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Susceptibility profiles and clinical efficacy of antifungals against *candida* bloodstream isolates from critically ill patients: Focus on intravenous itraconazole

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ABSTRACT

In vitro and clinical data were analysed to evaluate the susceptibility profile of itraconazole in light of the new cut-off points. The in vitro activity of itraconazole was compared with that of eight comparators against 119 *Candida* bloodstream isolates from 2015 to 2018. Minimum inhibitory concentrations (MICs) were measured by the colorimetric MICRONAUT-S assay. The content of wells without any color change was sub-cultured to measure killing efficacy. No major differences were found against *Candida albicans*. Itraconazole, posaconazole and amphotericin B were the most active agents against *Candida parapsilosis*. Of the 32 isolates of *C. parapsilosis* that were resistant to fluconazole, 96.9%, 78.1% and 93.8% were susceptible to itraconazole, voriconazole and posaconazole, respectively. The ratio of the minimum fungicidal concentration (MFC) to the MIC of itraconazole was lower than for the other azoles against *C. parapsilosis* and *C. glabrata*. Itraconazole achieved greater inhibition over-time of the growth of *C. parapsilosis* than fluconazole. Seventy-three critically ill patients who were unresponsive to antibiotics received intravenous empirical treatment with itraconazole (n = 28) or comparators (n = 45). Case-control matching was conducted for severity, comorbidities, risk factors for candidemia, administered antibiotics and days of antifungal treatment. Breakthrough candidemia was found in 3.6% of patients treated with itraconazole and in 32.1% of patients treated with comparators (*P*: 0.020); breakthrough candidemia by *C. parapsilosis* was found in 3.6% and 28.6% of patients, respectively. Results indicate that itraconazole retains a valuable susceptibility profile against *Candida* isolates, particularly *C. parapsilosis*. This superior profile may explain the clinical efficacy in the occurrence of breakthrough candidemia and warrants further clinical investigation.

1. Introduction

Candida species are the fourth most common cause of bloodstream infections in critically ill patients. The widespread use of fluconazole has led to the emergence of infections by non-*albicans* species and also by species resistant to fluconazole [1]. A survey of susceptibilities of *Candida* bloodstream isolates from Wurzburg over the 7-year period from 2005 to 2012 showed that although studied isolates remained susceptible to azoles, a shift of the distribution of minimum inhibitory concentrations (MICs) to lower values was noted for itraconazole but not for fluconazole [2]. Whether this shift of MICs really indicates superior clinical efficacy of itraconazole remains to be clarified. Moreover, it was

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recently proposed that the breakpoints of the MICs of itraconazole should be lower compared with fluconazole [3].

In the light of data indicating the high selective pressure exerted by the overconsumption of fluconazole, we investigated whether itraconazole was active against *Candida* species and could be a possible alternative treatment. The susceptibilities of bloodstream isolates infecting critically ill patients in Greece to nine antifungals were investigated. As antifungal treatment is often given empirically in critically ill patients who are unresponsive to antibiotics, this study followed a case-matching approach to compare the efficacy of intravenous itraconazole with other antifungals administered for this purpose.

2. Material and methods

2.1. Collection of isolates

Isolates used for the study were all species of *Candida* isolated from the blood of patients with sepsis at the prospective registry of the Hellenic Sepsis Study Group (HSSG) from January 2015 to July 2018. This prospective registry took place in 65 study sites (departments of Internal Medicine, departments of Surgery and Intensive Care Units [ICUs]) across Greece. The study protocol was approved by the Ethics Committee of the participating hospitals. Patients were enrolled after written informed consent was provided by themselves or by first-degree relatives in case of patients unable to consent. Consenting allowed the analysis of biosamples and patient clinical information. Study participants were suffering from lung infections, urinary tract infections, intra-abdominal infections or primary bloodstream infections aggravated by at least two signs of systemic inflammatory response syndrome (SIRS). Patients were excluded from the study if they had neutropenia, were infected with human immunodeficiency virus or had intake of corticosteroids at doses exceeding 0.5 mg/kg of equivalent prednisone daily for at least 15 consecutive days.

Blood was sampled within the first 24 h following the presentation of signs of SIRS after venipuncture of one peripheral vein under aseptic conditions. All study sites of the HSSG used similar blood culture and microbial identification techniques; 20 mL of blood was inoculated into ready-prepared culture vials (bioMérieux, Marcy l'Etoile, France) and incubated into the BacT/ALERT 3D automated system using BacT/ALERT iAST culture bottles. Grown fungal species were sub-cultured onto Sabouraud dextrose agar (Oxoid Ltd, London, UK) for 24 h at 37 °C under 5% CO_2. Grown *Candida* species were further identified by the classical germ tube test and API 20C AUC (bioMérieux). Colonies were stored in skimmed milk at -80 °C and transported to the central laboratory at the 4th Department of Internal Medicine of ATTIKON University Hospital.

