Update on invasive fungal infections: the last two years

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Several important changes are taking place in the field of invasive fungal infections. The increasing incidence of invasive fungal infections as a result of progress in areas of medicine such as organ transplantation, cancer therapy or intensive-care medical technology has provided the impetus for a search for a new and more favorable scenario. In addition, pharmaceutical companies have developed new, well-tolerated and more effective broad-spectrum antifungal agents. Therefore, during the last few years, several articles on invasive fungal infections have been published. This review focuses on the new insights in the literature on fungal infections during 2004 and 2005. Three areas of interest have been identified: (i) epidemiology and risk factors, (ii) new diagnostic procedures, and (iii) prevention and treatment. A review of the English-language and Spanish-language literature on invasive fungal infections has been made, and those articles considered essential reading have been reviewed and discussed in order to highlight their original aspects.

Key words: **Invasive fungal infections. Systemic mycoses. Organ transplantation**

Actualización de las infecciones fúngicas invasoras: los dos últimos años

En el campo de las infecciones fúngicas invasoras se están produciendo varios cambios importantes. El aumento en la incidencia de las infecciones fúngicas invasoras a consecuencia de los progresos que se han realizado en áreas de la medicina, como el trasplante de órganos, el tratamiento del cáncer y la tecnología de los cuidados médicos intensivos, ha estimulado la búsqueda de nuevos contextos más favorables. Además, las compañías farmacéuticas han desarrollado agentes antifúngicos de amplio espectro bien tolerados y más eficaces. Así, durante los últimos años se han publicado varios artículos relativos a las infecciones fúngicas invasoras. Esta revisión está fundamentada en las publicaciones relativas a las infecciones fúngicas que han aparecido en la bibliografía médica durante los años 2004 y 2005. Se han identificado tres áreas de interés: *1)* epidemiología y factores de riesgo; *2)* nuevos procedimientos diagnósticos, y *3)* prevención y tratamiento. Se ha realizado una revisión de la bibliografía médica en los idiomas inglés y español relativa a las infecciones fúngicas invasoras, con exposición y discusión de los artículos considerados de lectura obligada en el intento de poner de manifiesto sus aspectos originales.

Palabras clave: **Infecciones fúngicas invasoras. Micosis sistémicas. Trasplante de órganos.**

Introduction

Several important changes are taking place in the field of invasive fungal infections (IFI). The increasing incidence of IFI as a result of the progress in some areas of medicine such as organ transplantation, cancer therapy or intensive-care medical technology, has provided the impetus for a search for a new and more favourable scenario. As a logical result of the renewed interest in the treatment of IFI, pharmaceutical companies have developed new, well-tolerated and more effective broad-spectrum antifungal agents. Moreover, the availability of better antifungal drugs has also stimulated the search for new tools in the early diagnosis of IFI and new therapeutic strategies. Overall, there is increasing evidence that IFI should no longer be considered the final event of patients with severe underlying diseases, but a severe complication which may be prevented or cured.

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Below, we review what, in our opinion, are the most interesting articles in the IFI field during 2004 and 2005.

Epidemiology and risk factors

During the last two years, several articles on the epidemiology of IFI have been published in the literature. Population-based surveillance studies are able to identify all cases of IFI regardless of the setting. They decrease the bias resulting from the selection of only a subset of hospitals and permit newly affected patient groups to be identified. Therefore, population-based surveillance studies should be the standard for establishing the epidemiology of any infection and for studying the risk factors of the infected population. However, they are expensive and cumbersome, and, therefore, uncommon. As for *Candida* bloodstream infections (BSI), before 2004, several studies were reported in the United States[1,2], but in Europe the studies were limited to Iceland[3] and Finland[4]. In 2005, Almirante et al[5] reported the results of a population-based, active, prospective surveillance study for bloodstream infections caused by *Candida* in Spain, to determine the distribution of the species involved and the prevalence of resistance to antifungals, and to evaluate risk factors for mortality. Furthermore, in Denmark, a semi-national study of candidemia has also been reported[6], as well as other non-population-based prospective multi-center studies[7]. The Spanish study[5] was performed in the Barcelona area with a population of 3.9 million between 1 January 2002 and 31 December 2003. Fourteen hospitals participated (from 214 to 1,295 beds). All blood cultures from which a *Candida* species was isolated were reported and during the first week after the BSI was diagnosed, the infection was confirmed or ruled out. Three weeks later the case report form was completed and the outcome was recorded. Periodic audits of clinical laboratories were performed. To measure severity of illness, the Acute Physiology and Chronic Health Evaluation (APACHE) II score was used for adult patients admitted to ICUs. For adults outside an ICU, the Karnofsky performance status scale was used. However, for pediatric patients, a standardized score of severity of illness was not used. The isolation of any *Candida* species from the blood was defined as a case. A new case was defined as isolation of a *Candida* species > 30 days after the initial case. Cases occurring either prior to or within 2 days of admission were considered community-acquired. A case was defined as likely to be catheter-related when (i) semiquantitative culture of the catheter tip yielded more than 15 CFU of a *Candida* species, or (ii) simultaneous quantitative cultures of blood samples showed a ratio of ≥ 5:1 CFU of blood samples obtained through the catheter and a peripheral vein.

