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CLINICAL THERAPEUTICS

Optimization of Fluconazole Dosing for the Prevention and Treatment of Invasive Candidiasis Based on the Pharmacokinetics of Fluconazole in Critically Ill Patients

J. M. Boonstra,[a] A. G. Märtson,[a] I. Sandaradura,[b,c] J. G. W. Kosterink,[a,d] T. S. van der Werf,[e,f] D. J. E. Marriott,[g,h] J. G. Zijlstra,[i] D. J. Touw,[a] J. W. C. Alffenaar[b,c,j]

[a]University of Groningen, University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology, Groningen, the Netherlands
[b]The University of Sydney, Faculty of Medicine and Health, School of Pharmacy, Sydney, Australia
[c]Westmead Hospital, Sydney, Australia
[d]University of Groningen, Department of Pharmacy, Section Pharmacotherapy and Pharmaceutical Care, Groningen, the Netherlands
[e]University of Groningen, University Medical Center Groningen, Department of Internal Medicine, Groningen, the Netherlands
[f]University of Groningen, University Medical Center Groningen, Department of Pulmonary Diseases and Tuberculosis, Groningen, the Netherlands
[g]St. Vincent's Clinical School, University of New South Wales, Kensington, New South Wales, Australia
[h]Department of Clinical Microbiology and Infectious Diseases, St. Vincent's Hospital, Darlinghurst, New South Wales, Australia
[i]University of Groningen, University Medical Center Groningen, Department of Critical Care, Groningen, the Netherlands
[j]Marie Bashir Institute of Infectious Diseases and Biosecurity, University of Sydney, Sydney, Australia

J. M. Boonstra and A. G. Märtson contributed equally to this work. Author order was determined alphabetically by last name.

ABSTRACT The efficacy of fluconazole is related to the area under the plasma concentration-time curve (AUC) over the MIC of the microorganism. Physiological changes in critically ill patients may affect the exposure of fluconazole, and therefore dosing adjustments might be needed. The aim of this study was to evaluate variability in fluconazole drug concentration in intensive care unit (ICU) patients and to develop a pharmacokinetic model to support personalized fluconazole dosing. A prospective observational pharmacokinetic study was performed in critically ill patients receiving fluconazole either as prophylaxis or as treatment. The association between fluconazole exposure and patient variables was studied. Pharmacokinetic modeling was performed with a nonparametric adaptive grid (NPAG) algorithm using R package Pmetrics. Data from 33 patients were available for pharmacokinetic analysis. Patients on dialysis and solid organ transplant patients had a significantly lower exposure to fluconazole. The population was best described with a one-compartment model, where the mean volume of distribution was 51.52 liters (standard deviation [SD], 19.81) and the mean clearance was 0.767 liters/h (SD, 0.46). Creatinine clearance was tested as a potential covariate in the model, but was not included in the final population model. A significant positive correlation was found between the fluconazole exposure (AUC) and the trough concentration (C_{min}). Substantial variability in fluconazole plasma concentrations in critically ill adults was observed, where the majority of patients were underexposed. Fluconazole C_{min} therapeutic drug monitoring (TDM)-guided dosing can be used to optimize therapy in critically ill patients. (This study has been registered at ClinicalTrials.gov under identifier NCT02491151.)

KEYWORDS fluconazole, pharmacokinetics, invasive candidiasis, critically ill

Invasive candidiasis remains a common nosocomial infection, with high mortality rates even among patients receiving antifungal treatment (1, 2). Although prevention of *Candida* bloodstream infection is important, with routine change or removal of indwelling catheters and appropriate antifungal prophylaxis in specific high-risk groups (3–6),

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Address correspondence to J. W. C. Alffenaar, johannes.alffenaar@sydney.edu.au.

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timely initiation of treatment using adequate dosages of fluconazole is mandatory to improve the treatment outcome of invasive candidiasis (7, 8). Due to the increase of *Candida* species with reduced susceptibility or resistance to fluconazole, routine microbial surveillance of nonsterile and sterile sites in at-risk patients is suggested to permit clinicians to initiate effective treatment (7, 9, 10).

Echinocandins are recommended as primary antifungal agents for the treatment of invasive *Candida* infection (11). Treatment should be continued for at least 14 days following documented clearance of *Candida* species from the bloodstream and resolution of signs and symptoms attributable to infection (11). In addition to prophylaxis, fluconazole is used as a step-down treatment and targeted treatment against fluconazole-susceptible species, especially those with reduced susceptibility to echinocandins, such as *Candida parapsilosis* (11). Step-down treatment with fluconazole as early as 5 days after the start of intravenous treatment with an echinocandin appeared to be effective as antifungal treatment compared with an echinocandin administered for the full treatment course (12). Reduction of echinocandin use due to step-down treatment with fluconazole results in a decreased risk for the development of echinocandin-resistant microorganisms and significant cost savings (13).

