5th National Congress

Diagnosis and Treatment of Opportunistic Mycosis

new Microbiologica proceedings

President
Claudio Viscoli

June 25-27, 2009

Starhotel President
Corte Lambruschini, 4
Genoa

Program

Thursday, June 25

Session 1
Mycology laboratory
Chairpersons: E. Concia (*Verona*), S. Oliveri (*Catania*)

14.00 **Drug resistance in Fungi: An emergent problem?**
G. Morace (*Milan*), E. Borghi (*Milan*).....9
Discussant: S. Oliveri (*Catania*)

14.45 **Azole resistance in Aspergillus**
M. Sanguinetti (*Rome*), B. Posteraro (*Rome*) 13
Discussant: M.T. Montagna (*Bari*)

15.30 **Progress on antigen detection for diagnosis of invasive fungal infections**
F. Barchiesi (*Ancona*).....16
Discussant: E. Castagnola (*Genoa*)

16.15 **Antifungal susceptibility testing: state-of-the-art**
G.P. Gesu (*Milan*)
Discussant: F. Barchiesi (*Ancona*)

SYMPOSIUM: Clinical mycology update
Chairpersons: A. Bacigalupo (*Genoa*), C. Viscoli (*Genoa*)

17.30 **Comparing echinocandins. Pharmacologist's point of view**
F. Pea (*Udine*)

17.55 **Candida infections in the intensive care unit**
M. Bassetti (*Genoa*).....18

18.20 **Invasive Aspergillosis in acute Myeloid Leukemia: from Scientific Trials to Clinical Practice**
L. Pagano (*Rome*), M. Caira (*Rome*) 21

Friday, June 26

SYMPOSIUM: From laboratory to clinical practice: why do we need the 3rd echinocandin
Chairpersons: E. Concia (*Verona*)

14.00 **Why another echinocandin? Microbiological point of view**
G.P. Gesu (*Milan*)

14.25 **Why another echinocandin? PK/PD aspects**
F. Scaglione (*Rome*).....37

14.50 **From laboratory to bedside. Why a new echinocandin**
P.L. Viale (*Udine*).....40

Session 3
Clinical aspects II
Chairpersons: G. Carosi (*Brescia*), A.M. Nosari (*Milan*)

16.00 **Fungal endocarditis**
M. Venditti (*Rome*).....42
Discussant: P.L. Viale (*Udine*)

16.45 ***Pneumocystis* pneumonia: a fungal disease not to be neglected**
V. Del Bono (*Genoa*), E. Furfaro (*Genoa*) 45
Discussant: M. Venditti (*Rome*)

17.30 **Aspergillosis: community acquired or health care associated infection?**
S. Brusaferro (*Udine*) 47
Discussant: N. Petrosillo (*Rome*)

18.15 **An interisting case report**
E. Delfino (*Genoa*)

Saturday, June 27

Session 4:
Therapeutic aspects
Chairpersons: R. Cauda (*Rome*), F. Paradisi (*Florence*)

Presentazione

Le micosi invasive sono un'importante causa di morbilità e mortalità nel paziente critico ematologico, oncologico, trapiantato e in rianimazione e la loro incidenza è in crescita. Tali infezioni aumentano perché i progressi della medicina rendono i pazienti più immunocompromessi e quindi più vulnerabili ad essere colpiti dalle micosi opportunistiche.

La maggior parte delle micosi invasive è causata da Candida e Aspergillo, responsabili di oltre il 90% di tutte le infezioni fungine nosocomiali, anche se non bisogna sottovalutare altri patogeni, quali per esempio gli zigomiceti, che rappresentano una sgradita novità epidemiologica.

Per quanto riguarda le aspergillosi, si è osservato un aumento soprattutto a carico del polmone, sia nei pazienti in terapia per leucemia, sia in quelli con tumori solidi sottoposti a chemioterapie intensive o ad alte dosi. L'aspergillosi è, inoltre, la micosi gravata da più alto tasso di mortalità, specie nei soggetti con immunodepressione grave, e il suo accertamento diagnostico non è sempre facile. È opinione comune che un'accurata diagnosi precoce nei pazienti a rischio e la rapida istituzione di un'adeguata terapia antifungina, possano avere un ruolo cruciale nell'arginare l'evoluzione sfavorevole dell'infezione. Anche le candidosi invasive sostenute sempre più frequentemente da ceppi di Candida meno responsivi al fluconazolo rappresentano un importante problema ospedaliero. I punti cruciali nella gestione delle micosi invasive sono almeno tre:

