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Candidemia in hospitalized adults: current challenges and strategies of management

Guillermo Cuervo Requena

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UNIVERSIDAD DE BARCELONA

DEPARTAMENTO DE CIENCIAS CLÍNICAS

FACULTAD DE MEDICINA

CANDIDEMIA IN HOSPITALIZED ADULTS:

CURRENT CHALLENGES AND STRATEGIES OF MANAGEMENT

Memoria presentada por Guillermo Cuervo Requena

Para optar al grado de Doctor en Medicina

Dirigida por:

Jordi Carratalà

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El **Dr. Jordi Carratalà,** Profesor titular del Departamento de Ciencias Clínicas de la Facultad de Medicina de la Universidad de Barcelona, y la **Dra. Carolina Garcia-Vidal**, Facultativo Especialista del Servicio de Enfermedades Infecciosas del Hospital Universitario de Bellvitge, certifican que la tesis doctoral titulada:

"CANDIDEMIA IN HOSPITALIZED ADULTS: CURRENT CHALLENGES AND STRATEGIES OF MANAGEMENT"

que presenta el licenciado **Guillermo Cuervo-Requena** ha sido realizada bajo nuestra dirección en el Servicio de Enfermedades Infecciosas del Hospital Universitario de Bellvitge, en el marco del Programa de Doctorado "Medicina i Recerca Translacional" de la Universidad de Barcelona. La consideramos finalizada y autorizamos su presentación con el objetivo de poder ser juzgada por el tribunal que corresponda.

Para que así conste, firmamos la presente certificación en Barcelona, 7 de marzo 2016.

Dr. Jordi Carratalà **Dra. Carolina Garcia-Vidal**

"…Cree que tu arte es una cima inaccesible. No sueñes en domarla. Cuando puedas hacerlo, lo conseguirás sin saberlo tú mismo.

Ten fe ciega no en tu capacidad para el triunfo, sino en el ardor con que lo deseas…"

Horacio Quiroga

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SCIENTIFIC PRODUCTION

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A Simple Prediction Score for Estimating the Risk of Candidemia Caused by Fluconazole Non-susceptible Strains. Cuervo G, Puig-Asensio M, Garcia-Vidal C, Fernández-Ruiz M, Pemán J, Nucci M, Aguado JM, Salavert M, González-Romo F, Guinea J, Zaragoza O, Gudiol C, Carratalà J and Almirante B. *Clin Microbiol Infect* 2015 Jul;21(7):684.e1-9. doi: 10.1016/j.cmi.2015.02.029. Epub 2015 Mar 10. Impact Factor: 5.758

Breakthrough Candidemia in the Era of Broad-Spectrum Antifungal Therapies. Cuervo G, Garcia-Vidal C, Nucci M, Fernández-Ruiz M, Puchades F, Obed M, A. Manzur, C. Gudiol, J. Peman, J.M. Aguado, J. Ayats and J. Carratalà. *Clin Microbiol Infect* 2016 Feb;22(2):181-8. doi: 10.1016/j.cmi.2015.09.029. Epub 2015 Oct 13. Impact Factor: 5.758

Echinocandins for urinary source candidemia: a propensity score analysis. Cuervo G, Garcia-Vidal C, Puig-Asensio M, Vena A, Fernández-Ruiz M, González-Barberá E, M. J. Blanco-Vidal, A. Manzur, C. Gudiol, J. Pemán, J. Ayats, B. Almirante, J. Carratalà and the CANDIPOP Group. Submitted.

Effect of Statin Use on Outcomes of Adults with Candidemia. Cuervo G, Garcia-Vidal C, Nucci M, Puchades F, Fernández-Ruiz M, Mykietiuk A, Manzur A, Gudiol C, Peman J, Viasus D, Ayats J and Carratalà J. *PLoS ONE* 2013 8(10): e77317. doi:10.1371/journal.pone.0077317. Impact Factor: 3.534

COMMUNICATIONS

a) INVITED LECTURES

- **Candidemia de brecha.** *XX Congreso SEIMC. Barcelona*, 26-28 May 2016.
- **Tratamiento empírico de la candidemia**, V Scientific Meeting of the Grupo de Estudio de Micología Médica of the SEIMC (GEMICOMED), 27 February 2015.

b) SLIDE SESSIONS

- **The Impact of Guidelines-concordant Management on the Outcomes of Candidemia.** G. Cuervo; C. Garcia-Vidal; M. Puig-Asensio; P. Merino; A. Vena; A. Martín-Peña; M. Montejo; A. Ruiz; J. Fortún; M. Fernández-Ruiz; A. I. Suárez; J.M. Aguado; J. Pemán; M. Salavert; J. Garnacho-Montero; J.M. Cisneros; P. Muñoz; B. Almirante and J. Carratalà. 26th European Congress of Clinical Microbiology and Infectious Diseases. Amsterdam, Netherlands: 9-12 April 2016.
- **A Prediction Rule for Estimating the Risk of Candidemia Caused by Fluconazole non-Susceptible Strains.** Cuervo G; Puig-Asensio M; Garcia-Vidal C; Fernández-Ruiz M; PemánJ; Nucci M; Aguado JM; Salavert M; González-Romo F; Guinea J; Zaragoza O; Gudiol C; Carratalà J and Almirante B. *54th Interscience Conference on Antimicrobial Agents and Chemotherapy.* Control number M-1205. Washington (USA): 5-9 September 2014; Final program, page 146.
- **Características clínicas, tratamiento y pronóstico de la candidemia de origen urinario.** G. Cuervo; C. Garcia-Vidal; M. Fernández-Ruiz; J. Guitián; M. Obed; A. Manzur; C. Gudiol; J. Peman; J. Ayats; J. Carratalà. *XVIII Congreso SEIMC. Valencia*, 9-11 April 2014. Session 456. Final Program, page 114.

- **Características tratamiento y pronóstico de la candidemia de brecha en los pacientes en profilaxis antifúngica**. G. Cuervo; C. Garcia-Vidal; M. Nucci; F. Puchades; M. Fernández-Ruiz; A. Mykietiuk; A. Manzur; C. Gudiol; J. Peman; J. Ayats; J. Carratalà. *XVII Congreso SEIMC. Zaragoza*, 29-31 May 2013. Session 533. Final Program, page 123.
- **Candidemia en pacientes adultos: características clínicas, evolución y pronóstico en relación al uso de estatinas.** G. Cuervo; C. Garcia-Vidal; J. Ayats; C. Gudiol; A. Fernández; M. Bodro; J. Carratalà. *XVI Congreso SEIMC. Bilbao*, 9-11 May 2012. Session 316. Final Program, page 91.

c) POSTER SESSIONS

- **Echinocandins for urinary source candidemia: a propensity score analysis.** Autores: G. Cuervo; C. Garcia-Vidal; M. Puig-Asensio; M. Fernández-Ruiz; E. González-Barberá; A. Manzur; M. Obed; C. Gudiol; J. Pemán; J. Ayats; B. Almirante; J. Carratalà. 25th European Congress of Clinical Microbiology and Infectious Diseases. Copenhagen, Denmark: 25-28 April 2015.
- **Usefulness of a Score to Predict Candidemia Caused by Azole-Resistant Strains.** G. Cuervo, C. Garcia-Vidal, M. Nucci, M. Fernandez-Ruiz, M. Obed, A. Manzur, C. Gudiol, J. Peman, J. Ayats, J. Carratalà. *53rd Interscience Conference on Antimicrobial Agents and Chemotherapy,* September 10-132013; Denver (USA). Control number M-230.
- **Effect of statin use on the outcomes of adults with candidemia.** G. Cuervo, C. Garcia-Vidal, F. Puchades, M. Fernandez-Ruiz, A. Mykietiuk, A. Manzur, C. Gudiol, J. Peman, J. Ayats, J. Carratalà. *52th Interscience Conference on*

Antimicrobial Agents and Chemotherapy, 9-12 September 2012; San Francisco (USA). Control number M-329.

INDEX

1. INTRODUCTION

1.1. Candidemia: The Burden of the Problem

The yeast *Candida* is an environmental fungus usually recovered from soil, animals and food. It represents a normal commensal with humans, as it is commonly found on skin; throughout the entire gastrointestinal tract; in respiratory samples; genital tract, and in urine of catheterized patients (Mandell et al. 2014).

The earliest reports of oral lesions that were probably thrush predated the concept of a microbial pathogen. In *Of the Epidemics*, Hippocrates describes "mouths affected with aphthous ulcerations". But it has not been until 1839, when in the oropharyngeal and oesophageal thrush discovered during an autopsy of a patient with typhoid fever, German surgeon Langenbeck found that "the pseudomembranes consisted of an immense number of fungi" (translated from German in (Knoke & Bernhardt 2006)). After successive taxonomic re-classifications, the current name *Candida albicans* (the first described species) has come to be known since the mid-twentieth century (McCool n.d.).

Since the 1940's, with the widespread use of antibiotics, the history of *Candida* infections has become more noteworthy (Mandell et al. 2014). A sharp increase has been seen since the early 1980's, when fungi first emerged as major causes of human disease, especially among immunocompromised patients and those hospitalized with severe underlying diseases. *Candida,* nowadays, is the single most important cause of opportunistic mycoses worldwide (Pfaller & Diekema 2007).

From the initial cutaneous and mucosal presentations, we currently face an emergence of invasive forms of *Candida* infections, which are iatrogenic by definition (Pfaller &

Diekema 2007), being the spread into the bloodstream, also named candidemia, the most prominent form according to its frequency and clinical relevance.

Candidemia is, in fact, a common cause of infection worldwide, representing the seventh leading cause of bloodstream infections in hospitalized patients in Europe (Marchetti et al. 2004)(Fluit et al. 2000) and even the fourth leading cause in the United States (Wisplinghoff et al. 2004). This infection is associated with significant morbidity, prolonged hospital stay, increased health care cost, and high mortality (Horn et al. 2009)(Morgan et al. 2005). Despite advances in diagnosis and the development of new antifungal drugs, mortality at 30 days is still estimated to be between 30 and 40% of patients in Spain (Pemán et al. 2005)(M Puig-Asensio et al. 2014) . Previous studies have shown that both the delay in antifungal treatment and its inadequacy represent independent predictors for increased morbidity and mortality (Morrell et al. 2005)(Bassetti et al. 2014).

Hospitalized patients at increased risk for developing candidemia include: chemotherapy recipients with solid tumours or haematological cancers (with or without neutropenia or qualitative neutrophil dysfunction); recipients of stem cell (HSCT) and solid organ transplantations (SOT); those who receive corticosteroids or other immunosuppressive therapy; patients infected with HIV (not receiving HAART); patients undergoing major gastrointestinal surgery or who present severe pancreatitis; severely burnt patients; those with disruption of mucosal integrity; patients with chronic inflammatory disease receiving new biological therapies; elderly and critically ill patients; etc. (Pemán et al. 2013).

Given the substantial excess mortality due to candidemia, multi-level actions have been proposed to prevent the onset of this serious disease: adherence to hand hygiene protocols; proper catheters placement techniques; and, of utmost importance, a judicious use of antibiotics (Pfaller & Diekema 2007). Administration of antifungal prophylaxis in high-risk neutropenic patients is strongly recommended (Marr et al. 2000)(Ullmann et al. 2012), but its utility in non-neutropenic critically-ill patients is less defined (Pappas et al. 2009)(Cornely et al. 2012). Lastly, administration of an early antifungal therapy guided by surrogate non-culture methods, also known as pre-emptive therapy, has been a matter of intense research in the last few years but a strong consensus has yet to be reached (Nguyen et al. 2012)(Fortún et al. 2014).

In this setting, the increasing use of systemic antifungal drugs has led to a complex scenario of a changing epidemiology, in which non-*albicans* species predominate (Nguyen et al. 1996)(Kontoyiannis et al. 2002) with diverse antifungal resistance (Clancy et al. 2006)(Garnacho-Montero et al. 2010)(Fekkar et al. 2014)(Beyda et al. 2014). Therefore, treating physicians must balance for every single patient the need of an early and effective treatment, weighting also the putative ecological impact of wide-ranging antifungal drugs. Likewise, other aspects must be considered, such as pharmacodynamics [e.g. fungicidal activity in patients with septic shock (Reboli et al. 2011); efficacy against a particular species (Fernández-Ruiz et al. 2014)] ; and, pharmacokinetics [e.g. tissue penetration with drugs in the eye (Oude Lashof et al. 2011) or in the urine].

To add more trouble, a comprehensive approach to those patients affected should consider other non-pharmacologic, yet synergistic aspects of management, such as

proper drainage of collections (e.g. abscess or retained urine) or catheter removal, should this prove to be the source (Andes et al. 2012)(Mireia Puig-Asensio et al. 2014), as well as early detection of complicated forms of the infection, as in the case of *Candida* endocarditis (Baddley et al. 2008) or ocular involvement (Oude Lashof et al. 2011). In following with that line of thinking, there were proposed bundles of structured interventions but still of unproven utility (Antworth et al. 2013)(Takesue et al. 2014).

Finally, given the high mortality of this infection in spite of an appropriate therapy, there is interest in exploring the benefit of some immunomodulator molecules that could act in the acute phase of the illness, with improvement in its outcomes (Wiersinga 2011).

For all aforementioned reasons, candidemia still raises several interesting and unexplored aspects, representing daily challenges in handling affected patients. In the next sections, we will try to detail the rationale for our hypotheses and set the findings of our investigation within the context of current medical knowledge.

2. CURRENT CHALLENGES IN THE MANAGEMENT OF CANDIDEMIA IN ADULT PATIENTS

GENERAL DESCRIPTION AND RATIONALE OF OBJECTIVES:

2.1. Choice of empirical treatment

Candidemia remains a frequent cause of nosocomial bloodstream infection worldwide and is associated with significant morbidity, prolonged hospital stay, increased health care cost, and high mortality (Horn et al. 2009),(Morgan et al. 2005).

The empirical treatment of this life-threatening infection also remains a challenge for clinicians. Although fluconazole has typically been the preferred initial treatment, especially in hemodynamically stable patients without prior azole exposure (Pappas et al. 2009),(Karthaus et al. 2011), recent epidemiological studies raise concerns about the increasing prevalence of *Candida* species non-susceptible to fluconazole (Flu-NS) (Horn et al. 2009),(Arendrup et al. 2013). Accordingly, current european guidelines have relegated fluconazole to be used only as a step-down treatment option (Cornely et al. 2012),(Ullmann et al. 2012). However, in addition to the obvious rise in health care expenses, this management strategy could be followed by a substantial increase in echinocandin resistance, as suggested by some observational studies (Garcia-Effron et al. 2008),(Dannaoui et al. 2012),(Beyda et al. 2014). It has therefore become necessary to determine the current role of fluconazole in the empirical treatment of candidemia.

We aimed to develop and validate a fluconazole non-susceptibility (Flu-NS) prediction score that would be easy to use at the bedside, using only simple clinical criteria to assess the risk factors for Flu-NS candidemia. Ultimately, this tool could be useful when selecting an empirical antifungal treatment for patients with candidemia.

2.2. Specific clinical settings.

2.2.1. The problem of breakthrough candidemia

Invasive fungal infections (IFI) are increasing in frequency in developed countries mainly due to the rising number of immunocompromised patients who are at risk (Oren & Paul 2014). These infections are associated with significant morbid-mortality (Perfect et al. 2014) (Morgan et al. 2005). For these reasons, strategies to decrease the prevalence of IFI such as antifungal prophylaxis or pre-emptive antifungal therapy aimed to offer an early treatment option, have become common in selected populations (Ullmann et al. 2012),(Cornely et al. 2012). As a consequence, growing numbers of patients are diagnosed with *Candida* breakthrough infections (Arendrup et al. 2011) (Sandven et al. 2006).

Some studies have focused on the risk factors and epidemiology of breakthrough candidemia, most in the nineteen-nineties and early in this century. Nevertheless, current information regarding the clinical manifestations of breakthrough candidemia and its impact on the prognosis of affected patients in this era of broad-spectrum antifungal therapies is still scarce and mainly derived from studies performed in northern Europe or the USA.

We analyzed this issue in a recent multicenter cohort of candidemia patients from different countries. We sought to determine the proportion of patients with candidemia who developed breakthrough candidemia while receiving antifungal therapy and compare clinical and microbiological characteristics, the appropriateness of empiric therapy, and outcomes. We also assessed the risk factors for breakthrough candidemia.

2.2.2. Echinocandins for urinary source candidemia

As already mentioned, candidemia remains a common cause of bloodstream infection all over the world (Marchetti et al. 2004)(Fluit et al. 2000)(Wisplinghoff et al. 2004)(Bassetti et al. 2013) and is still associated with significant morbi-mortality (Horn et al. 2009)(Morgan et al. 2005)(M Puig-Asensio et al. 2014). Most candidemia episodes are considered to arise from an endogenous source or, less often, from the skin through the colonization of vascular catheters (Krause et al. 1969)(Nucci & Anaissie 2001). However, some patients with urinary colonization and urological obstructive disorders may have candidemia with the urinary tract being considered the source (Ang et al. 1993)(Fisher et al. 2011)(Huang et al. 2013). Recently, a number of matters have arisen regarding the management of urinary source candidemia (USC).

Current guidelines advise against the use of echinocandins for treating urinary tract infections due to *Candida spp.* (Cornely et al. 2012)(Pappas et al. 2016) because of its low concentration reached in urine (Felton et al. 2014) and recommend the use of fluconazole or amphotericin B. However, usefulness of those antifungal drugs may be limited by several drawbacks in this population. On one hand, recent epidemiological studies have raised concerns about the increasing prevalence of *Candida spp.* non-susceptible to fluconazole (Arendrup et al. 2013)(Wisplinghoff et al. 2014)(Cuervo et al. 2015)(Cuervo et al. 2016), with a lack of specific information on microbiology data of patients with USC. Moreover, patients with USC often are elderly and have severe comorbidities including renal function impairment, precluding the use of amphotericin B. Finally, although this source of candidemia has been classically considered as “low-risk” (Ang et al. 1993), current information on its outcomes is lacking.

The primary aim of our study is to assess whether the use of echinocandins as compared to fluconazole treatment has or not a negative impact on USC outcomes. We also provide current information on the epidemiology of USC from a large cohort of patients with candidemia.

2.3. Modulation of the immune response in patients with candidemia

In light of the incidence of candidemia and the high mortality of this infections in spite of an appropriate therapy (Pappas et al. 2009)(Flückiger et al. 2006)(Horn et al. 2009)(Morgan et al. 2005), there is interest in exploring the benefit of some immunomodulator molecules that could act in the acute phase of the illness, with the improvement of its outcomes (Wiersinga 2011).

Hydroxymethylglutaryl-CoA reductase inhibitors, also known as statins, are increasingly used in clinical practice to treat dyslipidemia. Statin therapy has been shown to decrease cardiovascular events and mortality from coronary artery disease (Brugts et al. 2009). Interestingly, statins exhibit potent anti-inflammatory, anticoagulant, and anti-oxidative effects called "pleiotropic properties"(Liao & Laufs 2005). Due to these properties, it has been suggested that these drugs may have beneficial effects during sepsis. Experimental and observational studies (Viasus et al. 2010)(Gao et al. 2008) have shown that statin therapy reduces inflammatory cytokines (Novack et al. 2009). There is an increasing interest in determining whether statins improve prognosis of patients with severe infections. The results of certain observational studies suggest that statins may reduce mortality in patients suffering from sepsis (Gao et al. 2008)(Almog et al. 2004)(Ma et al. 2012), bacteraemia (Liappis et al. 2001), community-acquired bacterial pneumonia (Viasus et al. 2010)(Thomsen et

al. 2008)(Khan et al. 2013) and even influenza (Bearman et al. 2010)(Kwong et al. 2009). On the other hand, in vitro studies have found that statins have an intrinsic antifungal effect, hindering fungal growth (Westermeyer & Macreadie 2007).

However, information evaluating the effects of statin therapy on clinical outcomes of patients with candidemia is scarce. The purpose of our study is to assess whether prior statin use is associated with a decreased risk of mortality in a large multicenter cohort of adult patients with candidemia.

3. HYPOTHESES

1. A clinical score constructed with variables readily available at bedside would be useful to predict a candidemia caused by a *Candida* strains not-susceptible to fluconazole.

2. Strains involved in breakthrough candidemia would probably be more resistant to antifungal drugs.

3. Empirical therapy for breakthrough candidemia would be more frequently inappropriate and the outcomes of patients affected would probably be poorer.

4. Echinocandin therapy in patients suffering a urinary source candidemia could be a risk factor for worse outcomes.

5. Clinical outcomes of patients who were receiving statin therapy when they suffered a candidemia would probably be better than those occurring in patients who were not.

4. OBJECTIVES

4.1. Study of the risk factors of candidemia caused by fluconazole non-susceptible strains: derivation and validation of a clinical score

- To identify independent risk factors for candidemia caused by fluconazole non-susceptible strains.
- To develop a simple prediction score to identify patients with an episode of candidemia caused by those strains from a multicentric derivation cohort (CANDIPOP survey).
- To validate that prediction score in another multicentric prospective cohort.

4.2. Descriptive study of breakthrough candidemia

- To determine in a recent multicenter cohort of candidemia patients from different countries the proportion who developed breakthrough candidemia while receiving antifungal therapy.
- To compare clinical and microbiological characteristics, appropriateness of empiric therapy and outcomes of breakthrough with non-breakthrough candidemia.
- To assess the risk factors for breakthrough candidemia.

4.3. Analysis of antifungal therapy of patients with urinary source candidemia

- To describe the clinical and microbiological characteristics of urinary source candidemia.
- To evaluate the risk factors for clinical failure of urinary source candidemia.
- To assess, by a propensity score analysis, whether initial echinocandin use was associated with worse outcomes.

