**Population Pharmacokinetics of Fluconazole in Liver Transplantation: Implications for Target Attainment for Infections with *Candida albicans* and non-*albicans* spp.**

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**Running title:** population pharmacokinetics of fluconazole in liver transplant patients

**Keywords:** fluconazole, population pharmacokinetics, liver transplantation, personalized therapy, efficacy

**Word count of the text:** 3587

**Word count of the abstract:** 250

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**Key points**

* Fluconazolestill remains the drug of choice for antifungal prophylaxis or treatment against *C. albicans*, *C. parapsilosis* and *C. tropicalis* in clinically stable liver transplant (LT) patients.
* Estimated CrCL does not have an impact on fluconazole clearance in the LT population.
* In LT patients in the first month from liver transplantation the attainment of a pharmacodynamic target for efficacy is possible with dosages as low as 100-200 mg daily.

**Abstract**

*Objectives:*To assess the population pharmacokinetics of fluconazole and the adequacy of current dosages and breakpoints against *Candida* *albicans* and non-*albicans* spp. in liver transplant (LT) patients.

*Patients and Methods:*Patients initiated i.v. fluconazole within one month from liver transplantation (LTx) for prevention or treatment of *Candida* spp. infections. Multiple assessments of trough and peak plasma concentrations of fluconazole were undertaken in each patient by means of therapeutic drug monitoring. Monte Carlo simulations were performed to define the probability of target attainment (PTA) with a loading dose (LD) of 400, 600 and 800mg at day 1, 7, 14 and 28 from LTx, followed by a maintenance dose (MD) of 100, 200 and 300mg daily of the pharmacokinetic/pharmacodynamic target of AUC24h/MIC ratio ≥55.2.

*Results:* Nineteen patients were recruited. A two-compartment model with first-order intravenous input and first-order elimination was developed. Patient’s age and time elapsed from LTx were the covariates included in the final model. At an MIC of 2 mg/L, a LD of 600 mg was required for optimal PTAs between days 1 to 20 from LTx, while 400 mg were sufficient from days 21 on. A MD of 200 mg was required for patients aged 40-49 years-old, while a dose of 100 mg was sufficient for patients aged ≥50 years.

*Conclusions:*Fluconazole dosages of100-200 mg daily may ensure optimal PTA against *C. albicans*, *C. parapsilosis* and *C. tropicalis*. Higher dosages are required against *C. glabrata*. Estimated creatinine clearance is not a reliable predictor of fluconazole clearance in LT patients.

**Introduction**

Invasive fungal diseases (IFDs) are a serious complication following liver transplantation (LTx), with mortality rates of 2-20% in the Model for End-Stage Liver Disease (MELD) era [[1](#_ENREF_1" \o "Saliba, 2013 #16), [2](#_ENREF_2" \o "Nagao, 2016 #17)]. The clinical presentation is frequently subtle and non-specific. Current diagnostic approaches are notoriously insensitive. Appropriate treatment is often delayed. Collectively, this leads to persistently poor clinical outcomes in many patients with IFDs and especially post-liver transplant (LT) patients [[3](#_ENREF_3)].

In LT recipients, *Candida* spp. accounts for approximately 70 % of IFDs. The proportion attributable to *Aspergillus* and *Cryptococcus* is less than 20% [[3](#_ENREF_3" \o "Hogen, 2017 #15)]. *Candida albicans* is the dominant species in most studies, although in recent years an increase in non-*albicans* species has been observed. A large prospective epidemiological survey in 15 US medical transplantation centers between 2001-2006 [[4](#_ENREF_4" \o "Andes, 2016 #18)] showed that among 261 cases of invasive candidiasis in LT patients, 47.5% were caused by *C. albicans*, 22.2% by *C. glabrata*, and 5.7% by *C. parapsilosis*. More recently, a multicenter, multinational retrospective study on invasive candidiasis that included 42 LT recipients, confirmed *C. albicans* as the most frequent etiological agent (59.5% of cases), followed by *C. tropicalis* and *C. parapsilosis*, whose prevalence rates were 11.9% and 7.1%, respectively [[5](#_ENREF_5)].