1) *la diagnosi precoce dell'infezione;*
2) *la sua gestione razionale, con identificazione dei focolai e del microrganismo in causa;*
3) *la verifica dell'efficacia delle terapie. A quest'ultimo riguardo è bene ricordare che si sono compiuti importanti progressi nel trattamento delle micosi invasive ottenuti con l'impiego di nuovi farmaci come le echinocandine e i triazolici, che si aggiungono ai polieni e agli altri azolici.*

In questo quinto congresso Nazionale sulla Diagnostica e Terapia delle Micosi opportunistiche cercheremo di aggiornare gli sviluppi degli ultimi due anni nella gestione di questa impegnativa patologia. Questo workshop si pone l'obiettivo di fare luce sulle ultime novità nella diagnosi, clinica e terapia delle infezioni fungine invasive, dedicando una parte sulla gestione clinica pratica di tali infezioni.

Nell'augurarci tre giorni di stimolante e proficuo confronto sugli argomenti in oggetto, desideriamo porgere a tutti i partecipanti un cordiale benvenuto.

Claudio Viscoli

DRUG RESISTANCE IN FUNGI: AN EMERGENT PROBLEM?

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Up to date, available antifungal drugs are mainly represented by three classes and by 5-flucytosine, characterized by different mechanisms of action and spectrum of activity. The physiologic key role of cell membrane together with the different sterols composition (ergosterol in fungal cells and cholesterol in mammalian ones) identify this fungal structure as a favourite target for antifungal drugs (polyenes and azoles), showing adequate activity. The synthesis of glucans of the cell wall (echinocandins) could be considered another useful metabolic target. The emergence of primary (innate) or secondary (acquired) antifungal resistance can be strictly related to the mechanism(s) of the toxic activity of the drug and could be considered the cause of unsuccessful therapy (1). However, antifungal resistance is more multifaceted, and a unsuccessful treatment could be due to several factors involving, in agreement with White, host, antimicrobial compound, and, obviously, the guilty fungus (2). The drug resistance could involve different molecules of the same class, depending on their major or minor target affinity, or molecules of different classes, depending on aspecific cellular mechanisms of toxic substances extrusion (efflux pumps).
Microbiological assessment of antifungal resistance is now possible thanks to the advent of reference methods for *in vitro* susceptibility testing for both yeasts and moulds and, although limited to fungi belonging to *Candida* genus, to the MIC breakpoints agreement for some drugs (Table 1).

TABLE 1. In vitro interpretation values concerning the potential therapeutic efficacy of some antifungal drugs on infections caused by Candida spp.

*Results interpretation values: MIC in μg/ml and inhibition diameter (mm)^*								
Drug	*Susceptible*		*Susceptible dose dependent*		*Intermediate*		*Resistant*	
	MIC	*mm*	*MIC*	*mm*	*MIC*	*mm*	*MIC*	*mm*
Anidulafungin*	2	ND°		ND			ND	ND
Caspofungin	2	ND		ND			ND	ND
Micafungin*	2	ND		ND			ND	ND
Fluconazole	≤ 8	≥ 19	16-32	15-18			≥ 64	≤ 14
Itraconazole	≤ 0.125	ND	0.25-0.5	ND			≥ 1	ND
Voriconazole	≤ 1	≥ 17	2	14-16			≥ 4	≤ 13
5-Flucytosine	≤ 4	ND			8-16	ND	≥ 32	ND

^Clinical Laboratory Standards Institute (CLSI) protocols (M27A3, M27S3)
*A recent paper by David Perlin group (AAC 2009, 53: 112-122) suggests a breakpoint ≤0.5 for these two echinocandins
°ND = Not Determined

Most of the studies concerning the molecular mechanisms of antifungal resistance have been performed on *C. albicans* clinical isolates.

Echinocandins

Echinocandins inhibit the synthesis of beta-1,3-D-glucan, essential component of fungal cell wall. This mechanism of action permits their use on both yeasts and moulds resistant to azoles, but results not useful for *Zygomycetes*, *Cryptococcus neoformans* and *Fusarium* spp. Echinocandins are fungicidal on yeasts and fungistatic on molds, blocking the hyphal apical growth, and they are active in vitro and in vivo against fungal biofilms (3). Reduced susceptibility to echinocandins could be related to FKS genes mutations (FKS1, FKS2, e FKS3), that influence aminoacidic substitutions of their target beta-1,3-D-glucan synthase (3).

