



Population pharmacokinetics of a posaconazole tablet formulation in transplant adult allogeneic stem cell recipients

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ABSTRACT

Background: Posaconazole is an antifungal agent extensively used as a prophylaxis for invasive fungal infections (IFIs) in allogeneic stem cell transplant (SCT) recipients. Low posaconazole concentrations have been associated with reduced clinical response. The aim of this study was to develop a population pharmacokinetic (popPK) model of a posaconazole tablet formulation in allogeneic SCT adult recipients for supporting model-informed precision dosing (MIPD).

Materials and method: Prospective observational study performed in adult allogeneic SCT recipients receiving posaconazole as prophylaxis for IFIs and followed up by a therapeutic drug monitoring (TDM) program. Posaconazole plasma concentrations were quantified using an ultra-high-performance liquid chromatography (UPLC) with UV detector. A popPK model was developed using NONMEM v.7.4.0. Deterministic and stochastic simulations were carried out with the final model to evaluate the differences across physiological variables with impact on drug exposure.

Results: A one-compartment model with sequential absorption (zero and first order) and first order elimination described adequately 55 posaconazole concentrations from 36 patients. Higher doses of posaconazole were found to be required by males and patients with lower values of total serum proteins. A nomogram to estimate the posaconazole daily dose based on pharmacokinetic/pharmacodynamic (PKPD) criterion for males and females for different values of total proteins was developed.

Conclusions: Gender and total serum proteins have been identified as covariates influencing posaconazole CL/F in adult allogeneic SCT recipients receiving the delay-released tablet formulation. Additional studies are required to better characterize the absorption of posaconazole and implications on dosage recommendations together with potential safety concerns.

1. Introduction

Invasive fungal infections (IFIs) remain a significant health threat in immunocompromised patients, including blood cancer patients and those undergoing haematopoietic stem cell transplantation. In view of the substantial disease burden associated with IFIs, primary antifungal prophylaxis is crucial in patients at high risk of prolonged neutropenia (Perfect et al., 2014).

Posaconazole is a triazole antifungal agent with a broad-spectrum

activity which is widely used for prophylaxis of invasive Aspergillus and Candida infections in high-risk patients for IFIs as well as for treating certain fungal infections (EMA, 2021). Low posaconazole concentrations have been associated with lower clinical efficacy (Stott and Hope, 2017). Posaconazole target trough concentrations (C_{min}) higher than 0.7 mg/L for patients receiving posaconazole for prophylaxis or higher than 1 mg/L for those with established infection have been proposed (Ashbee et al., 2014). Moreover, according to preclinical pharmacokinetic/pharmacodynamic (PKPD) models, area under the

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concentration-time curve and minimum fungicidal inhibitory concentration ratios (AUC/MIC) for posaconazole of 100 for prophylaxis and 200 for treatment of fungal infections have been proposed in order to improve efficacy (Dekkers et al., 2016). On the other hand, it is generally well tolerated and no correlation between toxicity and drug exposure has been found.

Posaconazole has been extensively used without therapeutic drug monitoring (TDM). However, several studies demonstrated that, under these circumstances, a high percentage of patients (36.0–90.5%) did not achieve the target concentrations, as posaconazole concentrations showed large inter- and intra-patient variability (Bryant et al., 2011; Eiden et al., 2012; Hoeningl et al., 2012; Mårtson et al., 2019; Thompson et al., 2009). Thus, nowadays, TDM, in combination with clinical assessment of response and determination of MIC, is already recommended by some scientific societies as a tool for personalizing posaconazole dosing, as is the case of the British Society for Medical Mycology (Ashbee et al., 2014) and the 6th European Conference on Infections in Leukemia (ECIL-6) (Tissot et al., 2017).

Posaconazole was initially approved as an oral suspension formulation. However, food has an important impact on the bioavailability of this formulation and many patients do not achieve optimal posaconazole target concentrations. Oral absorption appears to be saturable and is also strongly affected by gut motility and gastric acidity. Medications that increase gastric pH (eg. proton pump inhibitors, histamine H2-receptor antagonists), nausea and vomiting, the presence of mucositis or graft-versus-host disease (GVHD) of the gut, and the use of agents that promote gastrointestinal motility are other factors that contribute to high pharmacokinetic (PK) variability (Courtney et al., 2004, 2003; Ullmann et al., 2006).

For these reasons, a delayed-release solid tablet formulation was developed to maximize systemic absorption. However, although the oral bioavailability of posaconazole tablets is higher than the suspension formulation, considerable variability is still observed (Krishna et al., 2012). Moreover, inter-occasion variability (IOV) of posaconazole clearance has also been described, which further supports the benefits of posaconazole TDM (Petitcollin et al., 2017).

Posaconazole PK have been extensively studied in patients taking the suspension formulation (Dolton et al., 2014; Gubbins et al., 2006; Kohl et al., 2010; Krishna et al., 2012; Ullmann et al., 2006). However, few population PK (popPK) models to provide the basis for supporting model-informed precision dosing (MIPD) are available for the tablet formulation (Petitcollin et al., 2017; Van Iersel et al., 2018).

The objective of this study was to develop a popPK model of a posaconazole tablet formulation in allogeneic SCT adult recipients to support MIPD strategies.

