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SHORT COMMUNICATION

Itraconazole Serum Trough Concentrations Using Oral Capsules for the Treatment of Chronic Pulmonary Aspergillosis: What is the Target?

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Abstract
Background In regions where there is only itraconazole capsule as a therapeutic option for treatment of chronic pulmonary aspergillosis (CPA), measuring the serum concentrations becomes even more important for therapeutic success.
Objective Evaluate the initial itraconazole serum trough concentrations after the administration of oral capsule of itraconazole for the treatment of CPA.
Methods The measurement was performed at least 7-days after initiation of therapy. The standard treatment at our institution was a 200 mg capsule every 12 h. We defined that an adequate serum trough concentration of itraconazole during treatment was 1–4 mg/L.
Results This study recruited 28 patients. The median value was 0.30 mg/L (IQR 0.01–0.70). Only 11% (n = 3) had adequate serum concentrations based on guideline recommendation. All patients with clinical deterioration had itraconazole serum levels $\leq$ 0.8 mg/L.
Conclusion The initial serum concentrations of itraconazole after capsule formulation administration were low. Increasing the dose should be considered when the itraconazole concentration is low, especially if it is $\leq$ 0.8 mg/L, and the patient presents with clinical deterioration. Larger studies are needed to evaluate the adequate concentrations recommended for CPA.

Keywords Chronic pulmonary aspergillosis · Itraconazole · Capsule · Serum trough concentrations

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Introduction

Chronic pulmonary aspergillosis (CPA) is a disease caused by filamentous fungi of the genus *Aspergillus* [1] , usually seen in immunocompetent or mildly immunosuppressed patients with underlying structural lung diseases [2, 3].

Antifungal treatment is based on azoles [4], of which itraconazole is the most frequently used [5]. The outcome may be affected by various determinants, such as susceptibility, timing of therapy, infection site, and itraconazole pharmacokinetics [6]. Plasma concentrations at steady state cannot be predicted based on the initial oral dose or the previous treatment due to pharmacokinetic variability [7].

Itraconazole is available in oral formulations: capsules and oral solution, and intravenous formulations [8]. The oral bioavailability of capsules is increased by gastric acidity and food [9]. Itraconazole has a 30% higher bioavailability as an oral solution than as capsules [10]. Therefore, in countries where only itraconazole capsules are available, which present erratic absorption [6, 11], measuring serum level is very important to assure therapeutic success [8].

Serum drug monitoring was proposed by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) as an alternative to improve efficacy, reduce toxicity, and to confirm that the patient remains in the therapeutic range during follow up [12]. However, the strongest evidence to support therapeutic drug monitoring (TDM) of itraconazole is for the prevention of invasive fungal infections in immunocompromised patients, and there is no evidence that same concentration targets are necessarily optimal for CPA [8].

Our objective is to evaluate the initial itraconazole serum trough concentrations after the administration of oral capsule of itraconazole for the treatment of CPA. A secondary objective is to evaluate the outcomes of treatment based on concentrations.

Methods

Study Design

This was a cross-sectional study of patients with proven CPA who underwent antifungal treatment with itraconazole capsules between January 2015 and December 2021.

We included all cases diagnosed with CPA and that had been treated with capsules of itraconazole (Traxonol®), that were followed up at outpatient clinics of Hospital das Clínicas, a tertiary-care hospital associated with the University of São Paulo, Brazil. Patients aged < 18 years and those who did not have itraconazole serum levels measured were excluded.

Ethical approval for the study was obtained from the local ethics committee (Approval date: 08/06/2021; approval file #49999021.0.0000.0068). The informed consent was waived because this study was retrospective, with the review of medical records.

Definition of CPA

CPA was defined as the presence of findings suggestive of aspergillosis on chest computerized tomography (CT) for at least 3 months; or a subacute invasive aspergillosis for at least 1 month with serological or microbiological evidence of *Aspergillus* spp., and the exclusion of alternative diagnoses [4].