Mortality was classified as follows: (i) early mortality, defined as death occurring 3 to 7 days after diagnosis and, (ii) late mortality, defined as death occurring between days 8 to 30. Mortality during days 1 and 2 were excluded from the analysis. For the late mortality study, adequate treatment was defined as ≥ 5 days of any antifungal medication in addition to catheter removal.

Candida isolates were identified by standard procedures and antifungal susceptibility testing was performed following EUCAST methodology[8,9]. Isolates were classified as showing decreased susceptibility to fluconazole when the MIC was ≥ 16 mg/L.

Statistical analysis was performed using SAS, version 8.2 (SAS Institute, Cary, N.C.). The Chi-square or Fisher exact test was used to compare categorical variables. Univariate and multivariate analyses were performed using the LOGISTIC procedure.

We detected 341 patients with *Candida* BSI. Four patients had a recurrent episode, resulting in a total of 345 cases. The average annual incidence of candidemia was 4.3 cases per 100,000 inhabitants. The incidence rate was highest in infants (38.8 cases per 100,000 inhabitants), and in those aged > 65 years (12 cases per 100,000 inhabitants). Community acquisition accounted for 11% of cases and only 33% of cases of candidemia were diagnosed in an ICU.

Regarding species distribution, the most common isolate was *C. albicans* (51%), followed by *C. parapsilosis* (23%), *C. tropicalis* (10%), *C. glabrata* (9%), and *Candida krusei* (4%). Other *Candida* species were isolated in 11 cases (3%).

In a multivariate analysis excluding *C. parapsilosis* (due to significant differences in demographics and crude mortality), previous treatment with fluconazole (odds ratio [OR] 3.3; 95% confidence interval [CI] 1.8-6.1; $p < 0.01$) was the only exposure significantly associated with non-*C. albicans* candidemia. In addition, previous antibiotic use was a protective factor for non-*C. albicans* candidemia compared with *C. albicans* candidemia (OR 0.4; 95% CI 0.2-0.8; $p < 0.01$).

Mortality within the first 30 days was 44%, and 22% died within 7 days of culture. Multivariate analysis indicated that treatment with an antifungal and having the catheter removed as a part of treatment were independently associated with a lower odds ratio of early death (treatment with an antifungal: OR 0.05; 95% CI, 0.01-0.2; $p < 0.01$; catheter removal: OR 0.3; 95% CI, 0.1-0.9; $p = 0.04$). The same results were obtained for late mortality. Thus, in the multivariate analysis, only intubation (OR 7.5; 95% CI, 2.6-21.1; $p < 0.01$) and adequate treatment (OR 0.2; 95% CI, 0.08-0.8; $p < 0.01$) were independent predictors of late mortality. Multivariable analysis indicated that treatment with an antifungal and having the catheter removed as a part of treatment were independently associated with a lower odds of early death (treatment with antifungal: OR 0.05; 95% CI, 0.01-0.2; $p < 0.01$; catheter removal: OR 0.3; 95%CI, 0.1-0.9; $p = 0.04$.). On the contrary having a haematological malignancy was associated with greater odds (OR 3.5; 95% CI, 1.1-10.4; $p = 0.03$). Same results were obtained for late mortality. Thus, in multivariate analysis, only intubation (OR 7.5; 95% CI, 2.6-21.1; $p < 0.01$) and adequate treatment (OR 0.2; 95% CI, 0.08-0.8; $p < 0.01$) were independent predictors of late mortality.

Antifungal susceptibility testing is well recorded in a study by Cuenca-Estrella et al[10], in which amphotericin B and flucytosine were active in vitro against all strains. A total of 24 strains (6.8%) showed decreased susceptibility to fluconazole (MIC ≥ 16 mg/L) and 43 (12.3%) showed decreased susceptibility to itraconazole (MIC ≥ 0.25 mg/L). Voriconazole and caspofungin were active in vitro against most isolates, even those that were resistant to fluconazole.

In summary, with regard to the epidemiology of candidemia, the following issues can be highlighted: (i) the incidence of candidemia in diverse geographic locations is different. Thus, in the USA, incidence ranged from 6 to 10 cases per 100,000 inhabitants[1,2,11], while in Europe it ranged from 1.7 to 11 cases per 100,000 inhabitants[3,4,6]. The Barcelona study showed a rate of 4.3 cases per 100,000 inhabitants[5]; (ii) incidence rates are related to age. Thus, in infants (38 per 100,000 inhabitants) and in adults > 65 years (12 per 100,000 inhabitants), these are higher than in people aged between 1 and 64 years (2.4 per 100,000 inhabitants)[5]; (iii) species distribution is geographically-related and age-related. Thus, a significant increase in the incidence of *C. glabrata* BSI has been reported in the United States, Denmark and Finland[3,6,12], but not in other European studies[7,13]. Infants have a higher rate of *C. parapsilosis* in Spain than in the USA[1,2,5]; (iv) mortality is still very high, but there are also differences between infants (lower) and adults[5]; (v) catheters are a relevant source of candidemia[5], and removal plus antifungal treatment is independently associated with a decreased risk for both early and late mortality[5]; (vi) for those species of yeasts naturally susceptible to antifungals such as *C. albicans*, *C. tropicalis* and *C. parapsilosis*, the rates of resistance are low[1,6,7,10,13,14]. As expected, a high level of resistance was detected among *C. krusei* isolates. Sixteen percent of *C. glabrata* isolates had fluconazole MICs > 8 mg/L[10]; this is consistent with other reports[6,7,13,14,28].