MICs of itraconazole, fluconazole, voriconazole, posaconazole, caspofungin, anidulafungin, micafungin, amphotericin B and 5-flucytosine were measured using a microdilution technique in microtiter plates with known concentrations of antifungals (Micronaut-S, Merlin Diagnostika, GmbH, Berlin, Germany) at a final volume of 0.2 mL using 5×10^5 cfu/mL of each isolate and RPMI1640 growth medium. The MIC was considered the lowest concentration leading to colorimetric changes after 48 to 72 h of incubation at 35 °C under ambient atmosphere. Results were interpreted using the EUCAST2018 susceptibility breakpoints for each fungal species.

To measure minimum fungicidal concentrations (MFCs), the content of wells without any color change were plated onto Sabouraud dextrose agar. MFC was considered as the lowest antifungal concentration killing 99.9% of the plated inoculum. The MFC to MIC ratio was calculated for each azole and expressed by mean ± standard error (SE).

To investigate the time-kill effect of itraconazole on *C. albicans* and *C. parapsilosis*, one 1×10^5 cfu/mL log-phase inoculum of eight isolates of *C. albicans* and nine isolates of *C. parapsilosis* were exposed over time into tubes of 10 mL RPMI1640 with or without 2% hydroxypropyl-β-cyclodextrin or 0.5 μg/mL itraconazole diluted in 2% hydroxypropyl-β-cyclodextrin or 10 μg/mL fluconazole (Hospital Line, Athens, Greece). The selected concentrations of fluconazole and itraconazole correspond to mean serum concentrations after administration of conventional doses [4,5]. Tubes were incubated at 37 °C in a shaking water bath and fungal outgrowth was determined at baseline and after 4, 24, 48 and 72 h of incubation. More precisely, 0.1 mL of the tube content was removed and diluted five serial times 1:10 in 0.9% NaCl, and 0.1 mL of each dilution was plated onto Sabouraud dextrose agar. The number of colonies was multiplied by the dilution factor and expressed as log10.

2.2. Clinical data

The data registry of the HSSG was searched for patients hospitalized from January 2017 to July 2018 in Intensive Care Units (ICUs) who were originally diagnosed with clinically documented sepsis and remained non-responsive after at least 7 days of empirical antibiotic treatment and then received empirical antifungal treatment. The decision to administer empirical antifungals was based on the presence of at least two risk factors for candidiasis. Patients who had received any prior antifungals were excluded from the study. Considered risk factors for candidiasis were: abdominal surgery, acute pancreatitis, at least one site (oral cavity, rectum, urine, tracheal secretions) colonized by *Candida* spp, intake of total parenteral nutrition, history of type 2 diabetes mellitus, hemodialysis, and solid tumor malignancy or lymphoma. Participation in the study was limited to ICUs that could contribute at least five patients who had been empirically treated with itraconazole. Selected comparators were patients hospitalized in the same ICUs in the same time period who were empirically administered echinocandins or other azoles. The diluent for the administered itraconazole preparation was hydroxypropyl-β-cyclodextrin (Micronazol, Hospital Line, Athens, Greece). The following information was analysed for each patient: demographics, sequential organ failure assessment (SOFA) score and acute physiology and chronic health evaluation (APACHE) II score on the day of start of antifungals, comorbidities and Charlson's comorbidity index (CCI), administered antibiotics, administered antifungals and follow-up microbiology.

The primary endpoint was the development of breakthrough candidemia over the first 28 days of follow-up from the start of empirical antifungals. The secondary endpoints were the development of candidemia by *C. parapsilosis* and the time until the development of breakthrough candidemia.

2.3. Statistical analysis

Statistical correlations between the MICs were conducted according to Spearman rank of order. MFC:MIC ratios were compared using the Student's paired t-test with post-hoc corrections by Bonferroni. The change of log10 fungal growth over-time was calculated and compared by analysis of variance (ANOVA) with post-hoc Bonferroni corrections.

Clinical data were recorded for 73 patients, 28 treated with itraconazole and 45 treated with comparators; 28 comparators were chosen to match the itraconazole group for age, APACHE II score, SOFA score, CCI, number of risk factors, administered antibiotics and days of antifungal treatment. Case-control matching was conducted with the relevant procedure in SPSS v.25 and did not pose any difficulties because nearly all the parameters required for

Table 1
Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of the 119 studied *Candida* species against nine antifungal agents.