Fluconazole has time- and concentration-dependent fungistatic activity and efficacy associated with the ratio of the area under the concentration-time curve (AUC) to the MIC (14). For treatment, an AUC for 24 h ($AUC_{0–24\ h}$) of 400 mg · h/liter and for prophylaxis an $AUC_{0–24\ h}$ of 200 mg · h/liter are considered appropriate exposure for management of infections with fluconazole-susceptible species (15–18). Although therapeutic drug monitoring (TDM) is not routinely recommended for fluconazole (19), detection of underexposure of fluconazole in specific pediatric patient populations (20) and obese patients (21) has been demonstrated to be beneficial. Higher weight-based loading doses (12 mg/kg body weight) and maintenance doses (6 or 12 mg/kg/day, depending on renal function) are required to prevent underexposure (22). The heterogeneity of patients in the intensive care unit (ICU) results in differences in drug exposure, and therefore, standard dosing may not be appropriate for every patient (23). Furthermore, the dosage must be adapted to renal function, which may fluctuate significantly over time and is very difficult to estimate in ICU patients (24). Based on the highly variable drug exposure and the relationship between drug exposure and efficacy/toxicity, TDM could benefit ICU patients (25–28).

The aim of this study was to evaluate fluconazole drug concentration variability in ICU patients and to develop a pharmacokinetic (PK) model to support personalized fluconazole dosing to achieve adequate exposure in ≥95% of the patients after loading and during steady state.

RESULTS

In total, 49 patients were included in the study: 28 patients (57%) received fluconazole prophylaxis, and 21 patients (43%) received fluconazole treatment for invasive candidiasis (with 17 patients receiving fluconazole as initial therapy and 4 receiving fluconazole as step-down therapy) (Table 1). All prophylaxis recipients had at least two documented risk factors for invasive candidiasis, and 28 patients reported three or more risk factors. A total of 76.2% (16/21) of all patients receiving fluconazole as treatment underwent major surgery prior to the diagnosis of invasive candidiasis. The majority (93.8% [15/16]) underwent surgery for an indication other than solid organ transplantation, which consisted primarily of intestinal resection or bowel surgery.

Therapy and therapy outcome. The median dose in mg per kg per day, the $AUC_{0–24\ h}$, and the trough concentration (C_{min}) of fluconazole after a loading dose and during steady state (day 5 of fluconazole treatment) are displayed in Table 2. Despite a loading dose, the fluconazole exposure was significantly lower after 24 h compared with fluconazole exposure in steady state. Most patients (36/49 [73.4%]) ceased fluconazole after completion of successful therapy. Prophylaxis with fluconazole failed in 3 patients (10.7%) due to a breakthrough infection ($n = 3$ patients with *Candida glabrata*), and treatment with caspofungin or liposomal amphotericin B

TABLE 1 Patient demographics[a]

Demographic	Result for patient group:		
	All (n = 49)	Prophylaxis (n = 28)	Treatment (n = 21)
Female, no. (%)	13 (27.0)	7 (24.1)	6 (28.6)
Age, median yr (IQR)	60 (52–66)	56 (45–62)	66 (59–73)
Wt, median kg (IQR)	80.0 (71.0–88.0)	81.0 (73.3–88.5)	77.0 (71.0–93.5)
BMI, median (IQR)	24.9 (23.6–27.6)	25.3 (23.7–27.5)	25.2 (23.6–30.1)
Underlying condition, no. (%)			
Major surgery	38 (77.5)	22 (78.6)	16 (76.2)
Solid organ transplant	20 (40.1)	19 (67.9)	1 (4.8)
Severity of disease score, median (IQR)			
APACHE II	19 (15–24)	19 (14–21)	20 (16–24)
APACHE IV	62 (50–88)	61 (45–88)	72 (51–89)
LODS	6 (3–9)	6 (3–8)	7 (3–10)
SAPS	40 (34–56)	38 (33–54)	45 (38–57)
Risk factor exposure, no. (%)			
Central venous catheter	45 (91.8)	27 (96.4)	18 (85.7)
Total parenteral nutrition	8 (16.3)	3 (10.7)	5 (23.8)
Mechanical ventilation	48 (98.0)	27 (96.4)	21 (100.0)
Immunosuppressive therapy	20 (40.8)	19 (67.9)	1 (4.8)
Dialysis	14 (28.6)	9 (32.1)	5 (23.8)
Corticosteroid therapy	25 (51.0)	21 (75.0)	4 (19.0)
Broad-spectrum antibiotic use	46 (93.9)	25 (89.3)	21 (100.0)

[a]Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; APACHE IV, Acute Physiology and Chronic Health Evaluation IV; LODS, Logistic Organ Dysfunction System; SAPS, Simplified Acute Physiology Score.

was initiated. Three patients (10.7%) discontinued prophylaxis with fluconazole due to lack of effectiveness (colonization of the gastrointestinal [GI] tract: two with *C. glabrata* and one with *Candida albicans*) and switched to amphotericin B orally. In the fluconazole treatment group, 6 patients (28.6%) died during fluconazole treatment. Death was related to the underlying condition in all patients, where 3 patients died from cardiac comorbidity, 2 patients from pancreatitis and 1 patient from advanced liver cirrhosis. A significant positive correlation was found between the fluconazole exposure (AUC) and the C_{min} and fluconazole dose (Table 3). The correlation between the fluconazole exposure was significantly stronger (R^2 = 0.9619 and 0.9854) than the exposure-dosage relationship (R^2 = 0.3311 and 0.2512). Patients on dialysis, patients who underwent solid organ transplantation, and