2. Materials and methods

2.1. Study design

This was a prospective observational study performed in allogeneic SCT recipients receiving posaconazole as prophylaxis for IFIs who are being followed up by TDM program at the Hematology Service and the Clinical Pharmacokinetics Unit of the Pharmacy Service of the University Hospital of Salamanca (Spain). The study was conducted between July 2020 and April 2021.

Posaconazole was orally administered as Noxafil® tablets with a starting loading dose of 300 mg (three 100 mg delayed-release tablets) twice a day on the first day of treatment, followed by a maintenance dose of 300 mg once a day thereafter. Patients were treated for 100 days post-transplant or until breakthrough of an IFI, an adverse event requiring discontinuation, or death (mainly due to underlying GVHD). Non-hospitalized patient adherence was assessed by reviewing medication dispensing records and ratified with a personal care interview with the patient. Non-adherent patients were excluded from the study.

The study protocol was authorized by the Ethics Committee of

Clinical Research of the University Hospital of Salamanca (CEIC number: PI2020/03/460), and all patients signed informed consent regarding their willingness to participate in the study.

2.2. Data collection

The following information was recorded for each patient: age, gender, height, total body weight (TBW), body surface area (BSA) which was calculated as $\text{weight (Kg)} \cdot 0.425 \cdot \text{height (cm)} \cdot 0.725 \cdot 0.007184$ (Du Bois and Du Bois, 1989), body mass index (BMI) calculated as $\text{weight (kg)} / \text{height}^2$ (m), diagnosis, albumin, total proteins, hepatic and digestive GVHD status (defined by clinical and histological criteria in 4 grades) (Glucksberg et al., 1974), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), γ -glutamyltransferase (GGT), total bilirubin, urate, creatinine, estimated glomerular filtration rate (eGFR) calculated with CKE-EPI in ml/min, C-reactive protein (CRP); hemoglobin, and absolute neutrophil counts (ANC).

Diagnosis was categorized for evaluation in the popPK covariate model building procedure in leukemia, lymphomas and others. Sampling time and dosing regimen available at the time of the extraction were also recorded. The patients' concomitant medication was reviewed to identify drugs with potential inference in drug absorption.

2.3. Therapeutic drug monitoring

Blood samples were collected in tubes with EDTA. The plasma was separated by centrifugation (3700 rpm for 3 min) and frozen below -20° for storage until concentration measurement.

The number of samples per patient varied depending on our ability to collect them. In hospitalized patients at the beginning of treatment, PK sampling times starting day 2 were the following: trough concentration 1 h, 3.5 h and 24 h post-dose. In non-hospitalized patients, one sample was drawn between 12 and 24 h after drug administration.

Plasma concentrations of posaconazole were measured by reverse-phase ultra-high-performance liquid chromatography (UPLC) using a Luna Omega C18 column (1.6 μm ; 2.1 mm x 50 mm, Phenomenex Company). The mobile phase used for the elution consisted of a 55/45 mixture of 0.5% formic acid and acetonitrile; the flow rate was 0.5 ml/min and the UV detector was set at 259 nm. Before the chromatographic injection, the samples were treated with protein precipitation with acetonitrile and later centrifugation at 5000 rpm for 5 min, injecting the supernatant after the filtration through 0.22 μm .

The method was adequately validated for specificity, linearity, precision, accuracy and stability according to FDA Guidance and EMA Guidelines on bioanalytical method validation (FDA, 2018; Smith, 2012). The calibration range of the assay was 0.2–15.0 $\mu\text{g/ml}$. The accuracy (percentage difference from actual) at low, medium, and high quality control samples was 5.3%, 2.2% and 2.7%, respectively. The precision (coefficient of variation, CV) at low, medium, and high quality control samples was 7.0%, 3.5% and 3.1%, respectively. The lower limit of quantification (LLOQ) was 0.2 $\mu\text{g/ml}$.

2.4. Population pharmacokinetic analysis

Non-linear mixed effects modeling approach using the first-order conditional estimation (FOCE) method with interaction was used to develop the popPK model using NONMEM® version 7.4.0 (Icon Development Solutions, Ellicott City, MD, USA) (Beal et al., 2009). Data visualization and statistical analyses, including evaluation and representation of model and simulation results were carried out in R version 4.0.2. (R Core Team, Vienna, Austria, 2012).

The PK of the posaconazole tablet formulation was initially described by a one-compartment model with sequential absorption (zero order followed by first order processes). Posaconazole plasma concentrations after oral and IV administration were not available and consequently bioavailability was not estimated, and PK parameters were considered

apparent. The model was parametrized in terms of apparent clearance (CL/F), apparent volume of distribution (V/F), duration of zero order absorption process (D1) and first order absorption rate constant (k_a). Reduced absorption models, due to the lack of PK information in the absorption phase, were evaluated such as first order and/or absorption process fixed to previous values reported. Inter-individual variability (IIV) of PK parameters estimated were assumed to follow a log-normal distribution. Residual unknown variability (RUV) was evaluated following a proportional, additive or combined (proportional and additive) error model. The magnitude of IIV and RUV was expressed approximately as a coefficient of variation. Correlation between random parameters and IOV were evaluated. Time-varying of posaconazole elimination since allogeneic transplant was also studied following a longitudinal (power and sigmoid models) or categorical (significant change in CL/F after six months since the allogeneic SCT) relationship.

