function as measured by low total bilirubin, ALT and AST, in order to avoid ineffectiveness of the prophylaxis/treatment and its consequences (fungal infections, especially invasive aspergillosis). However, therapeutic drug monitoring is still necessary in pediatric bone marrow transplant patients.

In conclusion, this study has demonstrated that there is a large inter-individual variability in the pharmacokinetics of voriconazole in pediatric bone marrow transplant patients. A weight-adjusted and fixed dosing regimen leads to widely variable exposure of voriconazole in pediatric bone marrow transplant patients. A population pharmacokinetic model was developed for voriconazole in pediatric bone marrow transplant patients in pre-transplant and early post-transplant period. Bioavailability of voriconazole is substantially lower in pediatric bone marrow

transplant patients (46.5%) than non-transplant adult subjects (96%), and significantly decreased with decreased indices of liver function tests. Clearance of voriconazole significantly decreased with increased indices of liver function tests. Given the large variability in the pharmacokinetics and the good correlation between AUC and trough concentrations, trough concentrations should be used to individualize voriconazole dose.

Chapter VII Conclusions and Future Directions

7.1 Discussion and Summary

The objective of the work carried out in this dissertation was to characterize the pharmacokinetics of voriconazole in transplant patients, to identify factors that are associated with the variability in the pharmacokinetics using population pharmacokinetic modeling, and to apply the finding for developing adequate dosing guidelines for transplant patients.

In this research work, we studied the pharmacokinetics of voriconazole in liver transplant, lung transplant and pediatric bone marrow transplant patients. Multiple blood samples were collected within one dosing interval. Voriconazole plasma concentrations were measured using HPLC. Non-compartmental analysis was performed using WinNonlin. Nonlinear mixed-effects pharmacokinetic models were developed using NONMEM. The association between pharmacokinetic parameters and patient- and donor-specific variables was evaluated. Several key findings were generated in this work, which are summarized in the following section.

In the first part of the study, we studied the pharmacokinetics of voriconazole in liver transplant patients. We characterized the pharmacokinetics of voriconazole in liver transplant patients, evaluated the potential correlations between pharmacokinetic parameters and patient variables, externally validated the model, and explore limited sampling strategies (LSS) using Bayesian

approaches. We demonstrated that there was a good correlation between AUCo-∞ and trough voriconazole plasma concentrations. T1/2, Cmax, trough level, AUCo-∞, AUMCo-∞ and MRTo-∞ were significantly correlated with postoperative time. T1/2, λ, AUCo-∞ and CL/F were significantly correlated with indices of liver function (AST, total bilirubin and INR). Cmax, Clast, AUMCo-∞ and MRTo-∞ are significantly lower in the presence of deficient *CYP2C19*2* alleles. In the population analysis, a one-compartment model with an absorption lag time (Tlag) adequately described the data. Population estimates of CL/F and Vd/F were 7.92L/hr and 248L. Levels of CL/F, Vd/F and Tlag decreased with post-operative time and converged to stable levels in about 7 post-operative days. CL/F significantly decreased with increased INR. Co-administration of pantoprazole, race and ALT were also significantly associated with pharmacokinetic parameters but ultimately excluded in the final model. VPC showed that most of the data fell within the 90% prediction interval and were symmetrically distributed around the median. Additional 52 samples from 19 patients were collected for external validation. MPE was 0.206ug/ml (not significantly different from zero) and MAPE was 0.99ug/ml. Compared to trough levels, LSS using two samples or one sample at a different time provided better MPE, MAPE and correlation (R^2) between the real and LSS-predicted AUC. These findings suggested that a fixed dosing regimen of voriconazole results in a highly variable exposure of voriconazole in liver transplant patients. Given that trough voriconazole concentration is a good measure of drug exposure (AUC), voriconazole dose can be individualized based on trough concentrations measurements in liver transplant patients. There is a significant association of voriconazole pharmacokinetics with post-operative time and liver function. Donor characteristics had no significant correlation with the pharmacokinetics of voriconazole. Our observations also

suggested a need for intravenous administration of voriconazole in the immediate post-operative period before an oral dose can be administrated.