TABLE 2 Therapy and therapy outcome[a]

Parameter	Result for recipients of:	
	Prophylaxis	Treatment
Dose and exposure, median (IQR)		
Loading dose, mg/kg/day	4.7 (2.8–5.3)	5.6 (3.8–8.8)
$AUC_{0-24\ h}$ on day 1, mg · h/liter	137.0 (11.5–169.7)	174.0 (108.9–345.6)
C_{min} on day 1, mg/liter	4.5 (3.4–6.0)	6.4 (3.9–12.7)
Dose on day 5, mg/kg/day	1.4 (1.1–4.0)	3.8 (2.7–6.7)
$AUC_{0-24\ h}$ on day 5, mg · h/liter	183.9 (70.3–469.4)	405.0 (239.2–587.9)
C_{min} on day 5, mg/liter	6.5 (2.0–15.5)	13.3 (7.7–20.7)
Therapy discontinuation, no. (%)		
Successful therapy	21 (75.0)	15 (71.4)
Breakthrough infection	3 (10.7)	NA
Adverse event	0 (0.0)	0 (0.0)
Lack of effectiveness	3 (10.7)	0 (0.0)
Death	1 (3.6)	6 (28.6)

[a]Abbreviations: AUC, area under the curve; C_{min}, trough concentration; NA, not applicable.

TABLE 3 Association between patient characteristics, clinical parameters, and fluconazole exposure ($AUC_{0–24\ h}$)[a]

Parameter	Correlation coefficient	*P* value
Day 1		
C_{min} (mg/liter)	0.951	<0.001
Loading dose (mg/kg/day)	0.678	<0.001
Body wt	0.112	0.446
BMI	0.195	0.180
Dialysis use (CVVH)	−0.319	0.025
SOT	−0253	0.080
Diabetes	−0.090	0.541
Corticosteroid use	−0.248	0.085
APACHE II	−0.004	0.979
APACHE IV	−0.088	0.583
LODS	0.042	0.776
MPM0	0.006	0.972
MPMII	0.134	0.405
SAPS	0.135	0.353
Creatinine	−0.201	0.166
ALP	0.184	0.237
ALAT	−0.196	0.208
ASAT	−0.182	0.249
γGT	0.144	0.357
Day 5		
C_{min} (mg/liter)	0.993	<0.001
Dose (mg/kg/day)	0.736	<0.001
Body wt	0.103	0.596
BMI	0.109	0.573
Dialysis use (CVVH)	−0.526	0.003
SOT	−0.481	0.008
Diabetes	0.012	0.951
Corticosteroid use	−0.494	0.006
APACHE II	0.015	0.943
APACHE IV	−0.181	0.386
LODS	−0.065	0.737
MPM0	−0.148	0.479
MPMII	−0.055	0.793
SAPS	0.112	0.564
Creatinine	−0.135	0.486
ALP	0.098	0.642
ALAT	−0.140	0.504
ASAT	−0.122	0.563
γGT	0.072	0.731

[a]The Spearman coefficient was used for analysis. Abbreviations: C_{min}, trough concentration; BMI, body mass index; CVVH, continuous veno-venous hemofiltration; SOT, solid organ transplant; APACHE II, Acute Physiology and Chronic Health Evaluation II; APACHE IV, Acute Physiology and Chronic Health Evaluation IV; LODS, Logistic Organ Dysfunction System; SAPS, Simplified Acute Physiology Score; ALP, alkaline phosphatase; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; γGT, γ-glutamyl transferase.

patients receiving immunosuppressive and corticosteroid therapy had significantly reduced fluconazole exposure. In the multiple linear regression analysis, variables obtained by the univariable analysis ($P < 0.100$) were included. Patients on dialysis and solid organ transplant patients had significantly reduced fluconazole exposure after multivariate analysis (Table 4).

Pharmacokinetic modeling. For the development of the population pharmacokinetic (PK) model, data from 33 patients with 561 fluconazole concentrations were used. Patients on dialysis ($n = 13$) and receiving fluconazole orally ($n = 3$) were excluded due to the possible different pharmacokinetic profile and missing detailed data on dialysis. This population was best described with a one-compartment pharmacokinetic model. Creatinine was tested as a possible covariate on clearance (CL); however, including it did not improve the goodness of fit or other parameters of the model. The final population pharmacokinetic model estimates are described in Table 5. The individual-

TABLE 4 Multiple linear regression model of factors significantly correlated with fluconazole exposure ($AUC_{0–24\ h}$) on day 5[a]

Factor	Effect	95% CI	*P* value
Dialysis use	−0.442	−410.859 to −68.279	0.008
SOT	−0.382	−344.177 to −35.525	0.020

[a]R^2 of model 1 = 0.421, and R^2 of model 2 = 0.407, compared with the model with all variables included.
Abbreviations: CI, confidence interval; SOT, solid organ transplant.

predicted and population-predicted versus observed goodness-of-fit plots are presented in Fig. 1. Figure 1B shows that the fit could not be described for some subpopulations by covariates, even after excluding patients on dialysis. The developed model showed good precision when estimating Bayesian posterior predictions (R^2 = 0.79). The external validation is presented in Fig. 2.