Data Collection

The standard treatment at our institution was a 200 mg capsule every 12 h. We did not prescribe a loading dose. Patients were instructed to take the drug with acidic drinks or with meals, and drug interactions were checked routinely in medical appointments. We also collected demographic and clinical data at the time of the diagnosis of CPA, such as age, sex, body mass index (BMI), pulmonary tuberculosis, nontuberculous mycobacterial (NTM) lung disease, asthma, chronic obstructive pulmonary disease (COPD), immunosuppression, and clinical outcomes after 12 months. When blood was collected for monitoring, creatinine, liver enzymes, and bilirubin were also evaluated. The outcomes were classified as: improvement, stability, and deterioration, based on the assessment by the attending physician. Clinical improvement was considered as the disappearance or reduction of constitutional and pulmonary symptoms. Clinical deterioration was defined by progression of systemic and respiratory signs.

Itraconazole Concentration

A blood sample was collected at least 7 days after starting treatment, one hour before drug administration, to ensure that steady-state conditions had been achieved. Serum values of itraconazole were not measured again during the antifungal treatment.

We defined an adequate serum trough concentration of itraconazole during treatment as being between 1 and 4 mg/L, based on recommendations for invasive aspergillosis [12]. This was used to determine the frequency of adequate serum levels. Concentrations > 4 mg/L were considered toxic [12].

Serum samples were analysed using liquid chromatography-mass spectrometry (LC–MS), with a coefficient of variation of 6.89%, and a lower limit of detection of < 0.007 mg/L.

Itraconazole was measured by a Waters® Acquity TQD LC–MS. A Waters Corporation TQD was used in positive ionization mode with an electrospray source. Samples were collected in serum, and the calibrators and controls used by Chromsystems®. Samples and calibrators were extracted by addition of zinc sulfate 0.1 M and acetonitrile (50µL de serum + 20µL of 2.5 µg/mL IS Mix (Itraconazole-D6) + 200µL of 0.1 M de ZnSO4 in 20% MeOH/80%H20 + 500µL of acetonitrile LC–MS Grade) followed by centrifugation (1440 g) and separation of the supernatant. Temperature column 40 °C, Acquity UPLC ®BE C18 1.7 µm × 2.1 µm × 150 mm, Waters®. Mobile phase at flow rate 0.50 mL/min; gradient; Mobile phase [C]: Ammonium acetate (HCOONH4)10 mM in water with 0.1% formic acid (HCOOH), and [D]: Ammonium acetate (HCOONH4 10 mM) in methanol with 0.1% formic acid (HCOOH). The triple quadrupole mass detections with multiple reaction monitoring mode were used to monitor the ion transitions (m/z) 705.40 > 119.10 for itraconazole. The deuterated internal standards for each antimycotic drug were also quantified and added in all analyzed samples. The method was validated following the CLSI C62-A document. The method was shown to be simple, fast, accurate, and reproducible.

Statistical Analysis

All analyses were performed using the RStudio software (1.4 version) with the gtsummary package. Continuous variables were presented as median and interquartile range (IQR), and categorical variables were presented as percentages and total counts. Frequencies of categorical variables were calculated.

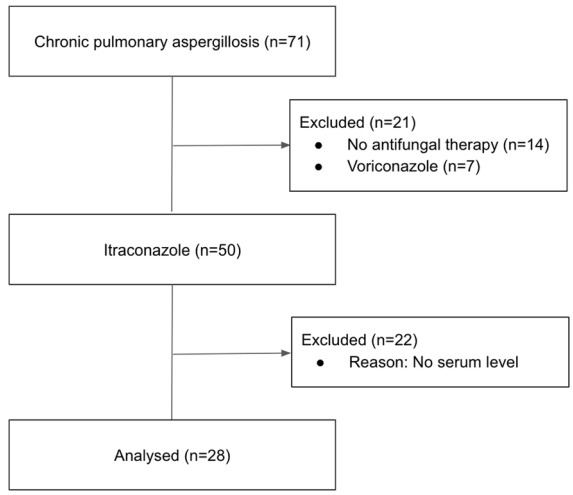

Fig. 1 Patient selection for evaluation of trough concentrations of itraconazole after administration of oral capsules for the treatment of chronic pulmonary aspergillosis in Hospital das Clínicas, University of São Paulo, between 2015 and 2021

Results

Initially, there were 71 patients diagnosed with CPA of which 21 were excluded due to treatment with voriconazole or absence of antifungal treatment. Fifty received treatment with itraconazole capsules. Twenty-two patients were excluded because they did not have serum levels of itraconazole measured. Therefore, 28 patients were included in the study (Fig. 1).