Antifungal prophylaxis following LTx reduces fungal colonization, invasive disease and attributable mortality [[6](#_ENREF_6)]. Guidelines from the Infection Diseases Society of America (IDSA) and the European Society of Clinical Microbiology and Infectious Disease (ESCMID) suggest prophylaxis against *Candida* spp. in those patients who have recently undergone abdominal surgery and who have additional risk factors for invasive candidiasis such as recurrent gastrointestinal perforations or anastomotic leakages [[7](#_ENREF_7), [8](#_ENREF_8)].

Fluconazole is a triazole antifungal agent with activity against most *Candida* species with the exception of *C. krusei* and possibly *C. glabrata*. There is no activity against moulds [[9](#_ENREF_9" \o "Sabatelli, 2006 #77)]. Fluconazole has a favorable pharmacokinetic profile with a high oral bioavailability, renal elimination and relatively few drug-drug interactions due to its limited hepatic metabolism [[10](#_ENREF_10" \o "Bellmann, 2007 #26)]. Its high tolerability and low cost make it the drug of choice for antifungal prophylaxis or treatment against *Candida* spp. in clinically stable LT patients [[8](#_ENREF_8)].

Recently, echinocandins have emerged as attractive alternatives to fluconazole, with some advantages such as in the context of fluconazole resistant strains of *Candida,* in patients undergoing dialysis [[11](#_ENREF_11)] and in critically ill patients, even if data supporting their role in the LT population are still scarce [[12](#_ENREF_12)].

The aim of this study was to develop a population pharmacokinetic model of fluconazole for LT patients in the first month post-LTx. The probability of pharmacodynamic target attainment against different *Candida* species was determined to reflect on the adequacy of current fluconazole regimens and breakpoints.

**Methods**

**Study design**

This was a retrospective study that included all those patients admitted at the Intensive Care Unit of the Santa Maria della Misericordia University Teaching Hospital of Udine, Italy, immediately following LTx between January 2006 and December 2016, and in whom fluconazole was commenced within one month from LTx. The study was approved by the Regional Ethics Committee. Informed written consent was waived due to the observational nature of the investigation.

Patients received fluconazole for prophylaxis, pre-emptive treatment or targeted treatment of *Candida* infections. Prophylaxis was defined as the administration of fluconazole in the presence of risk factors for invasive candidiasis without clinical signs and symptoms of infection [[13](#_ENREF_13" \o "Scudeller, 2014 #66)]. Pre-emptive treatment was defined as the administration of fluconazole with evidence of significant *Candida* colonization without the proof of invasive fungal infection [[14](#_ENREF_14" \o "Viscoli, 2009 #1), [8](#_ENREF_8" \o "Cornely, 2012 #47)].

In all patients, fluconazole was administered intravenously. Fluconazole was initiated by the attending physician with a 400 mg loading dose (LD) at day 1, followed by a maintenance dose (MD) of 200 mg daily from day 2. Dose adjustments were applied on the basis of TDM from day 3 of therapy, by means of clinical pharmacological advice made available through the hospital intranet system. TDM of fluconazole has been provided by the Institute of Clinical Pharmacology three times a week, to achieve target trough concentrations (Cmin) ≥ 10 mg/L and peak concentrations (Cmax) 0.5 hours after 1-hour i.v. infusion ≥ 15 mg/L. Fluconazole dose was usually reduced when Cmin was more than 2-fold the target level. These target concentrations ensure the attainment of an AUC24h/MIC ratio ≥ 55.2 against all strains of *Candida* spp. susceptible to fluconazole (i.e., with an MIC ≤ 2 mg/L, using EUCAST methodology). This PK/PD index was adopted according to the study of Pai et al. [[15](#_ENREF_15)], who showed at CART analysis that an AUC24h/MIC ratio breakpoint of 55.2 was significantly associated with survival among 77 patients with candidemia treated with fluconazole in a large multicenter clinical trial. Exclusion criteria were age < 18 years, the use of any type of renal replacement therapy [[16](#_ENREF_16)] and the inaccuracy of TDM sampling times. A more aggressive PK/PD target of an AUC24h/MIC ratio ≥ 100 has also been considered, even if it was derived from a cohort of patients affected mainly by oropharyngeal candidiasis [[17](#_ENREF_17)].