As far as invasive aspergillosis (IA) is concerned, Morgan et al[15], performed a study for estimating the incidence among 4621 hematopoietic stem cell transplants (HSCT) and 4110 solid organ transplants (SOT) at 19 locations in the United States during a 22-month period. At 12 months, the aggregate cumulative incidence of aspergillosis was 0.5% after autologous HSCT, 2.3% after allogeneic HSCT from an HLA-matched related donor, 3.2% after transplantation from an HLA-mismatched related donor, and 3.9% after transplantation from an unrelated donor. After HSCT, the mortality of IA was very high, and at 3 months ranged from 53.8% of autologous transplants to 84.6% of unrelated-donor transplants. In SOT, the aggregate cumulative incidence of aspergillosis at 12 months was 2.4% after lung transplantation, 0.8% after heart transplantation, 0.3% after liver transplantation, and 0.1% after kidney transplantation. Mortality was also very high among SOT recipients. At three months after diagnosis of aspergillosis, mortality ranged from 20% for lung transplants to 66.7% for heart and kidney transplants. The species of *Aspergillus* involved in infections after HSCT included *A. fumigatus* (56%), *A. flavus* (18.7%), *A. terreus* (16%), *A. niger* (8%), and *A. versicolor* (1.3%). Those associated with infections after SOT included *A. fumigatus* (76.4%), *A. flavus* (11.8%), and *A. terreus* (11.8%). In summary, although the incidence of IA in this group of patients is low, mortality remains very high and therefore this infection is a fearsome complication. Lass-Flörl et al[16] illustrate how, in certain geographical areas, some species more frequently cause invasive infections. For unknown reasons (the authors demonstrate that this was not an outbreak), *A. terreus* are detected frequently as a cause of IA in hematological patients in Tyrol, Austria[16]: 67 cases of proven IA were analyzed retrospectively, revealing that 32 patients were infected by *A. terreus* and 35 by other species of *Aspergillus*. Both groups were comparable, with leukemia being the most common underlying disease. *A. terreus* was disseminated in 63% of cases, and other species of *Aspergillus* in 32%. In addition, the response of *A. terreus* infection to amphotericin was 20% compared with 47% for patients with non-*A. terreus* infection. This poor response was associated with high amphotericin MICs for *A. terreus*. In summary, *A. terreus* can be considered an emerging pathogen in certain areas showing in vitro and in vivo resistance to amphotericin B.

The study of the risk factors of IA in SOT recipients is necessary in order to design strategies to prevent of this frightening infection. Gavalda et al[17] carried out a retrospective case-control study including 156 cases of proven or probable IA in SOT recipients. The cases were recruited from 11 Spanish institutions. The main objective of the study was to analyze the risk factors of two different populations of patients: (i) Patients with early-onset aspergillosis (first three months after transplantation) and; (ii) Patients with late-onset aspergillosis (13 months after transplantation). Fifty seven patients had early-onset IA. The significantly associated risk factors were (i) a more complicated postoperative period, (ii) repeated bacterial infections or cytomegalovirus disease, and (iii) renal failure or the need for dialysis. On the other hand, 43% of cases had a late-onset infection. The significantly associated risk factors were (i) older age than patients with an early-onset IA, (ii) extremely immunosuppressed state because of chronic transplant rejection or allograft dysfunction, and (iii) post transplantation renal failure. In summary, the risk factors of the two groups of patients were different, and this may have implications for prevention.

It is well known that hematological malignancies and SOT are major risk factors for IA. However, critically ill patients are also at risk. In particular, those with chronic obstructive pulmonary disease (OR 2.9; 95% CI, 1.06-8.08; p= 0.03) or those treated with steroids (OR 4.5; 95% CI, 1.73-11; p = 0.002) seem to have an increased incidence of this infection[18]. In addition, a recent report highlights that IA can be diagnosed in critically ill patients without predisposing risk factors according to our current concepts, and that it is associated with extremely high mortality[19].