The median age was 51 years (IQR 42–59), and 16 (57%) patients were male. The median BMI was 20.8 kg/m^2 (IQR 17.6–23.6). The most common underlying lung disease was tuberculosis (82%, n = 23). Sixteen (57%) patients had a history of smoking. Only five (18%) patients used immunosuppressant drugs (Table 1).

The median serum itraconazole concentration was 0.30 mg/L (IQR 0.01–0.70) (Table 2). Most patients had concentrations lower than recommended (Fig. 2). Only 11% (n = 3) had adequate serum concentrations. Three patients had no detectable serum itraconazole. None of the patients presented toxic concentrations. Only one patient had kidney failure, and all had

Table 1 Characteristics of patients with chronic pulmonary aspergillosis treated with capsules of itraconazole

Characteristics	Total (n = 28)
Age median (IQR), years	51 (42–59)
Male	16 (57%)
BMI (kg/m^2)	20.8 (17.6–23.6)
Pulmonary TB	23 (82%)
NTM lung disease	3 (11%)
COPD	4 (14%)
Asthma	2 (7%)
Smoking	16 (57%)
Alcoholism	7 (25%)
Immunosuppressant drugs	5 (18%)
Diabetes mellitus	5 (18%)

TB tuberculosis, *COPD* chronic obstructive pulmonary disease, *NTM* nontuberculous mycobacterial

normal liver function tests (Table 2). Median values for creatinine were 0.83 mg/dL (IQR 0.67–0.89); aspartate aminotransferase 16 U/L (IQR 13–17); alanine aminotransferase 14 U/L (IQR 10–16); and total bilirubin 0.41 mg/dL (IQR 0.3–0.45).

Regarding the outcomes, most patients showed clinical improvement (n = 15, 55%) and clinical stability (n = 8, 30%), while only 4 patients (15%) presented clinical deterioration (Table 2). The outcome of one patient could not be evaluated due to incomplete treatment. The median value of serum itraconazole was low in all the groups—clinical improvement, stability and deterioration were, respectively, 0.30 mg/L (IQR 0.12–0.70), 0.20 mg/L (IQR 0.01–0.47), and 0.05 mg/L (IQR 0.01–0.27). All patients with clinical deterioration had itraconazole serum levels $\leq$ 0.8 mg/L.

Discussion

Trough serum concentrations of itraconazole during treatment with capsules of itraconazole was low in the majority of patients with CPA. If we consider as adequate the concentrations recommended in guidelines for acute invasive disease (1–4 mg/L), most treatments would have been considered inadequate. Unexpectedly, most patients with low concentration presented clinical improvement and stability. This makes us question if the recommendation for invasive aspergillosis is applicable to CPA.

The serum concentrations using capsules of itraconazole are lower when compared with other formulations [13]. This could be explained by the erratic absorption of this formulation [14]. Additionally, the complexities associated with pelletization technology impact the gastrointestinal absorption and in vivo bioavailability of itraconazole in CPA, ultimately influencing its serum levels and efficacy [15–17]. However, it is possible that the treatment of CPA may be achieved with lower concentrations of itraconazole than recommended for invasive disease. In the dermatophytic infections, the cut-off serum level for successful therapeutic outcome was also lower than recommended for invasive mycoses [18]. There is no evidence to determine optimal concentration targets for CPA [8], that should be achieved by studying the correlation between itraconazole concentrations and outcomes.