Probability of target attainment. The probability of target attainment is presented in Table 6 and Fig. 3. The lowest dose that achieved a target attainment of more than 95% was for a fixed dosing regimen of 1,200 mg loading + 1,000 mg daily (98% on the first day and 100% on the third and fifth days); for a weight-based dosing regimen, the lowest dose for target attainment of more than 95% was 13 mg/kg loading + 8 mg/kg daily (96% on the first day and 100% on the third and fifth days). None of the simulated patients receiving a fixed dosing regimen (1,200 mg loading + 1,000 mg daily) reached a fluconazole blood concentration of 80 mg/liter after the loading dose, 4% of the patients reached 80 mg/liter after day 3, and 7% of the simulated patients reached 80 mg/liter after day 5 of fluconazole dosing. A lower percentage of patients dosed with a weight-based dosing regimen of 13 mg/kg loading + 8 mg/kg daily reached the 80-mg/liter cutoff value (0% after the loading dose, 1% after day 3, and 5% after day 5 of dosing). Using the linear model AUC∼C_{min}, the C_{min} to be targeted on day 3 of therapy to achieve adequate AUC values was 14 mg/liter (90% confidence interval [CI] of 13.7 to 14.3 mg/liter).

DISCUSSION

This study demonstrated large variability in the fluconazole exposure in critically ill adults. Almost 50% of the patients did not reach the target for treatment (400 mg · h/liter) or prophylaxis (200 mg · h/liter) when Pfizer Summary of Product Characteristics (SPC) recommended fluconazole dosages were applied (24).

Leftover clinical samples from critically ill adults were used to construct concentration-time curves and to develop a pharmacokinetic model (29, 30). This approach results in no additional burden for the patient. Before applying this strategy for other drugs, stability during processing and storage needs to be confirmed.

As clinical samples are frequently collected in ICU patients, we had a large number of plasma samples, and the resulting pharmacokinetic model was able to predict fluconazole concentrations in an external data set with good precision of R^2 = 0.79, supporting the strategy. Although our model was able to predict the drug exposure in most of the patients, a number of patients did not fit the model; this could not be explained by covariate analysis. Due the heterogeneity of the ICU population, the sample size of our study was possibly too small, and this could be considered a limitation of the study (31).

Our study population was diverse, reflecting a "real-life" population. Analyzing subgroups, we noted that solid organ transplant recipients and patients on renal replacement therapy (RRT; usually, continuous veno-venous hemofiltration [CVVH]) were asso-

TABLE 5 Final one-compartment model population pharmacokinetic estimates[a]

Parameter	Mean (SD)	CV (%)	Median
V	51.52 (19.81) liters	38.45	50.54 liters
CL	0.767 (0.46) liter/h	60.21	0.82 liter/h

[a]Abbreviations: SD, standard deviation; CV, coefficient of variation; *V*, volume of distribution; CL, mean clearance.

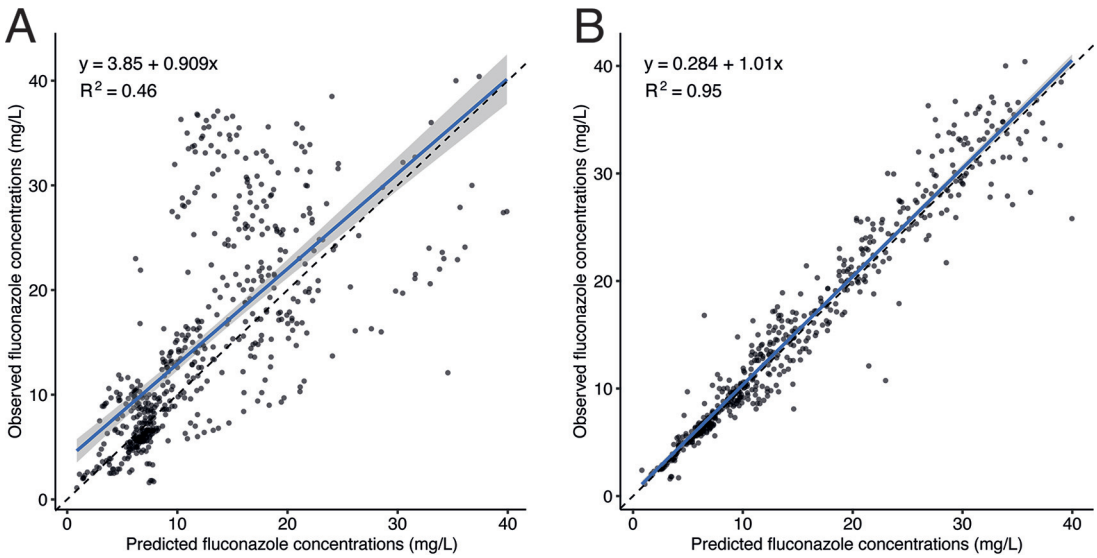

FIG 1 (A) The population-predicted versus observed goodness-of-fit plots for the final one-compartment model. (B) The individual predicted versus observed goodness-of-fit plots for the final one-compartment model.