After selecting the base (structural and stochastic) model, potential relationships between estimated individual PK parameters and physiological meaningful variables were explored graphically. In addition, the most physiologically plausible covariates were selected among highly correlated ones (i.e., estimated glomerular filtration rate over serum creatinine). Only covariates showing statistical significance (p -value <0.05) and those physiological plausible were considered as potentially clinically relevant and were further tested following a stepwise covariate methodology (forward p -value <0.05 ; backward p -value <0.01).

A decrease of 3.84 (p -value <0.01) of the minimum objective function value (MOFV) assuming a chi-squared distribution together with the reduction of IIV and RUV, an adequate precision and bias of parameter estimates (residual standard error, RSE, and shrinkage, respectively) and the improvement of the goodness-of fit plots were considered for model selection criteria.

The final popPK model developed was internally evaluated through visual predictive check (VPC). A total of 1000 replicates of the original dataset were generated through simulations in NONMEM (*post-hoc*). The 5th, 50th and 95th percentiles of the observed posaconazole plasma concentrations, as well as the 5th, 50th and 95th percentiles together with their 95% confidence interval (CI) for the corresponding model-based predicted concentrations computed for each bin across time and replicates were graphically represented and evaluated. A non-parametric bootstrap resampling method was used to evaluate the stability of the final model and the precision of parameter estimates. The bootstrapping procedure, which was based on 1000 resamples generated from the original dataset and following the same structure of the final model, was conducted in PsN toolkit version 4.9. (Lindbom et al., 2005). Uncertainty in PK parameter estimates was quantitatively assessed by calculating the 95% CI for parameter estimates. The model developed was considered stable if the median parameters estimated with the original dataset were found in the 2.5th and 97.5th percentiles built with the datasets generated by resample technique (Byon et al., 2013).

2.5. Simulations

Deterministic and stochastic simulations were carried out in R with the final posaconazole popPK model to evaluate the differences across physiologically variables identified with a significant impact on drug PK and exposure.

Stochastic model-based simulations were carried out for each scenario ($n = 5000$). Posaconazole AUC at steady state was calculated for a standard oral administration of 300 mg once daily. Probability of target attainment (PTA) of the efficacy PKPD criterion selected at the steady state (AUC/MIC ≥ 200) was calculated for the following pathogens and MIC: 1) MIC=0.06 mg/L *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis* and *Candida tropicalis*, 2) MIC=0.25 mg/L for *Aspergillus fumigatus* and *Aspergillus terreus*, and 3) MIC=0.5 mg/L for *Aspergillus flavus*, *Aspergillus nidulans* and *Aspergillus niger* (EUCAST, 2021; Sime et al., 2019).

Posaconazole doses in mg/day to achieve an AUC/MIC ≥ 200 for the possible scenarios considered, based on the physiological characteristics identified in the popPK analysis, were calculated and represented through deterministic simulations with R.

3. Results

A total of 55 posaconazole concentrations from 36 patients were included in the analysis. The baseline patients' characteristics and study data are summarized in Table 1. Patients included in this study had undergone an allogeneic SCT from healthy donors (related or unrelated). The most frequent diagnosis was acute myeloid leukemia ($n = 17$) and the median time since allogeneic transplant was 57 days. All of them received 300 mg once daily of posaconazole fasting with tablet presentation for prophylaxis of IFIs. In 9 hospitalized patients, posaconazole concentrations were obtained 1 h, 3.5 h and 24 h post-dose; 6 of these subjects started treatment in the hospital. Posaconazole concentrations in patients already in treatment at enrolment ($n = 27$) were obtained mainly prior to the drug administration (C_{min}). All samples were adequately quantified with the analytical method ($>LLOQ$).

A one-compartment model with sequential absorption (zero and first order) and first order elimination described adequately the posaconazole concentrations. The model included IIV on CL/F and V/F and a RUV following a proportional error model. First order absorption model, estimation of sequential absorption PK parameters with or without IIV did not fulfill the statistical criteria for model selection. This fact is most likely due to the data not being sufficiently informative to support estimation of absorption models. Therefore, the absorption PK parameters of a sequential model, D1 and k_a , were fixed to the values proposed by Van Iersel et al. (Van Iersel et al., 2018)) under comprehensive considerations together with similarities across the studied population. Inclusion of IOV on CL/F or correlation between IIV of CL/F and V/F did not improve model performance.

The following covariates were evaluated: TBW, BMI, BSA, bilirubin,

Table 1
Patient baseline characteristics and study data.