In the second part of the study, we studied the pharmacokinetics of voriconazole in lung transplant patients. We characterized the pharmacokinetics and bioavailability of voriconazole in adult lung transplant patients during early post-operative period, identified factors significantly associated with various pharmacokinetic parameters, and made recommendations for adequate dosing regimens. We demonstrated that there was a good correlation (R^2=0.98) between AUCo-∞ and trough concentrations. A two-compartment model adequately described the data. Population estimates of bioavailability, clearance, Vc and Vp were 45.9%, 3.45L/hr, 54.7L and 143L. Cystic fibrosis (CF) patients exhibited a significantly lower bioavailability (23.7%, n=3) than non-CF patients (63.3%, n=10). Bioavailability increased with post-operative time and reached steady levels in about one week. Vp increased with body weight. These findings suggested that bioavailability of voriconazole is substantially lower in lung transplant patients than non-transplant subjects, but significantly increases with post-operative time. CF patients exhibit significantly lower bioavailability and exposure of voriconazole, and therefore need higher doses. Weight-adjusted or fixed dosing regimens resulted in highly variable exposure of voriconazole. Voriconazole dose can be individualized based on trough concentrations as a good measure of drug exposure. Simulations demonstrated inadequacy of oral administration of voriconazole and adequacy of intravenous administration during the first post-operative day followed by oral doses.

In the third part of the study, we applied the two-portion absorption model to describe atypical voriconazole profiles. The simplified two-portion absorption model assumes discontinuous absorption of the available dose in two portions: F1 and F2 (F1+F2=1). Delayed transfer of each portion from the stomach to the gut and the sequential absorption was described by first-order processes with lag-times (Tlag1 and Tlag2) and transfer/absorption rate constants (ktra1 and ktra2). We demonstrated that a one-compartment model with first-order elimination in association with the simplified two-portion absorption model adequately described the data and showed superiority over one- and two-compartment models with an absorption lag time. The population estimates of F1, Tlag1, Tlag2, ktra1 and ktra2 were 0.27, 0.24 hours, 2.03 hours, 0.15 hr^{-1} and 0.004 hr^{-1}, respectively. Tlag1 was significantly smaller than Tlag2. These findings suggested that atypical voriconazole pharmacokinetic profiles were probably caused by impaired gastrointestinal functions that are common in the early post-transplant period, and could be reliably described by a simplified two-portion absorption model. Twenty-seven percent of the available dose seemed to be rapidly absorbed immediately, with the remainder being slowly absorbed. This model could be useful to understand the mechanisms of voriconazole atypical profiles and to improve estimation of voriconazole exposure in liver, lung and small intestine transplant patients.

In the final part of the study, we studied the pharmacokinetics of voriconazole in pediatric bone marrow transplant patients. We characterized the pharmacokinetics of voriconazole in pediatric bone marrow transplant patients during pre-operative and early post-operative period and identify factors significantly associated with various pharmacokinetic parameters. We demonstrated that there was a good correlation (R^2=0.94) between AUCo-∞ and trough

concentrations. A two-compartment model adequately described the data. Population estimates of bioavailability, clearance, Vc and Vp were 46.5%, 5.76L/hr, 19.4L and 58.8L. Clearance significantly decreased with increased indices of liver function tests. Volume of distribution significantly increased with body weight. Bioavailability significantly decreased with decreased indices of liver function tests. These findings suggested that bioavailability of voriconazole is significantly lower in pediatric bone marrow transplant patients than non-transplant adult subjects. Incorporation of patient variables associated with the pharmacokinetics of voriconazole may assist in optimizing the dosage regimen of voriconazole in pediatric bone marrow transplant patients. Voriconazole levels should be monitored and the dose can be individualized based on trough concentrations as a good measure of drug exposure.