ciated with a lower fluconazole exposure, which is consistent with earlier findings in patients on RRT and patients with various rates of creatinine clearance (CL_{CR}) (21, 22, 32, 33). Patel et al. found a significantly higher clearance of fluconazole in patients receiving continuous veno-venous hemodiafiltration (CVVHDF) compared with patients not undergoing continuous dialysis (32). Furthermore, significant underexposure due to increased fluconazole clearance in obese patients with CL_{CR} higher than 150 ml/min has been described by Alobaid et al. (21) These observations suggest that individualization of therapy for patients undergoing dialysis could be warranted.

Probability of target attainment (PTA) values for multiple dosing regimens were calculated using the developed population PK model. Higher dosing (1,200 mg loading + 1,000 mg daily or 13 mg/kg loading + 8 mg/kg daily) than currently recommended

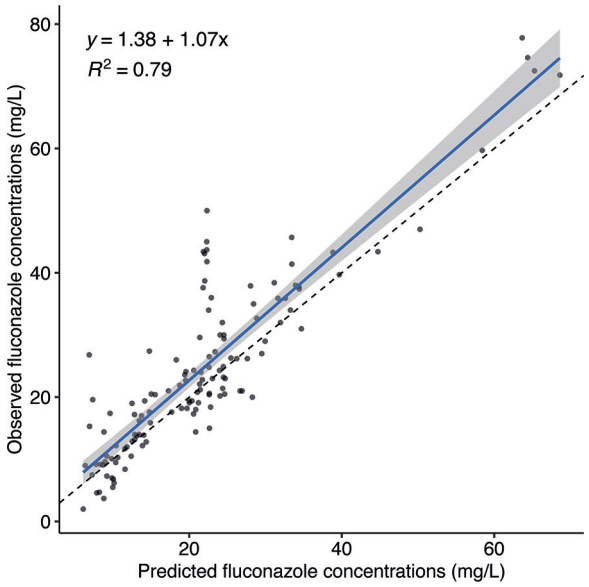

FIG 2 The Bayesian posterior predictions for the external data set.

TABLE 6 Probability of target attainment for seven dosing regimens with *f*AUC/MIC of >100 and MIC of ≥2 mg/liter for the first and third days of therapy[a]

	PTA for fixed dosing (%)				PTA for wt-based dosing (%)		
Fixed dosing regimen	Day 1	Day 3	Day 5	Wt-based dosing regimen	Day 1	Day 3	Day 5
400 mg daily	16	87	97	6 mg/kg daily	36	97	99
600 mg daily	60	98	100	7 mg/kg daily	58	98	100
800 mg loading + 400 mg daily	87	96	97	8 mg/kg daily	73	100	100
1,000 mg loading + 600 mg daily	94	100	100	9 mg/kg loading + 7 mg/kg daily	83	99	100
1,000 mg loading + 800 mg daily	94	100	100	12 mg/kg loading + 8 mg/kg daily	94	100	100
1,200 mg loading + 1,000 mg daily	98	100	100	13 mg/kg loading + 8 mg/kg daily	96	100	100

[a]Abbreviation: PTA, probability of target attainment.

appeared necessary to achieve a PTA of ≥95% throughout the treatment period. Although almost 50% of the patients were underexposed, a substantial portion of the patients (20%) could be considered overexposed, with AUCs ranging from 600 mg · h/liter to 800 mg · h/liter. Anaissie et al. (34) concluded that fluconazole dosing up to 1,600 mg daily was well tolerated, but toxic effects possibly related to higher fluconazole dosing in 38.5% of the patients were observed. Despite a good safety profile, hepatotoxicity has been associated with high fluconazole exposure, and fluconazole is recognized to have clinically relevant cytochrome P450 (CYP)-mediated drug-drug interactions. Routine administration of higher fluconazole dosages in ICU patients may be beneficial for patients at risk for underexposure, but result in an increased risk for adverse events in some of the critically ill adults. In our opinion, fluconazole TDM-guided dosing adjustments represent a more appropriate solution (33). Although data from randomized studies are lacking, TDM-guided fluconazole dosing in patients with deep intra-abdominal *Candida* infection, targeting a plasma concentration of 15 mg/liter for 14 days of therapy, was considered to have contributed to successful therapy outcome (35). In our study, fluconazole dose and fluconazole trough concentration (C_{min}) were associated with the fluconazole exposure (AUC), as shown in Fig. 4; however, only the C_{min} proved to be a good predictor for the AUC, making TDM very feasible. We believe that fluconazole could be an interesting and useful addition to the