The following demographic and clinical data were retrieved from patient clinical records: age, gender, weight, height, Simplified Acute Physiology Score II (SAPS II), date and reason for LTx, underlying diseases, reason for fluconazole treatment, fluconazole dose, date of TDM and duration of treatment. Serum creatinine, albumin and alanine-aminotransferase (ALT) concentrations were also collected at baseline and at each TDM session. Creatinine clearance (CrCL) was estimated using both the Cockcroft-Gault [[18](#_ENREF_18" \o "Cockcroft, 1976 #5)] and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [[19](#_ENREF_19)] formulas.

 Fluconazole concentrations were analyzed by means of a validated high-performance liquid chromatography (HPLC) method with UV detection [[20](#_ENREF_20" \o "Inagaki, 1992 #7)], with some modifications. Precision and accuracy were assessed by performing replicate analyses of quality control samples against calibration standards. Intra- and inter-assay coefficient of variations were < 10%. The lower limit of quantification was 1.0 mg/L.

**Population pharmacokinetic modelling**

One- and two-compartment models with zero-order administration and first-order elimination from the central compartment were created and fitted to the observed concentrations using the nonparametric adaptive grid (NPAG) approach embedded within the Pmetrics package for R (Laboratory of Applied Pharmacokinetics, Los Angeles, CA, USA) [[21](#_ENREF_21" \o "Neely, 2012 #9)]. Data were weighted using the Pmetrics “makeErrorPoly” function, which relates drug concentrations to the standard deviations of the observations. An additive lambda model (with starting value L=1) was chosen for the error model. Individual pharmacokinetic parameters (total clearance [CL], volume of distribution of the central compartment [V], first-order rate constant of elimination from the central to the peripheral compartment [kcp] and viceversa [kpc]) were computed by a maximum a posteriori (MAP) probability Bayesian technique.

 Initially, a 2-compartment base model without covariates that was parametrized only for fluconazole CL and V was developed. Potential relationships between the Bayesian estimates for CL and V from each patient with a number of covariates (age, weight, height, sex, SAPS II score, creatinine, albumin and ALT concentrations, CrCL, time from LTx [LTxtime]) were examined. A forward-backward selection process for covariate inclusion was adopted by using the Pmetrics “PMstep” function. A final multivariable model which included all the significant covariates was then constructed and refitted to the data.

The fit of various candidate models to the data was evaluated by computing the objective function value (OFV) and the Akaike information criterion (AIC). A decrease of at least 3.84 points of the OFV, coupled with the evaluation of the AIC, was considered significant for retaining a covariate in the final model. The goodness-of-fit and the coefficient of determination of the linear regression of the observed-versus-predicted plot for the population and individuals was evaluated. Model performance was evaluated by means of a visual predictive check (VPC), which compares the observed concentrations with model-predicted concentration-time profiles.

**Monte Carlo simulation analysis and determination of the probability of target attainment and of the cumulative fraction of response**

 The final multivariable population pharmacokinetic model was used to conduct 1000-subject Monte Carlo simulations in order to assess the probability of target attainment (PTA) of an AUC24h/MIC ratio ≥ 55.2 and/or ≥ 100 associated with three different LD (400, 600 and 800 mg) administered at day 1, 7, 14 and 21 from LTx, and three different MD (100, 200 and 300 mg daily). Variability in covariates was incorporated into these simulations. Simulated AUC24h were calculated using Bayesian estimates of concentration profiles obtained at 15 minute-intervals with the Pmetrics simulation engine. PTA ≥ 80% was considered as acceptable, while PTA ≥ 90% was considered as optimal [[22](#_ENREF_22" \o "Masterton, 2005 #67)].

 The cumulative fraction of response (CFR) achievable with the different MD of fluconazole were tested against the MIC distribution of *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata*, as reported elsewhere [[23](#_ENREF_23)]. This work was considered for our scopes, as it reported the wild-type distribution of fluconazole to a very high number of *Candida* species (n = 8059 for *C. albicans*, n = 2117 for *C. parapsilosis,* n = 1771 for *C. tropicalis* and n = 2240 for *C. glabrata*), which were all tested using broth microdilution method.