	Range of MICs (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	% inhibited	MFC_{50} (μg/mL)	MFC_{90} (μg/mL)	% killed
Candida albicans (n = 44)							
Itraconazole (0.064)*	0.032 – 0.25	0.032	0.032	95.5	2	>8	4.5
Fluconazole (2)*	0.25 – 8	0.5	1	95.5	8	32	22.7
Voriconazole (0.064)*	≤0.008 – 1	≤0.008	≤0.008	100	1	1	43.2
Posaconazole (0.064)*	≤0.008 – 1	≤0.008	≤0.008	97.8	1	1	18.2
Caspofungin	≤0.032 – >4	0.064	0.125		1	1	
Micafungin (0.016)*	0.016 – 0.5	0.032	0.032	47.7	0.064	1	4.5
Anidulafungin (0.032)*	0.016 – 0.5	0.032	0.032	93.2	0.125	1	13.6
5-Fluocytosine	≤0.064 – 0.25	≤0.064	≤0.064		0.125	0.5	
Amphotericin B (1)*	0.25 – 0.5	0.25	0.5	100	0.5	1	93.0
Candida parapsilosis (n = 51)							
Itraconazole (0.125)*	≤0.032 – >8	≤0.032	0.06	92.2	2	>8	21.6
Fluconazole (2)*	0.25-128	4	32	37.3	32	>128	3.9
Voriconazole (0.125)*	≤0.008 – >8	0.032	0.25	86.3	1	8	19.6
Posaconazole (0.064)*	≤0.008 – >8	≤0.008	0.02	96.1	1	1	35.3
Caspofungin	≤0.002 – 1	0.25	0.5		1	8	
Micafungin (0.002)*	0.016 – 0.5	0.5	0.5	2	1	4	0
Anidulafungin (0.002)*	0.032- >8	0.5	1	0	2	16	0
5-Fluocytosine	≤0.032 – 0.125	0.064	0.064		0.5	>2	
Amphotericin B (1)*	≤0.032 – 0.5	0.5	0.5	100	1	4	80.4
Candida glabrata (n = 17)							
Itraconazole	1 – >8	4	>8		8	>8	
Fluconazole (2)*	8 – >128	16	64	0	>128	>128	17.6
Voriconazole (0.125)*	0.064 – >8	0.25	4	41.2	4	>16	5.9
Posaconazole (0.064)*	0.25 – 1	0.5	1	0	16	>32	0
Caspofungin	0.064 – 0.25	0.064	0.125		0.5	>1	
Micafungin (0.032)*	0.016 – 0.032	0.016	0.016	100	0.064	>1	11.8
Anidulafungin (0.064)*	0.016 – 0.25	0.032	0.125	88.2	0.25	>1	17.6
5-Fluocytosine	≤0.064 – 0.125	≤0.064	≤0.064		0.125	0.25	
Amphotericin B (1)*	0.5 – 0.5	0.5	0.5	100	1	>8	70.6
Candida tropicalis (n = 7)							
Itraconazole (0.125)*	≤0.032 – 0.125	≤0.032	≤0.032	100	2	8	14.3
Fluconazole (2)*	1 – 8	4	8	42.9	32	>128	14.3
Voriconazole (0.125)*	0.032 – 0.25	0.064	0.25	85.7	2	4	14.3
Posaconazole (0.064)*	0.016 – 2	0.032	2	85.7	1	2	14.3
Caspofungin	0.064 – 4	0.125	4		0.5	>8	
Micafungin	0.032 – 2	0.064	2		0.5	2	
Anidulafungin (0.064)*	0.016 – 0.25	0.064	0.25	57.1	1	8	0
5-Fluocytosine	≤0.064 – 0.125	0.064	0.125		0.25	4	
Amphotericin B (1)*	0.25 – 1	0.5	1	100	1	4	71.4

* EUCAST susceptibility breakpoint.

matching were very similar in the two groups. Matching was accomplished by keeping the tolerances (fuzz factors) very close to zero, i.e. the matches were almost exact.

Quantitative variables were compared using the Student's t-test and qualitative variables were compared using the Fisher exact test. The odds ratios (OR) and 95% confidence intervals (CI) were calculated according to Mantel and Haenszel. Variables that differed between patients who developed and who did not develop breakthrough candidemia entered forward logistic regression analysis; ORs and CIs were provided. The time to breakthrough bacteremia was compared using the log-rank test. Any value of *P* below 0.05 was considered significant.

3. Results

3.1. *In vitro* susceptibility data

A total of 119 isolates were studied; 44 of *Candida albicans*; 51 of *Candida parapsilosis*; 17 of *Candida glabrata*; and seven of *Candida tropicalis*. The comparative susceptibilities to the nine studied antifungals are shown in Table 1. Notably, there were no major differences in susceptibilities against *C. albicans*; itraconazole, posaconazole and amphotericin B were the most active agents against *C. parapsilosis*; and itraconazole was more active than the other azoles against *C. tropicalis*. Thirty-two isolates of *C. parapsilosis* were resistant to fluconazole. Among these isolates, 31 (96.9%), 25 (78.1%) and 30 (93.8%) were susceptible to itraconazole, voriconazole and posaconazole, respectively.

Correlations of the distribution of the MICs of the four azole compounds for the 44 studied isolates of *C. albicans* and for the 51 studied isolates of *C. parapsilosis* are shown in supplementary Figs. 1 and 2, respectively. There was a clustering of inhibitory activity of itraconazole at lower MICs compared with fluconazole and other agents.