4.4. Study of the impact on outcomes of statin pre-treatment in patients with candidemia

- To compare, in a large multicenter cohort of adult patients with candidemia, the clinical and microbiological characteristics of those patients with prior statin use with the rest of them.
- To identify independent risk factors for early and late mortality in this multicenter cohort.
- To assess, by a propensity score analysis, whether prior statin use was associated with a decreased risk of mortality.

5. SETTING AND METHODOLOGY

5.1. Setting, patients and studies design

The Hospital de Bellvitge is a 900 beds university hospital for adult patients that serve a population of approximately 1.5 million of habitants, with more than 26,000 admissions and 100,000 emergency consultations each year. It is accredited as a tertiary centre with all the medical and surgical specialties except paediatrics and obstetrics and is located in Hospitalet de Llobregat, being the referral hospital of the west coast region of the Catalan Health System.

In this hospital since 2014 there is a prospective survey of all patients diagnosed with candidemia. The data is collected in a protocol and included in a database for analyzes. We also have retrospective information of episodes of candidemia from 2005 to 2013. The designs of the different studies are described below.

5.1.1. Study of the risk factors of candidemia caused by fluconazole non-susceptible strains: derivation and validation of a clinical score

For this study, a derivation cohort was extracted from the CANDIPOP survey (the Prospective Population Study on Candidemia in Spain), a prospective, multicenter, population-based surveillance program on candidemia conducted in 29 hospitals in five of the largest metropolitan areas of Spain (M Puig-Asensio et al. 2014).

A retrospective validation cohort was also analized and it included all adult patients diagnosed with candidemia between January 2005 and December 2012 in six tertiary hospitals in three countries: three in Spain (the Hospital de Bellvitge among them), two in Argentina, and one in Brazil.

5.1.2. Descriptive study of breakthrough candidemia

Retrospective multicenter study of all the episodes of candidemia that occurred in hospitalized adult patients between January 2005 and December 2012 at 6 tertiary teaching institutions in 3 different countries: three in Spain (Hospital de Bellvitge among them), two in Argentina and one in Brazil. Episodes of breakthrough candidemia were compared with those occurring in the remaining patients.

5.1.3. Analysis of antifungal therapy of patients with urinary source candidemia

From an extensive multicenter cohort of patients with candidemia, we performed a study of all episodes of USC occurring in hospitalized adult patients from January 2005 to April 2015. The study was conducted in 18 tertiary teaching institutions in two countries: 17 from Spain (16 included in the prospective CANDIPOP survey (M Puig-Asensio et al. 2014)) and one from Argentina. For the purpose of the study, we assessed the outcomes on patients treated with echinocandin as compared with those treated with fluconazole. We also provide current epidemiological information.

5.1.4. Study of the impact on outcomes of statin pre-treatment in patients with candidemia

Retrospective multicenter study of all episodes of candidemia occurring in hospitalized adult patients between January 2005 and December 2011 at six tertiary teaching institutions in three different countries: three in Spain (Hospital de Bellvitge among them), two in Argentina and one in Brazil. Episodes of candidemia occurring in statin users were compared with those occurring in statin non-users.

5.2. Clinical data and definitions

Candidemia was defined as the presence of at least one positive blood culture for Candida spp. in a patient with clinical signs and symptoms of sepsis. Catheter-related infection was defined on the basis of the Infectious Diseases Society of America guidelines (Mermel et al. 2009). Secondary candidemia was defined as a documented concurrent infection caused by the same *Candida* species at a site other than the catheter (Pittet & Wenzel 1995). The diagnosis of septic shock was based on a systolic blood pressure of less than 90 mm Hg and peripheral hypoperfusion and/or the need for vasopressors (Garcia-Vidal et al. 2010). Neutropenia was considered to occur when the granulocyte count was <500/ mm3. Empiric antifungal therapy was considered to be appropriate when the *Candida* isolated showed in vitro susceptibility to the antifungal drug administered. Not administering an empiric treatment was considered an inappropriate treatment. Clinical instability (at 48 hours after initiation of therapy) was defined as the presence of fever and / or hypotension or need of vasopressor drugs. The early and overall mortality were defined as death from any cause within 5 and 30 days of the onset of candidemia, respectively.

5.2.1. Study of the risk factors of candidemia caused by fluconazole non-susceptible strains: derivation and validation of a clinical score

The primary outcome variable was the presence of a *Candida* bloodstream infection caused by an isolate non-susceptible to fluconazole (Flu-NS). For the purpose of this study, *Candida* species were divided into two groups according to minimum inhibitory concentration (MIC) values obtained by Clinical and Laboratory Standards Institute (CLSI) procedure: isolates fully susceptible to fluconazole (Flu-S; i.e., those with an MIC

< 4 mg/l) and isolates non-susceptible to fluconazole (Flu-NS; i.e., those with an MIC ≥ 4 mg/L in addition to *C. krusei*, *C. glabrata* and *C. guilliermondii* regardless of their MIC value). The MIC cut-off value was selected according to CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria for *Candida* species-specific clinical breakpoints (Pfaller et al. 2010). For episodes in which two different *Candida* spp. were recovered simultaneously in the blood culture, at least one strain was required to meet the criteria for Flu-NS.

Previous exposure to azoles was considered if it had occurred in the 30 days prior to drawing the first positive blood culture. The type, dose, and duration of prior antifungal therapy were recorded. Severity of candidemia was classified as sepsis, severe sepsis, or septic shock at presentation according to standard definitions (Levy et al. 2003). To account for local epidemiology and antifungal resistance patterns, we used a variable that reflected resistance rates at the ward level in each hospital. Wards with a prevalence of Flu-NS isolates greater than 15% were defined as high prevalence units (HPU).

5.2.2. Descriptive study of breakthrough candidemia

Breakthrough candidemia (BrC) was defined as the diagnosis of candidemia in a patient who had received at least 3 days of a systemic antifungal agent (Nucci & Colombo 2002). For the purposes of our analysis, we defined as severely immunosuppressed those patients who had received a prior transplantation (either hematopoietic stem cell transplantation, from here on: HSCT, or solid organ transplantation, from here on: SOT) and/or those suffering chemotherapy-induced neutropenia.

5.2.3. Analysis of antifungal therapy of patients with urinary source candidemia

Urinary source candidemia was considered that episode of candidemia occurring in a patient with concomitant candiduria by the same *Candida spp.* and with a significant urological comorbidity (obstruction or manipulation of the urinary tract). Episodes considered to be USC were reviewed by three study investigators who were part of an eight-member clinical review panel (GC, CGV, MPA, AV, MFR, EGB, MJBV and AM). All members of the clinical review panel were infectious disease specialists and had extensive clinical experience dealing with patients with candidemia. The reviewers were asked to check the microbiology results, the baseline urologic comorbidity and to exclude other potential source of candidemia. Acute renal failure was defined as an increase in serum creatinine concentration of 44.2 umol/L (if the baseline was less than 221 umol/L), an increase of more than 20% (if the baseline was more than 221 umol/L) or dialysis requirement at candidemia onset (Lameire et al.). Urologic procedure was defined as a urinary catheter exchange or any surgical, percutaneous or endoscopic drainage of the urinary tract performed within 48 h of candidemia onset. The early and overall mortality were defined as death from any cause within 7 and 30 days of the onset of candidemia, respectively. Persistent candidemia was defined as persistently positive blood cultures after ≥72h of treatment initiation (Nucci 2010). We defined clinical failure as a composite endpoint, including 7-day mortality and/or persistent candidemia.

5.2.4. Study of the impact on outcomes of statin pre-treatment in patients with candidemia

An episode of candidemia was considered to be nosocomially acquired, community-acquired or healthcare-associated as described elsewhere (Friedman et al. 2002). Statin use was considered to be present in those patients who were taking a statin (simvastatin, atorvastatin, lovastatin, pravastatin or rosuvastatin) within the 7 days prior to the candidemia episode. Seven days period was used due to pleiotropic effects of statins may persist despite temporary cessation of administration. The use of other cardiovascular drugs like aspirin, beta-blockers and angiotensin II-converting enzyme (ACE) inhibitors was considered to be present in patients who were taking these drugs within the 30 days prior to the candidemia episode. Empirical antifungal therapy was considered to be appropriate when the *Candida* isolates showed in vitro susceptibility to the antifungal drug administered. When antifungal susceptibility testing was not available, we considered fluconazole, amphotericin B or an echinocandin as appropriate empirical antifungal treatment for *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis* and *Candida lusitaniae*. For *Candida glabrata* and *Candida krusei*, empirical antifungal treatment was considered to be appropriate when an equinocandin or amphotericin B was administered. The early, 14 days and overall case-fatality rates were defined as death from any cause within five, fourteen and 30 days after the onset of candidemia respectively.

5.3. Microbiology

Two sets of two blood samples were collected from patients with a suspected bloodstream infection. The blood samples were processed using a BACTEC 9240 system (Becton–Dickinson Microbiology Systems, New Jersey, USA) or BacTAlert (BioMérieux SA, Marcy L'Etoile, France) with an incubation period of 5 days. If yeast cells were observed after microscopic examination of a Gram stain, blood bottles were subcultured onto Sabouraud agar plates (BD BBL StrackerTM PlatesTM, Heidelberg, Germany) and chromogenic media (ChromAgar BioMerieux SA, Paris, France). Yeast isolates were identified by conventional methods. The antifungal susceptibility of the isolates was classified in accordance with the Clinical and Laboratory Standards Institute M27-S3 document (CLSI 2009). In vitro antifungal activity was studied by applying a commercial microdilution method (YeastOne®Sensititre®, TREK Diagnostic Systems Ltd, Ohio, USA) or an E-test (BioMérieux SA, Marcy L'Etoile, France), following the manufacturer's instructions. Of note, those *Candida spp* isolates with a MIC ≥ 4 mg/L to fluconazole were considered non-susceptible to fluconazole, with the exception of *Candida krusei* and *Candida glabrata* isolates, which were considered non-susceptible to fluconazole regardless of their MIC value. For amphotericin B, isolates inhibited by 1 mg/L were considered resistant. Quality controls were performed in each centre using Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258.

5.3.1. Study of the risk factors of candidemia caused by fluconazole non-susceptible strains: derivation and validation of a clinical score

For the derivation cohort, *Candida* isolates were processed and stored at participating hospitals as described elsewhere (Guinea et al. 2014). Fluconazole susceptibility testing was performed according to the CLSI M27-A3 (CLSI 2009) and EUCAST (E.Def7.1 and E.Def7.2) (Anon 2008)(Arendrup et al. 2012) broth microdilution methods. The fluconazole MIC was defined as the lowest drug concentration that inhibited 50% of growth compared with the growth control after 24 hours of incubation at 35º C for all *Candida* species, except for *C. glabrata*, which was determined after 48 hours to prevent misclassification bias among the isolates (Ostrosky-Zeichner et al. 2008). CLSI and EUCAST procedures were carried out at the Clinical Microbiology Department of Gregorio Marañón Hospital and at the Spanish National Centre for Microbiology in Madrid, respectively, as previously reported (Guinea et al. 2014).

For the validation cohort, yeast identification and in vitro antifungal activity was assessed at participating hospitals using local routine methods. We used a commercial microdilution method (YeastOne®Sensitre®, TREK Diagnostic Systems Ltd, England) or an E-test (BioMerieux SA, Paris, France) according to the manufacturer's instructions.

5.4. Statistical analysis

Quantitative variables were reported as the median and interquartile range (IQR); categorical variables, as absolute numbers and percentages. To detect significant differences between groups, we used the Chi-square test or Fisher's exact test for categorical variables, and the Student t-test, Mann-Whitney test or ANOVA for continuous variables, as appropriate. All data were analyzed using SPSS software, version 15.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was established at α=0.05. All reported p-values are two-tailed.

5.4.1. Study of the risk factors of candidemia caused by fluconazole non-susceptible strains: derivation and validation of a clinical score

Potential predictors of Flu-NS isolates were identified by logistic regression analysis using the CLSI MIC clinical breakpoints. In order to facilitate score generation and application in clinical settings, continuous variables (e.g. days of previous azole exposure) were transformed into binary factors using the most discriminatory cut-off point. Statistically significant variables in univariate analysis were entered into a multivariate logistic regression model. Calibration of the model was assessed by the Hosmer–Lemeshow test. The final regression model was transformed into a point-based score to build the predictive score. The logistic regression coefficients for statistically significant predictors of Flu-NS isolates were rounded to integers to assign the value of each variable. It was decided *a priori* that septic shock would remain in the predictive score because it is usually taken into account when choosing empirical therapy using existing guidance (Pappas et al. 2009).

The discriminatory power of the developed score was evaluated by the area under the receiver operating characteristics (ROC) curve (AUC) and the 95% confidence interval

(CI). We selected the best cut-off value to estimate the diagnostic sensitivity and specificity in the validation set. The prediction score was internally assessed with the MIC values obtained by the EUCAST method in the CANDIPOP study and then applied to the external validation cohort. The discrimination ability of the score was again assessed by AUC of ROC curve analysis.

5.4.2. Descriptive study of breakthrough candidemia

Two groups of high-risk patients were deemed suitable for separate analysis: on one hand, severely immunosuppressed patients (according to the definition already given); and on the other hand, critically ill non-neutropenic patients admitted to an intensive care unit (ICU). We performed a multivariate logistic regression analysis of factors potentially associated with the occurrence of BrC in each risk group. The multivariate analysis included all significant variables (p value <0.05) in the univariate analysis and we added other factor considered relevant in the literature (presence of a central venous catheter for ≥48 h) (Nucci & Colombo 2002). The goodness-of-fit of the model was assessed by the Hosmer–Lemeshow test. The relative risks were expressed as adjusted odds ratios (AOR) and 95% confidence intervals (95%CI). A Kaplan-Meier curve was plotted to show the survival probabilities at 30 days, according to the appropriateness of antifungal therapy.

5.4.3. Analysis of antifungal therapy of patients with urinary source candidemia

Factors associated with clinical failure (defined as 7-day mortality and/or persistent candidemia) were evaluated by univariate and multivariate analysis. The multivariate analysis included all significant variables (p value <0.05) in the univariate analysis and all other factor considered relevant according to the literature. Given the lack of

aleatorization of initial therapies, a propensity score of receiving echinocandins (PS Echinocandins) was estimated using a backward stepwise logistic regression model including variables with *p* values ≤ 0.115 in the univariate analysis and other variables considered relevant in the decision-making of an empirical treatment. Variables included were: Age ≥ 70y, Dialysis, Solid Organ Transplantation (SOT); Chemotherapy; Corticosteroid therapy; Immunosuppressive therapy; Chronic Human Immunodeficiency Virus (HIV) infection and Intensive Care Unit (ICU) requirement at onset. The goodness of fit of the model was assessed by the Hosmer-Lemeshow test (P=.254). The discriminatory power of the score, evaluated by the area under the receiver operating characteristics (ROC) curve, was 0.83 (95 % CI, 0.74-0.91) showing thus a good ability to predict the use this drugs. The PS Echinocandins was then used as a covariate in a multivariate analysis to adjust for potential confounding factors associated with initial antifungal therapy (Austin 2011)(Austin 2007). Sensitivity analyses were performed by repeating the propensity score approach with different methods; 1:1 matching with replacement and a caliper of 0.25, and quintile stratification.

5.4.4. Study of the impact on outcomes of statin pre-treatment in patients with candidemia

A multivariate logistic regression analysis of factors potentially associated with mortality included all variables that were significant in the univariate analysis. Due to the baseline imbalances between patients, a propensity score for receiving statin therapy was added to the model. The relative risks were expressed as adjusted odds

ratios (AOR) and 95% confidence intervals. Goodness-of-fit of the final model was assessed by the Hosmer-Lemeshow test.

5.5 Ethical Issues

All the observational studies were conducted in accordance with the Declaration of Helsinki and were approved by the ethics committees of the participating institutions. To protect personal privacy, identifying information in the electronic database was encrypted for each patient. Informed consent was waived by the ethics committees because no intervention was involved and no patient identifying information was included, with the exception of the prospective CANDIPOP study in which written consent was obtained from all patients included.

6. RESULTS

6.1. Study of the risk factors of candidemia caused by fluconazole non-susceptible strains: derivation and validation of a clinical score

- Risk factors for candidemia caused by fluconazole non-susceptible strains.
- Derivation of a simple prediction score to identify patients with an episode of candidemia caused by those strains.
- Validation of the prediction score in another multicentric prospective cohort.
- Fitness of the score.

A simple prediction score for estimating the risk of candidaemia caused by fluconazole non-susceptible strains

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Abstract

We aimed to develop a simple prediction score to identify fluconazole non-susceptible (Flu-NS) candidaemia using simple clinical criteria. A derivation cohort was extracted from the CANDIPOP study, a prospective, multicentre, population-based surveillance programme on candidaemia conducted in 29 hospitals in Spain from April 2010 to May 2011. The score was validated with an external, multicentre cohort of adults with candidaemia in six tertiary hospitals in three countries. The prediction score was based on three variables selected by a logistic regression model together with the severity of disease. In total, 617 and 297 cases of candidaemia were included in the derivation and validation cohorts, respectively; of these, 134 (21.7%) and 57 (19.2%) were caused by Flu-NS strains. Factors independently associated with Flu-NS were transplant recipient status (adjusted odds ratio (AOR) 2.13; 95% CI 1.01–4.55; p 0.047), hospitalization in a unit with a high prevalence (15%) of Flu-NS strains (7.53; 4.68–12.10; p < 0.001), and previous azole therapy for at least 3 days (2.04; 1.16–3.62; p 0.014). The area under the receiver operating characteristics curve (AUC) was 0.76 (0.72–0.81), and using 2 points as the Flu-NS prediction score cut-off gave a sensitivity of 82.1%, a specificity of 65.6%, and a negative predictive value of 93%. The AUC in the validation cohort was 0.72 (95% CI 0.65–0.79). Hence, the Flu-NS prediction score helped to exclude Flu-NS *Candida* strains. This could improve the selection of empirical treatments for candidaemia in the future.
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Introduction

Candidaemia remains a frequent cause of nosocomial bloodstream infection worldwide and is associated with significant morbidity, prolonged hospital stays, increased healthcare costs and high mortality [1,2].

The empirical treatment of this life-threatening infection also remains a challenge for clinicians. Although fluconazole has

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typically been the preferred initial treatment, especially in haemodynamically stable patients without previous azole exposure [3,4], recent epidemiological studies raise concerns about the increasing prevalence of *Candida* species non-susceptible to fluconazole (Flu-NS) [1,5]. Accordingly, current guidelines have relegated fluconazole to be used only as a step-down treatment option [6,7]. However, some observational studies suggest that this management strategy could have an as-yet unknown ecological impact [8–10], in addition to the obvious rise in healthcare expenses. It has therefore become necessary to determine the current role of fluconazole in the empirical treatment of candidaemia.

We aimed to develop and validate a Flu-NS prediction score that would be easy to use at the bedside, using only simple clinical criteria to assess the risk factors for Flu-NS candidaemia. Ultimately, this tool could be useful when selecting an empirical antifungal treatment for patients with candidaemia.

Materials and methods

Study design, setting and patients

We extracted a derivation cohort from the CANDIPOP study (the Prospective Population Study on Candidaemia in Spain), a prospective, multicentre, population-based surveillance programme on candidaemia conducted in 29 hospitals in five of the largest metropolitan areas of Spain [11]. Between April 2010 and May 2011, blood cultures positive for *Candida* spp. were identified in the microbiology laboratories of participating hospitals and reported to regional study collaborators who collected clinical data using a standardized case report form as described elsewhere [11]. The study was approved by the ethics committees of each participating institution.

We created an external validation cohort that included all adult patients diagnosed with candidaemia between January 2005 and December 2012 in six tertiary hospitals in three countries (three in Spain, two in Argentina and one in Brazil). Information was retrospectively collected by detailed review of the medical records. The study was approved by the ethics committees of the participating institutions. The same inclusion and exclusion criteria were used as for the CANDIPOP study.

Variables and definitions

The primary outcome variable was the presence of a *Candida* bloodstream infection caused by a Flu-NS isolate. For the purpose of this study, *Candida* strains were divided in two groups according to MIC values obtained by CLSI procedure: isolates fully susceptible to fluconazole (Flu-S; i.e. those with an MIC <4 mg/L) and isolates non-susceptible to fluconazole (Flu-NS; i.e. those with an MIC 4 mg/L in addition to *Candida krusei*, *Candida glabrata* and *Candida guilliermondii* regardless of their MIC value), which included fluconazole-resistant as well as fluconazole dose-dependently susceptible isolates. The MIC cut-off value was selected according to CLSI criteria for *Candida* species-specific clinical breakpoints [12]. For species in which breakpoints are lacking, we considered as Flu NS the non-wild-type isolates. We recorded only nine cases of candidaemia caused by *C. guilliermondii* in the derivation cohort. Among them, more than 66% (6/9) displayed a MIC of fluconazole 4 mg/L. Nevertheless, we truly believe that these isolates should be classified as Flu-NS, as there is scarce evidence that *C. guilliermondii* is a good target for therapy with fluconazole. As the aim of the score is to provide a tool to safely choose an empirical treatment, we are persuaded that it was a cautious approach. For those episodes in which two different *Candida* spp. were recovered simultaneously in the blood culture, at least one strain was required to meet the criteria for Flu-NS. In a second analysis in the derivation cohort, we used the definition of Flu-S and Flu-NS strains according to the non-species-specific *Candida* breakpoints published in the EUCAST webpage (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Antifungal_breakpoints_v_7.0.pdf) as previously reported [13].

Previous exposure to azoles was considered if it had occurred in the 30 days before drawing the first positive blood culture. The type, dose and duration of previous antifungal therapy were recorded. Severity of candidaemia was classified as sepsis, severe sepsis, or septic shock at presentation according to standard definitions [14]. To account for local epidemiology and antifungal resistance patterns, we used a variable that reflected resistance rates at the ward level in each hospital. Wards with a prevalence of Flu-NS isolates >15% were defined as high prevalence units (HPU).