Emerging infections have been also a concern during the last two years, with zygomycosis as one of the most fearsome. Roden et al[20] published an excellent review on zygomycosis: it included 929 eligible cases published in the English-language literature since 1885. Sixty-five of the patients were male. Sinus infection accounted for 39% of cases, while pulmonary infection was present in 24% of cases, and cutaneous infection in 19%. Infection was disseminated in 23% of cases. Survival varied with the site of infection: only 4% of patients with disseminated disease survived, 15% with gastrointestinal infection survived, and 24% with pulmonary infection survived. Location of zygomycosis was also related to underlying disease: 60% (92 of 154) of patients with malignancy had pulmonary disease, whereas 66% (222 of 337) with diabetes had sinus disease. Rhinocerebral disease was detected more frequently in patients with diabetes (33%) than in patients with malignancy (4%). Hematogenous dissemination to the skin was rare, although 78 (44%) of 176 cutaneous infections were complicated by deep extension or dissemina-

tion. Mortality was very high (97%) for those patients who were not treated. Treatment with surgery decreases mortality to 43%, but antifungal treatment with amphotericin B deoxycholate was more effective and decreased mortality to 39%. Finally, a combination of antifungal therapy and surgery achieved 70% survival.

Diagnosis

The high mortality of IFI and the difficulties in obtaining a rapid and accurate diagnosis have stimulated the development of diagnostic methods aimed at detecting different fungal markers. Among these markers, galactomannan (GM) is a polysaccharide cell-wall component that is released by growing hyphae. The BIO-RAD Company has developed a double-sandwich EIA that incorporates the 15 galactofuranose-specific EBA2 monoclonal antibody as both the acceptor and detector for GM. Many studies have shown this EIA to be a promising diagnostic tool for invasive aspergilosis in neutropenic patients with cancer. However, different studies have found different sensitivity and specificity and some authors have suggested that diagnostic indices lower than those recommended by BIO-RAD should be used for a more accurate diagnosis of IA[21]. Marr et al[21] analyzed the variables that could affect the performance of the test. All enrolled patients were bone marrow transplant (BMT) recipients. Blood samples were collected prospectively and obtained weekly from the start of conditioning chemotherapy until day 75 after receipt of the stem-cell product. In cases of fever (temperature > 38.0 °C), blood samples were obtained daily and when fungal infection was diagnosed. All patients received antifungal prophylaxis with fluconazole (400 mg/day), which was administered for 75 days after BMT. Patients who had persistent fever despite antibiotics received amphotericin B (0.5-1.0 mg/kg or equivalent doses of a lipid preparation) until fever and neutropenia resolved. Routine procedures were followed to establish the diagnosis of fungal infections. After a diagnosis of IA, treatment included conventional amphotericin B, lipid formulations of amphotericin B, itraconazole, and investigational azole antifungal compounds. The diagnosis of IA followed the consensus criteria of the European Organization for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group[22], although these did not include GM EIA results.

A total of 986 serum samples from 67 patients were included in the analysis. Of these, 178 serum samples were from 13 patients with proven IA, 201 from 11 patients with probable IA, 135 from 8 patients with possible IA, and 472 from 35 control subjects. Only 5 samples obtained from 35 control patients were positive (≈ 1% of false positive results). Using a cut-off of 1.0, the sensitivity of the test in proven or probable cases was 54.2%. Because antifungal therapy was given to patients for prophylactic and empirical purposes, the analysis was performed after stratifying patients for receipt of mold-active antifungal therapy (itraconazole or amphotericin B formulations) within 2 weeks before the diagnosis of aspergillosis. This approach showed that the sensitivity of the test was lower (18.35%) in those patients receiving antifungals than in those who were untreated (83.75%). Thus, the test was performed with a lower cut-off and ROC curves were calculated. These showed that sensitivity increased substantially, with a minimal loss of specificity, when the index cut-off was decreased from 1.0 to 0.5. Furthermore, the sensitivity and specificity plots of patients with and without antifungal treatment showed that sensitivity remained high, regardless of the cut-off, in those patients not receiving antifungals. However, in those patients who were receiving antifungal therapy, a decrease in the cut-off to 0.5 increased the sensitivity to 81.8%, with a decrease in specificity from 100% to 77.1%. In addition, a cut-off of 0.5 increased the time between a positive test result and clinical diagnosis. Other studies have reached similar conclusions[23,24]. This test has good interlaboratory and intralaboratory reproducibility but extreme caution should be exercised when different lots are used with the same samples[25]. In summary, a re-evaluation of GM EIA is clearly needed, and a consensus from European and US groups would be welcome in order to provide a useful guideline.

Another marker that is potentially useful as a diagnostic adjunct for invasive fungal infections is βD-glucan. Recently, Associates of Cape Cod have introduced Fungitell, a kit able to detect circulating βD-glucan in serum. Odabasi et al[26] described the validation and performance of this marker in patients with acute myelogenous leukemia and myelodysplastic syndrome. They concluded that βD-glucan levels of ≥ 60 pg/mL might be a useful diagnostic adjunct for the diagnosis of IFI, particularly in high-risk populations. Based on this study, the interpretation of values was as follows: < 60 pg/ml, negative; 60 to 79 pg/ml, indeterminate; ≥ 80 pg/ml, positive. However, one report has raised concern about the performance of this test[27]. The authors found that 14 out of 25 bacteremic patients (10 with gram-positive bacteremia) were βD-glucan-positive. Therefore, until a more extensive study is performed, this diagnostic tool can be recommended to rule out a fungal infection caused by the fungi it detects. The test has good reproducibility[28]. As suggested for GM, an extensive collaborative study should be performed in order to produce clear guidelines for using these tools in the diagnosis of IFI.