The ratio of the minimum bactericidal concentration to the MIC is considered an expression of the microbicidal or microbiostatic effect of antibiotics. To our knowledge, a similar approach has not been published for antifungal agents. In the current study, the MFC:MIC ratio was compared between the four studied azoles. As shown in Fig. 1, this ratio was lower for itraconazole than comparators for *C. parapsilosis* and *C. glabrata*.

As shown in Fig. 2, itraconazole and fluconazole did not substantially change the over-time growth of *C. albicans*, whereas itraconazole inhibited significantly the over-time growth of *C. parapsilosis*.

3.2. Clinical data

A total of 73 cases who received intravenous empirical antifungal treatment in three ICUs were analysed; 28 received itraconazole 200 mg every 12 h on the first two days and then 200 mg once daily; two received voriconazole 200 mg twice daily; 21 received anidulafungin 200 mg on the first day and then

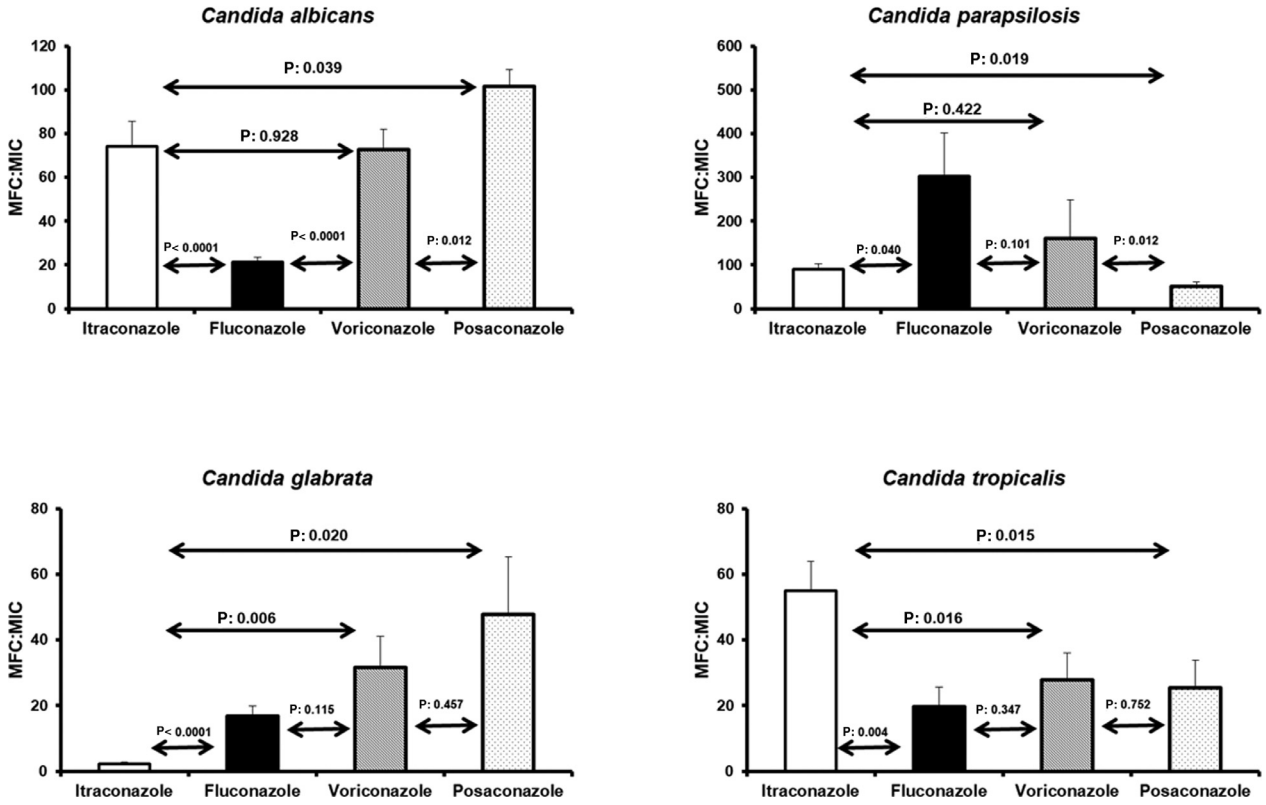

Fig. 1. Comparative ratios of minimum fungicidal concentration (MFC) to minimum inhibitory concentration (MIC) of itraconazole, fluconazole, voriconazole and posaconazole against the four studied *Candida* species. *P*-values of the indicated comparisons are provided.

100 mg daily; and 22 received 100 mg micafungin once daily. Demographic characteristics of patients under empirical treatment with itraconazole and with other antifungals after case-control matching are shown in Table 2. There were no differences between patients under treatment with itraconazole and patients under treatment with other antifungals whether matching was followed or not.