**Statistical analysis**

 The Kolmogorov-Smirnov test was used to assess whether patients’ data were normally or non-normally distributed. Accordingly, data were summarized as mean ± standard deviation (SD) or median with 25th-75th percentiles in the descriptive statistics. All statistical analysis and plotting were performed with R, version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

**Results**

**Patient characteristics**

A total of 19 patients were included in this study. Viral- and alcoholic-related cirrhosis accounted for 78.9 % (15/19) of the reasons for LTx. Table 1 summarizes patient demographics and their clinical characteristics. The median (IQR) CrCL and ALT concentrations were 67.1 mL/min/1.73 m2 (46.1-90.0) and 79.0 IU/L (54.5-162.0), respectively. Most patients received fluconazole for therapeutic purposes [7/19 (36.8%) as pre-emptive therapy and 4/19 (21.1%) as targeted therapy). In the majority of cases treatment was started during the first week from LTx (14/19; 73.7 %) with a median MD of 200 mg daily. Among patients who had repeated TDM interventions (68.4%, 13/19), the fluconazole dose was reduced to 100 mg daily in five cases. The median (IQR) duration of therapy was 16 days (13.5-19.5). In terms of clinical outcomes, 94.7 % (18/19) of patients had favorable response (cure and/or no development of infection). One patient died for post-operative complications. The fluconazole Cmin was slightly below the desired range (7.37 mg/L).

**Population pharmacokinetic modelling**

A total of 89 fluconazole plasma concentrations (53 Cmin and 36 Cmax) were included in the population pharmacokinetic analysis. A 2-compartment model performed better than a 1-compartment model (OFV and Akaike criteria 466.7 and 473, respectively, for the 1-compartment model, and 443.6 and 454.3, respectively, for the 2-compartment model). The covariates that improved the fit of the model to the data were the patient’s age on fluconazole CL and time from LTx on fluconazole V. After inclusion of these two covariates, OFV and Akaike further improved to 428.4 and 443.8, respectively.

 The final pharmacokinetic model was as follows: CL = θ1 - θ2•Age and V= θ3 - θ4•LTxtime, where CL and V represent the values of fluconazole clearance and volume of distribution, respectively, while the covariates “Age” and “LTxtime” are patient’s age and the time (in days) elapsed from LTx. The mean (± SD) pharmacokinetic estimates of the final multivariate model were CL = 0.55 (0.19) L/h and V = 27.02 (10.78) L.

 The relationship between fluconazole observed versus predicted concentrations on a population level (*r*2 = 0.45; bias = -0.89; imprecision = 4.43) and after Bayesian estimation (*r*2 = 0.92; bias = -0.02; imprecision = 0.79) are shown in Figure 1.

 The statistics of the population Bayesian pharmacokinetic parameter obtained with the final model are summarized in Table 2. The VPC plot showed that 97.8% of the observed concentrations resides within the 95% confidence intervals derived from model predictions (Figure 2). A plot of the weighted residual is also provided as Supplementary Material (Figure S).

**Monte Carlo simulation study**

The PTAs for an AUC24h/MIC ratio ≥ 55.2 with incremental LD of fluconazole of 400 mg, 600 mg and 800 mg was simulated at post-LTx days 1, 7, 14 and 21. Considering an MIC of 2 mg/L, an LD of 600 mg was required for optimal PTAs between days 1 to 20 from LTx, while an LD of 400 mg was sufficient from days 21 on (Figure 3).

 As far as the MD is concerned, Figure 4 shows the PTAs for an AUC24h/MIC ratio ≥ 55.2 with 100, 200 and 300 mg daily in relation to different classes of patient’s age (40-49, 50-59 and 60-69 year old). In order to achieve optimal PTAs at an MIC of 2 mg/L, a dose of 200 mg was required for patients aged 40-49 year-old, while a dose of 100 mg was sufficient for patients aged ≥ 50 years.

The PTAs for an AUC24h/MIC ratio ≥ 100 are reported as Supplementary Material. An LD of 800 mg was always needed for attaining optimal and/or acceptable PTAs at an MIC of 2 mg/L regardless of LTxtime (Table S1). The MD for achieving optimal PTAs at an MIC of 2 mg/L in LT patients aged 40-49, 50-59 and 60-69 years old were of 400 mg, 200 mg and 100 mg, respectively (Table S2).