Number of patients	36
Demographics	
Age [median in years (range)]	53 (27–73)
Gender [female (%)]	52.8
Total body weight [median in Kg (range)]	68.3 (40.0–103.5)
Body mass index [median in Kg/ m ² (range)]	24.0 (16.4–35.9)
Body surface area [median in m ² (range)]	1.8 (1.3–2.2)
Primary diagnosis [number of patients (%)]	
Acute myeloid leukemia	17 (47.2)
Myelodysplastic syndrome	5 (13.9)
Hodgkin's lymphoma	2 (5.6)
Non-Hodgkin's lymphoma	2 (5.6)
Myelofibrosis	1 (2.8)
Acute lymphoblastic leukemia	7 (19.4)
Chronic lymphocytic leukemia	1 (2.8)
T cell prolymphocytic leukemia	1 (2.8)
Other clinical characteristics (number of patients)	
Digestive GVHD status	
I	8
II	3
III	0
IV	1
Biochemical parameters [median (range)]	
Total bilirubin (mmol/L)	0.43 (0.19–1.28)
Aspartate-transaminase (IU/L)	24 (9–69)
Alanine-transaminase (IU/L)	38.5 (11–264)
Alkaline phosphatase (IU/L)	71.5 (41–207)
Gamma-glutamyltransferase (IU/L)	44.5 (11–280)
Creatinine clearance (mL/min) ^a	98.8 (43.6–141.0)
Albumin (mg/dL)	4.1 (2.7–4.8)
Total serum proteins (g/dL)	6.5 (4.8–7.8)

GVHD: disease of graft versus host disease.

^a calculated with CKE-EPI equation.

alkaline phosphatase, AST, ALT, GGT, age, eGFR, total protein, albumin, ANC, hemoglobin, sex, diagnosis, time since allogeneic transplant, hepatic and digestive GVHD status. The inclusion of gender and total serum proteins on CL/F explained 34% of its variability. The PK parameter estimates of the final model together with the internal validation results are summarized in Table 2. All PK parameters were estimated with adequate precision and lack of pronounced bias (RSE < 26% and shrinkage < 61% in all cases) and fell within the calculated 95% CI of the bootstrap analysis. Reliability and robustness of the parameter estimates were acceptable (57% of bootstrap samples successfully converged).

Goodness-of-fit plots of the final popPK model showed a lack of structural bias and a proper correlation between population and individual model-based predictions compared to the observed data (Fig. 1). The VPC showed an adequate description of the posaconazole concentration time course and its associated PK variability after oral administration to the patients with the model developed for the tablet formulation (Fig. 2).

The PTA of the standard posaconazole treatment of 300 mg once daily together with the posaconazole dose required to achieve the efficacy PKPD criterion selected (AUC/MIC \geq 200) are shown in Fig. 3. Higher doses of posaconazole were required in males and subjects with lower values of total serum proteins. These results show adequate expected treatment efficacy (PTA \geq 90%) of the posaconazole standard therapy (300 mg once a day) for MIC of 0.06 mg/L. In contrast, current standard dosage would be not sufficient to warrant treatment efficacy for IFI that required MIC of 0.5 mg/L, nor for MIC of 0.25 mg/L in males or in females with low serum protein values.

A nomogram with the posaconazole daily dose required to achieve the PKPD criterion defined for males and females for different values of total proteins are shown in Fig. 3. Adequate treatment success was obtained with the standard posaconazole dosage against *Candida sp.* infections (CMI=0.06 mg/L). Based on the deterministic simulations, more intensive regimens (up to 3–4 fold the standard one) would be required to achieve the PKPD criterion selected for *Aspergillus sp.* infections, especially in males with low plasma proteins values.

4. Discussion

Posaconazole is extensively used for prophylaxis for IFIs, especially in immunocompromised patients after allogeneic SCT. Nevertheless,

posaconazole treatments are overshadowed by the large PK variability, which might cause subtherapeutic exposures (Kraljevic et al., 2020). The use of popPK models integrated within a Bayesian forecasting framework have shown to have important advantages for providing individualized dose recommendations. This approach allows for characterizing population average PK parameters, intra- and inter-subject variability, and for identifying and quantifying key factors with influence on PK behavior. Having available appropriate and reliable popPK models is thus essential prior to the implementation of a posaconazole TDM program into routine clinical practice.

Although currently, posaconazole tablets are the main formulation used in therapy due to their improved oral bioavailability (Sime et al., 2019), studies in adult patients using the delayed-release tablet formulation by compartmental modeling are sparse, and available analysis use data from healthy volunteers and patients included in clinical trials (Boonsathorn et al., 2019; Petitcollin et al., 2017). Nevertheless, to the best of our knowledge, popPK studies developed with patients undergoing prophylaxis with posaconazole oral tablets in real-world practice are lacking (Petitcollin et al., 2017). Moreover, only a minor fraction of the overall variability in posaconazole exposure has been established with the available models in the literature.

This study characterized the posaconazole popPK in patients receiving prophylaxis of IFIs with the tablet formulation in the clinical setting. A one-compartment model with sequential absorption (zero and first order) with first order elimination and proportional error model provided an adequate description of the posaconazole delayed-release tablet formulation PK. The reduced data information during the absorption phase did not allow for properly estimating properly either ka nor D1 and both were fixed to previous values reported by the group of Van Iersel (Van Iersel et al., 2018). The estimated population CL/F (8.02 L/h) is aligned with those previously reported from posaconazole popPK studies in adult patients (7.30 - 8.36 L/h) (Petitcollin et al., 2017; Van Iersel et al., 2018). A significantly higher V/F (547 L) was found compared with previous studies (410 - 420 L). This increase in V/F could be associated with the population characteristic differences across the studies considered (allogeneic SCT adult recipients vs. other hematological patients and healthy volunteers).