Microbiological studies

Candida isolates corresponding to episodes recorded in the derivation cohort were processed and stored at participating hospitals as described elsewhere [13]. Fluconazole susceptibility testing was performed according to the CLSI M27-A3 [15] and EUCAST (E.Def7.1 and E.Def7.2) [16,17] broth microdilution methods. The fluconazole MIC was defined as the lowest drug concentration that inhibited 50% of growth compared with the growth control after 24 h of incubation at 35°C for all *Candida* species, except for *C. glabrata*, which was determined after 48 h to prevent misclassification bias among the isolates [18]. CLSI and EUCAST procedures were carried out at the Clinical Microbiology Department of Gregorio Marañón Hospital and at the Spanish National Centre for Microbiology in Madrid, respectively, as previously reported [13].

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For episodes recorded in the validation cohort, yeast identification and *in vitro* antifungal activity were assessed at participating hospitals using local routine methods. *In vitro* antifungal activity was studied by a commercial microdilution method (YeastOne®Sensitre®, TREK Diagnostic Systems Ltd, East Grinstead, UK) or by E-test (BioMérieux SA, Paris, France), in accordance with the manufacturer's instructions. Antifungal susceptibility of the isolates was classified according to the CLSI M27-A3 document [15].

Statistical analysis

Categorical variables were compared using the chi-square or Fisher exact tests; continuous variables were compared using the Student's t-test or Mann–Whitney *U*-test, as appropriate. All statistical tests were two-tailed, and significance was set at $p < 0.05$.

Potential predictors of Flu-NS isolates were identified by logistic regression analysis using the CLSI MIC clinical breakpoints. To facilitate score generation and application in clinical settings, continuous variables (e.g. days of previous azole exposure) were transformed into binary factors using the most discriminatory cut-off point. Statistically significant variables in univariate analysis were entered into a multivariate logistic regression model. Calibration of the model was assessed by the Hosmer– Lemeshow test. The final regression model was transformed into a point-based score to build the predictive score. The logistic regression coefficients for statistically significant predictors of Flu-NS isolates were rounded to integers to assign the value of each variable. It was decided *a priori* that septic shock would remain in the predictive score because it is usually taken into account when choosing empirical therapy using existing guidance [3].

The discriminatory power of the developed score was evaluated by the area under the receiver operating characteristics (ROC) curve (AUC) and the 95% CI. We selected the best cut-off value to estimate the diagnostic sensitivity and specificity in the validation set. The prediction score was internally assessed with the MIC values obtained by the EUCAST method in the CANDIPOP study and then applied to the external validation cohort. The discrimination ability of the score was again assessed by AUC of ROC curve analysis. Statistical analyses were performed with MICROSOFT SPSS-PC+, version 15.0 (SPSS, Chicago, IL, USA).

Results

Study population

In total, 773 episodes of candidaemia were documented in the CANDIPOP study. Of these, we excluded patients who declined to participate (n = 21), those with more than one episode (n = 23), and those <16 years old (n = 112). Consequently, 617 cases of candidaemia were enrolled in the derivation cohort. A total of 297 cases were analysed in the validation cohort. Table 1 shows the baseline characteristics of patients included in both the derivation and validation cohorts. The most frequent species isolated in both cohorts was *Candida albicans*. Flu-NS accounted for 134 strains isolated in the derivation cohort (21.7%) compared with 57 strains isolated in the validation cohort (19.2%).

Score derivation

The epidemiological and clinical characteristics of the patients with Flu-S and with Flu-NS strains in the derivation cohort are

TABLE 1. Demographic, clinical and microbiological characteristics among patients included in the derivation and external validation cohorts

	Derivation cohort (n = 617)	External validation cohort (n = 297)
Demographics		
Male sex, *n* (%)	367 (59.5)	174 (58.6)
Age (years), median (IQR)	67.8 (54.3–77.3)	62 (50–73.5)
Length of in-hospital stay (days), median (IQR)	20 (12–36)	15 (3–30)
High prevalence unit, *n* (%)	269 (43.6)	169 (56.9)
Comorbidities, *n* (%)		
Diabetes mellitus	153 (24.8)	89 (30)
Liver disease	95 (15.4)	58 (19.5)
COPD	74 (12)	69 (23.2)
Chronic renal disease	174 (28.2)	82 (27.6)
Dialysis	42 (6.8)	24 (8.1)
Cerebrovascular disease	50 (8.1)	40 (13.5)
Active malignancy	238 (38.6)	132 (44.4)
Transplant recipient[a]	40 (6.5)	44 (14.8)
HIV infection	16 (2.6)	13 (4.4)
Risk factors for candidaemia, *n* (%)		
ICU admission (at onset)	173 (28)	77 (25.9)
Central venous catheter	459 (74.6)	178 (59.9)
Total parenteral nutrition	281 (45.5)	127 (42.8)
Previous gastrointestinal surgery[b]	184 (29.8)	77 (25.9)
Neutropenia (<500)	26 (4.2)	30 (10.1)
Chemotherapy[b]	55 (8.9)	57 (19.2)
Corticosteroid therapy[b]	175 (28.4)	133 (44.8)
Previous antibiotics[b]	471 (76.4)	260 (87.5)
Previous antifungals[b]	100 (16.2)	22 (7.4)
Previous azole therapy (3 days) [b]	78 (12.6)	15 (5.1)
Severity, *n* (%)		
Septic shock	94 (15.2)	85 (28.6)
Source of infection, *n* (%)		
Primary	342 (55.4)	142 (47.8)
Catheter-related	206 (33.4)	57 (19.1)
Urological	38 (6.2)	26 (8.8)
Abdominal	25 (4.1)	21 (7.1)
Other	6 (1)	3 (1)
Microbiology		
Candida species, *n* (%)[c]		
C. albicans	291 (46.3)	141 (47.5)
C. parapsilosis	136 (21.7)	49 (16.5)
C. glabrata	97 (15.4)	37 (12.5)
C. tropicalis	52 (8.3)	40 (13.5)
C. krusei	14 (2.2)	13 (4.4)
Other	38 (6.1)	17 (5.7)
Flu-NS strains, *n* (%)	134 (21.7)	57 (19.2)

Abbreviations: COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; ICU, intensive care unit; IQR, interquartile range.
[a]Thirteen haematopoietic stem cell transplants and 27 solid organ transplants in the derivation cohort versus 17 and 27, respectively, in the validation cohort.
[b]Within the preceding month.
[c]Eleven cases of mixed candidaemia in the derivation cohort.

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compared in Table 2. Univariate analysis identified four variables that were significantly associated with Flu-NS, including epidemiological factors (HPU 80.6% versus 33.3% p < 0.001), co-morbidities (malignancy: 47% versus 36.3%, p 0.024; or previous solid organ or haematopoietic stem cell transplantation: 13.4% versus 4.6%, p 0.001) and previous antifungal therapy (azole therapy for at least 3 days in the previous month 23.1 versus 9.7%, p < 0.001). The results are equivalent for patients who received previous fluconazole therapy during >7 days or >14 days. Multivariate analysis summarized in Table 3 shows that the independent factors associated with Flu-NS were as follows: being a previous solid organ or haematopoietic stem cell transplantation recipient, hospitalization in an HPU, and azole therapy for 3 days. Hosmer–Lemeshov goodness-of-fit of the final model was 82%.

TABLE 2. Univariate analysis of patients with Flu-NS and Flu-S candidaemia in the derivation cohort

Flu-S (*n* = 483)		Flu-NS (*n* = 134)	p-value
Demographics			
Male sex, *n* (%)	289 (59.8)	78 (58.2)	0.735
Age (years), median (IQR)	67.4 (54.2–76.8)	69.3 (55.5–77.7)	0.490
Length of in-hospital stay (days), median (IQR)	20 (12–37.5)	21 (8.5–34)	0.146
High prevalence unit, *n* (%)	161 (33.3)	108 (80.6)	<0.001
Comorbidities, *n* (%)			
Diabetes mellitus	117 (24.2)	36 (26.9)	0.531
Liver disease	68 (14.1)	27 (20.1)	0.085
COPD	53 (11)	21 (15.7)	0.139
Chronic renal disease	143 (29.6)	31 (23.1)	0.141
Dialysis	35 (7.2)	7 (5.2)	0.411
Cerebrovascular disease	40 (8.3)	10 (7.5)	0.759
Active malignancy	175 (36.3)	63 (47)	0.024
Solid organ or haematopoietic stem cell transplant recipient	22 (4.6)	18 (13.4)	<0.001
HIV infection	10 (2.1)	6 (4.5)	0.121
Risk factors for candidaemia, *n* (%)			
ICU admission (at onset)	141 (29.1)	32 (23.9)	0.578
Central venous catheter	364 (75.4)	95 (71.4)	0.337
Total parenteral nutrition	220 (45.5)	61 (45.5)	0.996
Previous gastrointestinal surgery[a]	145 (30)	39 (29.1)	0.837
Neutropenia (<500)	18 (3.7)	8 (6)	0.253
Chemotherapy[a]	39 (8.1)	16 (11.9)	0.164
Corticosteroid therapy[a]	137 (28.4)	38 (28.4)	0.999
Previous antibiotics[a]	370 (76.6)	101 (75.4)	0.679
Previous antifungals[a]	67 (13.9)	33 (24.8)	0.002
Previous azole therapy (≥ 3 days)[a]	47 (9.7)	31 (23.1)	<0.001
Severity, *n* (%)			
Septic shock	67 (13.9)	27 (20.1)	0.074
Source of infection, *n* (%)			
Primary	268 (55.5)	74 (55.2)	0.957
Catheter-related	168 (34.8)	38 (28.4)	0.163
Urological	27 (5.6)	11 (8.2)	0.265
Abdominal	17 (3.5)	8 (6)	0.203
Other	3 (0.6)	3 (2.2)	0.120
Microbiology			
Candida species, *n* (%)[b]			
C. albicans	288 (58.3)	3 (2.2)	<0.001
C. parapsilosis	130 (26.3)	6 (4.57)	<0.001
C. glabrata	0	97 (72.3)	<0.001
C. tropicalis	50 (10.1)	2 (1.5)	<0.001
C. krusei	0	14 (10.4)	<0.001
C. guilliermondii	0	9 (6.7)	<0.001
Other	26 (5.3)	3 (2.2)	0.067

Abbreviations: Flu-NS, isolates non-susceptible to fluconazole (i.e. those with an MIC ≥ 4 mg/L plus *C. krusei*, *C. glabrata* and *C. guilliermondii*, regardless of their MIC value); Flu-S, isolates fully susceptible to fluconazole (i.e. those with an MIC <4 mg/L).
[a]Within the preceding month.
[b]Eleven cases of mixed candidaemia.

TABLE 3. Independent risk factors associated with Flu-NS candidaemia: multivariate analysis

Characteristic	AOR	95% CI	p-value
Malignancy	1.27	0.82–1.96	0.282
Solid organ or haematopoietic stem cell transplant recipient	2.13	1.01–4.55	0.047
High prevalence unit	7.53	4.68–12.10	<0.001
Previous azole therapy (≥ 3 days)[a]	2.04	1.16–3.62	0.014

Abbreviations: AOR, adjusted odds ratio; Flu-NS, isolates non-susceptible to fluconazole (i.e. those with an MIC ≥ 4 mg/L plus *Candida krusei*, *Candida glabrata* and *Candida guilliermondii*, regardless of their MIC value).
[a]Within the preceding month.

Results from the multivariate analysis were used to develop the clinical Flu-NS prediction score. According to the regression coefficients, we assigned 2 points to HPU and 1 point to each of the other independent parameters. We also added 1 point for patients with septic shock at presentation. The application of the score to patients enabled us to categorize subjects into six different classes: 269 (43.6%) had 0 points, 72 (11.7%) had 1 point, 172 (27.9%) had 2 points, 85 (13.8%) had 3 points, 17 (2.8%) had 4 points and 2 (0.3%) had 5 points. The incidence of Flu-NS by the CLSI reference procedure in these groups was 7.1%, 6.9%, 33.7%, 45.9%, 64.7%, and 100%, respectively. The ROC curve for the Flu-NS prediction score showed an accuracy measured by the AUC of 0.76 (0.72–0.81) (Fig. 1, curve A). When applied exclusively to the 26 neutropenic patients of this cohort, the Flu-NS score conserved its performance (ROC curve AUC 0.79 (0.59–0.98)). Diagnostic sensitivity, specificity and predictive values according to different cut-off values in the derivation cohort are shown in Table 4. A cut-off point of 2 for the Flu-NS score had a sensitivity of 82.1%, a specificity of 65.6% and a negative predictive value of 93% (Table 4). Using the MIC cut-off value obtained by the EUCAST procedure to classify episodes as Flu-NS in the same cohort, the score retained an AUC of 0.79 (95% CI 0.76–0.83) (Fig. 1, curve B). The application of the score when the MIC values were obtained by the EUCAST procedure enabled us to categorize subjects into six different classes: 221 (35.9%) had 0 points, 64 (10.4%) had 1 point, 218 (35.4%) had 2 points, 93 (15.1%) had 3 points, 18 (2.9%) had 4 points and 2 (0.3%) had 5 points. The incidence of Flu-NS in these groups was 2.7%, 3.1%, 36.2%, 46.2%, 66.7% and 100%, respectively.

External validation

The application of the score to patients in the validation cohort enabled us to categorize them into six different classes: 96 (32.3%) had 0 points, 28 (9.4%) had 1 point, 109 (36.7%) had 2 points, 47 (15.8%) had 3 points, 15 (5%) had 4 points and 2 (0.7%) had 5 points. The incidences of Flu-NS in the validation cohort ranged from 6% for patients without risk factors to 64%

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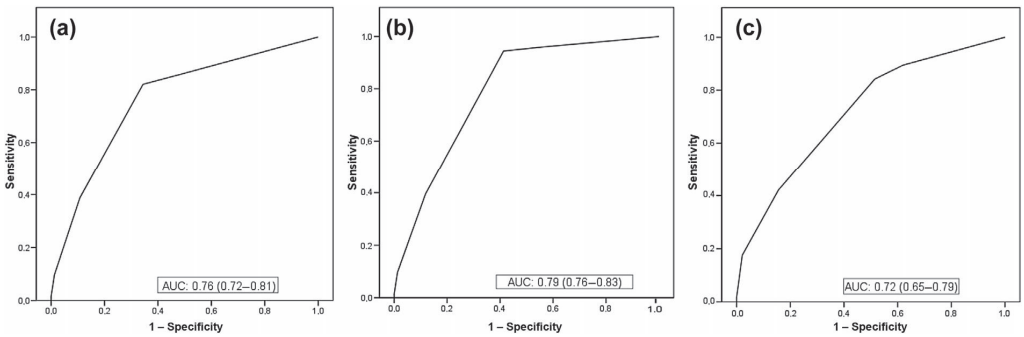

FIG. 1. Receiver operating characteristics (ROC) curve for the Flu-NS score in three cohorts: (a) derivation, (b) EUCAST internal validation and (c) external validation. Abbreviations: EUCAST, European Committee on Antimicrobial Susceptibility Testing; Flu-NS, isolates non-susceptible to fluconazole (i.e. those with an MIC 4 mg/L plus *Candida krusei*, *Candida glabrata* and *Candida guilliermondii*, regardless of their MIC value).

for subjects with 4 or more points on the score, with an AUC of 0.72 (95% CI 0.65–0.79) (Fig. 1, curve C). When applied exclusively to the 30 neutropenic patients of this cohort, the Flu-NS score conserved its performance (ROC curve AUC 0.71 (0.51–0.90)). Diagnostic sensitivity, specificity and predictive values according to different cut-off values in the validation cohort are also shown in Table 4.

Discussion

In the present study, we have created and validated a simple score that can estimate the risk of Flu-NS candidaemia using readily available clinical parameters at the bedside. Specifically, the presence of hospitalization to an HPU before transplantation, and at least 3 days of prior azole therapy as independent predictors of Flu-NS by multivariate analysis allowed us to create a valid prediction score. The absence of any of these predictive factors decreased the risk of Flu-NS candidaemia substantially, supporting the empirical use of fluconazole as the first antifungal agent. To our knowledge, this is the first investigation to have developed a clinically relevant tool to guide empirical antifungal therapy in this setting. Interestingly, the score appears to retain its applicability when using the EUCAST breakpoints.

Many previous studies have attempted to identify risk factors associated with azole resistance. Some have analysed non-albicans candidaemia as a surrogate marker of azole resistance and found risk factors such as chemotherapy [19], solid tumour [20] or previous surgery [21]. Other works with microbiological confirmation of resistance have described gastrointestinal surgery [22] or the cumulative length of hospitalization [23] as being relevant; however, these were single-centre studies limited to oncological or critically ill surgical patients, respectively. Another prospective study reported that neutropenia, chronic renal disease and previous fluconazole exposure were independently associated with such strains [24]. Previous azole therapy was also found to be a risk factor in other works [25,26], all those based on the old CLSI breakpoints. Furthermore, we found that both prior antifungal therapy and its duration were independent risk factors, a fact already demonstrated by other authors who observed a dose-dependent relationship between fluconazole consumption and breakthrough azole-resistant candidaemia [27]. This evidence supports the guideline recommendation of avoiding fluconazole as the initial empiric therapy in those patients with previous exposure [3].

TABLE 4. Receiver operating characteristic curve cut-off values for the derivation and validation cohorts

	Derivation cohort				EUCAST internal validation				External validation cohort			
Cut-off point	Sn	Sp	NPV	PPV	Sn	Sp	NPV	PPV	Sn	Sp	NPV	PPV
‡1	85.8	51.8	92.9	33	95.8	45.6	97.3	35	87.7	39.6	93.1	25.6
‡2	82.1	65.6	93	39.9	94.4	58.7	97.1	41	84.2	49.6	92.9	28.4
‡3	38.8	89.2	84	50	39.6	88.1	82.7	50.4	42.1	84.4	85.8	39.3
‡4	9.7	98.8	79.7	68.4	9.7	98.7	78.2	70	17.5	97.9	83.2	66.7
5	1.5	100	78.5	100	1.4	100	76.9	100	1.8	100	80.9	100

Abbreviations: EUCAST, European Committee on Antimicrobial Susceptibility Testing; NPV, negative predictive value; PPV, positive predictive value; Sn, sensitivity; Sp, specificity.

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In the present study, we stressed the importance of local epidemiology in the pre-test probability of developing Flu-NS candidaemia. We found that being hospitalized in a unit with a Flu-NS candidaemia prevalence of 15% had the highest weighting as a predictor in our score. In fact, data from the CANDIPOP survey showed a great difference in the prevalence of Flu-NS strains among the different participating cities [13]. This is clearly evident when we compare countries of northern Europe with Latin-America [28,29], or wider comparisons as the SENTRY Antimicrobial Surveillance Program [30]. Our results highlight the importance of local surveillance studies.

Our multivariate analysis found that transplantation recipient status was an independent predictor of Flu-NS candidaemia. Previous reports on haematological neutropenic patients receiving stem cell transplantation found similar results [31,32]. Nevertheless, information concerning solid organ transplantation is conflicting, with some authors reporting greater azole resistance [33] and others failing to report this association [34]. However, these findings are difficult to compare because of differences in the design, definitions and populations used in those studies.

To date, no evidence supports the argument that azole-resistant strains are associated with a greater incidence of septic shock. Despite this, the fungicidal activity of echinocandins against most *Candida* species is argued to be a reason for offering these drugs to unstable patients [3]. Further studies are needed to validate this hypothesis. However, the addition of septic shock to our clinical prediction score provides a safety factor for clinicians, who usually prefer broad-spectrum coverage in critically ill patients.

Our clinical prediction score faces physicians with quite different settings. On the one hand, there is a patient subgroup with low risk (score <1) in whom empirical fluconazole appears to be a safe initial treatment. On the other hand, there is a patient subgroup with several risk factors (score 2) and a high probability of Flu-NS candidaemia. Together with critically ill patients, those patients warrant broad-spectrum therapy, with fluconazole reserved as a step-down option. Finally, for those patients with 3 or more points on the Flu-NS score, the use of an echinocandin should be mandatory because of the high risk of Flu-NS isolates. To note, echinocandin-resistance (though not common) may be observed in some Flu-NS isolates.

The strengths of the current study include the prospective nature of the derivation cohort, the large number of consecutive patients evaluated, the comprehensive data collection, and the multicentre design. Moreover, it is also important that validation be provided in an international cohort, including patients from different geographic areas. Nevertheless, the predictive power of the score needs to be confirmed in even more diverse clinical settings to ensure its wider validity and reliability. The limitations of the present study include differences in MIC determination techniques between the derivation and validation cohorts. As the MIC breakpoints are method-specific, using the CLSI breakpoints for interpretation of the MICs generated by Sensititre YeastOne or Etest in the validation cohort may have led to discordant results in a number of isolates. Second, it is true that our score mostly identified Flu-NS strains corresponding to non-*albicans* species, with primary resistance or dose-dependent susceptibility to fluconazole. However, the Flu-NS score was also useful for patients with isolates with acquired resistance to fluconazole (11 isolates: three *C. albicans*, six *C. parapsilosis* and two *C. tropicalis*). Among them, more than 90% (10/11) had 2 points and more than 63% (7/11) had 3 points in the score. Third, it is certainly possible that the rapid evolution of molecular diagnostic methods [35] may lead to clinical tools such as this becoming redundant; however, the availability of such techniques will remain restricted in resource-limited settings. Finally, a major drawback is in the nature of a prediction score that aims to simplify a complex clinical situation. We stress that such a tool should support, but never replace, clinical judgement.

In summary, our easy to use, simple Flu-NS prediction score can estimate the risk of Flu-NS candidaemia using readily available clinical parameters at the bedside. This gives physicians a validated and reliable tool to enable the rational and safe choice of an initial antifungal agent in the management of candidaemia in adult patients.