**Cumulative fraction of response of fluconazole dosing regimens**

Table 3 summarizes the CFRs achievable against C. *albicans*, C. *parapsilosis*, C. *tropicalis* and *C. glabrata* with different fluconazole MD in the three tested classes of patient’s age. The dose of 100 mg daily resulted in CFR ≥ 90% across all the classes of patient’s age against C. *albicans* and C. *tropicalis,* and in patients aged ≥ 50 years against C. *parapsilosis*. A dose increase to 200 mg daily was required in patients younger than 50 years against *C. parapsilosis.* Conversely, only suboptimalPTAs may be achieved against *C. glabrata* across all classes of patient’s age.

Figure 5 depicts a dosing algorithm for choosing the most appropriate LD and MD of fluconazole that ensured a CFR ≥ 90% against C. *albicans*, C. *parapsilosis* and C. *tropicalis,* according to the variability of LTxtime and of patient’s age.

**Discussion**

This study developed a population pharmacokinetic model for fluconazole in LT patients who were in their first month from LTx, with the intention of investigating the potential clinical covariates affecting drug pharmacokinetics in this population. PTAs using an AUC24h/MIC ratio ≥ 55.2 and/or ≥ 100 with different incremental LD and MD of fluconazole were simulated and applied to a wide distribution of *Candida* spp. to identify the fluconazole regimens that might better enable optimal target attainment.

The final two-compartment population pharmacokinetic model accounted for up to 92% of the observed variability in patient concentrations over time, with acceptable measures of bias and precision. The values of pharmacokinetic parameters found in our analysis were different compared to those reported in other populations. In particular, mean fluconazole CL (0.55 L/h) was substantially lower than that reported in healthy volunteers (1.17 L/h) [[24](#_ENREF_24)] and in burn patients (1.25 L/h) [[25](#_ENREF_25)], but quite close to those estimated in critically ill patients (0.77 L/h) [[26](#_ENREF_26)] and in immunosuppressed HIV subjects (0.73 L/h) [[27](#_ENREF_27), [28](#_ENREF_28)]. The mean fluconazole V (27.02 L) was also lower than previously reported in healthy subjects [[24](#_ENREF_24)].

Covariate analysis showed that the V for fluconazole is affected by LTxtime, while CL is affected by patient’s age. The former relationship may be expected when considering the evolving pathophysiological changes with time. As the condition of patients improves with fewer episodes of infection and a reduced requirement for vasopressors, the V contracts [[29](#_ENREF_29" \o "Blot, 2014 #34)]. Furthermore, patients with a pre-existing cirrhosis usually experience an expanded plasma and blood volume, increased cardiac output and fluid retention [[30](#_ENREF_30" \o "Henriksen, 2002 #36)]. As most of this volume load is retained within the venous site of the circulatory system, which is by far more compliant than the arterial system, it may take some time for this excess fluid to redistribute.

Somewhat surprisingly, various measures of renal function (i.e. serum creatinine and estimated CrCL) were not found to have an impact on fluconazole CL. Bayesian posterior estimates of fluconazole CL of each patient were not significantly associated with CrCL estimates at regression analysis (r2=0.0635, power of the test=0.438). Although fluconazole is predominantly renally excreted [[24](#_ENREF_24" \o "Debruyne, 1993 #29)], this finding may be explained by poor estimates of glomerular filtration rate (eGFR) in LT patients. In a prospective study conducted in 1447 patients who underwent orthotopic LTx between 1984 and 2001, radionuclide-measured GFR and estimated GFR by means of the Cockcroft-Gault, Nankivell and Modification of Diet in Renal Disease (MDRD) formulas agreed no more than 50% at pre-LTx evaluation and no more than 43.3% at three month follow-up [[31](#_ENREF_31" \o "Gonwa, 2004 #37)]. Similarly, in 68 de novo LT patients in the context of a prospective multicenter clinical trial, there was poor association between measured GFR and serum creatinine (*r2*of 0.17) or eGFR estimated using the Cockcroft-Gault formula (*r2* of 0.35) or eGFR estimated using the MDRD formula (*r2* of 0.35) [[32](#_ENREF_32)]. The second aspect is related to the way fluconazole is eliminated by the kidneys. Specifically, fluconazole undergoes both a complete glomerular filtration and extensive reabsorption within the renal proximal tubule [[24](#_ENREF_24), [33](#_ENREF_33)]. As only a moderate correlation (*r2* of 0.44) was found between measured GFR and fluconazole CL among 40 patients who were tested with three probe drugs for tubular anion transport, reabsorption and cationic secretion, respectively, it is possible that differences in tubular activity that are unrelated to GFR may exist [[34](#_ENREF_34)]. Furthermore, all of our patients were administered calcineurin inhibitors (CNI) for immunosuppression following LTx. It is also possible that CNI-mediated nephrotoxicity, which affects both the glomerular structure and the tubular system of the kidneys [[35](#_ENREF_35)], could have contributed to alter the dynamics between renal filtration and tubular reabsorption of fluconazole.