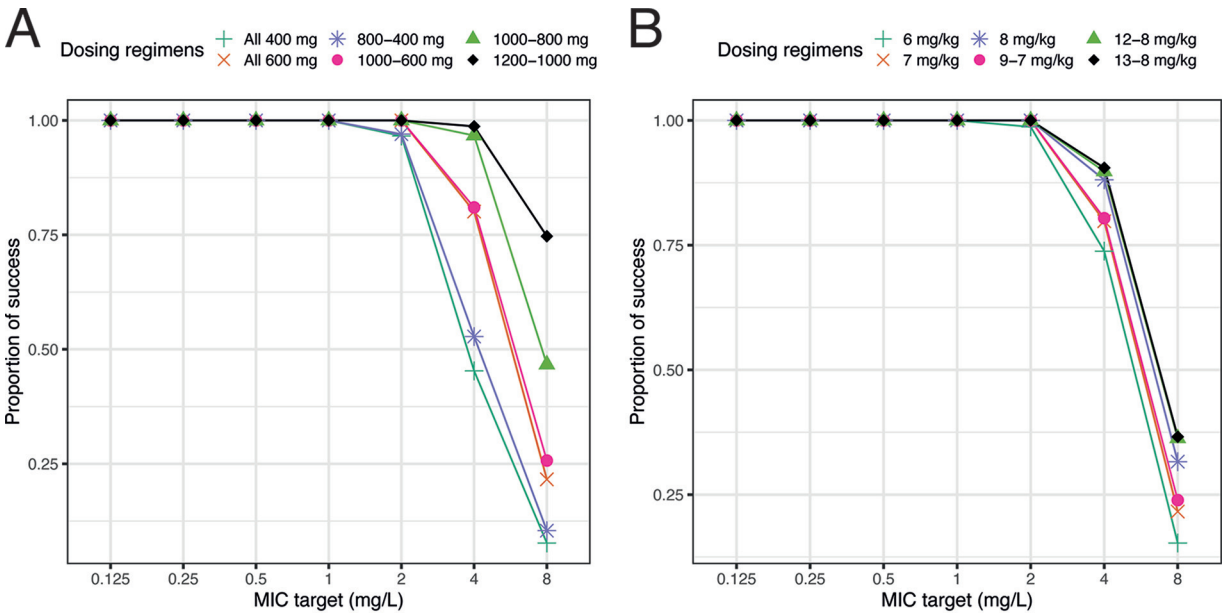

FIG 3 (A) The PTAs for an *f*AUC/MIC target of 100 for the third day of therapy for fixed dosing. The *x* axis presents the MIC targets, and the *y* axis presents the proportion of success. (B) The PTAs for an *f*AUC/MIC target of 100 for the third day of therapy for weight-based dosing. The *x* axis presents the MIC targets, and the *y* axis presents the proportion of success.