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Transparency declaration

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Ethics approval

This observational study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Comité Ético de Investigación Clínica of each participating hospital. To protect personal privacy, identifying information in the electronic database was encrypted for each patient. Informed consent was waived by the Clinical Research Ethics Committee for the validation cohort because no intervention was involved and no patient-identifying information was included.

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6.2. Descriptive study of breakthrough candidemia

- Frequency of breakthrough candidemia.
- Clinical and microbiological characteristics of patients with breakthrough candidemia.
- Risk factors for breakthrough candidemia.
- Appropriateness of empiric therapy and outcomes of patients with breakthrough candidemia.

Breakthrough candidaemia in the era of broad-spectrum antifungal therapies

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Abstract

We aimed to assess the characteristics, treatment, risk factors and outcome of patients with breakthrough candidaemia (BrC) in the era of broad-spectrum antifungal therapies. We carried out a multicentre study of hospitalized adults with candidaemia at six hospitals in three countries. BrC episodes were compared with the remaining episodes (non-BrC). Of 409 episodes of candidaemia, 37 (9%) were BrC. Among them, antifungal treatment was administered as prophylaxis in 26 severely immunosuppressed patients (70%) and as a fever-driven approach in 11 (30%). *Candida albicans* was significantly less common in patients with BrC (24% versus 46%, p 0.010) whereas *Candida krusei* was more frequent (16% versus 2.4%, p < 0.001). BrC was associated with infections caused by fluconazole non-susceptible isolates (50% versus 18%, p < 0.001). *Candida albicans* BrC was associated with previous fluconazole treatment whereas *Candida parapsilosis* candidaemia was mostly catheter-related and/or associated with previous echinocandin therapy. The empirical antifungal therapy was more often appropriate in the non-BrC group (57% versus 74%, p 0.055). No significant differences were found in outcomes (early and overall mortality: 11% versus 13% p 0.802 and 40% versus 40% p 0.954, respectively). Fluconazole non-susceptibility was independently associated with the risk of BrC (adjusted OR 5.57; 95% CI 1.45–21.37). In conclusion, BrC accounted for 9% of the episodes in our multicentre cohort. The *Candida* spp. isolated were different depending on the previous antifungal therapy: previous azole treatment was associated with fluconazole non-susceptible strains and previous echinocandin treatment was associated with BrC caused by *C. parapsilosis*. These results should be taken into account to improve the empirical treatment of BrC.
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Introduction

Invasive fungal infections are increasing in frequency in developed countries, mainly due to the rising number of immunocompromised patients who are at risk [1]. These infections are associated with significant morbidity, prolonged hospital stays, increased healthcare costs and high mortality [2–4]. For these reasons, strategies to decrease the prevalence of invasive fungal infections, such as antifungal prophylaxis or pre-emptive

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antifungal therapy that aims to offer an early treatment option, have become common in selected populations [5,6]. As a consequence, growing numbers of patients are diagnosed with Candida breakthrough infections [7–11].

Some studies have focused on the risk factors and epidemiology of breakthrough candidaemia (BrC), most in the 1990s and early twenty-first century. Nevertheless, current information regarding the clinical manifestations of BrC and the impact of BrC on the prognosis of affected patients in this era of broad-spectrum antifungal therapies is still scarce and mainly derived from studies performed in northern Europe or the USA.

Here we analyse this issue in a recent multicentre cohort of patients with candidaemia from different countries. We sought to determine the proportion of patients with candidaemia who developed BrC while receiving antifungal therapy and compare clinical and microbiological characteristics, the appropriateness of empirical therapy, and outcomes. We also assessed the risk factors for BrC.

Materials and Methods

Setting, patients and study design

We performed a retrospective multicentre study of all the episodes of candidaemia that occurred in hospitalized adult patients between January 2005 and December 2012 at six tertiary teaching institutions in three different countries: three in Spain, two in Argentina and one in Brazil. To have an estimation of time trend, we have arbitrarily divided the study period into two sections of 4 years each, 2005–2008 versus 2009–2012. The following information was collected from medical records: demographic characteristics, comorbidities, systemic antifungal therapy or prophylaxis and other concurrent medications, clinical features, sources of candidaemia, causative species, specific antifungal therapy and outcomes. For the purposes of this study, the episodes of BrC occurring in patients undergoing systemic antifungal therapy as a fever-driven approach or as prophylaxis (from here on: BrC patients) were compared with those occurring in the remaining patients (from here on: non-BrC patients).

Definitions

Breakthrough candidaemia was defined as the diagnosis of candidaemia in a patient who had received a systemic antifungal agent for at least 3 days [12]. Catheter-related infection was defined on the basis of the Infectious Diseases Society of America guidelines [13]. Secondary candidaemia was defined as a documented concurrent infection caused by the same *Candida* species at a site other than the catheter [14]. The diagnosis of septic shock was based on a systolic blood pressure <90 mmHg and peripheral hypoperfusion and/or the need for vasopressors [15]. Neutropenia was considered to occur when the granulocyte count was <500/mm^3. For the purposes of our analysis, we defined as severely immunosuppressed those patients who had received a transplant (either haematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT)) and/or those suffering chemotherapy-induced neutropenia. Empiric antifungal therapy was considered to be appropriate when the *Candida* isolated showed *in vitro* susceptibility to the antifungal drug administered. Not administering an empirical treatment was considered an inappropriate treatment. The modification of antifungal therapy in each patient of the BrC group was compared with the recommendations of therapeutic guidelines [5,6,16]. Clinical instability (at 48 h after initiation of therapy) was defined as the presence of fever and/or hypotension or need for vasopressor drugs. The early and overall mortality were defined as death from any cause within 5 and 30 days of the onset of candidaemia, respectively.

Microbiological studies

Two sets of two blood samples were collected from patients with a suspected bloodstream infection. The blood samples were processed using a BACTEC 9240 system (Becton–Dickinson Microbiology Systems, Franklin Lakes, NJ, USA) or BacTAlert (BioMérieux SA, Marcy L'Etoile, France) with an incubation period of 5 days. If yeast cells were observed after microscopic examination of a Gram stain, blood bottles were subcultured onto Sabouraud agar plates (BD BBL StrackerTM PlatesTM, Heidelberg, Germany) and chromogenic media (ChromAgar BioMerieux SA, Paris, France). Yeast isolates were identified by conventional methods. The antifungal susceptibility of the isolates was classified in accordance with the CLSI M27-S3 document [17]. Antifungal susceptibility testing was performed using a microdilution colorimetric Sensititre YeastOne® SYO-10 panel (TREK Diagnostic Systems, Cleveland, OH, USA) or by E-test (BioMérieux SA) following the manufacturer's instructions. CLSI species-specific clinical breakpoints were applied. Of note, those *Candida* spp. isolates with a MIC $\geq$4 mg/L to fluconazole were considered non-susceptible to fluconazole, with the exception of *Candida krusei* and *Candida glabrata* isolates, which were considered non-susceptible to fluconazole regardless of their MIC value. For amphotericin B, isolates inhibited by 1 mg/L were considered resistant. Quality controls were performed in each

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centre using *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 [18].

Statistical analysis

Quantitative variables were reported as the median and interquartile range (IQR); categorical variables, as absolute numbers and percentages. To detect significant differences between groups, we used the chi-square test or Fisher's exact test for categorical variables, and the Student's *t* test, Mann–Whitney test or analysis of variance for continuous variables, as appropriate. Two groups of high-risk patients were deemed suitable for separate analysis: on the one hand, severely immunosuppressed patients (according to the definition already given); and on the other hand, critically ill non-neutropenic patients admitted to an intensive care unit. We performed a multivariate logistic regression analysis of factors potentially associated with the occurrence of BrC in each risk group. The multivariate analysis included all significant variables ($p < 0.05$) in the univariate analysis and we added another factor considered relevant in the literature (presence of a central venous catheter for $\geq$48 h) [12]. The goodness-of-fit of the model was assessed by the Hosmer–Lemeshow test. The relative risks were expressed as adjusted odds ratios (aOR) and 95% CI. A Kaplan–Meier curve was plotted to show the survival probabilities at 30 days, according to the appropriateness of antifungal therapy. All data were analysed using SPSS software, version 15.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was established at $\alpha = 0.05$. All reported p-values are two-tailed.

Results

Patients and clinical characteristics

Over the study period we documented 409 episodes of candidaemia; 37 (9%) were BrC and 372 (91%) were non-BrC. There were no patients with more than one episode. Interestingly, the incidence of BrC was significantly higher in the second period (2005–2008 versus 2009–2012, 14 versus 23 patients, p 0.037). Appendix 1 exhaustively details the characteristics of each of the BrC patients. Antifungal treatment was administered as prophylaxis to 26 severely immunosuppressed patients (ten HSCT patients (27%), 12 SOT patients (32%) and four (11%) with chemotherapy-induced neutropenia) and as a fever-driven approach in 11 (30%) critically ill non-neutropenic patients. The median number of days of previous antifungal treatment was 10 days (IQR 5–20); 11 days (IQR 5–22) for prophylaxis patients and 6.5 days (IQR 3–9) for those patients receiving therapy as a fever-driven approach (p 0.060). Table 1 details the antifungal agent used before the diagnosis of candidaemia, the length of previous antifungal treatment and the breakthrough *Candida* species isolated. The epidemiological and clinical characteristics of the patients with and without BrC are compared in Table 2.

Candida species and antifungal susceptibility

Table 3 shows the *Candida* species and susceptibility by groups. The most frequent species isolated in both groups was *Candida albicans*. However, *C. albicans* was significantly less common in patients with BrC (24% versus 46%, p 0.010); whereas *C. krusei* was significantly more frequent in this group (16% versus 2.4%, $p < 0.001$). No significant differences were found in other species. Of note, BrC caused by *C. albicans* was only observed during either itraconazole treatment (one episode) or fluconazole treatment (eight episodes; cumulative dose: median 1900 mg; range 600–4000 mg).

Fluconazole susceptibility was determined in 356 isolates (28 isolates (76%) in the BrC group and 328 (88%) in the non-BrC group), echinocandin susceptibility in 213 isolates (16 (43%) in the BrC group and 197 (53%) in the non-BrC group) and amphotericin B susceptibility in 315 isolates (26 (70%) in the BrC

TABLE 1. Summary of patients with BrC

Prior antifungal treatment[a]	Patients (*n*)		Days of treatment Median (IQR) [b]	*Candida* species isolated (*n*)												Strains resistant to prior treatment *n* (%)	
				C. albicans		*C. parapsilosis*[c]		*C. glabrata*		*C. tropicalis*		*C. krusei*		Other			
	Px	FDA		Px	FDA	Px	FDA	Px	FDA	Px	FDA	Px	FDA	Px	FDA	Px	FDA
Fluconazole	18	10	9.5 (5–20)	2	6	4	1	3	1	2	1	4	0	3	1	8 (28.6)	1 (3.6)
Itraconazole	2	1	10 (3–11)	0	1	2	0	0	0	0	0	0	0	0	0	0	0
Posaconazole	4	0	14 (3–117)	0	0	0	0	2	0	1	0	1	0	0	0	3 (75)	0
Anidulafungin	5	1	13 (4–29)	0	0	2	0	0	1	1	0	2	0	0	0	1 (16.7)[d]	0
Caspofungin	1	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Voriconazole	1	0	24	0	0	0	0	0	0	0	0	1	0	0	0	1 (100)	0

Abbreviations: FDA, fever-driven approach in critically ill non-neutropenic patients; Px, prophylaxis in severely immunosuppressed patients.
[a]Six patients were previously treated with two different antifungal drugs: two patients with two azoles and four patients with an echinocandin and an azole.
[b]The days of prophylaxis or therapy for the fever-driven approach before the onset of candidaemia did not differ significantly between the different drugs used (p 0.216).
[c]The six patients with BrC caused by *C. parapsilosis* during azole treatment had catheter-related candidaemia.
[d]Susceptibility to echinocandins was only available in three patients previously treated with anidulafungin.

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TABLE 2. Clinical characteristics and probable sources of candidaemia for patients with BrC and non-BrC

	BrC (n = 37) (%)	Non-BrC (n = 372) (%)	p
Demographics			
Male sex	24 (64.9)	218 (58.6)	0.460
Age, median (IQR) years	53 (41–65)	62 (49–74)	0.002
Age > 70 years	3 (8.1)	132 (35.5)	<0.001
Comorbid conditions			
Chronic renal disease	9 (24.3)	103 (27.7)	0.648
Dialysis	5 (13.5)	30 (8.1)	0.263
Diabetes mellitus	12 (32.4)	107 (28.7)	0.662
Chronic obstructive pulmonary disease	5 (13.5)	78 (20.9)	0.273
Chronic heart disease	6 (16.2)	98 (26.3)	0.232
Cerebrovascular disease	0	59 (15.9)	0.003
Liver disease	11 (29.7)	62 (16.7)	0.051
Malignancy	23 (62.1)	151 (40.6)	0.012
Haematopoietic stem cell transplantation	10 (27)	15 (4.1)	<0.001
Graft-versus-host disease	3 (8.1)	5 (1.3)	0.028
Solid organ transplantation	12 (32.4)	30 (8.1)	<0.001
HIV infection	2 (5.4)	17 (4.6)	0.687
Charlson median (IQR)	3 (2–5)	4 (2–6)	0.159
Charlson >5 p	12 (32.4)	150 (48.2)	0.230
Risk factors (within the previous 30 days)			
Intensive care unit stay	15 (40.5)	165 (44.3)	0.705
Mechanical ventilation	13 (35.1)	134 (36.1)	0.849
Total parenteral nutrition	13 (35.1)	119 (31.9)	0.763
Previous surgery	15 (40.5)	159 (42.7)	0.765
Venous catheter placement (>48 h)[a]	36 (97.3)	322 (86.6)	0.103
Urinary catheter	19 (51.3)	197 (52.9)	0.810
Neutropenia	11 (29.7)	34 (9.1)	<0.001
Chemotherapy	12 (32.4)	65 (17.5)	0.029
Radiotherapy	0	13 (3.5)	0.614
Corticosteroid and/or immunosuppressive therapy	25 (67.6)	173 (46.5)	0.014
Clinical status (at candidaemia diagnosis)			
Fever	24 (64.9)	273 (73.4)	0.056
Hypotension	13 (35.1)	110 (29.6)	0.504
Vasopressor therapy requirement	11 (29.7)	107 (28.8)	0.972
Acute renal failure	9 (24.3)	110 (29.6)	0.396
Confusion	11 (29.7)	127 (34.1)	0.450
Probable source of infection			
Urinary tract	1 (2.7)	30 (8.1)	0.374
Digestive tract and abdomen	10 (27)	53 (14.2)	0.051
Catheter-related infection	8 (21.6)	80 (21.5)	0.941
Endocarditis	0	3 (0.8)	0.658
Unknown (primary candidaemia)	13 (35.1)	176 (47.3)	0.156
Other	5 (13.5)	30 (8.1)	0.287

Abbreviations: IQR, interquartile range. Solid Organ Transplantation. HIV: Human Immunodeficiency Virus. ICU: Intensive Care Unit.
[a]Data available for 405 patients.

TABLE 3. *Candida* species of patients with BrC and non-BrC

	BrC (n = 37) (%)	Non-BrC (n = 372) (%)	p
Candida species			
C. albicans	9 (24.3)	172 (46.2)	0.010
C. parapsilosis	8 (21.6)	73 (19.6)	0.771
C. glabrata	6 (16.2)	42 (11.3)	0.375
C. tropicalis	4 (10.8)	54 (14.5)	0.538
C. krusei	6 (16.2)	9 (2.4)	<0.001
C. lusitaniae	0 (0)	1 (0.3)	1
Other	4 (10.8)	21 (5.6)	0.267
Fluconazole non-susceptible strains [a]	14 (50)	58 (17.7)	<0.001
Echinocandin resistant strains [b]	2 (12.5)	9 (4.6)	0.195
Amphotericin B resistant strains [c]	1 (3.8)	5 (1.7)	0.406

[a]Fluconazole susceptibility was determined in 356 strains.
[b]Echinocandins susceptibility was determined in 213 strains.
[c]Amphotericin B susceptibility was determined in 315 strains.

group and 289 (78%) in the non-BrC group). Overall, candidaemia caused by fluconazole-non-susceptible strains was more frequent in the BrC group (50% versus 18%, p < 0.001), also in the group of patients who had received azole therapy previously (48% versus 15%, p < 0.001). Rates of resistance to amphotericin and echinocandins were similar for both groups. Table 1 summarizes the antifungal susceptibility of different strains in the BrC group according to previous antifungal treatment. Thirty-eight per cent of patients had an isolate that was non-susceptible to the previous antifungal drug used. That percentage was 75% (3/4) for patients receiving posaconazole and 28% (11/39) for patients treated with another antifungal (p 0.093).

Independent factors associated with BrC

The multivariate analysis is outlined in Table 4. We analysed the factors associated with BrC, first in the whole cohort and then in two different populations: severely immunosuppressed patients and critically ill non-neutropenic patients admitted to an intensive care unit. The goodnesses-of-fit of the final models were 74%, 93% and 99%, respectively. Independent risk factors associated with the occurrence of BrC in the whole cohort were SOT (aOR 19.34; 95% CI 4.10–91.33; p < 0.001) and fluconazole non-susceptibility (aOR 3.55; 95% CI 135–9.32; p 0.010). However, when the risk groups were analysed separately, the only factor that remained significantly associated with BrC was fluconazole non-susceptibility in severely immunosuppressed patients (aOR 5.57; 95% CI 1.45–21.37; p 0.012). No independent risk factor was identified for critically ill patients admitted to an ICU.

Therapeutic approaches and outcomes

A detailed description of the treatments and outcomes is provided in Table 5. Overall, 360 episodes (88%) were treated with specific antifungal therapy for candidaemia. The appropriateness of the empirical antifungal therapy was more frequent in the non-BrC patients (57% versus 74%, p 0.055). The time until an appropriate therapy was administered was shorter in the BrC group (median 1 versus 2 days, p 0.039). Fluconazole was the empirical antifungal agent most frequently administered as a single initial drug in both groups (49% versus 59%, p 0.155). However, echinocandins (32% versus 16%, p 0.020) and amphotericin B (16% versus 6.5%, p 0.036) were more commonly used in BrC patients. Within the first 48 h of the onset of candidaemia, only 13 patients (35%) with BrC received an antifungal treatment different from that used as prophylaxis or as a fever-driven approach. In those patients in

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TABLE 4. Independent risk factors for BrC

Risk factor	Whole cohort (n = 409) Adjusted OR (95% CI)	p	Severely Immunosuppressed (n = 94) Adjusted OR (95% CI)	p	Critically ill non-neutropenic (n = 148) Adjusted OR (95% CI)	p
Age ≥70 years	0.40 (0.10–1.64)	0.203	3.42 (0.42–27.68)	0.249	0 (0–0)	0.998
Cerebrovascular disease	0 (0–0)	0.997	0 (0–0)	0.999	0 (0–0)	0.998
Malignancy	1.81 (0.55–5.89)	0.327	1.83 (0.28–11.82)	0.528	4.11 (0.56–30.33)	0.166
Chemotherapy	1.88 (0.40–8.92)	0.429	1.44 (0.19–11.12)	0.729	0 (0–0)	0.999
Haematopoietic stem cell transplantation	3.47 (0.79–15.12)	0.098	4.53 (0.53–38.56)	0.167		
Solid organ transplantation	19.34 (4.10–91.33)	<0.001	19.97 (0.93–427.53)	0.055		
Neutropenia	2.08 (0.52–8.32)	0.302	3.53 (0.51–24.29)	0.201		
Central venous catheter placement (>48 h)	1.43 (.494–4.15)	0.508	0.78 (0.21–2.96)	0.719	0 (0–0)	0.999
Corticosteroid and/or Immunosuppressive therapy	0.34 (0.08–1.51)	0.156	0.31 (0.02–4.73)	0.398	0.36 (0.04–3.67)	0.391
Fluconazole non-susceptibility	3.55 (1.35–9.32)	0.010	5.57 (1.45–21.37)	0.012	1.72 (0.15–20.42)	0.667

TABLE 5. Treatment and clinical outcomes for patients with BrC and non-BrC

	BrC (n = 37) (%)	Non-BrC (n = 372) (%)	p
Appropriate empirical antifungal treatment[a]	16 (57.1)	242 (74)	0.055
Days until appropriate antifungal treatment, median (IQR)	1 (0–2)	2 (1–3)	0.039
Empirical antifungal drug selected			<0.001
Fluconazole	18 (48.6)	224 (59.1)	0.155
Itraconazole	2 (5.4)	1 (0.3)	0.015
Voriconazole	0	11 (2.9)	0.582
Posaconazole	1 (2.7)	0	0.160
Echinocandins	11 (32.4)	62 (16.4)	0.020
Anidulafungin	5 (13.5)	32 (8.6)	0.353
Caspofungin	6 (16.2)	25 (6.7)	0.045
Micafungin	0	5 (1.3)	0.956
Amphotericin B	5 (13.5)	25 (6.5)	0.036
Catheter removal	21 (58.3)	219 (68)	0.241
Outcomes			
Intensive care unit admission	10 (27)	113 (30.4)	0.573
Mechanical ventilation	11 (29.7)	101 (27.1)	0.766
Instability (at 48 h)	13 (35.1)	135 (36.3)	0.680
Persistent candidaemia	3 (8.1)	62 (16.7)	0.236
Septic metastases	1 (2.7)	22 (5.9)	0.663
Recurrence	0	13 (3.5)	0.506
Early mortality (5 days)	4 (10.8%)	50 (13.4%)	0.802
Overall mortality (30 days)	15 (40.5)	149 (40.1)	0.954

Abbreviations: IQR, interquartile range.
[a]Data available for 355 patients.