The magnitude of the AUC24h/MIC pharmacodynamic target for fluconazole varies among studies. In animal models of candidiasis a free AUC24h/MIC ratio of approximately 25 is associated with treatment success [[36](#_ENREF_36" \o "Andes, 2003 #65)], and it is now recognized that a value > 50 may be required [[15](#_ENREF_15" \o "Pai, 2007 #4)]. An even more aggressive target of 100 has been recently suggested by the British Society of Medical Micology especially for severe systemic infections or *Candida glabrata* related-infections [[37](#_ENREF_37)].

In our population, the attainment of the pharmacodynamic target of efficacy of an AUC24h/MIC ratio ≥ 55.2 was obtained with fluconazole LD of 400-600 at start of therapy and MD of 100-200 mg daily thereon, which are by far lower than those usually recommended by current guidelines (400-800 mg/day) for systemic infections [[37](#_ENREF_37" \o "Ashbee, 2014 #43)]. Indeed, at an MIC of 2 mg/L, which is the clinical breakpoint of susceptibility of fluconazole for *C. albicans*, *C. parapsilosis* and *C. tropicalis*, optimal PTAs (≥ 90%) were ensured with these low dosages. Moreover, as the MIC90 for fluconazole of *C. albicans*, *C. parapsilosis* and *C. tropicalis* are 0.25, 2 and 2 mg/L respectively [[23](#_ENREF_23" \o "Pfaller, 2010 #12)], optimal drug exposure might also be expected with these dosages when used for prophylaxis or in the empirical treatment of *Candida* infections in non-critically ill LT patients, which are the clinical contexts where fluconazole may be used instead of the echinocandins according to IDSA/ESCMID clinical guidelines [[8](#_ENREF_8" \o "Cornely, 2012 #47), [7](#_ENREF_7" \o "Pappas, 2016 #52)]. However, higher LD (800 mg) and MD (100-400 mg) of fluconazole may be needed if targeting AUC24h/MIC ratio at > 100.

The echinocandins have not shown a clear benefit over fluconazole against *Candida glabrata* infections both in prospective [[38](#_ENREF_38), [39](#_ENREF_39)] and retrospective studies [[40](#_ENREF_40)], and considering that adequate CFRs may be attained with fluconazole at dosages higher than 300 mg daily, it may be possible that fluconazole could be successfully used even in this scenario. This is likely due to the fact that the MIC50 for fluconazole against *C. glabrata* is 4 mg/L, but only 10.3% of strains have an MIC > 16 mg/L [[23](#_ENREF_23)]. In any case, more data are needed before drawing definitive conclusions, especially in the light of the high epidemiological cut-off value of *C. glabrata* against fluconazole, which is 32 mg/L [[23](#_ENREF_23)].