Finally, a brief comment about some recently published data regarding the activity of and resistance to antifungals. Pfaller et al[29] performed an extensive study including yeast strains from 39 countries. They analyzed susceptibility to fluconazole (140, 767 isolates) and voriconazole (79,343 isolates) by the CLSI (formerly NCCLS) M44-A disk diffusion method. Resistance to both azoles was negligible in *C. albicans* and *C. parapsilosis*. The resistance rate of *C. tropicalis* was ≈ 6% for both azoles. *C. glabrata* showed a higher rate of resistance, but for voriconazole, only 10% of isolates were resistant. *C. krusei*, intrinsically resistant to fluconazole, has low rates of resistance to voriconazole (≈ 7%). In conclusion, the most frequent species of human pathogenic yeasts can be treated with azole drugs, but special care should be taken with *C. glabrata*.

Anidulafungin is a new echinocandin antifungal agent that shows good activity against *Candida* spp. Pfaller et al[30] analyzed the in vitro activity of anidulafungin against 2235 clinical isolates of *Candida* spp. using the CLSI broth microdilution method described in document M27A2.

Anidulafungin was very active against *Candida* spp. With an MIC_{90} of 2 mg/L, *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. kefyr* were the most susceptible species of *Candida* (MIC_{90}, 0.06- 0.12 mg/L). *C. parapsilosis*, *C. lusitaniae*, and *C. guilliermondii* were the least susceptible (MIC_{90}, 0.5-2 mg/L). Anidulafungin is also active against fluconazole-resistant isolates. The authors include 315 fluconazole-resistant isolates, of which 99% were inhibited by ≤ 1 mg/L of anidulafungin.

Prevention and treatment

The new antifungal agents have proven to be safer and more efficacious than conventional amphotericin B, and are a landmark in the treatment of IFI. The availability of new drugs with different mechanisms of action, antifungal activity, and toxicity profiles has encouraged many doctors to offer antifungal therapy in clinical situations where, until now, the use of antifungal drugs was hampered by the toxicity and lack of activity of conventional amphotericin B. Therefore, the availability of new antifungal agents may not only increase the chances of surviving an established IFI, but also offer the possibility of using antifungal therapy in prophylaxis, pre-emptive, or empiric therapy to decrease fungal-associated mortality in selected populations.

Below, we summarize the most important reports on antifungal therapy.

Antifungal prophylaxis

We have selected two articles regarding the use of antifungal drugs in prophylaxis of IFI.

In the first paper, Marr et al[31] reported their experience in an open, randomized, single-institution study for the comparison of fluconazole with itraconazole in the prevention of IFI in allogeneic stem cell transplant recipients. A total of 304 patients were included in the study to receive fluconazole at 400 mg daily and itraconazole oral solution at 2.5 mg/kg/8 h or 200 mg/daily/IV during the first 180 days after transplantation, or until 4 weeks after discontinuation of graft-versus-host-disease. Proven and probable IFI were evaluated by intention-to-treat and on-treatment analysis. More patients in the itraconazole arm developed hepatotoxicity, and more patients suspended itraconazole because of toxicity or gastrointestinal intolerance (36% vs 16%, $p < 0.001$). Intention-to-treat analysis demonstrated no difference in the incidence of IFI during the study period (fluconazole 16% vs itraconazole 13%, $p = 0.46$); however, fewer patients in the itraconazole arm developed IFI on treatment (fluconazole 15% vs itraconazole 7%, $p = 0.03$). Itraconazole provided better protection against invasive mold infections (fluconazole 12% vs itraconazole 5%, $p = 0.03$), but similar protection against candidiasis (3% vs 2%, $p = 0.69$). There was no difference in overall or fungal-free survival.

Itraconazole appears to prevent mold infections in the subset of patients who tolerate the drug; however, toxicity and poor tolerability limit its success as prophylaxis. We also recommend the article by Winston et al[32], which completes the information on itraconazole prophylaxis in this setting.

In the second paper, Shorr et al[33] reported the results of a meta-analysis of randomized, placebo-controlled trials of fluconazole prophylaxis in the prevention of IFI in critically-ill surgical patients. They identified four randomized studies comparing fluconazole with placebo for the prevention of fungal infections in the surgical intensive care unit. The study enrolled 626 patients and used different dosing regimens of fluconazole. All trials were double-blind and two were multicenter studies. Fluconazole significantly reduced the incidence of fungal infections (pooled OR 0.44; 95% CI, 0.27-0.72; $p < 0.001$). However, fluconazole prophylaxis was not associated with a survival advantage (pooled OR for mortality 0.87; 95% CI, 0.59-1.28; p = NS). Generally, fluconazole appeared to be safe. The meta-analysis concludes that prophylactic fluconazole for the prevention of mycoses in critically-ill surgical patients decreases the rate of IFI, but this strategy does not improve survival. The absence of a survival advantage may reflect the few studies in this area and the possibility that this issue has not been adequately studied. A randomized, double-blind, multicenter study with a proper and uniform definition of the population to be included is urgently needed in this area.