Mutations on two hot-spot regions of FKS1 gene seem to correlate with a reduced in vitro susceptibility of *Candida albicans* and related species, whereas mutations of FKS2 gene seem to be strictly related to *C. glabrata* echinocandins-resistant strains. A spontaneous polymorphism of FKS1 hot-spot regions in *Candida parapsilosis*, *C. orthopsilosis,* and *C. metapsilosis*, seems to be responsible of reduced susceptibility to echinocandins (3). Because the mechanism is specific for echinocandins, such resistant strains retain susceptibility to azoles and amphotericin B (4). Up to date, *Aspergillus* spp resistance has never been reported in literature, however some authors were able to induce resistance in laboratory genetically modified strains of *A. fumigatus* (3).

5-flucytosine

5-flucytosine is a fungistatic drug that acts as an uracil competitive antimetabolite, inhibiting the thymidylate synthase and consequently the RNA synthesis.

A cellular permease is responsible of its antifungal activity, facilitating the access to fungal cell where the drug is transformed by cytosine deaminase in the active form, 5-fluorouracil.

The monotherapy with 5-flucytosine is limited by the presence of both primary and secondary resistance, that could be due to the permease loss, coded by FCy2 gene, resulting in lack of drug entry, or to alterations of other enzymes: cytosine deaminase, coded by FCy1 gene, or uracil-phosphoribosyltransferase, coded by FUR1 gene (4).

Polyenes

Polyenes (amphotericin B, nystatin) interact with ergosterol with conseguent formation of membrane pores and cell lysis. Up to date, amphotericin B, with their liposomal and lipid forms, represents the most utilized antifungal drug for the treatment of invasive infections in immunocompromised or critical patients.

The microbiologic resistance to polyenes is difficult to investigate in vitro because heavily dependent on test methods (4, 5).

Thus, the molecular mechanisms of amphotericin B resistance have been investigated on laboratory mutants instead of sequential clinical isolates during antifungal therapy and after its failure (2, 5). These experiments suggest some hypotheses: a complete lack of ergosterol in fungal membrane, a masking or a different ergosterol structure

that inhibits polyenes binding, an alteration of ergosterol (ERG3) biosynthetic pathway resulting in other sterols synthesis with minor affinity for polyenes (1, 4). *Candida lusitaniae* and *Aspergillus terreus* are thought to be naturally resistant to polyenes.

(Tri)azoles

The azole class is represented by various molecules (imidazoles with two nitrogen atoms and triazoles with three nitrogen atoms) that inhibit the sterol-14-alfa-demethylase, encoded by ERG11 in yeasts or CYP51 in moulds, key enzyme for ergosterol biosynthesis.

Among triazoles, fluconazole shows most restricted spectrum (yeasts and some dermatophytes), whereas the other three molecules (itraconazole, posaconazole, voriconazole) act on both yeasts and moulds, with the exception of *Zygomycetes*, that show only a partial species-specific posaconazole sensitivity. *Candida krusei* shows a primary resistance to fluconazole, but not to other triazoles.

Azoles resistance is reported for various fungi including *Aspergillus* spp, but the phenomenon has been extensively studied in *Candida albicans* strains sequentially isolated from HIV patients subjected to prolonged fluconazole treatment (2).

Various molecular mechanisms of fluconazole resistance demonstrated for *C. albicans* have been subsequently observed in other opportunistic fungal species. These mechanisms are:

a) upregulation or mutation of ERG11 gene;
b) overexpression of efflux pumps, transmembrane proteins with important detoxicant capability, resulting in a lack of intracellular drug access.

The efflux pumps involved belong to two main classes: ATP-Binding-Cassette (ABC) and Major Facilitators (MF).

In *C. albicans,* the MF pump, linked to fluconazole resistance, represents the gene product of MDR1, whereas the ABC pumps are encoded by CDR1 and CDR2. Homologues efflux pumps have been discovered in clinical isolates of *Candida glabrata* (CgCDR1, CgCDR2, CgSNQ2), *C. dubliniensis* (CdCDR1, CdCDR2, CdMDR1), *C. tropicalis* (CtMDR1). Mutation of ERG11 have been reported in clinical resistant strains of *C. glabrata, C. krusei* e *C. tropicalis* (2).

ERG genes alterations, particularly in ERG3, could be also responsible of azole resistance (1, 2, 4). Recent studies are mainly focused on transcriptional factors that orchestrate the promoter region of these genes, and demonstrate that deletions in genes coding for transcriptional factors could revert resistant strains in sensitive to antifungal drug targeting ergosterol (2, 4).

The secondary azole resistance in filamentous fungi is less frequent and most of literature findings are referred to *Aspergillus fumigatus* isolates (4-6). The molecular mechanisms utilized by *Aspergillus* spp. are similar to those described for *Candida* spp. and involve upregulation of efflux pumps and modifications of sterol-14-*alfa*-demethylase (6).