Flip-flop kinetics can occur with sustained-release formulations and create difficulties to determine and interpret pharmacokinetic parameters if not recognized. Nevertheless, it is important to note that the Ka of the posaconazole delayed-release tablet formulation has been reported as ranging from 0.59 to 0.85 1/h (Chen, 2020). According to our model, posaconazole elimination constant rate (Ke) is predicted to be between 0.005 and 0.021 1/h in our population. Therefore, it is not expected that Ka will be slower than Ke, and consequently elimination processes are properly characterized in the popPK model presented.

The popPK model presented in this work has evaluated the influence of a wide number of factors with a potential impact on posaconazole exposures. Gender was found to have a significant impact on posaconazole CL/F most likely related to body size. However, gender improved fitting compared with other evaluated body size metrics, such as TBW, BMI and BSA. This fact could be related to differences between males and females due to gender hormone-related effects on the drug disposition process, particularly those involving drug-metabolizing enzymes and transporters (Sheth et al., 2015). In fact, several studies suggested lower activities of isoenzymes of the uridine 5-diphosphate glucuronosyltransferase (UGT) superfamily, responsible for the metabolism of posaconazole, in women versus men (Soldin and Mattison, 2009). Moreover, posaconazole is a substrate for P-glycoprotein, the membrane transporter best characterized in the literature regarding sex differences in expression. Furthermore, the hepatic P-glycoprotein expression has been described to be two-fold lower in women compared to men (Schuetz et al., 1995), which could explain a lower rate of posaconazole CL in women versus men.

Total serum proteins were also found to have a significant impact on posaconazole CL/F. Posaconazole CL/F decreased and, thus, exposure

Table 2

Posaconazole population pharmacokinetic parameters.

Parameters	Final Model			Bootstrap	
	Estimate	RSE (%)	Shrinkage (%)	Median	95% CI
CLpop (L/h)	8.02	8.10	–	7.98	6.25–9.36
CLsex (L/h)	0.613	11.5	–	0.623	0.450–0.780
CLprot(L/h)	–1.48	23.0	–	–1.47	–2.59 to –0.680
Vpop (L)	548	26.8	–	582	362–855
Ka (1/h)	0.795 [#]	–	–	0.795 [#]	0.795–0.795
D1 (h)	2.62 [#]	–	–	2.62 [#]	2.62–2.62
IIVCL (CV,%)	28.9	22.9	16.7	0.070	0.004–0.139
IIVV (CV,%)	52.4	18.0	67.6	0.235	0.009–0.446
RUVprop (CV,%)	21.6	23.0	26.7	0.042	0.013–0.087

$$CL (L/h) = CL_{pop} \times CL_{sex}^{SEX} \times (PROT/6.4)^{CL_{prot}}$$

CI, confidence interval; CLpop, clearance of the typical subject (men, PROT=6.4 g/dL); CLsex, magnitude of the effect of sex on CL; CLprot, magnitude of the effect of total proteins on CL; CLsex, magnitude of the effect of sex on CL; D1, duration of zero order absorption process; IIVCL, interindividual variability on clearance; IIVV, interindividual variability on apparent volume of distribution; Ka: absorption rate constant; PROT, total proteins in g/dL; RSE, residual standard error; RUVprop, proportional error of residual variability; SEX, 0 for men and 1 for women; Vpop, volume of distribution of the typical subject; #, parameter fixed.

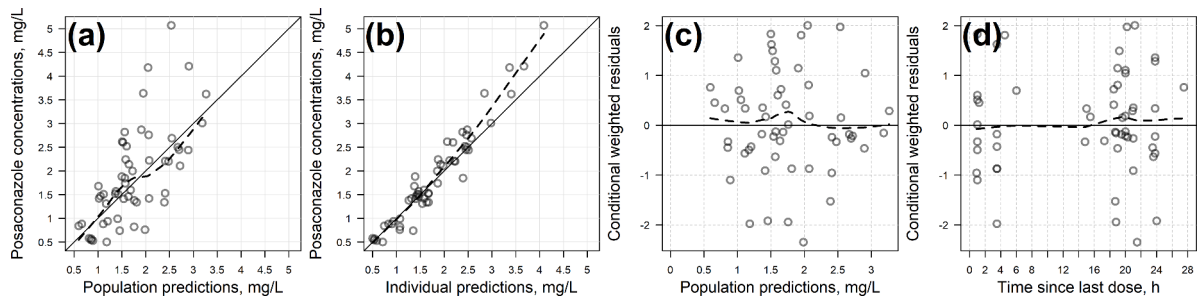


Fig. 1. Goodness-of-fit plots of the final model developed. (a) observed concentrations versus population predicted concentrations; (b) observed concentrations versus individual predicted concentrations; (c) conditional weighted residuals versus time; (d) conditional weighted residuals versus observed concentrations. Solid line: identity line; open circles: posaconazole concentrations; dashed lines: locally weighted scatterplot smoothing (LOWESS).