Pre-emptive therapy with antifungal agents

The term "pre-emptive therapy" was introduced by Robert Rubin in 1991[34] for the prevention of CMV pneumonia in allogeneic bone-marrow transplant recipients using the asymptomatic shedding of CMV as a marker to start ganciclovir. The recent availability of the above-mentioned non-culture-based methods for the early diagnosis of IFI has opened the possibility of using this approach in IFI infection.

Maertens et al[35] recently published a very interesting report in which they evaluated the feasibility of pre-emptive antifungal therapy based on non-invasive methods such as GM detection and pulmonary CT-scan in consecutive high-risk neutropenic patients who had received fluconazole prophylaxis. A total of 136 treatment episodes for patients who were at risk of acquiring IFI were screened for the presence of GM. A diagnostic evaluation including thoracic CT scanning (HRCT) and bronchoscopy with lavage was performed on the basis of well-defined clinical, radiological, and microbiological criteria. Only seropositive patients and patients with a positive microbiological test result plus supportive radiological findings received liposomal amphotericin B, and traditional empirical antifungal therapy of persistent neutropenic fever was specifically avoided. Neutropenic fever developed in 117 episodes, of which at least 41 episodes (35%) satisfied existing criteria for empirical antifungal therapy. However, the protocol-driven pre-emptive approach reduced the rate of antifungal use for these episodes from 35% to 7.7% (a 78% reduction) and led to the early initiation of antifungal therapy in 10 episodes (7.3%) that were not clinically suspected of being IFI. No undetected cases of IA were identified but 1 case of zygomycosis was missed. Breakthrough candidemia was diagnosed by conventional culture techniques and was treated successfully. With a pre-emptive approach, the 12-week survival rate for patients with IFI was 63.6% (it was 63.1% for those with IA). This important study concluded that pre-emptive therapy based on enzyme immunoassay and HRCT reduced the exposure to

antifungal drugs and offered effective antifungal control, but it failed to detect non-*Aspergillus* IFI.

Two important questions arise from this study: Are we seeing the end of traditional empirical antifungal therapy of neutropenic fever? and Can other non-invasive tests such as glucan detection improve the results obtained with the detection of GM?

Piarroux et al[36] performed a single-institution study to evaluate the efficacy of pre-emptive antifungal therapy in preventing proven candidiasis in critically-ill surgical patients. The study compared non-contemporary groups with a two-year retrospective analysis and a two-year prospective intervention group. During the prospective period, systematic mycological screening was performed on all patients admitted to a surgical intensive care unit (SICU), immediately on admission and then weekly until discharge. A corrected colonization index, as defined by Pittet et al[37], was used to assess the intensity of *Candida* mucosal colonization. Patients with a corrected colonization index > 0.4 received early pre-emptive antifungal therapy (intravenous fluconazole: loading dose 800 mg, then 400 mg/day for 2 wks). During the retrospective period, 32 patients of 455 (7%) presented with proven candidiasis: 22 (4.8%) were community-acquired and 10 (2.2%) were SICU-acquired. During the prospective period, 96 patients with a corrected colonization index > 0.4 of 478 received pre-emptive antifungal treatment and only 18 cases (3.8%) of proven candidiasis were diagnosed; all were community-acquired infections. *Candida* infections occurred more frequently in the control cohort (7% vs 3.8%; p = 0.03). Incidence of SICU-acquired proven candidiasis decreased significantly from 2.2% to 0% (p < 0.001, Fisher test). Incidence of proven imported candidiasis remained unchanged (4.8% vs 3.8%; p = 0.42). No emergence of azole-resistant *Candida* species (especially *Candida glabrata*, *Candida krusei*) was noted during the prospective period. In conclusion, pre-emptive antifungal therapy may prevent acquisition of proven candidiasis in SICU patients with no apparent selection of fluconazole-resistant yeasts. Other studies using non-culture-based methods for the early diagnosis of *Candida* infection would be of great interest in this setting.

Empirical antifungal therapy

IFI are a major cause of death in patients with prolonged neutropenia. Empirical antifungal therapy is a standard of care in patients with neutropenic fever who do not defervesce after several days of broad-spectrum antibiotic therapy. Walsh et al[38] evaluated for the first time the efficacy and safety of an echinocandin in this clinical indication. The design of this trial was similar to that of the two previous trials published by these authors[39,40] regarding the use of liposomal amphotericin B and voriconazole in patients with persistent neutropenic fever.