7.2 Clinical implications

1. Both fixed and weight-adjusted dosing regimens of voriconazole resulted in a highly variable exposure of voriconazole, and therefore routine therapeutic drug monitoring of voriconazole trough plasma concentration is necessary in transplant patients.
2. Voriconazole dose can be individualized based on trough concentrations as a good measure of drug exposure in transplant patients given that there was a good correlation between AUCo-∞ and trough voriconazole plasma concentrations in transplant patients.
3. Oral administration only of voriconazole is inadequate in transplant patients. Voriconazole should be administrated intravenously during the early period of time after transplant followed by oral doses in transplant patients.

4. For liver transplant and lung transplant patients, the pharmacokinetics of voriconazole changed significantly with post-operative time, and eventually reached a steady level in about one week. Levels of CL/F, Vd/F and Tlag in liver transplant patients decreased with post-operative time and reached steady levels in about one week. Bioavailability of voriconazole is substantially lower in lung transplant patients than non-transplant subjects, but significantly increased with post-operative time and reached a steady level in about one week. Therefore voriconazole dose for liver transplant and lung transplant patients should be low during the early period of time after transplant and then gradually increased with time.
5. Cystic fibrosis patients exhibited significantly lower bioavailability and exposure of voriconazole, as indicated by significantly reduced trough levels and AUC of voriconazole, and therefore need higher voriconazole doses.
6. For pediatric bone marrow transplant patients, higher oral dose should be administrated due to reduced bioavailability.

7.3 Limitations and Future Directions

1. These studies involved intense blood sampling in a single dosing interval in a small group of relatively homogenous transplant patients due to clinical constraints of the standard patient care. The small sample size may reduce the power of the statistical analysis and conclusions.

2. These studies were conducted in a certain pre-transplant or post-transplant period due to clinical constraints of the standard patient care. The homogeneity of the subjects may limit the applicability of the conclusions to the entire population.
3. Pharmacodynamic modeling was not applied in these studied. The first reason was that simple efficacy measure for molds are not quite available yet. So far there have only been data in animals for Candida showing a predictive pharmacodynamic parameter (AUC/MIC) and a potential target value (2) with no equivalent data for molds. The second reason was that all the transplant patients in our institution who receive voriconazole for prophylaxis go through routine clinical therapeutic monitoring program, based on which the dose of voriconazole is constantly adjusted to avoid infections and toxicity. Therefore pharmacodynamic endpoints such as infections and toxicity were not available.
4. A potentially significant reduction in the exposure of voriconazole has been observed in patients receiving concomitant pantoprazole, as indicated by significantly reduced trough levels, Cmax, AUC and AUMC of voriconazole. *In vivo* and *in vitro* studies are warranted to illustrate the effects of pantoprazole on the pharmacokinetics of voriconazole, especially its effects on the absorption of voriconazole, and to explore the underlying mechanisms of these effects.
5. Limited sampling strategies developed using Bayesian approaches in this study have shown potential to accurately and precisely estimate voriconazole exposure with one or two blood samples and no rigid sampling time or dosage regimens required, but definitely required external validation before used in practice to individualize voriconazole dosage.

6. A definitely significant reduction in bioavailability and exposure of voriconazole has been observed in cystic fibrosis patients, as indicated by significantly reduced trough levels and AUC of voriconazole. *In vivo* and *in vitro* studies are warranted to illustrate the effects of cystic fibrosis on the pharmacokinetics of voriconazole, especially its effects on the absorption of voriconazole, and to explore the underlying mechanisms of these effects.
7. Due to clinical constraints, bioavailability of voriconazole was only assessed once in each patient in all the studies in this dissertation, which limited the comparison of bioavailability of voriconazole before and after transplant and limited the demonstration of the change in bioavailability of voriconazole with post-operative time. Future studies should assess bioavailability of voriconazole at multiple times in each patients in order to compare bioavailability of voriconazole before and after transplant and to demonstrate the change in bioavailability of voriconazole with post-operative time

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