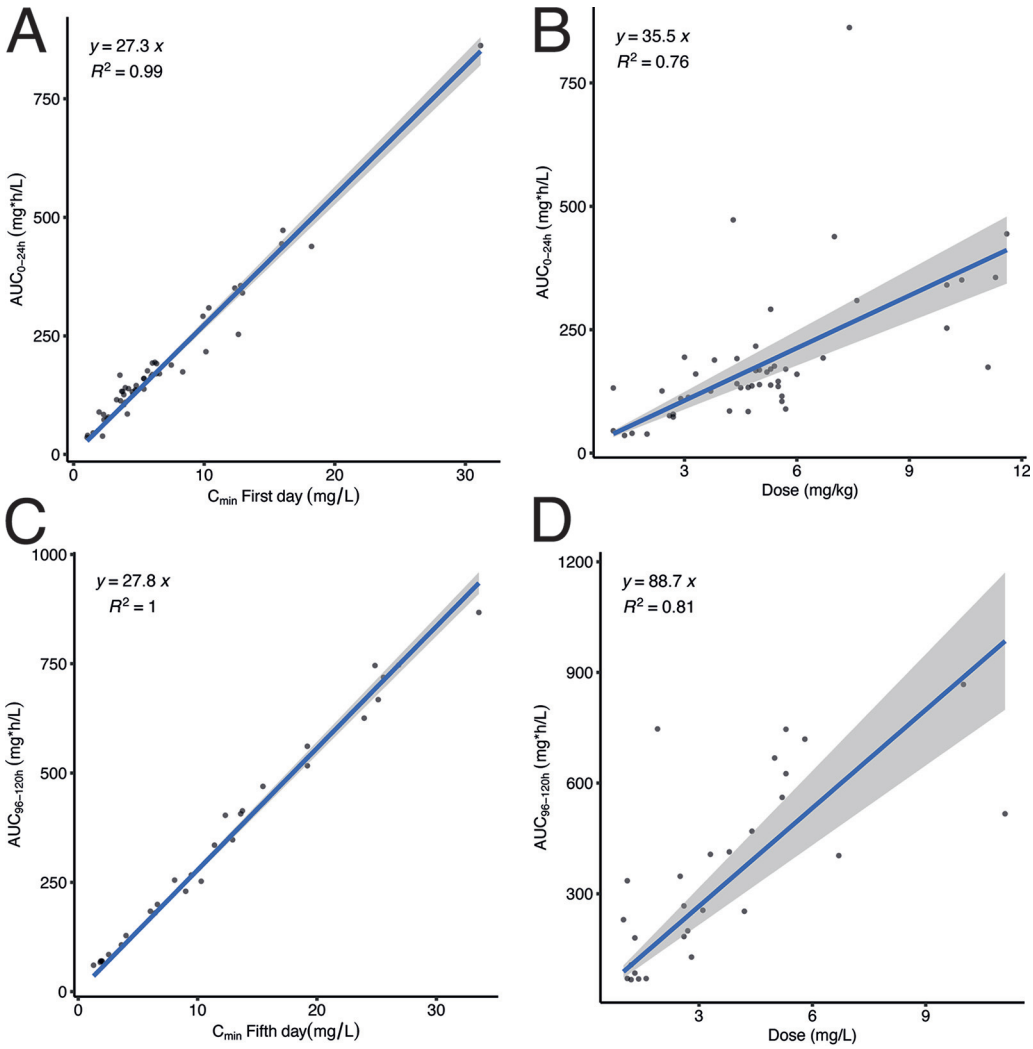

FIG 4 (A) The fluconazole exposure after a loading dose correlated with the trough concentration. (B) The fluconazole exposure after a loading dose correlated with the dose. (C) The fluconazole exposure after 5 days of dosing correlated with the trough concentration. (D) The fluconazole exposure after 5 days of dosing correlated with the dose.

panel of drugs for which a pharmacy-based active TDM service is an effective tool to optimize drug dosing (as demonstrated for aminoglycoside antibiotics), resulting in higher antimicrobial efficacy, shorter hospitalization, and a reduced incidence of adverse events (36).

Over the last 2 decades, multiple studies have demonstrated significant variability in fluconazole exposure in different patient populations. Increased fluconazole dosing is proposed to avoid underexposure, but we demonstrated the potential risk for unnecessary overexposure in a heterogeneous patient population. A randomized clinical trial with critically ill patients receiving fluconazole to compare different dosing strategies (e.g., SPC dosing [800 mg loading + 400 mg maintenance]), TDM-guided dosing, higher fixed dosing (e.g., 1,200 mg loading + 600 mg maintenance), and weight-based dosing (e.g., 13 mg/kg loading + 8 mg/kg maintenance) will provide stronger evidence for optimal dosing with fluconazole. However, it is unlikely that such a trial will be funded for a generic drug. The use of TDM for fluconazole remains, therefore, at the discretion of clinicians to be used in selected cases. In this study, leftover blood samples were collected to obtain plasma samples to determine the fluconazole plasma concentrations in each patient. The use of leftover samples for a stable compound such as fluconazole has been reported in other studies, and comparable results to scheduled PK sampling were demonstrated (29, 30). This noninvasive approach to obtain plasma

concentrations for other compounds could be very useful in patient populations that are difficult to study. Before using a scavenge sample approach for other drugs, the stability of the proposed drug must first be established for the conditions under which the drug will be stored and processed.

In conclusion, we observed substantial variability in fluconazole plasma concentrations in critically ill adults. Fluconazole exposure was strongly correlated with fluconazole trough concentration. Considering the large variability in exposure and in particular the observed underexposure in ICU patients, fluconazole C_{min} TDM-guided dosing could be a valuable tool to optimize antifungal therapy with fluconazole in critically ill patients, solid organ transplant recipients, and patients on dialysis.

MATERIALS AND METHODS

This prospective study was conducted at the University Medical Center Groningen. Patients were eligible for inclusion if they were at least 18 years of age, were admitted to the ICU, and received antifungal therapy or prophylaxis with fluconazole. This study was evaluated by the local ethics committee (Institutional Review Board 2014, METc 2014.363), and according to Dutch law, a waiver was obtained for this study according to the Medical Research Involving Human Subjects Act due to its noninvasive nature: i.e., fluconazole plasma concentrations were determined in discarded samples. Patients were included between October 2014 and February 2017 (ClinicalTrials.gov identifier NCT02491151).

Data collection. Data were collected (J.B.) using a standardized case report form and verified by a second investigator (A.-G.M.). Data included demographics, clinical data, and therapy related data: i.e., age, gender, weight, length, underlying condition, length of stay in the ICU, leukocyte count, *Candida* species, and MIC. The presence of risk factors for invasive candidiasis was reviewed, including the presence of a central venous catheter, total parenteral nutrition, mechanical ventilation, dialysis, corticosteroid use, previous use of broad-spectrum antibiotics, and immunosuppressive therapy (37). Information was collected about the indication for antifungal therapy (prophylaxis or treatment), fluconazole dose (mg/day), route of administration, fluconazole concentrations, and dose adjustments. Leftover samples from routinely collected blood specimens were used for analysis. Whole-blood samples were collected on the ICU and stored directly at 2 to 8°C. Blood samples were processed within 24 h of collection, and plasma was stored at −20°C until analysis to determine the fluconazole concentration. This strategy has been used before for fluconazole and showed comparable results to scheduled PK sampling (29, 30). Total (bound and unbound) fluconazole concentrations were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay (38).