The study was a prospective randomized, double-blind, multinational trial in which the authors compared the efficacy and safety of caspofungin with that of liposomal amphotericin B as empirical antifungal therapy. At study entry, patients were stratified according to risk and to whether they had previously received antifungal prophylaxis. A successful outcome was defined as the fulfillment of all components of a five-part composite end point which included successful treatment of any baseline fungal infection, absence of any breakthrough fungal infection during therapy or within seven days after the completion of therapy, survival for seven days after the completion of therapy, no premature discontinuation of study therapy because of drug-related toxicity or lack of efficacy, and resolution of fever (defined as a temperature below 38 °C for at least 48 hours) during neutropenia. Secondary efficacy assessments consisted of assessments of each component of the primary end point. Survival times were also assessed for the modified intention-to-treat population. Efficacy was evaluated in 1095 patients (556 receiving caspofungin and 539 receiving liposomal amphotericin B). After adjustment for strata, the overall success rates were 33.9 percent for caspofungin and 33.7% for liposomal amphotericin B (95.2% CI for the difference, 5.6 to 6.0%), thus fulfilling statistical criteria for the noninferiority of caspofungin. Among patients with baseline fungal infections, a higher proportion of those treated with caspofungin had a successful outcome (51.9% vs 25.9%, p = 0.04). The proportion of patients who survived at least seven days after therapy was greater in the caspofungin group (92.6% vs 89.2%, p = 0.05). Premature study discontinuation occurred less often in the caspofungin group than in the amphotericin B group (10.3% vs 14.5%, p = 0.03). The rates of breakthrough fungal infections and resolution of fever during neutropenia were similar in the two groups. Fewer patients who received caspofungin had a nephrotoxic event (2.6% vs 11.5%, p < 0.001), an infusion-related event (35.1% vs 51.6%, p < 0.001), a drug-related adverse event or drug-related discontinuation.

In conclusion, caspofungin was as efficacious as liposomal amphotericin B in patients with persistent fever and neutropenia, and was better tolerated overall than liposomal amphotericin B. Thus, caspofungin provides a new option for empirical antifungal therapy in these patients.

This is the largest study ever on empirical antifungal therapy of patients with persistent neutropenic fever and provides important information on the use of a new and promising class of antifungals. However, the use of a composite end-point in which unspecific variables such as fever and tolerance were very important is a serious drawback in this type of trial. In fact we recommend a careful reading of the important editorial accompanying the article[41] to ascertain the exact relevance of the information provided by the Walsh trial.

Treatment of established invasive fungal infections

As mentioned above, the early administration of antifungal therapy is generally associated with a more favorable outcome, but not many recent articles have studied this issue in the clinical setting. The goals of the study by Morrell et al[42] were to identify the prevalence of the delay of empiric antifungal treatment for patients with a *Candida* BSI until after the results of blood cultures were known, and to determine whether this delay influenced the clinical outcomes in patients with *Candida* BSI.

The authors retrospectively identified 157 patients with a *Candida* BSI over a 4-year period (January 2001 through December 2004): 50 (31.8%) died during hospitalization and 134 had begun empiric antifungal therapy after the results of fungal cultures were known. After the first positive blood sample was drawn for culture, 9 (5.7%) pa-

tients received antifungal treatment within 12 hours, 10 (6.4%) patients received antifungal treatment between 12 and 24 hours, 86 (54.8%) patients received antifungal treatment between 24 and 48 hours, and 52 (33.1%) patients received antifungal treatment after 48 h. Multiple logistic regression analysis identified APACHE scores (one-point increments) (adjusted odds ratio [AOR] 1.24; 95% CI, 1.18-1.31; p < 0.001), prior antibiotic treatment (AOR 4.05; 95% CI, 2.14-7.65; p = 0.028), and administration of antifungal treatment 12 h after having the first positive blood sample for culture (AOR 2.09; 95% CI, 1.53-2.84; p = 0.018) as independent determinants of hospital mortality. Administration of empiric antifungal treatment 12 h after a positive blood sample is drawn is common among patients with *Candida* BSI and is associated with greater hospital mortality.

Development of non-culture based tests to complement traditional cultures may decrease the delay in antifungal administration and thus improve the outcome. Alternatively, identification of patients with a very high-risk of *Candida* BSI should prompt the early initiation of antifungals.

Voriconazole has proven efficacy in the treatment of aspergillosis and other mold infections and esophageal candidiasis. Kullberg et al[43] compared the efficacy of voriconazole and conventional amphotericin B followed by fluconazole as first-line therapy in the treatment of candidemia in non-neutropenic patients. Their study was designed as a multi-center, open, randomized, non-inferiority trial which compared voriconazole with amphotericin B followed by fluconazole after 3-7 days. Non-neutropenic patients with a positive blood culture for a species of *Candida* and clinical evidence of infection were enrolled. Patients were randomly assigned, at a ratio of 2:1, to voriconazole (n = 283) or amphotericin B followed by fluconazole (n = 139). The primary efficacy analysis was based on the clinical and mycological response 12 weeks after the end of treatment, as assessed by an independent data-review committee unaware of treatment assignment. Of the 422 patients randomized, 370 were included in the modified intention-to-treat population. Voriconazole was not inferior to amphotericin B/fluconazole in the primary efficacy analysis, with successful outcomes in 41% of patients in both treatment groups (95% CI for difference 10.6%-10.6%). At the last evaluable assessment, outcome was successful in 162 (65%) patients assigned to voriconazole and 87 (71%) assigned to amphotericin B/fluconazole (p = 0.25). Voriconazole cleared blood cultures as quickly as amphotericin B/fluconazole (median time to negative blood culture, 2.0 days). Treatment discontinuations due to all-cause adverse events were more frequent in the voriconazole group, although most discontinuations were due to non-drug-related events and there were significantly fewer serious adverse events and cases of renal toxicity than in the amphotericin B/fluconazole group.