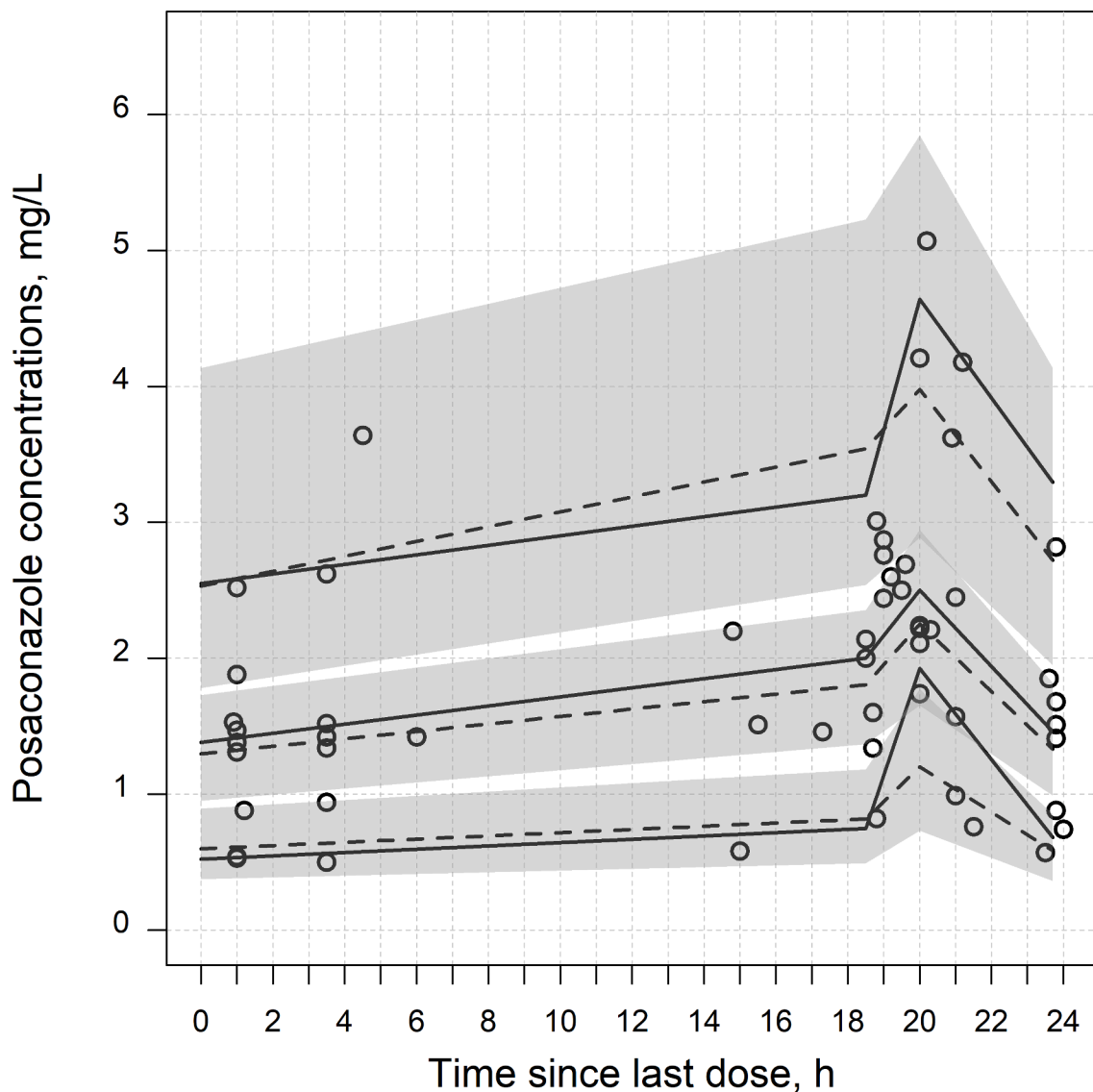


Fig. 2. Visual predictive check (VPC) for the concentration-time after-dose profiles of posaconazole in the studied population. The figure shows the empirical median and 5th and 95th empirical percentiles (solid lines), the theoretical median and the 5th and 95th theoretical percentiles (dashed lines), the 95% confidence interval of the theoretical median and percentiles (shaded areas), and the observed posaconazole concentrations (open circles).

augmented with increasing total proteins. Given the extensive binding of posaconazole (99%) to plasma proteins (Chen et al., 2020), decreased protein binding may occur in patients with low protein concentrations, which might result in an increase in free drug concentrations. Since only free drugs are available for delivery to the tissues, including those from

organs implied in metabolism and excretion, protein binding may have a restrictive effect upon posaconazole elimination. On the other hand, although posaconazole is predominantly bound to albumin, no association was found for this plasma protein and drug PK parameters. It must be taken into account that other plasma binding proteins, such as alpha