There are several limitations of this study. The retrospective design of the investigation led to the development of a model based on unplanned analysis of TDM data. The small sample size and the lack of microbiologic evaluation at the end of treatment are other relevant limits. It would be prudent to limit the generalizability of the conclusions only to populations with clinical characteristics very similar to ours, considering that non-parametric Monte Carlo simulation may replicate only the characteristics of the study population. Since no study patient had CrCL < 35 mL/min/1.73 m2, our model could not rule out the effect of renal failure on fluconazole CL. Moreover, the pharmacodynamic target of AUC24h/MIC ratio ≥ 55.2 was validated for fluconazole efficacy only in patients with candidemia. This could limit the generalizability of the findings in case of abdominal candidiasis, considering that the penetration rate in ascitic fluid and bile of LT patients was of 0.5-0.8 [[41](#_ENREF_41" \o "Pea, 2014 #68)]. Finally, we recognize that this PK/PD target was applied for both prophylaxis and treatment of fungal infections.

Nevertheless, we believe that our study is important because it shows that in LT patients in the first month from LTx the attainment of a pharmacodynamic target for efficacy is possible with dosages as low as 100-200 mg daily. The need for low fluconazole doses is particularly important in the setting of LT patients, as it may minimize the risk of hepatotoxicity. Although fluconazole was shown to have a better hepatic safety profiles than the other triazoles [[42](#_ENREF_42" \o "Kyriakidis, 2017 #73)], it has been shown that sometimes it may cause dose-dependent elevation of liver enzymes [[43](#_ENREF_43" \o "Wang, 2010 #74)] and rarely severe hepatotoxicity [[44](#_ENREF_44" \o "Bronstein, 1997 #75), [45](#_ENREF_45" \o "Wells, 1992 #76)]. Moreover, we showed that estimated CrCL may not be a fair predictor of fluconazole CL in the LT population. In this subpopulation of patients, TDM may represent a valuable tool for optimizing fluconazole exposure.

**Compliance with Ethical Standards**

**Fundings** This study was conducted as part of our routine work.

**Conflict of interest** W. H. holds or has recently held research grants with F2G, AiCuris, Astellas Pharma, Spero Therapeutics, Matinas Biosciences, Antabio, Amplyx, Allecra, Auspherix and Pfizer and he holds awards from the National Institutes of Health, Medical Research Council, National Institute of Health Research, FDA and the European Commission (FP7 and IMI). W. H. has received personal fees in his capacity as a consultant for F2G, Amplyx, Ausperix, Spero Therapeutics, Medicines Company, Gilead and Basilea and he is an Ordinary Council Member for the British Society of Antimicrobial Chemotherapy. M. B. has participated in advisory boards and/or received speaker honoraria from Achaogen, Angelini, Astellas, AstraZeneca, Bayer, Basilea, Gilead, Menarini, MSD, Pfizer, The Medicines Company, Tetraphase and Vifor. F. P. has received speaker honoraria from and attended advisory boards for Basilea Pharmaceutics, Gileads, MSD and Pfizer. All other authors have none to declare.

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| **Table 1**. Demographic and clinical characteristics of the population. |
| Total number of patients | 19 |
| Age (years) | 60.0 (50.5 – 62.0) |
| Gender (male/female) | 14/5 |
| Weight (kg)  | 75.0 (67.0 – 80.0) |
| BMI (kg/m2) | 25.3 (23.9 – 27.6) |
| Creatinine clearance (mL/min/1.73 m2) | 67.1 (46.1 – 90.9) |
| Albumin (g/L) | 23.0 (21.0 – 27.0) |
| Alanine-aminotransferase (UI/L) | 79.0 (54.5 – 162.0) |
| SAPS II | 46.0 (37.0 – 52.0) |
| Fluconazole therapy |  |
|  | Time of starting therapy from transplantation (days) | 2.0 (0.0 – 6.0) |
|  | Fluconazole dose (mg/daily) | 200.0 (200.0 – 200.0) |
|  | Fluconazole AUC24h (mg∙h/L) | 268.7 (233.5 – 384.1) |
|  | Duration of therapy (days) | 16.0 (13.5 – 19.5) |
| Reason for fluconazole prescription |  |
|  | Prophylaxis | 8 (42.1) |
|  | Pre-emptive therapy | 7 (36.8) |
|  | Targeted therapy | 4 (21.1) |
| Reasons for liver transplantation |  |
|  | Viral-related cirrhosis  | 10 (52.6) |
|  | Alcoholic-related cirrhosis | 5 (26.3) |
|  | Cryptogenic cirrhosis | 2 (10.5) |
|  | Primary biliary cirrhosis | 1 (5.3) |
|  | Primary sclerosing cholangitis | 1 (5.3) |
| Data for continuous variables are presented as median (IQR) and data for dichotomous variables are presented as number (%).  |