Conclusions

In vitro, the drug resistance of an etiologic agent could correlate with the therapeutic failure only if the susceptibility/resistance profile is determined by means of

affordable methods and if the in vitro resistance is subsequent to target modifications or to the lack of drug access into the fungal cell.
Certainly, the knowledge of the molecular mechanisms responsible of antifungal drug resistance represents an important scientific progress, but its real role in the clinical outcome of invasive fungal infections is still unclear.
On the whole, a reasoned use of antifungal drugs in clinical practice could contain the selection of resistant strains, and could avoid the emergence of fungal species "naturally" resistant to the few available molecules.

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AZOLE RESISTANCE IN ASPERGILLUS

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With respect to other fungal species commonly causing invasive fungal infections (IFI), *Aspergillus* species are increasingly responsible for serious life-threatening infections, especially in patients with cancer and severe neutropenia and in hematopoietic stem cell transplant recipients (1). The widespread use of fluconazole prophylaxis in such patients means that invasive aspergillosis (IA) and other filamentous fungal infections, rather than infection by *Candida* species, cause the majority of deaths from IFI (1) with IA mortality exceeding 50% in most reports. *Aspergillus fumigatus* still accounts for the majority of cases of IA; however, less susceptible to antifungal agents non-*fumigatus* aspergilli have emerged in recent years. Clinical failure of IA may due to many factors including the weak defenses of the immunocompromised host and poor bioavailability of the administered drug, antifungal resistance accounts, at least partially, for the poor outcomes in IA. Thus, reports of drug-resistant clinical isolates of *Aspergillus* have emerged in recent years, as well as studies dealing in antifungal resistance mechanisms (2).

Currently there are four classes of antifungal agents with activity against *Aspergillus*: the polyenes, such as amphotericin B deoxycholate and its lipid formulations; the triazoles, including itraconazole (ITC), voriconazole (VRC), and the newer posaconazole (PSC), albaconazole; the echinocandins, such as caspofungin, micafungin, and anidulafungin; and the allylamines such as terbinafine. Although the echinocandins, the most recently developed class of antifungals, are fungistatic for *Aspergillus* and other filamentous fungi, caspofungin has been approved as salvage therapy for patients with IA. Conversely, while the triazole fluconazole is ineffective against *A. fumigatus*, ITC has been the first azole introduced for the treatment of aspergillosis, and VRC is superior to amphotericin B for the treatment of IA and has become the primary treatment choice.

The azole antifungals interfere with the biosynthesis of ergosterol from lanosterol, by inhibiting the cytochrome P450 14-α demethylase (Cyp51p), encoded by the *ERG11* (*CYP51* in *Aspergillus*) gene. This inhibition depletes the membranes of ergosterol and results in the accumulation of toxic sterol pathway intermediates, which arrest fungal growth. Unlike *Candida* species, primary resistance does not appear to be a problem, since most *Aspergillus* species are highly susceptible to these drugs, whereas a significant number of clinical isolates of *A. fumigatus* displaying secondary acquired resistance to ITC and other triazoles have been described (3). Very recently, clinical *A. fumigatus* isolates with multiple resistance to VRC, ITC, RVC, and PSC were also recognized (4).

Triazole resistance mechanisms in *Aspergillus* mainly involve alterations of the *CYP51* gene (5). Although in *A. fumigatus* there are two distinct but related Cyp51 proteins encoded by *CYP51A* and *CYP51B*, only mutations in *CYP51A* are important

for clinical resistance. These mutations, which include amino acid substitutions at the methionine (Met) 220, glycin (Gly) 54, Gly 138, Gly 448, and leucin (Leu) 98, coupled with a tandem repeat (TR) of a 34-bp sequence in the *CYP51* gene promoter, confer different susceptibility profiles as listed in Table 1.

TABLE 1. Amino acid substitutions in the *CYP51A* gene conferring triazole resistance in *A. fumigatus*

Amino acid changes	*Antifungal(s)*	*Reference*
M220I, M220V, M220K, M220T	ITC	Qiao et al, 2008
G54R, G54E, G54K, G54V	ITC, PSC	Qiao et al, 2008
G138C, G448S	ITC, VRC	Garcia-Effron et al, 2008; Bellete et al, 2009
L98H	ITC, PCS, VRC, RVC*	Qiao et al, 2008; Garcia-Effron et al, 2008

*This pattern of cross-resistance is due to a higher *CYP51A* expression produced by the 34-bp TR in the gene promoter, in combination with the amino acid substitution L98H as indicated.