Breakthrough candidemia occurred in one of 28 patients treated with itraconazole (3.6%) compared to 12 of 45 unmatched comparators (26.7%). The OR for breakthrough candidemia in itraconazole-treated patients was 0.10 (95% CIs: 0.01-0.83; *P*: 0.033). After comparing baseline characteristics of the unmatched cohort between patients who developed and those who did not develop breakthrough candidemia, the only variables that differed were APACHE II score and itraconazole treatment (supplementary Table 1).

Breakthrough candidemia occurred in one itraconazole-treated patient (3.6%) compared with nine matched comparators (32.1%); in one (3.6%) and eight (28.6%) patients, respectively, breakthrough isolates were species of *C. parapsilosis*; and in zero (0%) and one (3.6%) patient, respectively, breakthrough isolates were species of *C. albicans*. The OR for breakthrough candidemia with itraconazole treatment was 0.08 (95% CIs: 0.01-0.67; *P*: 0.020). After comparing baseline characteristics of patients who developed and those who did not develop breakthrough candidemia, the only variables that differed were APACHE II score, intake of linezolid and itraconazole treatment (supplementary Table 1). These variables entered forward logistic regression analysis and this showed itraconazole treatment to be the only variable significantly protective from breakthrough candidemia (Table 3). One of nine isolates causing breakthrough candidemia by *C. parapsilosis* in the comparator group was resistant to fluconazole. All other isolates were susceptible to all antifungals.

The time to breakthrough candidemia was significantly prolonged under itraconazole compared with treatment with other antifungals (Fig. 3). Mortality after 28 days from the start of antifungal treatment was 42.9% (n = 12 patients) in the itraconazole group and 32.1% (n = 9 patients) in the comparator group (*P*: 0.582).

4. Discussion

This study showed that despite the decrease in the itraconazole susceptibility cut-off point, itraconazole retained similar inhibitory activity to the other azoles against *C. albicans* and had a superior susceptibility profile over comparators against *C. parapsilosis*. When itraconazole was given as empirical treatment in critically ill patients who were unresponsive to antibiotics, the rate of breakthrough candidemia was lower than that in patients who received prophylaxis with other antifungals. Data on antifungal prophylaxis or previous use of antifungals in the patients from which these strains were isolated were not provided. This information might be important to explain the reported fluconazole resistance.

All 119 bloodstream *Candida* species in the HSSG registry during 2015 to 2018 were analysed. Distribution of the isolated species was similar to that of other series from China, Spain and Mexico, with more than half the cases caused by non-*albicans* species [6-8]. These and other studies [9,10] generally agree with the current results on the susceptibility profile of itraconazole. In all but one of the above studies, species of *C. parapsilosis* were susceptible to fluconazole. In one study of 83 critically ill Chinese patients infected by *C. parapsilosis* [8], reported activities of fluconazole and itraconazole were 48.2% and 92.8%, respectively, which is also in agreement with the current results on the in vitro superiority of itraconazole over fluconazole against *C. parapsilosis*.