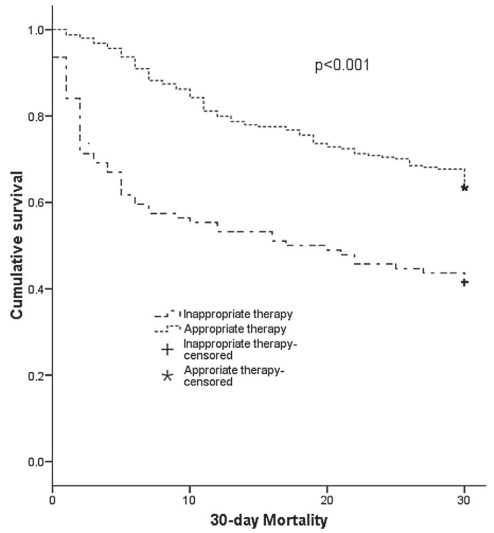

FIG. 1. Kaplan–Meier 30-day survival curves according to appropriateness of therapy.

whom the therapy was modified following guideline recommendations, appropriate empirical treatment increased to 78%. Venous catheters were removed with a similar frequency in both groups. No significant differences were found in outcomes. The Kaplan–Meier curve in Fig. 1 shows that patients receiving inappropriate antifungal therapy had poorer survival at 30 days than those who received appropriate antifungal therapy ($p < 0.0001$).

Discussion

In this multicentre study involving a large number of patients with candidaemia from three different countries we found that BrC accounted for 9% of the episodes. Most of them occurred among severely immunosuppressed patients and were caused by non-*albicans* species. BrC was associated with infections due to fluconazole non-susceptible isolates. The *Candida* spp. isolated in BrC were different depending on the previous antifungal therapy. Interestingly, BrC caused by *C. albicans* was only observed among patients receiving itraconazole or fluconazole, whereas *C. parapsilosis* was mostly catheter-related and/or associated with previous echinocandin therapy. Fluconazole non-susceptibility appeared as an independent risk factor for BrC.

Our study concurs with others in reporting patients with BrC as acutely ill, most with serious comorbid conditions,

frequently neutropenic or previously treated with corticosteroids or other immunosuppressive drugs [12,19–24]. A shift to non-*albicans* species had also been encountered in this population [19–22,25], in particular caused by fluconazole non-susceptible strains, probably as a result of antifungal pressure, a fact demonstrated by other investigators who documented a dose-dependent relationship between fluconazole consumption and BrC due to azole-resistant strains [23]. Our findings provide new and relevant information: (1) BrC caused by *C. albicans* was only observed in patients treated with itraconazole or fluconazole and not in patients treated with other antifungals; (2) patients who had been on posaconazole treatment had a high prevalence of BrC caused by fluconazole non-susceptible strains; and (3) BrC caused by *C. parapsilosis* was mostly catheter-related or associated with previous echinocandin therapy.

In this contemporary study, we identified fluconazole non-susceptibility as an independent factor related with BrC. Previous studies had documented neutropenia, corticosteroid therapy or central venous catheters as risk factors for BrC [12,20,26]. However, our analysis distinguishes immunosuppressed patients and critically ill non-neutropenic patients, and did not identify these risk factors.

An important finding of our multinational study is the poor compliance to therapeutic guidelines: only a third of BrC patients received an antifungal treatment different from the previous one within 48 h of diagnosis. Those patients who received a proper change of drug saw an improvement in the appropriateness of the empirical therapy up to similar percentages as for non-BrC patients.

In our study, patients with BrC had similar outcomes to the other patients. In an attempt to explain this counterintuitive finding, we can argue that patients with BrC more often received inappropriate empirical treatment, but the length of time before receiving an appropriate antifungal therapy was shorter than in the other patients. Moreover, patients with BrC more frequently received echinocandins, fungicidal drugs with a potential superiority over azoles in the treatment of candidaemia [27,28]. Finally, it is tempting to speculate that the more resistant *Candida* species involved in BrC might be less virulent, as reported in some animal models of systemic candidiasis [29].

In spite of the multicentre nature and the large number of candidaemia episodes analysed in this study, it has some limitations that should be acknowledged. First, it was retrospective and the sample size of BrC patients was small, a drawback also shared with other similar studies. Second, the small number of BrC episodes observed in patients with previous posaconazole or echinocandin treatment and the lack of data about the denominator population made any further analysis or correlation between those antifungals and the risk of BrC difficult. Finally, some lost data on the susceptibility of the strains analysed also interfered with the interpretation.

In conclusion, our multicentre study found that BrC accounted for 9% of the episodes, most of them occurring among severely immunosuppressed patients. The *Candida* species found in BrC were different depending on previous antifungal therapy. Remarkably, previous exposure to posaconazole was associated with fluconazole non-susceptible strains and previous echinocandin therapy, with BrC caused by *C. parapsilosis*. These results could help clinicians to improve the empirical treatment of BrC.

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Transparency Declaration

The authors declare no conflict of interest.

Ethics Approval

This retrospective observational study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Comité Ético de Investigación Clínica del Hospital Universitari de Bellvitge (Clinical Research Ethics Committee, Hospital Universitari de Bellvitge). To protect personal privacy, identifying information referring to each patient in the electronic database was encrypted. Informed consent was waived by the Clinical Research Ethics Committee because no intervention was involved and no information identifying patients was included.

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APPENDIX 1. Detailed description of BrC patients

No.	Gender/Age	Indication	Prior antifungal treatment	Days of previous treatment	Other prior antifungal treatment	*Candida* species isolated	Resistance to previous treatment	Empirical therapy	Appropriateness of empirical therapy	Directed therapy	EM (5 days)	LM (30 days)
1	M/36	Px	Fluconazole	20	N	*C. glabrata*	Y	Caspofungin	Y	Caspofungin	N	N
2	M/66	FDA	Fluconazole	7	N	*C. albicans*	N	Fluconazole	Y	Fluconazole	Y	Y
3	F/70	Px	Fluconazole	5	N	*C. krusei*	Y	Fluconazole	N	Fluconazole	N	Y
4	M/48	Px	Fluconazole	23	N	*C. krusei*	Y	Fluconazole	N	Amphotericin B	Y	Y
5	M/65	Px	Fluconazole	10	N	*C. glabrata*	Y	Fluconazole	N	Amphotericin B	N	N
6	M/53	FDA	Fluconazole	5	N	*Other*	Y	Fluconazole	N	Fluconazole	Y	Y
7	M/53	FDA	Fluconazole	13	N	*C. albicans*	N	Fluconazole	Y	Amphotericin B	N	Y
8	M/55	FDA	Fluconazole	12	N	*C. albicans*	UK	Fluconazole	UK	Fluconazole	N	N
9	M/66	FDA	Fluconazole	3	N	*C. tropicalis*	UK	Caspofungin	UK[a]	Fluconazole	N	N
10	F/40	Px	Fluconazole	10	N	*Other*	UK	Fluconazole	UK	Amphotericin B	N	N
11	M/44	Px	Fluconazole	16	N	*C. krusei*	Y	Fluconazole	N	Amphotericin B	N	Y
12	M/54	Px	Fluconazole	30	N	*C. tropicalis*	UK	Amphotericin B	UK	Amphotericin B	UK	UK
13	M/50	Px	Fluconazole	46	N	*C. parapsilosis*	UK	Fluconazole	UK	Amphotericin B	N	Y
14	M/37	Px	Fluconazole	20	N	*C. albicans*	UK	Caspofungin	UK[a]	Fluconazole	N	N
15	F/44	Px	Fluconazole	39	N	*Other*	UK	Fluconazole	UK	Fluconazole	N	N
16	M/56	Px	Fluconazole	5	N	*Other*	Y	Anidulafungin	N	Amphotericin B (liposomal)	N	N
17	M/65	FDA	Fluconazole	6	N	*C. albicans*	N	Anidulafungin	Y	Fluconazole	N	Y
18	F/54	FDA	Fluconazole	9	Anidulafungin	*C. glabrata*	Y	Fluconazole	N	Anidulafungin	N	N
19	M/77	Px	Fluconazole	3	N	*C. albicans*	N	Fluconazole	Y	Anidulafungin	N	Y
20[c]	F/46	Px	Fluconazole	6	N	*C. glabrata*	Y	Fluconazole	N	Fluconazole	N	N
21	M/60	FDA	Fluconazole	3	N	*C. albicans*	N	Fluconazole	Y	Fluconazole	N	Y
22	M/64	Px	Fluconazole	>3	N	*C. parapsilosis*	UK	Amphotericin B (liposomal)	UK	Amphotericin B (liposomal)	N	N
23	F/69	FDA	Fluconazole	7	N	*C. albicans*	N	Fluconazole	Y	Fluconazole	N	N
24	M/56	FDA	Fluconazole	>3	N	*C. parapsilosis*	N	Fluconazole	Y	Anidulafungin	N	N
25	F/42	FDA	Itraconazole	3	N	*C. albicans*	UK	Itraconazole	UK	Fluconazole	N	N
26	F/37	Px	Itraconazole	10	Fluconazole	*C. parapsilosis*	N	Itraconazole	Y	Voriconazole	N	N
27	M/33	Px	Itraconazole	11	N	*C. parapsilosis*	N	Caspofungin	Y	Anidulafungin	N	Y
28	F/50	Px	Posaconazole	10	N	*C. glabrata*	Y	Anidulafungin	Y	Amphotericin B	N	Y
29	M/67	Px	Posaconazole	150	N	*C. glabrata*	Y	Posaconazole	N	Amphotericin B (liposomal)	N	N
30	M/29	Px	Posaconazole	18	N	*C. tropicalis*	N	Caspofungin	Y	Amphotericin B	N	N
31	F/65	Px	Posaconazole	1	Voriconazole	*C. krusei*	Y	Amphotericin B (liposomal)	Y	Caspofungin	N	N
32	F/41	Px	Caspofungin	3	Fluconazole	*C. parapsilosis*	N	Caspofungin	Y	Amphotericin B (liposomal)	N	Y
33	M/20	Px	Anidulafungin	4	Fluconazole	*C. krusei*	Y[b]	Fluconazole	N	Anidulafungin	N	N
34	M/60	Px	Anidulafungin	13	N	*C. parapsilosis*	Y	Anidulafungin	N	Amphotericin B (liposomal)	N	N
35	F/18	Px	Anidulafungin	22	N	*C. parapsilosis*	UK[a]	Amphotericin B	Y	Anidulafungin	N	N
36	F/72	Px	Anidulafungin	35	Fluconazole	*C. tropicalis*	UK[a]	Amphotericin B	N	Amphotericin B	N	Y
37	M/55	Px	Anidulafungin	4	N	*C. krusei*	N	Anidulafungin	Y	Anidulafungin	Y	Y

Abbreviations: EM, Early Mortality (5 days); LM, late mortality (30 days); Px, prophylaxis in severely immunosuppressed patients; FDA, fever-driven approach in critically ill non-neutropenic patients; Y, yes; N, no; UK, unknown.

[a]Echinocandins susceptibility not tested.

[b]Strain resistant to fluconazole but susceptible to echinocandins.

[c]A renal transplant recipient who suffered a *C. glabrata* candidaemia (MIC 8 μg/mL) in the context of an abdominal invasive candidiasis. She underwent surgical intervention with a proper drainage and was treated with high doses of fluconazole.

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6.3. Analysis of antifungal therapy of patients with urinary source candidemia

- Clinical and microbiological characteristics of urinary source candidemia.
- Risk factors for clinical failure in patients with urinary source candidemia.
- Outcomes of echinocandin-treated patients with urinary source candidemia.

MAJOR ARTICLE

Echinocandins Compared to Fluconazole for Urinary Source Candidemia: a Propensity Score Analysis

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Key Words: Candidemia, urinary source, echinocandin therapy, propensity score.

Running Title: Echinocandins for Urinary Source Candidemia

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40-word summary: Echinocandins reach low urinary concentrations. However, echinocandin therapy for patients with urinary source candidemia was not associated with clinical failure. Acute renal failure was a predictor of worse outcomes, whereas performance of an early urologic procedure was a protective measure.

ABSTRACT

Background: Although current guidelines advice against using echinocandins for treating urinary source candidemia (USC), clinical evidence to support this recommendation is lacking. We aimed to assess the impact of echinocandin as compared to fluconazole treatment on USC outcomes and to provide current epidemiological information.

Methods: Multicenter study of adult patients with candidemia conducted in 18 hospitals. USC was defined as a candidemia occurring in a patient with concomitant candiduria by same *Candida spp*. and with significant urological comorbidity. The primary outcome was clinical failure (defined as 7 days mortality and/or persistent candidemia) assessed in patients treated either with echinocandins or fluconazole. A propensity score to receive echinocandins was calculated and entered into the regression model.

Results: Of a total of 2044 episodes of candidemia, 107 were USC (5.23%). Most cases were caused by *C. albicans* (56.4%), followed by *C. glabrata* (25.9%) and *C. tropicalis* (12.9%). Clinical failure occurred in 6 (21.4%) patients treated with an echinocandin and in 11 (15.3%) treated with fluconazole (p=.518). Acute renal failure (adjusted odds ratio [AOR]: 6.71, 95% CI: 1.68-26.74) was the only independent factor associated with clinical failure whereas performing a urologic procedure appeared as a protector (AOR: 0.05, 95% CI: 0.01-0.28). Neither univariate nor multivariate analysis showed that echinocandin therapy had any impact on the risk of clinical failure.

Conclusions: In patients with USC, initial echinocandin therapy was not associated with clinical failure. Acute renal failure was a predictor of worse outcomes, whereas performance of an early urologic procedure was a protective measure.

INTRODUCTION

Candidemia is a common cause of bloodstream infection all over the world [1][2][3][4] and is still associated with significant morbi-mortality [5][6][7]. Most candidemia episodes are considered to arise from an endogenous source or, less often, from the skin through the colonization of vascular catheters [8][9]. However, some patients with urinary colonization and urological obstructive disorders may have candidemia with the urinary tract being considered the source [10][11][12]. Recently, a number of matters have arisen regarding the management of urinary source candidemia (USC).

Current guidelines advise against the use of echinocandins for treating urinary tract infections due to Candida spp. [13][14] because of its low concentration reached in urine [15] and recommend the use of fluconazole or amphotericin B. However, usefulness of those antifungal drugs may be limited by several drawbacks in this population. On one hand, recent epidemiological studies have raised concerns about the increasing prevalence of Candida spp. non-susceptible to fluconazole [16][17][18][19], with a lack of specific information on microbiology data of patients with USC. Moreover, patients with USC often are elderly and have severe comorbidities including renal function impairment, precluding the use of amphotericin B. Finally, although this source of candidemia has been classically considered as “low-risk” [10], current information on its outcomes is lacking.

The primary aim of our study is to assess whether the use of echinocandins as compared to fluconazole treatment has or not a negative impact on USC outcomes. We also provide current information on the epidemiology of USC from a large cohort of patients with candidemia.

METHODS

Setting, patients, and study design

From an extensive multicenter cohort of patients with candidemia, we performed a study of all episodes of USC occurring in hospitalized adult patients from January 2005 to April 2015. The study was conducted in 18 tertiary teaching institutions in two countries: 17 from Spain (16 included in the prospective CANDIPOP survey [7]) and one from Argentina. The following information was collected from medical records: demographic characteristics, comorbidities, clinical features, causative species, antifungal therapy and outcomes. For the purpose of the study, we assessed the outcomes on patients treated with echinocandin as compared with those treated with fluconazole. We also provide current epidemiological information.

Definitions

Candidemia was defined as the presence of at least one positive blood culture for Candida spp. in a patient with clinical signs and symptoms of sepsis. Only the first episode of candidemia for each patient was analyzed. We defined USC as an episode of candidemia occurring in a patient with concomitant candiduria by the same Candida spp. and with a significant urological comorbidity (obstruction or manipulation of the urinary tract). Episodes considered to be USC were reviewed by three study investigators who were part of an eight-member clinical review panel (GC, CGV, MPA, AV, MFR, EGB, MJBV and AM). All members of the clinical review panel were infectious disease specialists and had extensive clinical experience dealing with patients with candidemia. The reviewers were asked to check the microbiology results, the baseline urologic comorbidity and to exclude other potential source of candidemia.

The diagnosis of septic shock was based on a systolic blood pressure of less than 90 mmHg and peripheral hypoperfusion and/or the need for vasopressors. Neutropenia was considered to occur when the granulocyte count was <500/ mm3. Initial antifungal therapy was considered to be appropriate when the Candida spp. isolated showed in vitro susceptibility to the antifungal drug administered. Acute renal failure was defined as an increase in serum creatinine concentration of 44.2 umol/L (if the baseline was less than 221 umol/L), an increase of more than 20% (if the baseline was more than 221 umol/L) or dialysis requirement at candidemia onset [20]. Urologic procedure was defined as a urinary catheter exchange or any surgical, percutaneous or endoscopic drainage of the urinary tract.

The primary outcome of our study was clinical failure defined as a composite endpoint, including 7-day mortality and/or persistent candidemia. Persistent candidemia was defined as persistently positive blood cultures after ≥72h of treatment initiation [21]. The early and overall mortality were defined as death from any cause within 7 and 30 days of the onset of candidemia, respectively.

Microbiological studies

Two sets of two blood samples were collected from patients with a suspected bloodstream infection. The blood samples were processed using a BACTEC 9240 system (Becton–Dickinson Microbiology Systems, New Jersey, USA) or BacTAlert (BioMerieux SA, Marcy L'Etoile, France) with an incubation period of 5 days. If yeast cells were observed after microscopic examination of a Gram stain, blood bottles were subcultured onto Sabouraud agar plates (BD BBL StrackerTM PlatesTM, Heidelberg, Germany) and chromogenic media (ChromAgar BioMerieux SA, Paris, France). Yeast isolates were identified by conventional methods. The antifungal susceptibility of the

isolates was classified in accordance with the Clinical and Laboratory Standards Institute M27-S3 document [23]. In vitro antifungal activity was studied by applying a commercial microdilution method (YeastOneR SensititreR, TREK Diagnostic Systems Ltd, Ohio, USA) or an E-test (BioMerieux SA, Marcy L'Etoile, France), following the manufacturer's instructions. Of note, those *Candida spp.* isolates with a MIC ≥ 4 mg/L to fluconazole were considered non-susceptible to fluconazole, with the exception of *C. krusei, C. glabrata* and *C. guilliermondii* isolates, which were considered nonsusceptible to fluconazole regardless of their MIC value. Quality controls were performed in each centre using *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258.

Statistical analysis

Quantitative variables were reported as the median and interquartile range (IQR); categorical variables, as absolute numbers and percentages. To detect significant differences between groups, we used the Chi-square test or Fisher's exact test for categorical variables, and the Student t-test or Mann-Whitney test for continuous variables, as appropriate. For the purposes of the study, factors associated with clinical failure were evaluated by univariate and multivariate analysis. The multivariate analysis included all significant variables (p value <0.05) in the univariate analysis and all other factor considered relevant according to the literature. Given the lack of aleatorization of initial therapies, a propensity score of receiving echinocandins (PS Echinocandins) was estimated using a backward stepwise logistic regression model including variables with p values ≤ 0.115 in the univariate analysis and other variables considered relevant in the decision-making of an empirical treatment. Variables included were: Age ≥ 70y, Dialysis, Solid Organ Transplantation (SOT); Chemotherapy;

Corticosteroid therapy; Immunosuppressive therapy; Chronic Human Immunodeficiency Virus (HIV) infection and Intensive Care Unit (ICU) requirement at onset. The goodness of fit of the model was assessed by the Hosmer-Lemeshow test (P=.254). The discriminatory power of the score, evaluated by the area under the receiver operating characteristics (ROC) curve, was 0.83 (95 % CI, 0.74-0.91) showing thus a good ability to predict the use this drugs. The PS Echinocandins was then used as a covariate in a multivariate analysis to adjust for potential confounding factors associated with initial antifungal therapy [22][23]. Sensitivity analyses were performed by repeating the propensity score approach with different methods; 1:1 matching with replacement and a caliper of 0.25, and quintile stratification. All data were analyzed using SPSS software, version 15.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was established at α=0.05. All reported p-values are two-tailed.

RESULTS

Demographics and epidemiology

Of a total of 2044 candidemia episodes within the study period, 107 (5.23%) were USC, 68 (63.6%) in men and 60 (56.1%) in patients older than 70 years. Chronic kidney disease (51.4%), neoplasms (50.5%) and diabetes mellitus (40.2%) were the most frequent comorbidities. Regarding other risk factors of candidemia within the previous month, antibiotic therapy (89.7%), undergoing a surgical intervention (44.9%), corticosteroid therapy (27.1%) and the use of other immunosuppressive drugs (11.2%) were the most commonly found, whereas chemotherapy (8.4%) and neutropenia (2.8%) were less frequent.

The majority of cases of USC were caused by *C. albicans* (n=61; 56.4%), followed by *C. glabrata* (n=28; 25.9%), *C. tropicalis* (n=14; 12.9%), *C. parapsilosis* (n=3; 2.8%), *C. krusei* (n=1; 0.9%) and other *Candida spp.* (n=1; 0.9%). One patient has a mixed *albicans-glabrata* episode. Candidemia caused by fluconazole non-susceptible strains were observed in 31 (28.7%) isolates. Rate of resistance to echinocandins was low (n=3; 2.8%).

Therapeutic approaches and outcomes

Fluconazole was the most frequently drug administered (n=72; 67.3%) whereas echinocandins were used in 28 patients (26.1%). Other treatments administered were voriconazole (1 patient; 0.9%) and liposomal amphotericin B (1 patient; 0.9%). Five patients (4.7%) did not receive antifungal treatment. A urologic procedure was performed in more than a half of patients (n=58; 54.2%). Early mortality, clinical failure and overall mortality were 5.6, 18.7 and 15.9%, respectively.