This study indicates that voriconazole is a suitable alternative to amphotericin B/fluconazole, and echinocandins for the treatment of candidiasis in non-neutropenic patients. The choice of antifungal should be based on local epidemiology patterns and toxicity profiles to allow a more personalized treatment of candidemia.

Two recent papers have evaluated the efficacy of antifungals as salvage therapy in patients who are intolerant of or refractory to first-line antifungal treatment. Maertens et al[44] reported for the first time the efficacy and safety of a new class of antifungal compounds: the echinocandins. The echinocandins are a novel class of parenterally administered semi-synthetic lipopeptides with a pathogen-specific mechanism for non-competitive inhibition of biosynthesis of 1,3-β-glucans in the fungal cell wall. In this paper, the authors investigated the efficacy and safety of caspofungin in the treatment of IA. Ninety patients who were refractory to or intolerant of amphotericin B, lipid formulations of amphotericin B, or triazoles were enrolled to receive caspofungin. Efficacy was assessed for 83 patients who had infection consistent with definitions of IA and who received more than one dose of study drug. Common underlying conditions included hematologic malignancy (48% of patients), allogeneic blood and marrow transplantation (25% of patients), and SOT (11% of patients). Seventy-one patients (86%) were refractory to and 12 patients (14%) were intolerant of previous therapy. A favorable response to caspofungin was observed in 37 (45%) of 83 patients, including 32 (50%) of 64 with pulmonary aspergillosis and 3 (23%) of 13 with disseminated aspergillosis. Two patients discontinued caspofungin therapy because of drug-related adverse events. Drug-related nephrotoxicity and hepatotoxicity occurred infrequently. This study was crucial in the licensing process of caspofungin and showed that echinocandins are safe and probably effective drugs in the treatment of IA. Ideally, a proper comparison between voriconazole and caspofungin as first-line therapy of IA would be of great interest.

In the second paper, Patterson et al[45] completed the information provided by Herbrecht et al[46] who demonstrated the superiority of voriconazole over conventional amphotericin B in the treatment of IA. Patterson et al[45] evaluated the outcome of the patients who, in the previous trial were switched from voriconazole or amphotericin B to other licensed antifungal therapy (OLAT) because of drug intolerance or clinical failure. Fewer patients in the voriconazole group (52 [36%] of 144) switched to OLAT, compared with patients in the amphotericin B deoxycholate group (107 [80%] of 133). Lipid formulations of amphotericin B were the most common OLAT (38% of patients). Switches were made because of intolerance or insufficient response in 70% for patients in the amphotericin B deoxycholate group, compared with 24% of patients in the voriconazole group. Favourable responses to OLAT in the amphotericin B deoxycholate group occurred in only 19% of patients with initial insufficient response and 38% of patients with intolerance. Salvage therapy with a lipid formulation of amphotericin B after initial treatment with amphotericin B deoxycholate was successful for only 30% of patients (14 of 47). Treatment success among patients randomized to receive amphotericin B, including those whose treatment was switched to OLAT, was 32%, compared with 55% in patients who received voriconazole alone p < 0.001.

Both studies showed the poor response rate obtained with antifungal salvage therapy including therapy with lipid formulations of amphotericin B, and highlight the importance of effective initial therapy in this infection.

In view of the suboptimal responses of antifungal agents in the treatment of mold infections, combination antifungal therapy has been proposed, among other strategies, to improve outcome. Moreover, combination antifungal ther-

apy is particularly appealing for agents with distinct mechanisms of action, such as the azoles and echinocandins.

Apart from anecdotic reports, Marr et al[47] reported the first experience comparing combination therapy with conventional antifungal therapy in the treatment of IA. They compared two non-contemporary cohorts of patients who developed IA after allogeneic hematopoietic stem-cell transplantation. These patients experienced failure of initial antifungal therapy with amphotericin B formulations and received voriconazole (n = 31) or combination therapy with voriconazole plus caspofungin (n = 16) for salvage therapy. The combination of voriconazole and caspofungin was associated with an improved three-month survival rate, compared with voriconazole alone (hazard ratio [HR] 0.42; 95% CI, 0.17-1.1; p = 0.048). In multivariate models, salvage therapy with the combination of voriconazole and caspofungin had a lower mortality than voriconazole (HR 0.28; 95% CI, 0.28-0.92, p = 0.011) independently of other prognostic variables (e.g., receipt of transplant and type of conditioning therapy). The probability of death due to aspergillosis was lowest in patients who received the combination regimen.

In summary, the availability of several new antifungals has deeply changed the ominous outcome of IFI. Relevant and appropriate clinical trials should be designed to extend our knowledge in the evolving field of IFI.

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