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| **Table 2**. Parameter estimates of fluconazole for the final covariate two-compartment population pharmacokinetic model. |
|  | Mean | Standard deviation | Coefficient of variation (%) | Median |
| CL (L/h) = θ1 - θ2•Age |
|  | θ1 | 1.28 | 0.46 | 36.00 | 1.33 |
|  | θ2 | 0.01 | 0.009 | 66.06 | 0.014 |
| V(L) = θ3 - θ4•LTxtime |
|  | θ3 | 29.36 | 8.47 | 28.87 | 35.06 |
|  | θ4 | 0.325 | 0.315 | 96.81 | 0.27 |
| *k*cp (h-1) | 39.65 | 11.24 | 28.35 | 46.67 |
| *k*pc (h-1) | 32.22 | 13.45 | 41.74 | 39.72 |
| Age, patient’s age; CL, total clearance of fluconazole; *k*cp and *k*pc, first-order inter-compartmental transfer rate constant connecting the central and peripheral compartments; LTxtime, time (in days) elapsed from liver transplantation; V, volume of distribution of the central compartment. θ1 and θ2 are the intercept and slope estimates, respectively, of the linear regression between CL and Age. θ3 and θ4 are the intercept and slope estimates, respectively, of the linear regression between V and LTxtime. |

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| **Table 3**. Cumulative fraction of response of the fluconazole dosages of 100, 200 and 300 mg every 24 h in order to attain an AUC24h/MIC ratio ≥ 55.2 against the fluconazole MIC distribution as reported elsewhere [[23](#_ENREF_23)]. |
| Class of patient’s age(years) | Fluconazole dose(mg) | CFR (%) |
| *C. albicans* | *C. parapsilosis* | *C. tropicalis* | *C. glabrata* |
| 40-49 | 100 every 24h | 99 | 88 | 96 | 32 |
|  | 200 every 24h | 99 | 92 | 98 | 63 |
|  | 300 every 24h | 99 | 94 | 98 | 66 |
|  |
| 50-59 | 100 every 24h | 99 | 90 | 97 | 42 |
|  | 200 every 24h | 99 | 93 | 98 | 71 |
|  | 300 every 24h | 99 | 94 | 99 | 80 |
|  |
| 60-69 | 100 every 24h | 99 | 92 | 97 | 53 |
|  | 200 every 24h | 99 | 94 | 98 | 75 |
|  | 300 every 24h | 99 | 95 | 99 | 82 |

**Figure Legend**

**Figure 1.** Diagnostic plot for the final covariate model. Shown are observed versus population predicted concentrations (left) and individual predicted concentrations (right) in plasma. Solid lines refer to linear regression between observed and predicted concentrations. Dashed lines are the identity lines between observed and predicted concentrations.

**Figure 2.** Visual predictive check (VPC) of fluconazole plasma concentration versus time for the final covariate model. Grey shading displays predicted intervals of simulated data.

**Figure 3.**  Probability of target attainment (PTA) of AUC24h/MIC ratio ≥ 55.2 after fluconazole loading doses of 400, 600, 800 mg administered at 1, 7, 14, 21 days from liver transplantation. Horizontal dotted lines identify the threshold for optimal PTA (90%).

**Figure 4.**  Probability of target attainment (PTA) of AUC24h/MIC ratio ≥ 55.2 with fluconazole maintenance doses of 100, 200 and 300 mg every 24 h according to classes of patient’s age (40-49, 50-59, 60-69 years old). Horizontal dotted lines identify the threshold for optimal PTA (90%).

**Figure 5.** Dosing algorithm for the most appropriate loading dose (LD) and maintenance dose (MD) of fluconazole after liver transplantation in relation to time from transplantation and classes of patient’s age, which ensured a ≥ 90% probability of attaining the pharmacodynamic target of efficacy of an AUC24h/MIC ratio ≥ 55.2 for the empirical treatment of infections caused by *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis.*