Demographics, microbiology and outcome of patients treated either with echinocandins or fluconazole

Table 1 display a detailed description of demographic and clinical characteristics of 28 patients with USC treated with echinocandins and 72 patients treated with fluconazole. Patients treated with echinocandins received more frequently chemotherapy (21.4 vs. 2.8%, p=.006), corticosteroids (53.6 vs. 16.9%, p<.001) and immunosuppressive drugs (28.6 vs. 4.2%, p=.001) within the last month. The presence of fever, septic shock, acute renal failure and ICU requirement at candidemia onset was similar for patients receiving echinocandins or fluconazole as initial therapy.

Table 2 details the microbiologic results, therapy and outcomes of the both groups. No differences in *Candida spp.* strains isolated were found. Patients treated with echinocandins received a more frequent appropriate initial antifungal therapy (100 vs. 72.2%, p=.001), whereas performance of a urologic procedure was less often achieved in this group (35.7 vs. 58.3%, p=.030). Univariate analysis did not find differences in outcomes between patients receiving echinocandins or fluconazole as initial therapy.

Predictors of clinical failure (7 days mortality and/or persistent candidemia) in patients treated either with echinocandins or fluconazole

Only acute renal failure at onset was an independent predictor of clinical failure (AOR: 6.71, 95% CI: 1.68-26.74; p=.007) in the multivariate analysis displayed in Table 4. On the other hand, performing a urologic procedure appeared as a protective measure (AOR: 0.05, 95% CI: 0.01-0.28; p=.001). Neither univariate nor multivariate analysis showed that the initial use of echinocandin therapy had any impact on the risk of clinical failure. The incorporation of the PS Echinocandins into the model did not change this finding (Hosmer-Lemeshow goodness of fit of the model: .941). The

consistency of this result was confirmed by repeating the propensity score analyses by 1:1 matching with replacement and a caliper of 0.25, and quintile stratification (data not shown).

Discussion

If echinocandins could be used in patients with USC was the main point of our research. Our study highlights the pertinence of solving this issue, since we found that more than a half of patients with USC were elderly (≥ 70 years old), had chronic renal disease and nearly a third of this infections were caused by a fluconazole non-susceptible strain. Consequently, the use of fluconazole may be limited due to antifungal resistance and the use of amphotericin B might imply unacceptable side effects.

The successful use of caspofungin for the treatment of Candida cystitis [25] and USC caused by Candida albicans [26] has been documented in few reports. It is known that echinocandins reach poor concentration in urine, however their accumulation in renal parenchyma is high [15]. This fact might play a role in limiting the translocation from urine to blood and could explain the favorable outcomes observed in some single case reports [26][27]. We did not find differences in clinical failure among those patients with USC treated with echinocandins and those receiving fluconazole. Although ours is not a randomized study and definitive conclusions cannot be drawn, we have added further information supporting the use of echinocandins in patients with USC. These drugs probably should be a suitable option to consider in elderly patients with comorbidities and USC caused by azole-resistant strains.

There is only one previous observational study that described patients with USC [10]. That study included a low number of patients (26 cases of USC of a total cohort of 249 episodes of candidemia; 10.4%) and was performed in the early 90's (pre-echinocandin era). Our multicenter study with 2044 candidemia episodes

found that currently less than 6% of them could be attributed to this source. Both studies had similar finding with regards to the etiology. It seems logical, according to the plausible pathogenesis, that the most common species found were C. albicans and C. glabrata —usual colonizers of the gut and perineum— with C. parapsilosis being rare [9]. Remarkably, we found that non-albicans species represented nearly a half of cases and fluconazole non-susceptible strains were isolated in a third part of USC, even though only 8.4% of patients had received prior antifungal therapy. Finally, Ang et al. [10] described an overall mortality of 19%, whereas our study documented rates of early and overall mortality of 5.6 and 15.9%, respectively. These rates are high, despite they are lower than that documented in several cohorts of candidemia of all sources (close to 40%) [7] [28].

Independent prognostic factors in patients with USC have not been previously defined. We demonstrated that early drainage of urinary tract was a protector for clinical failure, indicating that a key aspect for the management of USC is the rapid reduction of the Candida spp. Inoculum. The finding of acute renal failure at onset as an independent predictor of clinical failure is likely an expression of the initial severity of sepsis and a marker of an unsolved urologic obstructive disorder.

In spite of the multicenter design and the large number of USC episodes analyzed, the main weaknesses of the study are that there is a lack of a generally accepted definition of USC and that the number of patients of our cohort treated with echinocandins was small. We are aware that the USC definition we used rests partly on subjective criteria and some episodes of endogenous candidemia may have been consequently misclassified as USC. However, we sought to minimize this

bias by using a precise definition that were applied in a uniform manner by experienced specialists on the management of candidemia from a panel of infectious diseases physicians.

In conclusion, our multicenter study of a large cohort of patients with USC found that initial therapy with echinocandins was not associated with clinical failure. Our findings should be confirmed in further studies involving a larger number of patients with USC treated with these drugs. However, given the relative rarity of USC, a randomized controlled trial is unlikely to be feasible or practical.

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Transparency declarations

CG-V has received grant support from Astellas and the Instituto de Salud Carlos III, and she has received honoraria for talks on behalf of Pfizer, Merck Sharp and Dohme and Astellas. **MF-R** has received honoraria for talks on behalf of Pfizer. **JP** has received grant support from Astellas Pharma, Gilead Sciences, MSD and Pfizer, and has received honoraria for talks on behalf of Astellas Pharma, Gilead Sciences, MSD and Pfizer. **JMA** has received grant support from Astellas Pharma, Gilead Sciences, MSD, Pfizer, Instituto de Salud Carlos III (Spanish Ministry of Economy and Competitiveness) and the Mutua Madrileña Foundation; has been an advisor/consultant to Astellas Pharma, Gilead Sciences, MSD and Pfizer; and has received honoraria for talks on behalf of Gilead Sciences, MSD, Pfizer and Astellas Pharma. **BA** has received grant support from Gilead Sciences, Pfizer and the Instituto de Salud Carlos III, and he has received honoraria for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas and Novartis. The remaining authors declare no conflict of interest.

ETHICS APPROVAL

This observational study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Comité Ético de Investigación Clínica of each participating hospital. To protect personal privacy, identifying information in the electronic database was encrypted for each patient. Informed consent was obtained for all patients included in the CANDIPOP Study. It was waived by the Clinical Research Ethics Committee for the remaining patients included because no intervention was involved and no patient-identifying information was included.

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Table 1: Demographic and clinical characteristic of 100 patients with USC according to initial antifungal treatment

	Echinocandin n=28 (%)	*Fluconazole n=72 (%)*	*p value*
Demographics			
Male Sex	19 (67.9)	44 (61.1)	.530
Age, median (IQR) years	67.4 (58-75)	74 (65-79)	.084
Age ≥ 70 years	12 (42.9)	45 (62.5)	.075
Comorbid conditions			
Chronic renal disease	16 (57.1)	35 (48.6)	.443
Dialysis	2 (7.1)	1 (1.4)	.192
Diabetes mellitus	12 (42.9)	31 (43.1)	.986
COPD	3 (10.7)	14 (19.4)	.383
Chronic heart disease	9 (32.1)	13 (18.1)	.127
Cerebrovascular disease	2 (7.1)	6 (8.3)	.999
Liver disease	2 (7.1)	2 (2.8)	.312
Malignancy	15 (53.6)	36 (50)	.748
SOT	3 (10.7)	1 (1.4)	.065
HIV infection	1 (3.6)	0	.280
Charlson > 5 p	10 (35.7)	22 (30.6)	.539
Risk factors (within 30 days)			
Prior surgery	12 (42.9)	32 (44.4)	.886
Neutropenia	0	3 (4.2)	.560
Chemotherapy	6 (21.4)	2 (2.8)	**.006**
Radiotherapy	0	3 (4.2)	.556
Corticosteroid therapy	15 (53.6)	12 (16.7)	**<.001**
Immunosuppressive therapy	8 (28.6)	3 (4.2)	**.001**

Prior antibiotic therapy	25 (89.3)	66 (91.7)	.707
Prior antifungal therapy	4 (14.3)	4 (5.6)	.215
Clinical characteristics (at onset)			
Fever	22 (78.6)	61 (84.7)	.462
Hypotension	8 (28.6)	24 (33.3)	.647
Vasopressors therapy requirement	9 (32.1)	20 (27.8)	.666
Acute renal failure	10 (35.7)	30 (41.7)	.585
ICU requirement	3 (10.7)	19 (26.4)	.111

IQR: Interquartile range. **COPD**: Chronic Obstructive Lung Disease. **HSCT:** Hematopoietic Stem Cell Transplantation. **SOT**: Solid Organ Transplantation. **HIV**: Human Immunodeficiency Virus. **ICU**: Intensive Care Unit.

Table 2: Microbiology, therapy and outcomes of patients with USC according to initial antifungal treatment

	Echinocandin n=28 (%)	*Fluconazole n=72 (%)*	*p value*
***Candida* species ***			
C. albicans	13 (46.4)	43 (58.9)	.365
C. glabrata	9 (32.1)	19 (26)	.714
C. tropicalis	4 (14.3)	9 (12.3)	.750
C. parapsilosis	2 (7.1)	1 (1.4)	.185
Fluconazole non-susceptible strains **	10 (35.7)	20 (27.4)	.564
Echinocandin resistant strains ***	1 (3.6)	2 (2.7)	.999
Appropriate initial antifungal treatment	28 (100)	52 (72.2)	**.001**
Median days until appropriate antifungal treatment (IQR)	2.5 (1.5-3)	2 (1-3)	.111
Urologic procedure	10 (35.7)	42 (58.3)	**.030**
Outcomes			
Persistent candidemia	6 (21.4)	9 (12.5)	.261
Septic metastases	2 (7.1)	7 (9.7)	.987
Early mortality (7d)	1 (3.6)	2 (2.8)	.999
Clinical Failure	6 (21.4)	11 (15.3)	.518
Overall mortality (30d)	6 (21.4)	8 (11.1)	.166

* One patient in the fluconazole group had a mixed *albicans-glabrata* candidemia
* Fluconazole susceptibility was determined in 99 strains
** Echinocandins susceptibility was determined in 79 strains
IQR: Interquartile range. **ICU**: Intensive Care Unit.

Table 3: Independent Risk Factors for Clinical Failure

Risk Factor	*Adjusted odds ratio (95% CI)*	*p-value*
Malignancy	1.74 (0.44-6.84)	.426
Acute Renal Failure	**6.71 (1.68-26.74)**	**.007**
Urologic procedure	**0.05 (0.01-0.28)**	**.001**
Initial Echinocandin therapy	0.47 (0.09-2.56)	.383
PS Echinocandins	5.91 (0.23-152.61)	.284

PS Echinocandins: Propensity Score of receiving Echinocandin therapy

6.4. Study of the impact on outcomes of statin pre-treatment in patients with candidemia

- Clinical and microbiological characteristics of patients with prior statin use.
- Independent risk factors for early and late mortality.
- Association of prior statin use with the risk of mortality.

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PLOS ONE

Effect of Statin Use on Outcomes of Adults with Candidemia

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Abstract

Background: Statins have immunomodulatory properties and hinder *Candida* growth. However, it is unknown whether they may improve prognosis in patients with candidemia. We sought to determine the effect of prior statin use on the clinical outcomes of patients suffering candidemia.

Methods and Findings: Multicenter cohort study of hospitalized adults with candidemia between 2005 and 2011 in six hospitals in Spain, Brazil and Argentina. Of 326 candidemias, 44 (13.5%) occurred in statin users and 282 (86.5%) in statin non-users. The median value of APACHE II at candidemia diagnosis was similar between groups (18 vs. 16; p=.36). *Candida albicans* was the most commonly isolated species, followed by *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, and *C. krusei*. There were no differences regarding appropriate empirical antifungal treatment. Statin users had a lower early (5 d) case-fatality rate than non-users (4.5 vs. 17%; p=.031). This effect was not observed with other cardiovascular drugs (aspirin, beta blockers and ACE inhibitors). Independent factor related to early case-fatality rate was APACHE II score (AOR, 1.08; 95% CI, 1.03–1.14; p=.002). An appropriate empirical antifungal therapy (AOR, 0.11; 95% CI, 0.04–0.26; p=<.001) and prior statin use were independently associated with lower early case-fatality (AOR, 0.17; 95% CI, 0.03–0.93; p=.041). Fourteen days (14d) and overall (30d) case-fatality rates were similar between groups (27% vs. 29%; p=0.77 and 40% vs. 44%; p=.66).

Conclusions: The use of statins might have a beneficial effect on outcomes of patients with candidemia. This hypothesis deserves further evaluation in randomized trials.

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Introduction

Candidemia is a common cause of nosocomial bloodstream infections worldwide [1,2] and is associated with significant morbidity, prolonged hospital stay, high mortality and increased health care costs [3]. Importantly, some investigators report that overall mortality has not decreased over the past decades [1-4].

Hydroxymethylglutaryl-CoA reductase inhibitors, also known as statins, are increasingly used in clinical practice to treat dyslipidemia. Statin therapy has been shown to decrease cardiovascular events and mortality from coronary artery disease [5]. Interestingly, statins exhibit potent anti-inflammatory, anticoagulant, and anti-oxidative effects called "pleiotropic properties" [6]. Due to these properties, it has been suggested that these drugs may have beneficial effects during sepsis. Experimental and observational studies [7,8] have shown that statin therapy reduces inflammatory cytokines [9]. There is an increasing interest in determining whether statins improve prognosis of patients with severe infections. The results of certain observational studies suggest that statins may reduce mortality in patients suffering from sepsis [8,10,11],

bacteremia [12], community-acquired bacterial pneumonia [7,13,14] and even influenza [15,16]. On the other hand, in vitro studies have found that statins have an intrinsic antifungal effect, hindering fungal growth [17].

However, information evaluating the effects of statin therapy on clinical outcomes of patients with candidemia is scarce. The purpose of this study is to assess whether prior statin use is associated with a decreased risk of mortality in a large multicenter cohort of adult patients with candidemia.

Methods

Setting, patients, and study design

We performed a retrospective multicenter study of all episodes of candidemia occurring in hospitalized adult patients between January 2005 and December 2011 at six tertiary teaching institutions in three different countries: three in Spain, two in Argentina and one in Brazil. Only the first episode of candidemia for each patient was analyzed. The following information was carefully collected from medical records: demographic characteristics, comorbidities, statin use and other concurrent cardiovascular medications (aspirin, beta blockers and angiotensin II-converting enzyme inhibitors), clinical features, sources of candidemia, causative species, antifungal therapy and outcomes. Episodes of candidemia occurring in statin users were compared with those occurring in statin non-users. To protect personal privacy, identifying information of each patient in the electronic database was encrypted. Informed consent was waived by the Clinical Research Ethics Committee because no intervention was involved and no patient identifying information was included.

Definitions

Candidemia and catheter-related candidemia were defined on the basis of the guidelines of the Infectious Diseases Society of America [1]. Secondary candidemia was defined as a documented concurrent infection caused by the same *Candida* species at a site other than the catheter [18]. An episode of candidemia was considered to be nosocomially acquired, community-acquired or healthcare-associated as described elsewhere [19]. Statin use was considered to be present in those patients who were taking a statin (simvastatin, atorvastatin, lovastatin, pravastatin or rosuvastatin) within the 7 days prior to the candidemia episode. Seven days period was used due to pleitropic effects of statins may persist despite temporary cessation of administration. The use of other cardiovascular drugs like aspirin, beta-blockers and angiotensin II-converting enzyme (ACE) inhibitors was considered to be present in patients who were taking these drugs within the 30 days prior to the candidemia episode. The diagnosis of septic shock was based on a systolic blood pressure of less than 90 mm Hg and peripheral hypoperfusion with the need for vasopressors [20]. Neutropenia was considered when the granulocyte count was <500/ mm^3. Empirical antifungal therapy was considered to be appropriate when the *Candida* isolates showed in vitro susceptibility to the antifungal drug administered. When antifungal susceptibility testing was not available, we considered fluconazole, amphotericin B or an equinocandin as appropriate empirical antifungal treatment for *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis* and *Candida lusitaniae*. For *Candida glabrata* and *Candida krusei*, empirical antifungal treatment was considered to be appropriate when an equinocandin or amphotericin B was administered. The early, 14 days and overall case-fatality rates were defined as death from any cause within five, fourteen and 30 days after the onset of candidemia respectively.

Microbiological studies

Two sets of two blood samples were drawn from patients with suspected bloodstream infection. Blood samples were processed by the BACTEC 9240 system (Becton Dickinson Microbiology Systems, Franklin Lakes, NJ, USA) with an incubation period of five days. If yeast cells were observed after microscopic examination of Gram stain, blood bottles were subcultured onto Sabouraud agar plates (BD BBL StrackerTM PlatesTM, Heidelberg, Germany) and chromogenic media (CAN2 ChromID™Candida Agar, BioMerieux, Paris, France). Yeast isolates were identified by conventional methods. In vitro antifungal activity was studied by a commercial microdilution method (YeastOne®Sensitre®, TREK Diagnostic Systems Ltd, England) or by E-test (BioMerieux SA, Paris, France), in accordance with the manufacturer's instructions. Antifungal susceptibility of isolates was classified according to the Clinical and Laboratory Standards Institute M27-A3 document [21].

Statistical analysis

The results were analyzed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). To detect significant differences between groups, we used the Chi-square test or Fisher's exact test for categorical variables and the Student t-test or Mann-Whitney test for continuous variables, as appropriate. We performed a multivariate logistic regression analysis of factors potentially associated with mortality included all variables that were significant in the univariate analysis. Due to the baseline imbalances between patients, a propensity score for receiving statin therapy was added to the model. The propensity score (PS), probability of receiving statins, was calculated using multivariate logistic regression model and included the following variables: age, Charlson index, place of acquisition (inpatient or outpatient, with the first as a reference), neutropenia and urinary catheter. The model showed a P value of 0,646 for the Hosmer-Lemeshow test and an area under curve of 0.73, showing good predictive ability. The relative risks were expressed as adjusted odds ratios (AOR) and 95% confidence intervals. Goodness-of-fit of the final model was assessed by the Hosmer-Lemeshow test. Statistical significance was established at $\alpha=0.05$. All reported p-values are two-tailed.

Results

Patient characteristics

Over the study period we documented 326 candidemias, 44 (13.5%) occurring in statin users and 282 (86.5%) in statin non-users. The epidemiological and clinical characteristics of the patients are outlined in Table 1. Comparing with statin non-users, statin users were older and more frequently had chronic renal disease, diabetes mellitus and chronic heart disease. The presence of a urinary catheter was also more frequent in this group. Conversely, statin users had less chronic liver disease and neutropenia. The median value of APACHE II at candidemia diagnosis was similar in the two groups.

Candida species

Among the species, the most frequent was *Candida albicans* followed by *C. parapsilosis, C. tropicalis, C. glabrata*, and *C. krusei* without significant differences between statin users and statin non-users. The place of acquisition, the likely source of infection and the species isolated are detailed in Table 2.

Treatment and outcomes

The treatment and clinical outcomes are detailed in Table 3. More than 70% of patients received an appropriate empirical antifungal treatment without significant differences between groups. The most frequently used drug was fluconazole, followed by anidulafungin. No significant differences were found in catheter removal between groups. No differences were observed in persistent candidemia, instability after 48 hours of treatment or septic metastases. The early case fatality rate was lower among statin users (n=2: 4.5% vs. n=48: 17%; p=.031). After adjusting for Propensity Score, the statins also decrease the probability of early case-fatality rate significantly (p = 0.014). This effect was not observed with any of the other cardiovascular drugs analyzed, including aspirin (odds ratio [OR], 1.5; 95% confidence interval [CI], 0.65–3.5; p=0.34), beta blockers (OR, 0.69; 95% CI, 0.23–2.0; p=0.49) or with ACE inhibitors (OR, 0.69; 95% CI, 0.3–1.5; p=0.36). Fourteen days (14d) and overall (30d) case-fatality rates were similar between groups (27% vs. 29%; p=0.77 and 40% vs. 44%; p=.66, respectively). The results were equivalent when neutropenic patients were excluded.

Independent factors associated with early case-fatality rate

Multivariate analysis adjusted for risk factors associated with mortality and including the propensity score is described in Table 4. The APACHE II score was an independent factors related to mortality (AOR, 1.08; 95% CI, 1.03-1.14; p=.002). An appropriate empirical antifungal therapy (AOR, 0.11; 95% CI, 0.04-0.26; p=<.001) and prior statin use were independently associated with lower early case-fatality (AOR, 0.17; 95% CI, 0.03–0.93; p=.041).

Table 1. Clinical characteristics of patients by statin group.