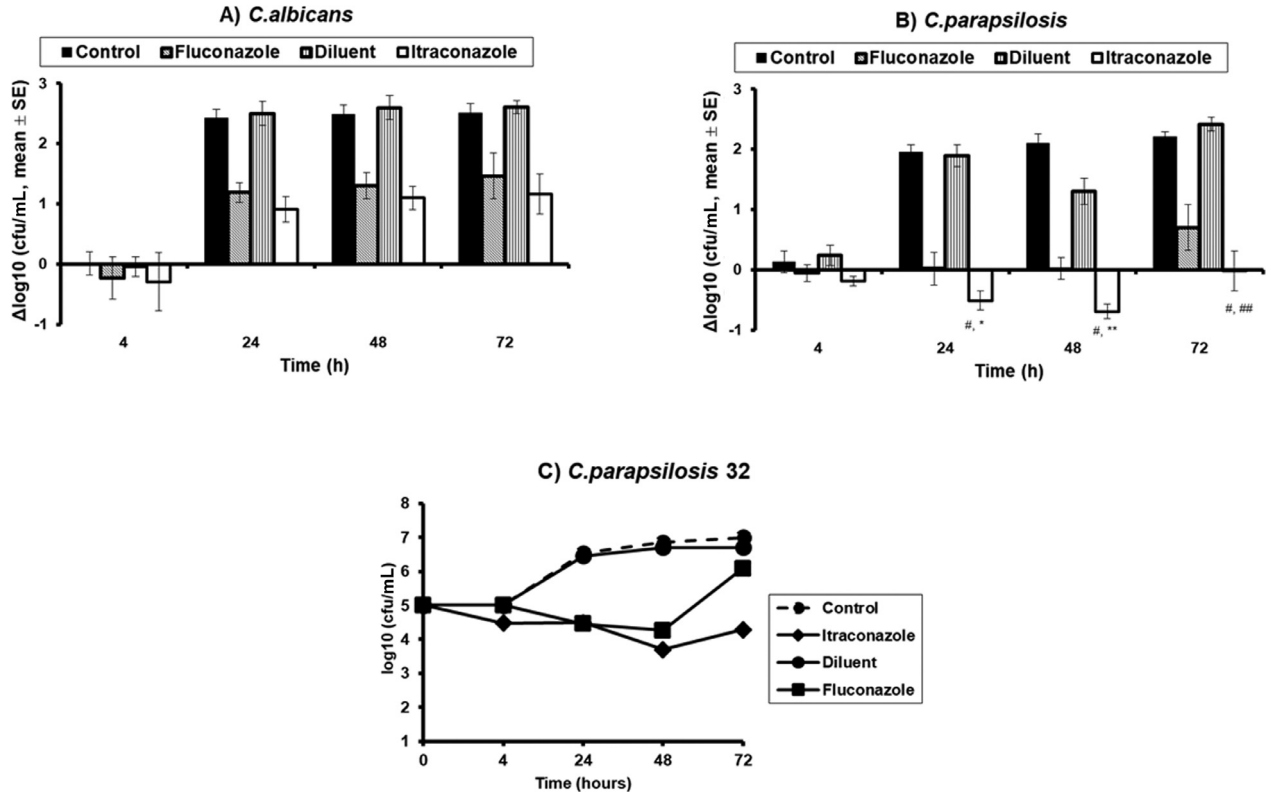

Fig. 2. Effect of itraconazole and fluconazole on fungal growth. (A) Cumulative over-time change of fungal outgrowth on eight *Candida albicans* isolates; (B) cumulative over-time change of fungal outgrowth on nine *Candida parapsilosis* isolates; and (C) time growth of *C. parapsilosis* isolate 32 under fluconazole, itraconazole and itraconazole diluent.
[#]$P<0.0001$ versus change of fungal growth of the respective control or itraconazole diluent.
[*]$P<0.0001$ vs. the respective growth under fluconazole.
[**]P: 0.033 vs. the respective growth under fluconazole.
[##]P: 0.045 vs. the respective growth under fluconazole.

Table 2
Baseline characteristics of patients treated empirically with intravenous itraconazole and matched comparators.

	Itraconazole (n = 28)	Comparators* (n = 28)	*P*
Age (years, mean ± SD)	62.2 ± 17.4	60.8 ± 16.8	0.767
APACHE II score (mean ± SD)	20.6 ± 4.7	19.3 ± 6.2	0.389
SOFA score (mean ± SD)	9.32 ± 3.34	9.14 ± 3.48	0.846
CCI (mean ± SD)	3.04 ± 2.46	3.07 ± 2.26	0.955
Number of risk factors	2.71 ± 0.98	2.71 ± 0.89	1.00
Abdominal surgery (n, %)	5 (17.9)	6 (21.4)	1.00
Acute pancreatitis (n, %)	0 (0)	0 (0)	1.00
Colonization by *Candida* spp (n, %)	4 (14.3)	0 (0)	0.111
Total parenteral nutrition (n, %)	2 (7.1)	4 (14.3)	0.669
Type 2 diabetes mellitus (n, %)	7 (25.0)	5 (17.9)	0.746
Solid tumor malignancy (n, %)	2 (7.1)	2 (7.1)	1.00
Intake of antibiotics (n, %)	28 (100)	28 (100)	1.00
3rd generation cephalosporins	8 (28.6)	6 (21.4)	0.758
Piperacillin/tazobactam	7 (25.9)	12 (42.9)	0.259
Carbapenems	4 (14.3)	7 (25.0)	0.503
Fluoroquinolones	10 (35.7)	3 (10.7)	0.055
Tigecycline	7 (25.0)	9 (32.1)	0.768
Colistin	16 (57.1)	8 (28.6)	0.058
Glycopeptides	6 (21.4)	5 (17.9)	1.00
Linezolid	2 (7.1)	6 (21.4)	0.252
Voriconazole (n, %)	0 (0)	2 (7.1)	0.491
Anidulafungin (n, %)	0 (0)	12 (42.9)	<0.0001
Micafungin (n, %)	0 (0)	14 (50.0)	<0.0001
Days of antifungal treatment (mean ± SD)	9.14 ± 7.31	11.32 ± 6.22	0.235

APACHE: acute physiology and chronic health evaluation; CCI: Charlson's comorbidity index; SD: standard deviation; SOFA: sequential organ failure assessment
* Matching was done for age, APACHE II score, SOFA score, CCI, the number of risk factors, the administered antibiotics and the days of antifungal treatment.