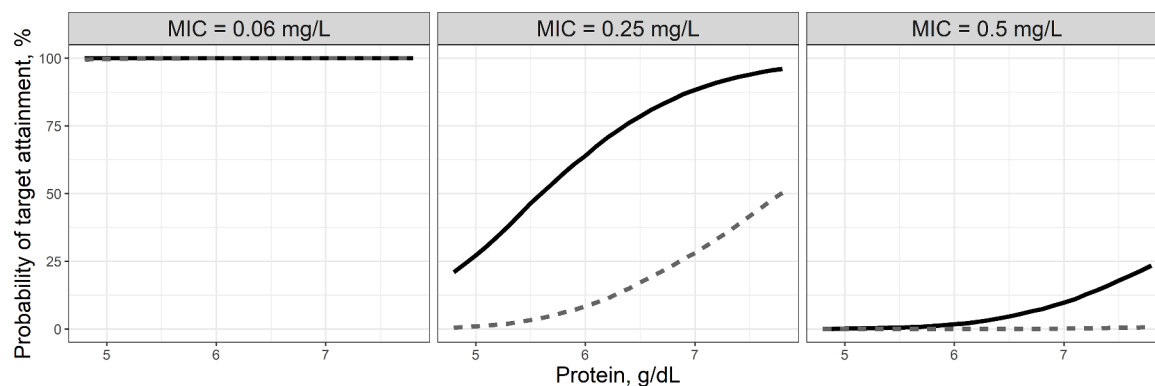


Fig. 3. Probability of target attainment (PTA) to reach an $AUC/MIC \geq 200$ with standard posaconazole treatment (300 mg q24h) for three different minimum inhibitory concentrations (MIC), according to possible serum protein values for females (black solid lines) and males (gray dashed lines).

1-acid glycoprotein and globulins, were not determined in our study (Lignell et al., 2011).

Several of the significant covariates identified during model development were not finally retained. This is the case with digestive GVHD, which did not fulfill the statistical criteria to be included as a covariate on CL/F in the final popPK model. Gastrointestinal damage secondary to GVHD has been previously described as responsible for decreased posaconazole concentrations (Krishna et al., 2009). Accelerated emptying of the gut caused by diarrhea associated with GVHD may contribute to the lower concentrations observed in these patients and resulting in a reduced bioavailability.

Although a positive relationship between CL/F and GVHD was initially observed in our study, this covariate could not finally be included maybe due to the lack of data to adequately characterize the drug absorption phase. Significant effects on apparent clearance (CL/F) were also found by other authors for presence of diarrhea (Dolton et al., 2014; Vehreschild et al., 2012).

As is a common finding of PK studies, we found that posaconazole V/F was influenced by some of the body size covariates that were tested. In particular, BSA and TBW were found to be positively correlated with posaconazole V/F. Higher TBW has also been significantly associated with a larger V/F in previous studies (Kohl et al., 2010; Vehreschild et al., 2012). Given that posaconazole is a highly lipophilic drug, this observation is most probably related to an extensive distribution into the adipose tissue thus decreasing total observed concentration. In fact, a recent popPK study in 16 obese patients receiving posaconazole showed that, in order to reach adequate drug concentrations, the normal maintenance dose for treatment of 300 mg administered once daily needed to be increased to 400 mg and 500 mg for patients weighting between 120 and 170 kg, and more than 170 kg, respectively (Wasmann et al., 2020). Moreover, in patients with hematological malignancies receiving posaconazole delayed-release tablets, lower trough concentrations have been observed in patients weighting 90 kg or more compared to those weighting less than 90 kg (0.65 vs. 1.29 mg/L) (Miceli et al., 2015).

Post-transplant time has been included as a covariate with influence on different PK parameters in some models developed for other azoles (Han et al., 2010; Lin et al., 2018). Physiological factors that determine drug PK (e.g., liver function, gastrointestinal function) are highly variable soon after the transplant, but usually recover and improve over time towards normal population values, which may explain the time-varying PK behavior after the allogeneic SCT transplant. In our study, we found no association between post-transplant time and posaconazole clearance. This may be due to the fact that most of the patients were in the acute post-transplant period.

Besides, other authors found that concomitant use of proton pump inhibitors was associated with a reduction in relative bioavailability with both suspension and tablets (Boonsathorn et al., 2019). In our study

this effect was not observed, because all patients were under treatment with these drugs.

Since our results show that gender and total protein appear to be important considerations for appropriate posaconazole dosing, deterministic and stochastic simulations were carried out in order to quantify the impact of these covariates on dose requirements. As illustrated in Fig. 3, higher dose requirements have been described for males and for patients with hypoproteinaemia. Since dose escalation appears necessary in some patients, the nomogram proposed in Fig. 3 could be useful for selecting the posaconazole initial dose, in order to avoid unnecessary underexposure. Moreover, no safety issues have been shown for posaconazole intravenous and suspension formulations administered up to 1200 mg (Cornely et al., 2013; Jang et al., 2010). Thus, no major safety concerns are expected for posaconazole delayed-release tablet formulations at higher doses than the standard one (300 mg once a day). However, additional studies are required to confirm the safety profile of posaconazole delayed-release tablet formulation. Fig. 4.