In our study, most patients had improved or stable clinical outcomes despite low serum concentrations. Because of this, no increase in dose of itraconazole was made. Increasing the dose should be considered when the itraconazole concentration is low, especially if it is $\leq$ 0.8 mg/L, and the patient presents with clinical deterioration. More studies are needed to correlate worse outcomes with low serum levels during treatment.

Itraconazole capsules are the only oral presentation of the drug in middle-income countries, thus the importance of understanding how this formulation may be used to treat different forms of aspergillosis. Our study contributed by characterizing trough concentrations using capsules. However, our study has limitations. Itraconazole was not dosed systematically during treatment, and the number of patients and the study design limited the ability to evaluate the correlation between concentrations and outcome. Furthermore, it is a retrospective study, which relies on information in medical records.

In conclusion, trough serum concentrations of itraconazole administered in capsules were low. However, most patients with CPA presented clinical improvement or stability. It is not clear whether the

Table 2 Serum concentrations of itraconazole, outcomes, and drug toxicities of patients with chronic pulmonary aspergillosis treated with itraconazole capsules

Itraconazole concentrations, outcomes, and toxicities	Total (n = 28)
Concentration (mg/L), median (IQR)	0.30 (0.01–0.70)
Number of patients with concentration 1–4 mg/L	3 (11%)
Concentration (mg/L) in patients with clinical improvement (n = 15), median (IQR)	0.30 (0.12–0.70)
Concentration (mg/L) in patients with clinical stability (n = 8), median (IQR)	0.20 (0.01–0.47)
Concentration (mg/L) in patients with clinical deterioration (n = 4), median (IQR)	0.05 (0.05–0.70)
Liver failure	0 (0%)
Kidney failure	1 (4%)

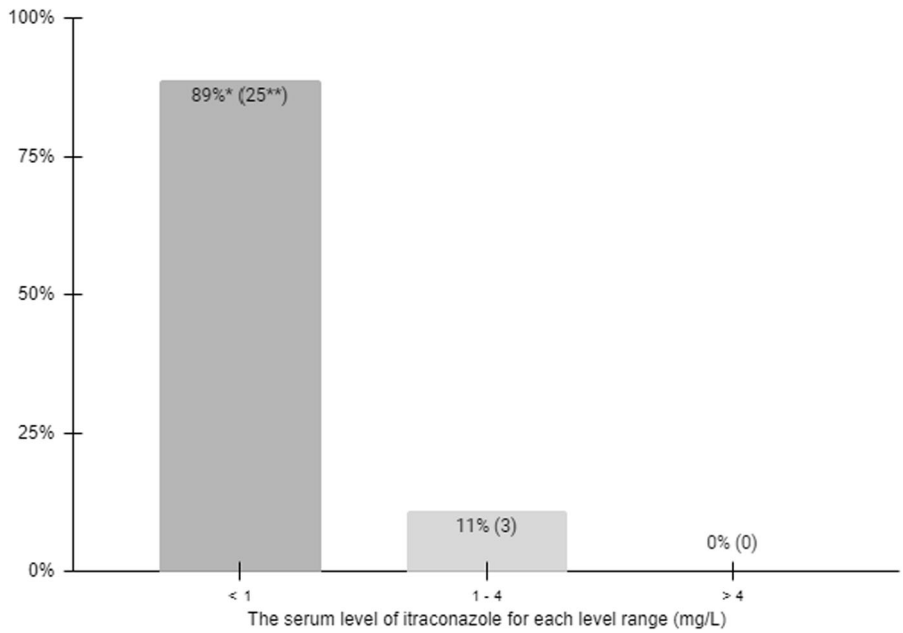

Fig. 2 The frequency based on the range of serum levels of itraconazole after oral capsule administration in the treatment of 28 patients with chronic pulmonary aspergillosis. *Proportions of patients; **numbers of patients

concentrations recommended for invasive aspergillosis are applicable to CPA. Large, prospective studies are needed to evaluate the adequate concentrations recommended for CPA.

Authors Contributions VFO and MMCM designed the study and analysed the data. VFO wrote the first draft. All authors have approved the final manuscript draft.

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Declarations

Conflict of Interest The authors whose names are listed above certify that they have no conflict of interest.

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