	Statin users (n=44) No. (%)	Statin non-users (n=282) No. (%)	*p*-value
Demographics			
Male sex	24 (54.5)	165 (58.5)	.620
Age, median (IQR) years	64 (56-74)	57 (43-70)	**.006**
Comorbid conditions			
Chronic renal disease	18 (40.9)	63 (22.3)	**.008**
Dialysis	6 (13.6)	17 (6)	.067
Diabetes mellitus	22 (50)	67 (23.8)	**<.001**
COPD	10 (22.7)	57 (20.2)	.710
Chronic heart disease	25 (56.8)	61 (21.6)	<.001
Cerebrovascular disease	8 (18.2)	40 (14.2)	.505
Liver disease	2 (4.5)	52 (18.4)	**.021**
Malignancy	16 (36.4)	111 (39.4)	.692
Stem cell transplantation	2 (4.5)	20 (7.1)	.394
Graft versus Host disease	0 (0)	8 (2.8)	.273
Solid organ transplantation	6 (13.6)	26 (9.2)	.364
HIV infection	0 (0)	14 (5)	.131
Risk factors			
ICU stay	20 (45.5)	132 (46.8)	.802
Mechanical ventilation	18 (40.9)	103 (36.5)	.636
Total parenteral nutrition	13 (29.5)	88 (31.2)	.768
Vasopressor therapy	10 (22.7)	78 (27.7)	.430
Previous surgery	23 (52.3)	112 (39.7)	.120
Catheter placement (>48h)	41 (93.2)	248 (87.9)	.360
Urinary catheter	32 (72.7)	130 (46.1)	**.002**
Neutropenia	0 (0)	42 (14.9)	**.006**
Chemotherapy	4 (9.1)	58 (20.6)	.070
Radiotherapy	1 (2.3)	9 (3.2)	.735
Corticosteroid therapy	15 (34.1)	133 (47.2)	.122
Prior antibiotic therapy	41 (93.2)	256 (90.8)	.801
Immunosuppressive therapy	10 (22.7)	65 (23)	.953
Antifungal prophylaxis	2 (4.5)	35 (12.4)	.120
Clinical characteristics (at candidemia diagnosis)			
Fever	38 (86.4)	213 (75.5)	.169
Hypotension	26 (59.1)	125 (44.3)	.293
Vasopressor therapy requirement	16 (36.4)	83 (29.4)	.402
Acute renal failure	18 (40.9)	81 (28.7)	.127
Confusion	20 (45.5)	98 (34.8)	.217
APACHE II, median (IQR)	18 (11-23)	16 (11-23)	.365

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Discussion

In this multicenter study involving a large number of hospitalized adults with candidemia, we found that patients who had received statins had lower early case-fatality rate compared with those who were not receiving statins. Interestingly, the survival benefit observed persisted after adjustment for confounders by multivariate analysis.

To date, candidemia remains to be associated with significant morbidity and mortality. In the present study, the overall case-fatality rate exceeded 40%, similar to those

Table 2. Sources of candidemia and *Candida* species according to statin groups.

	Statin users (n=44) No. (%)	Statin non-users (n=282) No. (%)	*p*-value
Site of acquisition *			.018
Nosocomially-acquired	37 (84.1)	242 (85.8)	
Community-acquired	7 (15.9)	15 (5.3)	
Healthcare-associated	0 (0)	23 (8.2)	
Source of infection			.615
Urinary tract	8 (18.2)	29 (10.3)	
Digestive tract and abdomen	4 (9.1)	44 (15.6)	
Catheter related infection	9 (20.5)	64 (22.7)	
Skin and soft tissue	1 (2.3)	3 (1.1)	
Surgical site infection	1 (2.3)	3 (1.1)	
Endocarditis	0 (0)	3 (1.1)	
Unknown	21 (47.7)	134 (47.5)	
Others	0 (0)	2 (0.7)	
***Candida* species**			
C. albicans	21 (47.7)	121 (42.9)	.549
C. parapsilosis	13 (29.5)	53 (18.8)	.099
C. glabrata	3 (6.8)	32 (11.3)	.367
C. tropicalis	6 (13.6)	41 (14.5)	.874
C. krusei	1 (2.3)	11 (3.9)	.594
C. lusitaniae	0 (0)	2 (0.7)	.575
Others	0 (0)	22 (7.8)	.055

*. The site of acquisition was not known in 2 patients.

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Table 3. Treatments and clinical outcomes of patients by statin groups.

	Statin users (n=44) No. (%)	Statin non-users (n=282) No. (%)	*p*-value
Appropriate empirical antifungal treatment	34 (77.3)	203 (71.9)	.464
Antifungal drug selected			.864
Fluconazole	21 (61.7)	117 (57.6)	
Itraconazole	0 (0)	1 (0.5)	
Voriconazole	1 (2.9)	7 (3.4)	
Anidulafungin	4 (11)	22 (10.8)	
Caspofungin	5 (15)	18 (8.8)	
Micafungin	0 (0)	10 (4.9)	
Amphotericin B deoxycholate	3 (8.8)	20 (9.8)	
Liposomal Amphotericin B	0 (0)	8 (3.9)	
Catheter removal	33 (75)	157 (55.7)	.138
Outcomes			
ICU admission	13 (29.5)	88 (31.2)	.734
Mechanical ventilation	12 (27.3)	85 (30.1)	.596
Instability (at 48h)	15 (34.1)	103 (36.5)	.840
Persistent candidemia	5 (11.4)	41 (14.5)	.645
Septic metastases	2 (4.5)	13 (4.6)	.529
Early case-fatality rate (5d)	2 (4.5)	48 (17)	**.031**
Fourteen days case-fatality rate (14d)	12 (27)	83 (29)	.77
Overall case-fatality rate (30d)	18 (40.1)	124 (44)	.663

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Table 4. Independent factors associated with early case-fatality rate: multivariate analysis.

Characteristic	Adjusted odds ratio (AOR)	95% confidence interval	*p*-value
Age	0.98	0.95-1.005	.104
Sex	1.50	0.63-3.60	.360
Charlson Comorbidity Index	1.09	0.93-1.27	.307
APACHE II score	**1.08**	**1.03-1.14**	**.002**
Aproppiate empirical antifungal therapy	**0.11**	**0.04-0.26**	**<.001**
PS	**260**	**1.06-63525**	**.048**
Statin therapy	**0.17**	**0.03–0.93**	**.041**

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reported in most series [3,22–26]. The early case-fatality rate was 4.5%. This figure is difficult to compare with others obtained in previous series because little information is available regarding frequency and associated factors. We found that APACHE II score was an independent factors related to mortality whereas an appropriate empirical antifungal therapy and prior statin use were independently associated with lower early case-fatality.

Statins were also associated with a lower overall (30d) case-fatality rate in ICU patients with candidemia in a previous single-center study conducted by Forrest et al [27]. That study had a small sample size (45 patients, including 15 statin users) and the exposure groups presented significant differences in APACHE II score. However, the overall survival benefit was not statistically significant when adjusted for APACHE II score. Another recent single-center study [28] did not show any benefit in the outcomes of patient with candidemia receiving statins. It was a small study (14 statin users) and did not evaluate the differences in the severity of the disease between groups. None of these studies analyzed the effect of statins in early mortality.

It might be speculated that the lower early case-fatality rates observed in statin users are due to the pleiotropic effects of statins. There is substantial evidence from basic science of the immunonodulatory role of statins in patients with sepsis, who present reductions in proinflammatory cytokines (TNF-α and IL-6) [9], induction of haem oxygenase, direct alteration of leucocyte-endothelial cell interaction and a reduction in the expression of MHC II [29]. Previous investigations have noted the role of statins in the maintenance of microvascular integrity with restoration of the normal endothelium functioning, and the inhibition of cell adhesion molecules [30–32]. Thus statins may have a critical role in the early course of candidemia. Moreover, statins have demonstrated a direct antifungal effect: the inhibition of HMG-CoA reductase affects the synthesis of

ergosterol, which strongly inhibits the growth of *Candida* species. Statins can also cause deletions in the mitochondrial genome of yeasts, hampering fungal growth [17]. Furthermore, synergy between statins and fluconazole has been reported, although not at clinically achievable concentrations [33].

Our study did not shown significant differences in overall case fatality rate between statins users and non-statins users. It should be noted, however, that host factors are the most important factors related with late death in patients with infection [34–36]. Poor prognosis within the first 30-days of candidemia is a marker of the fragile status of patients with candidemia. Therefore, it seems reasonable that a potential immunomodulatory treatment have not effect in late deaths.

Some researchers have suggested that the beneficial effects of statins observed in infectious diseases may actually reflect a healthy user bias. If this was true, this "healthy user behaviour" would result in apparent benefit for all classes of cardiovascular drugs [7,13]. However, none of the concomitant cardiovascular drugs (aspirin, beta-blockers and ACE inhibitors) were independently associated with mortality in the present study.

Our study has some limitations that should be noted. Firstly, it was retrospective and ha a small sample size of patients receiving statins. Secondly, most patients received empirical treatment with fluconazole. This practice may not necessarily reflect antifungal empirical choices at this time, after ESCMID recommendations for equinocandins use [37,38]. Thirdly, it did not specifically account for types of statins. Fourth, we also understand that the gut tolerance needed for statin administration could select a subgroup of patients in better conditions, even in the absence of differences in the APACHE II score between groups. Finally, the ideal timing for initiating statins with respect to the onset of sepsis is still unknown. Our patients were on chronic treatment with statins at onset of candidemia. The role of statins administered *de novo* in the context of a *Candida* sepsis should be analyzed in further studies.

In conclusion, the results of this multicenter study with a large cohort of hospitalized patients showed that prior statins use may improve the early case fatality rate in patients with candidemia. However, overall mortality was not different between patients receiving statins and those without this drug. This early beneficial effect of statins deserves to be evaluated in randomized trials.

Ethics Approval

This retrospective observational study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Comité Ético de Investigación Clínica del Hospital Universitari de Bellvitge (Clinical Research Ethics Committee, Hospital Universitari de Bellvitge). To protect personal privacy, identifying information of each patient in the electronic database was encrypted. Informed consent was waived by the Clinical Research Ethics Committee because no intervention was involved and no patient identifying information was included.

Author Contributions

Conceived and designed the experiments: GC CGV MN FP MFR A. Mykietiuk A. Manzur CG JP DV JA JC. Performed the experiments: GC CGV MN FP MFR A. Mykietiuk A. Manzur) CG JP DV JA JC. Analyzed the data: GC CGV. Contributed reagents/materials/analysis tools: GC CGV MN FP MFR A. Mykietiuk A. Manzur CG JP DV JA JC. Wrote the manuscript: GC CGV.

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7. DISCUSSION

7.1. A simple prediction score for estimating the risk of candidemia caused by fluconazole non-susceptible strains

We developed and validated a simple score that can estimate the risk of Flu-NS candidemia using readily available clinical parameters at the bedside. Specifically, the presence of hospitalization to a HPU, prior transplantation, and at least 3 days of prior azole therapy as independent predictors of Flu-NS by multivariate analysis allowed us to create a valid prediction score. The absence of any of these predictive factors decreased the risk of Flu-NS candidemia substantially, supporting the empirical use of fluconazole as the first antifungal agent. To our knowledge, this is the first investigation which have created a clinically relevant tool to guide empirical antifungal therapy in this setting. Interestingly, the score appears to retain its applicability when using the EUCAST breakpoints.

Many previous studies have attempted to identify risk factors associated with azole resistance. Some have analyzed non-albicans candidemia as a surrogate marker of azole resistance and found risk factors such as chemotherapy (Shigemura et al. 2014), solid tumour (Davis et al. 2007) or prior surgery (Playford et al. 2008). Other works with microbiological confirmation of resistance have described gastrointestinal surgery (Slavin et al. 2010) or the cumulative length of hospitalization (Kourkoumpetis et al. 2011) as being relevant; however, these were single-centre studies limited to oncological or critically-ill surgical patients, respectively. Another prospective study reported that neutropenia, chronic renal disease, and prior fluconazole exposure were independently associated with such strains (Garnacho-Montero et al. 2010). Prior azole therapy was also found to be a risk factor in other works (Shah et al.

2012),(Tumbarello et al. 2009), all those based on the old CLSI breakpoints. Furthermore, we found that both prior antifungal therapy and its duration were independent risk factors, a fact already demonstrated by other authors who observed a dose-dependent relationship between fluconazole consumption and breakthrough azole-resistant candidemia (Clancy et al. 2006). This evidence supports the guideline recommendation of avoiding fluconazole as the initial empiric therapy in those patients with prior exposure (Pappas et al. 2009).

Importantly, we stressed the relevance of local epidemiology in the pre-test probability of developing Flu-NS candidemia. We found that being hospitalized in a unit of with a Flu-NS candidemia prevalence of 15% or greater had the highest weighting as a predictor in our score. In fact, data from CANDIPOP survey showed a great difference in the prevalence of Flu-NS strains among the different participating cities (Guinea et al. 2014). This is clearly evident when we compare countries of northern Europe with Latin-America (Arendrup et al. 2011),(Nucci et al. 2013), or wider comparisons as the SENTRY Antimicrobial Surveillance Program (Pfaller et al. 2011). Our results highlight the importance of local surveillance studies.

In a multivariate analysis we found that transplantation recipient status was an independent predictor of Flu-NS candidemia. Previous reports on haematological neutropenic patients receiving stem cell transplantation found similar results (Marr et al. 2000),(Gamaletsou et al. 2014). Nevertheless, information concerning solid organ transplantation is conflicting, with some authors reporting greater azole resistance (Raghuram et al. 2012) and others failing to report this association (van Hal et al.

2009). However, these findings are difficult to compare because of differences in the design, definitions, and populations used in those studies.

To date, no evidence supports the argument that azole-resistant strains are associated with a greater incidence of septic shock. Despite this, the fungicidal activity of echinocandins against most *Candida* species is argued to be a reason for offering these drugs to unstable patients (Pappas et al. 2009). Further studies are needed to validate this hypothesis. However, the addition of septic shock to our clinical prediction score provides a safety factor for clinicians, who usually prefer broad-spectrum coverage in critically-ill patients.

Our clinical prediction score faces physicians with quite different settings. On the one hand, there is a patient subgroup with low risk (score < 1) in whom empirical fluconazole appears to be a safe initial treatment. On the other hand, there is a patient subgroup with several risk factors (score ≥ 2) and a high probability of Flu-NS candidemia. Together with critically ill patients, those cases warrant broad-spectrum therapy, with fluconazole reserved as a step-down option. Finally, for those patients with 3 or more points on the Flu-NS score, the use of an echinocandin should be mandatory because of the high risk of Flu-NS isolates. To note, echinocandin-resistance (though not common) may be observed in some Flu-NS isolates.

The strengths of the study include the prospective nature of the derivation cohort, the large number of consecutive patients evaluated, the comprehensive data collection, and the multicenter design. Moreover, it is also important that validation was provided in an international cohort, including patients from different geographic areas. Nevertheless, the predictive power of the score needs to be confirmed in even more

diverse clinical settings to ensure its wider validity and reliability. The limitations of the present study include differences in MIC determination techniques between the derivation and validation cohorts. As the MIC breakpoints are methodspecific, using the CLSI breakpoints for interpretation of the MICs generated by Sensititre YeastOne or Etest in the validation cohort may have led to discordant results in a number of isolates. Second, it is true that our score mostly identified Flu- NS strains corresponding to non-albicans species, with primary resistance or dose-dependent susceptibility to fluconazole. However, the Flu-NS score was also useful for patients with isolates with acquired resistance to fluconazole (11 isolates: three C. albicans, six *C. parapsilosis* and two *C. tropicalis*). Among them, more than 90% (10/11) had 2 points and more than 63% (7/11) had 3 points in the score. Third, it is certainly possible that the rapid evolution of molecular diagnostic methods (Nguyen et al. 2012) may lead to clinical tools such as this becoming redundant; however, the availability of such techniques will remain restricted in resource-limited settings. Finally, a major drawback is in the nature of a prediction score that aims to simplify a complex clinical situation. We stress that such a tool should support, but never replace, clinical judgment.

In summary, our easy to use, simple Flu-NS prediction score can estimate the risk of Flu-NS candidemia using readily available clinical parameters at the bedside. This gives physicians a validated and reliable tool to enable the rational and safe choice of an initial antifungal agent in the management of candidemia in adult patients.

7.2. Breakthrough candidemia in the era of broad-spectrum antifungal therapies

In a multicenter study involving a large number of patients with candidemia from three different countries we found that BrC accounted for 9% of the episodes. Most of them occurred among severely immunosuppressed patients and were caused by non-*albicans* species. BrC was associated with infections due to fluconazole non-susceptible isolates. The *Candida spp.* isolated in BrC were different depending on the prior antifungal therapy. Interestingly, BrC caused by *C. albicans* was only observed among patients receiving itraconazole or fluconazole, whereas *C. parapsilosis* was mostly catheter-related and/or associated with prior echinocandin therapy. Fluconazole non-susceptibility appeared as an independent risk factor for BrC.

Our study concurs with others in reporting patients with BrC as acutely ill, most with heavy comorbid conditions, frequently neutropenic or previously treated with corticosteroids or other immunosuppressive drugs (Nucci & Colombo 2002),(Nguyen et al. 1996) (Imhof et al. 2004). A shift to non-*albicans* species had also been encountered in this population (Nguyen et al. 1996)(Girmenia et al. 1996),(Colombo et al. 2014), in particular caused by fluconazole non-susceptible strains. Our findings provide new and relevant information: 1) BrC caused by *C. albicans* was only observed in patients treated with fluconazole and not in patients treated with other antifungal; 2) patients who had been on posaconazole treatment had a high prevalence of BrC caused by fluconazole non-susceptible strains; and 3) BrC caused by *C. parapsilosis* was mostly catheter-related or associated with prior echinocandin therapy.

In the study, we identified fluconazole non-susceptibility as an independent factor related with BrC. Prior studies had documented neutropenia, corticosteroid therapy or

central venous catheters as risk factors for BrC (Nucci & Colombo 2002),(Uzun et al. 2001),(Rex 1996). However, our analysis distinguishes immunosuppressed patients and critically-ill non-neutropenic patients, and did not identify these risk factors.

An important finding of our multinational study is the poor compliance to therapeutic guidelines: only a third of BrC patients received an antifungal treatment different than the previous one within 48 h of diagnosis. Those patients who received a proper change of drug saw an improvement in the appropriateness of the empirical therapy up to similar percentages as for non-BrC patients.

In our study, patients with BrC had similar outcomes to the other patients. In an attempt to explain this counterintuitive finding, we can argue that patients with BrC more often received inappropriate empirical treatment, but the length of time before receiving an appropriate antifungal therapy was shorter than in the other patients. Moreover, patients with BrC more frequently received echinocandins, fungicidal drugs with a potential superiority over azoles in the treatment of candidemia (Reboli et al. 2011),(Andes et al. 2012). Finally, it is tempting to speculate that the more resistant *Candida* species involved in BrC candidemia might be less virulent, as reported in some animal models of systemic candidiasis (Arendrup et al. 2002).

In spite of the multicenter nature and the large number of candidemia episodes analyzed in this study, it has some limitations that should be acknowledged. Firstly, it was retrospective and had the sample size of BrC patients was small; a drawback also shared with other similar studies. Secondly, the limited number of BrC episodes observed in patients with prior posaconazole and echinocandin treatment made difficult any further analysis of those groups. Finally, some lost data on the susceptibility of the strains analyzed also interfered with the interpretation.

In conclusion, our multicenter study found that BrC accounted for 9% of the episodes, most of them occurring among severely immunosuppressed patients. The *Candida* species found in BrC were different depending on prior antifungal therapy. Remarkably, prior exposure to posaconazole was associated with fluconazole non-susceptible strains and prior echinocandin therapy, with BrC candidemia caused by *C. parapsilosis*. These results could help clinicians to improve the empirical treatment of BrC.

7.3. Echinocandins for urinary source candidemia: a propensity score analysis

If echinocandins could be used in patients with USC was the main point of our research. Our study highlights the pertinence of solving this issue, since we found that more than a half of patients with USC were elderly (≥ 70 years old), had chronic renal disease and nearly a third of this infections were caused by a fluconazole non-susceptible strain. Consequently, the use of fluconazole may be limited due to antifungal resistance and the use of amphotericin B might imply unacceptable side effects.

The successful use of caspofungin for the treatment of *Candida* cystitis (Sobel et al. 2007) and USC caused by *Candida albicans* (Vaidyanathan et al. 2013) has been documented in few reports. It is known that echinocandins reach poor concentration in urine, however their accumulation in renal parenchyma is high (Felton et al. 2014). This fact might play a role in limiting the translocation from urine to blood and could explain the favorable outcomes observed in some single case reports (Vaidyanathan et al. 2013)(Haruyama et al. 2006). We did not find differences in clinical failure among those patients with USC treated with echinocandins and those receiving fluconazole. Although ours is not a randomized study and definitive conclusions cannot be drawn, we have added further information supporting the use of echinocandins in patients with USC. These drugs probably should be a suitable option to consider in elderly patients with comorbidities and USC caused by azole-resistant strains.

There is only one previous observational study that described patients with USC (Ang et al. 1993). That study included a low number of patients (26 cases of USC of a total cohort of 249 episodes of candidemia; 10.4%) and was performed in the early 90's

(pre-echinocandin era). Our multicenter study with 2044 candidemia episodes found that currently less than 6% of them could be attributed to this source. Both studies had similar finding with regards to the etiology. It seems logical, according to the plausible pathogenesis, that the most common species found were *C. albicans* and *C. glabrata* —usual colonizers of the gut and perineum— with *C. parapsilosis* being rare (Nucci & Anaissie 2001). Remarkably, we found that non-*albicans* species represented nearly a half of cases and fluconazole non-susceptible strains were isolated in a third part of USC, even though only 8.4% of patients had received prior antifungal therapy. Finally, Ang *et al*. (Ang et al. 1993) described an overall mortality of 19%, whereas our study documented rates of early and overall mortality of 5.6 and 15.9%, respectively. These rates are high, despite they are lower than that documented in several cohorts of candidemia of all sources (close to 40%) (M Puig-Asensio et al. 2014) (Bassetti et al. 2015).

Independent prognostic factors in patients with USC have not been previously defined. We demonstrated that early drainage of urinary tract was a protector for clinical failure, indicating that a key aspect for the management of USC is the rapid reduction of the *Candida spp.* Inoculum. The finding of acute renal failure at onset as an independent predictor of clinical failure is likely an expression of the initial severity of sepsis and a marker of an unsolved urologic obstructive disorder.

In spite of the multicenter design and the large number of USC episodes analyzed, the main weaknesses of the study are that there is a lack of a generally accepted definition of USC and that the number of patients of our cohort treated with echinocandins was small. We are aware that the USC definition we used rests partly on subjective criteria and some episodes of endogenous candidemia may have been consequently

misclassified as USC. However, we sought to minimize this bias by using a precise definition that were applied in a uniform manner by experienced specialists on the management of candidemia from a panel of infectious diseases physicians.