This study has some limitations. First, the sample size was small and, as previously mentioned, the analysis mainly involved trough concentrations, which was not sufficiently informative to adequately characterize the absorption phase in the studied population and made it necessary to fix the absorption parameters to previous reported values. Since the gastro-resistant tablet presentation was developed to maximize systemic absorption, and only less than a quarter of patients included in our study suffered from this GVHD, most of them with a grade I status, and no other physiopathological factors influencing this process were present in the study group, no major differences in comparison with other groups of hematological patients were expected. Second, since additional patients were not available, it was not possible to perform an external evaluation of the model, which would be of special interest in order to confirm the absorption model assumed in the final popPK model developed. Moreover, the difference in posaconazole exposure according to genetic polymorphisms has not been studied. The formation of posaconazole is mediated by UGT enzymes, especially UGT1A4, and gene polymorphisms are a key factor in the regulation of the content and activity of these enzymes. In fact, UGT1A4*3 genetic polymorphism has been associated with low posaconazole plasma concentrations in patients with hematological malignancies. (Suh et al., 2018). Unfortunately, genetic information was not available for the patients studied and therefore the impact of differences in UGT enzymes on posaconazole elimination has not been evaluated. Finally, it would be of interest to evaluate the potential relationship between additional plasma proteins, such as alpha 1-acid glycoprotein and globulins, and posaconazole CL/F.

This study presents several strengths. The popPK model proposed was performed with real-world patients rather than in a clinical trial framework, which adds valuable information regarding posaconazole tablet formulation PK in the clinical routine setting. The developed

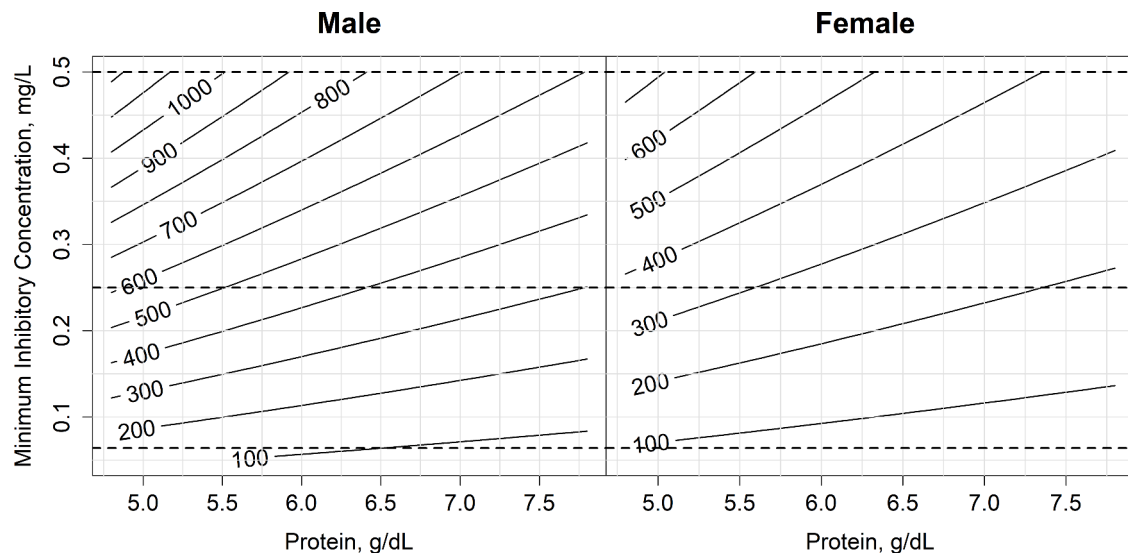


Fig. 4. Posaconazole model-informed precision dosing nomogram. The solid lines represent the posaconazole daily dose (mg) required to achieve the efficacy PKPD criterion selected ($AUC/MIC \geq 200$) for males (left panel) and females (right panel) at different minimum fungicidal inhibitory concentrations (MIC) and total serum proteins; dashed lines, MIC of *Candida sp.* (0.06 mg/L), *Aspergillus fumigatus* and *Aspergillus terreus* (0.25 mg/L) and *Aspergillus flavus*, *Aspergillus nidulans* and *Aspergillus niger* (0.5 mg/L).

popPK model adequately described the posaconazole concentrations available of allogeneic SCT recipients, which had not previously been described. Moreover, the main findings of this study are the physiological factors identified with a significant impact on posaconazole PK, leading to the nomogram proposed based on PKPD criterion which could be of interest for individualizing initial dose selection of the posaconazole tablet presentation in allogeneic SCT recipients.

5. Conclusions

Gender and total serum proteins have been identified as covariates influencing posaconazole CL/F in adult allogeneic SCT recipients receiving the delay-released tablet formulation. A nomogram is presented as a useful tool to support MIPD strategies. Additional studies are required to better characterize the absorption of posaconazole on the studied population and implications on drug elimination and dosage recommendations, together with potential safety issues of the higher posaconazole doses proposed.

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CRediT authorship contribution statement

Diego Peña-Lorenzo: Conceptualization, Writing – original draft, Data curation, Validation. **Noemí Rebollo:** Conceptualization, Writing – original draft, Supervision. **José Germán Sánchez-Hernández:** Conceptualization, Writing – original draft. **Aránzazu Zarzuelo-Castañeda:** Writing – review & editing. **Lourdes Vázquez-López:** Writing – review & editing. **María José Otero:** Conceptualization, Writing – review & editing, Supervision. **Jonás Samuel Pérez-Blanco:** Data curation, Methodology, Visualization, Formal analysis, Software, Validation, Writing – original draft, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

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