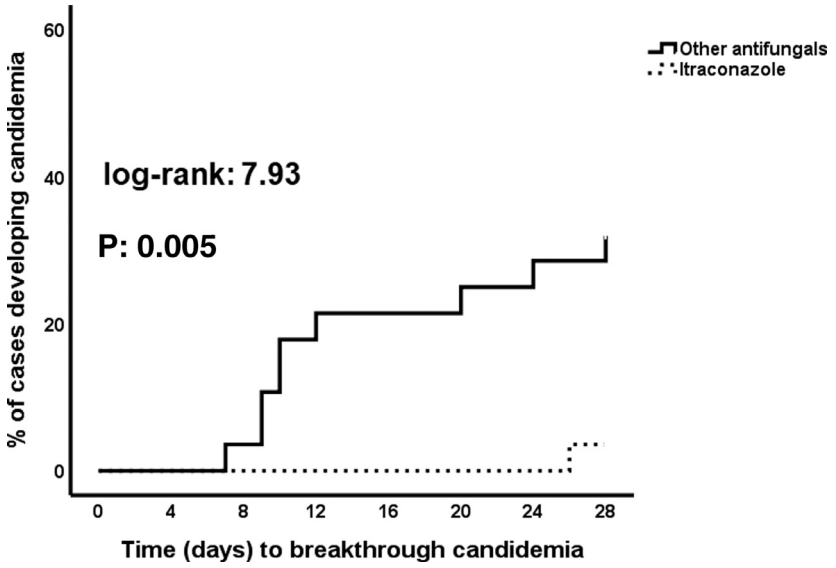

Fig. 3. Time to breakthrough candidemia in patients treated with intravenous itraconazole or other comparator antifungals. The *P*-value of the respective comparison is provided.

Table 3
Forward conditional logistic regression analysis of variables associated with the development of breakthrough candidemia Only significant variables described in supplementary Table 1 were entered in the equation.

	Odds ratio	95% confidence intervals	*P*
APACHE II	0.86	0.75-1.00	0.054
Itraconazole treatment	0.09	0.01-0.78	0.029

APACHE: acute physiology and chronic health evaluation.

In a retrospective survey from Mexico, 149 episodes of bloodstream *Candida* infections were analysed. Multivariate analysis failed to trace inappropriate antifungal therapy as an independent factor associated with unfavorable outcome [11]. Effective antifungal treatment mandates killing of the pathogens. Killing efficacy is approached here using the MFC:MIC ratio, which translates to the antifungal concentration that needs to be delivered in the tissue to achieve clinical efficacy. In this aspect, itraconazole is more active than the other azoles against *C. parapsilosis* and *C. glabrata*. In one of our studies in neutropenic rats, itraconazole constrained experimental infection by *C. parapsilosis* more effectively than fluconazole [12]. Intravenous itraconazole treatment led to significant decrease of fungal outgrowth in the lungs, spleens and kidneys compared with fluconazole. When rats were co-administered ceftriaxone to suppress bacterial translocation of enterobacteria from the gut, significant survival benefit from itraconazole treatment was shown [12].

Administration of antifungals as empirical therapy in patients who are unresponsive to antibiotics has also been proposed by others. In a recent multicenter study in 19 ICUs in France, patients with sepsis who were not responsive to antibiotics, with at least one new organ dysfunction and at least one site of *Candida* colonization, were randomized to placebo treatment or treatment with micafungin. The two groups did not differ in their primary endpoint, i.e. 30-day mortality [13]. The authors suggested that this failure to meet the primary endpoint might be associated with the failure of micafungin to achieve the pharmacokinetic/pharmacodynamic criteria of efficacy with the conventional 100 mg daily dose. This failure was particularly prominent against isolates of *C. parapsilosis* [14]. The superiority of itraconazole over other antifungals reported here should be interpreted in the light of its superior in vitro activity against the species of *C. parapsilosis*, which was the most common cause of breakthrough candidemia. Only one randomized clinical study has been published on the clinical efficacy of itraconazole in adult populations [15]. The study was conducted in patients with febrile neutropenia who were blindly allocated to intravenous itraconazole or classical amphotericin B. Respective clinical efficacy at the end of treatment was 47% vs. 38%, whereas the frequency of drug-associated adverse events was 5% and 54% [15]. A similar study using the less toxic liposomal amphotericin B derivative or echinocandins as comparators has not been conducted.

The clinical part of the current study has several limitations: a) the study uses an intravenous formulation of itraconazole that is not available in many European countries; b) the retrospective design does not allow conclusions on side effects and drug interactions; and c) power analysis was not conducted but only available cases were analysed. This could also influence analysis of 28-day mortality.

The results of the current study indicate that itraconazole retains a valuable susceptibility profile against recent bloodstream *Candida* isolates. This profile is similar to that for the other azoles against *C. albicans* but superior to fluconazole over *C. parapsilosis*. The MFC:MIC ratio of itraconazole was superior against *C. parapsilosis* and *C. glabrata*. Case-control analysis of retrospective data also indicate valuable efficacy in reducing cases of breakthrough candidemia when administered as empirical treatment to critically ill patients who were unresponsive to empirical antimicrobials. Although results may indicate that intravenous itraconazole may be an option for empirical therapy in ICUs with a high prevalence of *C. parapsilosis* infections, they also indicate the need for prospective and randomized clinical studies to validate the clinical efficacy of intravenous itraconazole.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.06.019.