In conclusion, our multicenter study of a large cohort of patients with USC found that initial therapy with echinocandins was not associated with clinical failure. Our findings should be confirmed in further studies involving a larger number of patients with USC treated with these drugs. However, given the relative rarity of USC, a randomized controlled trial is unlikely to be feasible or practical.

7.4. Effect of statin use on outcomes of adults with candidemia

In a multicenter study involving a large number of hospitalized adults with candidemia, we found that patients who had received statins had lower early case-fatality rate compared with those who were not receiving statins. Interestingly, the survival benefit observed persisted after adjustment for confounders by multivariate analysis.

To date, candidemia remains to be associated with significant morbidity and mortality. In the present study, the overall case-fatality rate exceeded 40%, similar to those reported in most series (Horn et al. 2009)(Almirante et al. 2005)(Pemán et al. 2005)(Nace et al. 2009)(Pappas et al. 2003)(Gudlaugsson et al. 2003). The early case-fatality rate was 4.5%. This figure is difficult to compare with others obtained in previous series because little information is available regarding frequency and associated factors. We found that APACHE II score was an independent factors related to mortality whereas an appropriate empirical antifungal therapy and prior statin use were independently associated with lower early case-fatality.

Statins were also associated with a lower overall (30d) case-fatality rate in ICU patients with candidemia in a previous single-centre study conducted by Forrest et al (Forrest et al. 2010). That study had a small sample size (45 patients, including 15 statin users) and the exposure groups presented significant differences in APACHE II score. However, the overall survival benefit was not statistically significant when adjusted for APACHE II score. Another recent single-centre study (Welch et al. 2013) did not show any benefit in the outcomes of patient with candidemia receiving statins. It was a small study (14 statin users) and did not evaluate the differences in the severity of the disease between groups. None of these studies analyzed the effect of statins in early mortality.

It might be speculated that the lower early case-fatality rates observed in statin users are due to the pleiotropic effects of statins. There is substantial evidence from basic science of the immunonodulatory role of statins in patients with sepsis, who present reductions in proinflammatory cytokines (TNF-α and IL-6) (Novack et al. 2009), induction of haem oxygenase, direct alteration of leucocyte-endothelial cell interaction and a reduction in the expression of MHC II (Terblanche et al. 2007). Previous investigations have noted the role of statins in the maintenance of microvascular integrity with restoration of the normal endothelium functioning, and the inhibition of cell adhesion molecules (Subramani et al. 2009)(Alvarez de Sotomayor et al. 2008). Thus statins may have a critical role in the early course of candidemia. Moreover, statins have demonstrated a direct antifungal effect: the inhibition of HMG-CoA reductase affects the synthesis of ergosterol, which strongly inhibits the growth of *Candida* species. Statins can also cause deletions in the mitochondrial genome of yeasts, hampering fungal growth (Westermeyer & Macreadie 2007). Furthermore, synergy between statins and fluconazole has been reported, although not at clinically achievable concentrations (Nash et al. 2002)[33].

Our study did not shown significant differences in overall case fatality rate between statins users and non-statins users. It should be noted, however, that host factors are the most important factors related with late death in patients with infection (AUSTRIAN & GOLD 1964)(Garcia-Vidal et al. 2008)(Wiersinga 2011). Poor prognosis within the first 30-days of candidemia is a marker of the fragile status of patients with candidemia. Therefore, it seems reasonable that a potential immunomodulatory treatment have not effect in late deaths.

Some researchers have suggested that the beneficial effects of statins observed in infectious diseases may actually reflect a healthy user bias. If this was true, this "healthy user behaviour" would result in apparent benefit for all classes of cardiovascular drugs (Viasus et al. 2010)(Thomsen et al. 2008). However, none of the concomitant cardiovascular drugs (aspirin, beta-blockers and ACE inhibitors) were independently associated with mortality in the present study.

Our study has some limitations that should be noted. Firstly, it was retrospective and ha a small sample size of patients receiving statins. Secondly, most patients received empirical treatment with fluconazole. This practice may not necessarily reflect antifungal empirical choices at this time, after ESCMID recommendations for equinocandins use (Ullmann et al. 2012)(Cornely et al. 2012). Thirdly, it did not specifically account for types of statins. Fourth, we also understand that the gut tolerance needed for statin administration could select a subgroup of patients in better conditions, even in the absence of differences in the APACHE II score between groups. Finally, the ideal timing for initiating statins with respect to the onset of sepsis is still unknown. Our patients were on chronic treatment with statins at onset of candidemia. The role of statins administered *de novo* in the context of a *Candida* sepsis should be analyzed in further studies.

The results of this multicenter study with a large cohort of hospitalized patients suggest that prior statins use might improve the early case fatality rate in patients with candidemia. However, overall mortality was not different between patients receiving statins and those without this drug. This early beneficial effect of statins deserves to be evaluated in randomized trials.

Some relevant new studies regarding the immunomodularor effects of statins have been added. Specifically, a number of randomized clinical trials were run to test the putatively beneficial effect of statin therapy, observed in retrospective studies, for patients suffering from community acquired pneumonia (Viasus et al. 2015), acute exacerbations of COPD (Criner et al. 2014) and sepsis-associated Acute Respiratory Distress Syndrome (McAuley et al. 2014)(Truwit et al. 2014). All of those studies invariably obtained negative results.

In light of those recent findings, it appears that the “healthy user effect” could be a bias difficult to overcome, even when trying to do so by a propensity score analysis. To conclude, we are currently much more sceptical about the beneficial effect of statins for patients suffering an episode of candidemia.

8. CONCLUSIONS

8.1. Study of the risk factors of candidemia caused by fluconazole non-susceptible strains: derivation and validation of a clinical score

- A simple prediction score can estimate the risk of candidemia caused by fluconazole non-susceptible strains using readily available clinical parameters at the bedside.

- This score gives physicians a validated and reliable tool to enable the rational and safe choice of an initial antifungal therapy in the management of candidemia in adult patients.

8.2. Descriptive study of breakthrough candidemia

- Our multicentre study found that breakthrough candidemia accounted for 9% of the episodes, most of them occurring among severely immunosuppressed patients.

- The *Candida* species found in breakthrough candidemia were different depending on prior antifungal therapy.

- Prior exposure to posaconazole was associated with fluconazole non-susceptible strains.

- *Candida parapsilosis* candidemia was mostly catheter-related and/or associated with prior echinocandin therapy.

- The empirical antifungal therapy was more often inappropriate in patients with breakthrough candidemia.

8.3. Analysis of antifungal therapy of patients with urinary source candidemia

- Initial therapy with echinocandins was not associated with clinical failure in patients with urinary source candidemia.

- Our findings should be confirmed in further studies involving a larger number of patients with USC treated with these drugs.

8.4. Study of the impact on outcomes of statin pre-treatment in patients with candidemia

- Although we found that prior statins improved the early case-fatality rate in patients with candidemia, overall case-fatality rate was not different between patients with and without previous treatment with these drugs.

- This early beneficial effect of statins should be regarded with caution and be confirmed in randomized controlled trials.

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10. ANNEXES

10.1. Study Protocols

PROTOCOLO CANDIDEMIAS

A) DATOS DEMOGRÁFICOS

Número de protocolo: I_I_I_I Iniciales: I_I_I_I

Hospital de Origen:

Edad: I_I_I Fecha de nacimiento: I_I_I_I_I_I_I_I

Sexo: 0. masculino; 1. femenino I_I Fecha del ingreso actual: I_I_I_I_I_I_I_I

Servicio donde se encuentra ingresado el paciente (Anexo 1): I_I_I

Fecha de extracción de los hemocultivos: I_I_I_I_I_I_I_I

Días desde el ingreso hasta la candidemia: I_I_I_I

Adquisición de la candidemia: I_I

1. Intrahospitalaria 2. Health-care 3. Extrahospitalaria

B) COMORBILIDADES

Nefropatía: 0. no; 1. sí I_I

Diálisis: 0. no; 1. sí I_I

DM: 0. no; 1. sí I_I

EPOC: 0. no; 1. sí I_I

Cardiopatía: 0. no; 1. sí I_I

Enfermedad cerebrovascular: 0. no; 1. sí I_I

Hepatopatía: 0. no; 1. sí I_I

Enfermedad neoplàsica: 0. no; 1. sí I_I

Tipo de neoplasia: 1. hematológica; 2. tumor sólido I_I

Trasplante de MO: 0.no; 1. sí; 2. desconocido I_I

Presencia de GVHD 0.no; 1. sí; 2. desconocido I_I

Neoplasia sólida:

Localización primaria (Anexo 2) I_I_I

Respuesta al tratamiento de la enfermedad de base: I_I

0. No tratada 1. Remisión completa 2. Remisión parcial

3.Fracaso/progresión 4. QMT paliativa

Transplante de Órgano Sólido: 0. no; 1. sí I_I

Órgano: I_I

1.Corazón 2.Corazón-Pulmón 3.Pulmón 4.Hígado 5.Riñón 6.Riñón-páncreas 7. Otros (especificar):

HIV/SIDA: 0. no; 1. sí I_I

Score de comorbilidad de Charlson:

C) FACTORES DE RIESGO (en los 30 días previos a la candidemia)

Estancia en UCI: 0. no; 1. sí I_I

Ventilación Mecánica: 0. no; 1. sí I_I

Nutrición Parenteral: 0. no; 1. sí I_I

Intervención quirúrgica : 0. no; 1. sí I_I

Tipo de cirugía: I_I

1.Abdominal 4.Neuroquirúrgica

2.Toráxica 5.Otras

3.Cabeza y cuello

Portador de vía venosa > 48h? 0. no; 1. sí I_I

Cateter corto > 48h? 0. no; 1. sí I_I

Cateter venoso largo acceso central > 48h? 0. no; 1. si I_I

Porth-A-Cath > 48h? 0. no; 1. sí I_I

Sondaje vesical: 0. no; 1. sí I_I

Otras manipulaciones (especificar): 0. no; 1. sí I_I

Neutropenia: 0. no; 1. sí I_I

Infección bacteriana concomitante: 0. no; 1. sí I_I

Localización (Anexo 3): Microorganismo aislado (Anexo 4):

En el mes previo a la candidemia ha recibido:

Quimioteràpia: 0.no; 1. sí I_I

Radioteràpia: 0. no; 1. sí I_I

Corticoides: 0.no; 1. sí I_I

Antibióticos: 0. no; 1. sí I_I

Antibiótico nº 1 recibido (Anexo 5) I_I_I_I

Antibiótico nº 2: I_I_I_I

Antibiótico nº 3: I_I_I_I

Tratamiento inmunosupresor 0.no; 1. sí I_I

Antiácidos, bloq. H2 o inhibidores BP 0.no; 1. sí I_I

Estatinas 0.no; 1. sí I_I; (tipo y dosis) Aspirina 0.no; 1. sí I_I

IECA's 0.no; 1. sí I_I;Betabloqueantes 0.no; 1. sí I_I

Nutrición enteral 0.no; 1. sí I_I

Tto antifúngico previo? 0. no; 1. sí I_I

Droga (Anexo 6): I_I (dosis y días de tto previo)

D) SITUACIÓN CLÍNICA DEL PACIENTE AL MOMENTO DE LA CANDIDEMIA (día del aislamiento)

Temperatura ≥38º C el dia de la candidemia 0.no; 1. sí I_I

TA < 90 mmHg 0.no; 1. sí I_I Necesidad de inotropos/vasopresores: 0.no;1. sí I_I

Deterioro de F. Renal *de novo:* 0.no; 1. sí I_I Confusión: 0.no; 1. Sí

Ingreso en UCI: 0.no; 1. sí I_I Necesidad de VM: 0.no; 1. sí I_I

Score de PITT (Anexo 7):

E) MICROBIOLOGÍA

Especie aislada (Anexo 8):

Antifungigrama (CIM):

Fluconazol	Anidulafungina	Anfotericina B
Itraconazol	Micafungina	Flucitocina
Posaconazol	Caspofungina	
Voriconazol	Ketoconazol	

Otros aislamientos de cándida (colonización): 0. no; 1. sí; 3.no cultivado I_I

Muestra (Anexo 9): ¿Identidad con aislamiento en sangre?: 0. no; 1. Sí I_I

Localización de la infección responsable: (Anexo 10) I_I_I

F) TRATAMIENTO DEL EPISODIO DE CANDIDEMIA ACTUAL

¿Ha recibido tratamiento antifungico empírico para el episodio actual?

0.no; 1. sí I_I

¿Correcto según resultados microbiológicos? 0.no; 1. sí I_I

Antifúngico nº 1 empírico: (Anexo 6) I_I_I_I

Antifúngico nº 2 empírico: I_I_I_I

Antifúngico nº 3 empírico: I_I_I_I

Antifúngico nº 1 según resultado microbiológico: I_I_I_I

Antifúngico nº 2 según resultado microbiológico: I_I_I_I

Antifúngico nº 3 según resultado microbiológico: I_I_I_I

Días hasta el inicio del Antifúngico correcto I_I_I

Persistencia de fiebre o inestabilidad hemodinámica a 48-72h del inicio del Antifúngico

0.no; 1. sí; 2. desconocido I_I

Días totales de Antifúngico recibido para el episodio: I_I_I

Número de hemocultivos positivos del episodio: I_I_I

¿Ha persistido la candidemia >48h desde el inicio de un tratamiento correcto?: I_I

0. no 1. sí 2. desconocido (no nuevos hemos)

¿Metastasis sépticas secundarias a la candidemia? 0.no; 1. sí; 2. desconocido I_I

¿Cuáles?

¿Se ha retirado el catéter? 0.no; 1. sí; 2. desconocido I_I

¿Se han realizado otros procedimientos mecánicos?

0.no; 1. sí; 2. desconocido I_I

¿Cuál? I_I

1. Retirada de sonda vesical.
2. Drenaje.
3. Cirugía mayor.
4. Otros (¿cuáles?)

G) PRONÓSTICO

¿Se ha curado el episodio infeccioso? 0.no; 1. sí; 2. desconocido I_I

Èxitus en los 30d posteriores a la candidemia 0.no; 1. sí; 2. desconocido I_I

Causa del éxitus I_I

0. No relacionada.

1. Probablemente no relacionada

2.Probalemente rel. (Atribuíble)

3. Contribuible

4.Desconocido Número de días desde la candidemia a la muerte

I_I_I

Recurrencia de la candidemia 0.no; 1. sí; 2. desconocido I_I

ANEXOS

ANEXO nº 1 Servicio en el que se encuentra el paciente.

1. Ang.C. Vascular (ACV)
2. Cardiologia (CAR)
3. Cirug.Cardiaca (CCA)
4. Cg Digestiva (CGD)
5. Endocrinología (END)
6. Gastroenterolog (GAS)
7. Hemat.Clin. Dir (HCL)
8. M.Infecciosas (INF)
9. Medic. Interna (MIR)
10. Medic.Intensiva (MIV)
11. Neurocirugia (NCR)
12. Nefrologia (NEF)
13. Neumologia (NML)
14. Neurologia (NRL)
15. Oncologia (ONC)
16. Otorrino (ORL)
17. Reanimacion Urg (REAU)
18. Rea.Postquirurg (RPQ)
19. Traumatologia (TRA)
20. U.Corta Est.Urg (UCEU)
21. Urgencias (URG)
22. Urologia (URO)
23. Unidad de cuidados paliativos (UCP)
24. Unidad de transplante hepático (UTH)
25. Otro: especificar

ANEXO nº 2: localización primaria de neoplasia sólida

1. Piel
2. Cabeza y cuello
3. Pulmón.
4. Mama
5. Esófago
6. Estómago
7. Intestino delgado
8. Colon/recto
9. Hígado o vía biliar
10. Páncreas
11. Vejiga o vía urinaria
12. Riñon
13. Próstata
14. Testículo
15. Útero
16. Ovario
17. Sarcomas
18. SNC
19. Otros
20.

ANEXO nº 3: Localización de la infección bacteriana concomitante.

1. Traqueobronquitis
2. Neumonía/Empiema
3. Gastroenterocolitis
4. Enterocolitis neutropénica
5. PBE
6. Colangitis
7. Inf. abd. localizada (absceso)
8. Inf. abd. difusa (peritonitis)
9. Prostatitis/ Pielonefritis
10. Divericulitis
11. Infección perianal
12. Infección de herida quirúrgica
13. Infección de piel y partes blandas
14. Bacteriemia primaria (end´gena)
15. Bacteriemia de catéter
16. ORL (sinusitis, o titis, parotiditis...)
17. Mucositis/cavidad oral
18. Infección SNC

19. Infección diseminada
20. Desconocido
21. Otros

ANEXO nº 4 (microorganismos aislados en los cultivos)

1- E coli
2- Pseudomonas aeruginosa
3- Klebsiella pneumoniae
4- Klebsiella oxytoca
5- Proteus mirabilis
6- Enterobacter aerogenes
7- Enterobacter cloacae
8- Citrobacter freundi
9- Citrobacter koseri
10- Morganella morgagnii
11- Aeromonas hydrophila
12- Schewanella putrefaciens
13- Salmonella enteritidis
14- Citrobacter amalonatycus
15- Capnocytophaga ochracea
16- Escherichia fergusoni
17- Brnahamella catharralis
18- Moraxella oslonensis
19- Serratia marcescens
20- Acinetobacter baumanii
21- Haemophilus influenzae
22- Neumococ
23- S aureus
24- MARSA
25- Estafilococo coagulasa negativo
26- SEPI
27- Corynebacterium jeikeium
28- Corynebacterium amycolatum
29- Corynebacterium urealyticum
30- Strepto viridans
31- Strepto mitis
32- Strepto anginosus
33- Srtepto salivarius
34- Srtepto sanguis
35- Strepto intermedius
36- Abiotrophia sp
37- Peptoestreptococ
38- Prevotella melaninogenica
39- Fusobacterium
40- Bacteroides fragilis
41- Bacteroides thetaiotamicron
42- Bacteroides sp
43- Closrtidium sp
44- Clostridium septicum
45- Strepto bovis I i II
46- Enterococ durans
47- Strepto agalactiae
48- Bacillus cereus
49- Enterococ faecalis
50- Enterococ faecium
51- Enterococ gallinarum
52- Listeria monocytogenes
53- Clostridium perfringens

ANEXO nº 5 (antibióticos)

1. Penicilina G sódica
2. Amoxicilina
3. Ampicilina
4. Amoxicilina-clavulánico
5. Piperacilina/Ticarcilina
6. Cloxacilina
7. Cefuroxima
8. Ceftriaxona
9. Ceftazidima
10. Cefotaxima
11. Cefepime
12 Norfloxacino
13 Ciprofloxacino
14 Levofloxacino
15 Moxifloxacino
16 Claritromicina
17 Azitromicina
18 Telitromicina
19 Clindamicina
20 Metronidazol
21 Ornidazol
22 Amicacina
23 Tobramicina
24 Gentamicina
25 Imipenem
26 Meropenem
27 Ertapenem
28 Aztreonam
29 Vancomicina
30 Teicoplanina
31 Doxiciclina
32 Linezolid
33 Daptomicina
34 Sinercid
35 Tygeciclina
36 Cotrimoxazol

37 Colistina
38 Rifampicina
39 Otros (especificar)

ANEXO nº 6: Antifúngico Empleado

1. Fluconazol
2. Itraconazol
3. Voriconazol
4. Posaconazol
5. Flucytosina
6. Amfotericina B
7. Amfo B liposomal
8. Caspofungina
9. Micafungina
10. Anidulafungina
11. Otro

ANEXO nº 7: Score de PITT (Chow et al. 1991)(Paterson et al. 2004)

Temperatura	
≤35ºC	2 puntos
35,1-36ºC	1 punto
36,1-38,9ºC	0 puntos
39-39,9ºC	1 punto
≥40ºC	2 puntos
Tensión arterial	
Caída de 30 mmHg TAS o 20 mmHg TAD	2 puntos
Drogas vasoactivas	2 puntos
TAS < 90 mmHg	2 puntos
Ventilación Mecánica	2 puntos
Parada Cardíaca	4 puntos
Estatus Mental	
Alerta	0 puntos
Desorientado	1 punto
Estuporoso	2 puntos
Coma	4 puntos

ANEXO nº 8 Especie de *Candida* aislada

1. *C. albicans*
2. *C. tropicalis*
3. *C. parapsilosis*
4. *C. glabrata*
5. *C. lusitaniae*
6. *C. krusei*
7. Otra (especificar)

ANEXO nº 9: Otros aislamientos de Cándida

1. Urocultivo
2. Esputo
3. Aspirado traqueal/ BAL
4. Biopsia
5. Frotis de herida quirúrgica
6. Otros

ANEXO nº 10 (localización de la infección responsable de la candidemia)

1. Desconocido (Candidemia primaria).
2. Candidemia relacionada con el catéter venoso.
3. Urinaria
4. Endocarditis
5. Otros (especificar)

10.2. Certificate of Foreign rotation

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OF YESHIVA UNIVERSITY

Science at the heart of medicine

Susan Ann Nuccio
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To Whom It May Concern:

This is to certify that Dr. Guillermo Cuervo was a Visiting Predoctoral Fellow in our Department of Microbiology and Immunology at the Albert Einstein College of Medicine for three months, from October to December 2014.

During this time period, Dr. Cuervo worked in the area of fungal pathogenesis. Specifically, he developed work with colleagues in the laboratory and joined in the ongoing projects on *Candida albicans* pathogenesis, extracellular vesicles secretion, melanin and antibody function.

Academically, he attended the weekly Lab Meetings and weekly Work in Progress (WIP) conferences. He also prepared an oral presentation about the results of the experiments performed during his basic research training.

Yours faithfully,

Susan A. Nuccio

Susan A. Nuccio,
Administrator