Therapy and therapy outcome. Prophylaxis with fluconazole for 5 days was administered only to patients who underwent major abdominal surgery according to the hospital protocol. These patients received doses of 400 mg on day 1, 200 mg on day 2, and 100 mg on days 3, 4, and 5, starting on the day of surgery. Based on the clinical status of the patient and microbiological surveillance data from nonsterile sites, prophylaxis was continued after day 5 with 100 mg fluconazole daily until clearance of *Candida* from the nonsterile sites or the absence of complications due to the surgical intervention. The response to fluconazole prophylaxis was determined 14 days after initiation; it was considered successful if there was no breakthrough infection or if therapy was not switched to another antifungal agent due to lack of clinical effectiveness. Patients receiving fluconazole for the treatment of invasive candidiasis were dosed with 800 mg on day 1, followed by 400 mg daily as the maintenance dose, according to the Pfizer Summary of Product Characteristics (SPC) (24). Initiation of treatment with fluconazole was based on growth of *Candida* species susceptible to fluconazole at sterile sites. For therapy outcome, possible reasons for therapy discontinuation were determined, including death, switching to another antifungal agent due to lack of efficacy, successful treatment, or the onset of an adverse event.

Pharmacokinetic modeling. Pharmacokinetic modeling was performed with a nonparametric adaptive grid (NPAG) algorithm using R package Pmetrics, R (Los Angeles, CA, USA) (39). One- and two-compartment pharmacokinetic models were parameterized with central volume of distribution (V; liters), clearance (CL; liters/h), rate constant for fluconazole distribution from the peripheral to the central compartment (k_{pc}; h^{-1}), and rate constant for fluconazole distribution from the central to the peripheral compartment (k_{cp}; h^{-1}). The goodness of fit of individual pharmacokinetic models was analyzed using the individual-predicted and population-predicted versus observed goodness-of-fit plots, the Akaike information criterion (AIC), the Bayesian information criterion (BIC), the log-likelihood value, and the bias and imprecision of the observed-predicted plots. Age, gender, weight and creatinine (μmol/liter) were tested as potential covariates to be included in the model. Covariates were included in the final model if the stepwise linear regression resulted in $P < 0.05$ or if other population pharmacokinetic parameters improved. The model was validated using an independent external data set with 132 samples from 30 patients (40). For the validation, the final model was used as a nonuniform prior, the validation data set was used as data, and cycles were set to 0. This created only posterior Bayesian predictions.

Probability of target attainment. Using the final model, probability of target attainment (PTA) was calculated using Monte Carlo simulations (n = 1,000). The pharmacokinetic/pharmacodynamic (PK/PD) target of unbound fluconazole AUC to the MIC (*f*AUC/MIC) was set to 100, as defined by the European Committee for Antimicrobial Susceptibility and Testing (EUCAST) (41). Fluconazole plasma protein binding was estimated to be 12% in all simulations (24). Different dosages were simulated to explore the probability of reaching the highest target: thus, higher-than-routine dosing regimens were tested. A wide

dosing range was examined where six fixed dosing regimens were simulated: 400 mg daily, 600 mg daily, 800 mg loading + 400 mg daily, 1,000 mg loading + 600 mg daily, 1,000 mg loading + 800 mg daily, and 1,200 mg loading + 1,000 mg daily. Six weight-based dosing regimens were simulated: 6 mg/kg daily, 7 mg/kg daily, 8 mg/kg daily, 9 mg/kg loading + 7 mg/kg daily, 12 mg/kg loading + 8 mg/kg daily, and 13 mg/kg loading + 8 mg/kg daily. The regimen was designed to cover *C. albicans* and *Candida tropicalis*, and a MIC of 2 mg/liter was used for PTA calculations, as the epidemiological cutoff (ECOFF) value for each of these pathogens is 2 mg/liter, respectively (41). The PTA target was to achieve adequate exposure in ≥95% of the patients after day 1 of fluconazole therapy. The number of patients reaching a toxic exposure (80 mg/liter) was simulated for the proposed dosing regimens (34). A linear model was used to determine the proposed C_{min} range for day 3 in our data set: 400 mg · h/liter for AUC∼C_{min} (41).

Statistical analysis. For the univariable analysis, a Spearman correlation coefficient was calculated to determine correlations between 2 continuous variables. For comparison of 2 groups, the Mann-Whitney U test was used. Variables obtained from univariable analysis ($P < 0.1$) were included in multiple linear regression analysis using a backward procedure, thereby removing nonsignificant variables, starting with the one with the highest *P* value.

All statistical analyses were performed using SPSS for Windows, version 20.0 (IBM SPSS, Chicago, IL), and R version 3.6.1. A *P* value of <0.05 was considered statistically significant.

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