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References

[1] Posteraro B, Spanu T, Fiori B, De Maio F, De Carolis E, Giaquinto A, et al. Antifungal susceptibility profiles of bloodstream yeast isolates by sensititre YeastOne over nine years at a large Italian teaching hospital. Antimicrob Agents Chemother 2015;59:3944–55.

[2] Huyke J, Mertin R, Walther G, Weber M, Kaerger K, Bougnoux ME, et al. *Candida albicans* bloodstream isolates in a German university hospital are genetically heterogeneous and susceptible to commonly used antifungals. Int J Med Microbiol 2015;305:742–7.

[3] Morris AJ, Rogers K, McKinney WP, Roberts SA, Greeman JT. Antifungal susceptibility testing result of New Zealand yeast isolates, 2001-2015: impact of recent CLSI breakpoints and epidemiological cut-off values for *Candida* and other yeast species. J Glob Antimicrob Res 2018;14:72–7.

[4] Kim H, Shin D, Kang HJ, Yu KS, Lee JW, Kim SJ, et al. Successful empirical antifungal therapy of intravenous itraconazole with pharmacokinetic evidence in pediatric cancer patients undergoing hematopoietic stem cell transplantation. Clin Drug Investig 2015;35:437–46.

[5] Sinnollareddy MG, Roberts JA, Lipman J, Akova M, Bassetti M, De Waele JJ, et al. Pharmacokinetic variability and exposures of fluconazole, anidulafungin, and caspofungin in intensive care unit patients: Data from multinational Defining Antibiotic Levels in Intensive care unit (DALI) patients Study. Crit Care 2015;19:33.

[6] González GM, de Treviño-Rangel RJ, Palma-Nicolás JP, Martínez C, González JG, Ayala J, et al. Species distribution and antifungal susceptibility of bloodstream fungal isolates in paediatric patients in Mexico: a nationwide surveillance study. J Antimicrob Chemother 2013;68:2847–51.

[7] Guinea J, Zaragoza O, Escribiano P, Martin-Mazuelos E, Pemán J, Sánchez-Reus F, et al. Molecular identification and antifungal susceptibility of yeast isolates causing fungemia collected in a population-based study in Spain in 2010 and 2011. Antimicrob Agents Chemother 2014;58:1529–37.

[8] Liu W, Tan J, Sun J, Xu Z, Li M, Yang Q, et al. Invasive candidiasis in intensive care units in China: in vitro antifungal susceptibility in the China-SCAN study. J Antimicrob Chemother 2014;69:162–7.

[9] Huang YT, Liu CY, Chung KP, Sheng WH, Hsueh PR. Antifungal susceptibilities of *Candida* isolated causing bloodstream infections at a medical center in Taiwan, 2009-2010. Antimicrob Agents Chemother 2014;58:3814–19.

[10] Xiao M, Fan X, Chen SCA, Wang H, Sun ZY, Laio K, et al. Antifungal susceptibilities of *Candida glabrata* species complex, *Candida krusei, Candida parapsilosis* species complex and *Candida tropicalis* causing invasive candidiasis in China: 3 year national surveillance. J Antimicrob Chemother 2015;70:802–10.

[11] González-Lara MF, Torres-González P, Cornejo-Juárez P, Velázquez-Acosta C, Martinez-Gamboa A, Rangel-Cordero A, et al. Impact of inappropriate antifungal therapy according to current susceptibility breakpoints on Candida bloodstream infection mortality, a retrospective analysis. BMC Infect Dis 2017;17:753.

[12] Carrer DP, Samonis G, Droggiti DI, Tsaganos T, Pistiki A, Giamarellos-Bourboulis EJ. Intravenous itraconazole against experimental neutropenic *Candida parapsilosis* infection: efficacy after suppression of bacterial translocation. J Infect Chemother 2013;19:1080–6.

[13] Timsit JF, Azoulay E, Schwebel C, Charles PE, Cornet M, Souweine B, et al. Empirical micafungin treatment and survival without invasive fungal infection in adults with ICU-acquired sepsis, *Candida* colonization, and multiple organ failure. JAMA 2016;316:1555–64.

[14] Julien V, Azoulay E, Schwebel C, Le Saux T, Charles PE, Cornet M, et al. Population pharmacokinetics of micafungin in ICU patients with sepsis and mechanical ventilation. J Antimicrob Chemother 2017;72:181–9.

[15] Boogaerts M, Winston DJ, Bow EJ, Garber G, Reboli AC, Schwarer AP, et al. Intravenous and oral itraconazole versus intravenous amphotericin B deoxycholate as empirical antifungal therapy for persistent fever in neutropenic patients with cancer who are receiving broad-spectrum antibacterial therapy. Ann Intern Med 2001;135:412–22.