Reduced intracellular accumulation of the drug due to increased expression of efflux pumps has been noticed in itraconazole-resistant *A. fumigatus*. However, despite the large number of genes encoding transporters in *A. fumigatus* as predicted by using the genome analysis, there is only some evidence concerning the relationship between overexpression of the transporter genes and triazole resistance in *A. fumigatus*, making necessary to give further insight into this phenomenon.

Susceptibility testing for clinical isolates of *Aspergillus* species is time-consuming and rarely performed, which represents an obstacle to effective therapy. In an effort to improve the identification of triazole resistance in primary isolates of *A. fumigatus*, Garcia-Effron et al. (3) described the development of a real-time PCR assay utilizing allele-specific molecular beacons to rapidly and accurately assess *A. fumigatus CYP51A* gene mutations conferring resistance to triazole drugs, thereby providing a comprehensive evaluation of drug resistance in this fungal pathogen.

Lastly, to test the hypothesis that azole resistance might develop through azole exposure in the environment rather than in azole-treated patients, Snelders et al. (6) compared antifungal susceptibility, resistance mechanisms, and genetic relatedness of ITC-resistant *A. fumigatus*, cultured from the indoor hospital environment or the soil in proximity to the hospital, with those of azole-resistant clinical isolates. While cross-resistance was observed for VRC, PSC, and the azole fungicides metconazole and tebuconazole, a single dominant resistance mechanism was present in both the clinical and 13 of 15 environmental isolates. Interestingly, environmental and clinical isolates were genetically clustered apart from non-resistant isolates, suggesting that patients with azole-resistant aspergillosis might have been colonized with azole-resistant isolates from the environment.

In conclusion, although certain azole resistance mechanisms has been defined in *A. fumigatus*, further understanding is strongly needed in *A. fumigatus* and non-*fumigatus Aspergillus* species. This should aid in maximizing the clinical utility of current drugs and in developing new antifungal agents and treatment strategies.

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PROGRESS ON ANTIGEN DETECTION FOR DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS

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In high-risk patient cohorts, such as patients after allogeneic stem-cell or solid-organ transplantation, or patients with acute leukaemia, early diagnosis of invasive fungal infections (IFIs) is essential. The limited sensitivity and specificity of conventional assays for the detection of IFI and the growing number of immunocompromised patients who are at risk for opportunistic fungal infections have led to the development of new assays. These methods include antigen detection systems, such as galactomannan (GM) immunoassay for the diagnosis of invasive aspergillosis (IA) and the (1→3)-β-D-glucan (BG) assay for the diagnosis of invasive disease caused by *Aspergillus* and *Candida* species and other opportunistic fungi. GM is a cell-wall polysaccharide specific to *Aspergillus* species that is detectable in serum and other body fluids during IA. In July 2003, the Platelia *Aspergillus* EIA test (Bio-Rad Laboratories) was approved by the US FDA for use in the diagnosis of IA in HSCT recipients and in patients with leukemia.

Data presented to the FDA cited a sensitivity of 80.7% and specificity of 89.2% for the diagnosis of IA, based on a multicenter study conducted on serially collected serum samples from 179 HSCT recipients and patients with leukemia, 31% of whom developed proven or probable IA. Parameters used in the study to determine a positive assay result included an OD index value of ≥0.5 and two positive assay results for an aliquot of the same serum sample (1). Recent literature reviews underlines that GM assay has moderate accuracy for diagnosis of invasive aspergillosis in immunocompromised patients. The test is more useful in patients who have hematological malignancy or who have undergone hematopoietic cell transplantation than in solid-organ transplant recipients (2).

The galactomannan enzyme immunoassay test has also been proposed as a surrogate marker for outcome evaluation. The variability in assay performance is considered to be multifactorial and includes issues affecting the release of the antigen from the hypha (e.g., fungal strain and stage of growth), leakage of the antigen from the site of infection into the blood, binding of the antigen by blood substances, host factors (e.g., location and extent of fungal disease, antifungal treatment, and age), and methodological factors, such as OD cutoff, definition of a positive "test" (single vs. sequential positive results). False-positive GM assay results have been reported for patients receiving piperacillin-tazobactam and amoxicillin-clavulanate. Measurements of GM in body fluids other than serum, such as bronchoalveolar lavage (BAL) fluid, urine, and cerebrospinal fluid, has also been proposed, but OD cutoff has not been fully investigated (1). BG is a cell-wall constituent of many pathogenic fungi, including *Aspergillus* and *Candida* species, and is detectable in patients' serum during invasive disease due to these organisms. In addition to patients with IA and candidiasis, BG is also detectable in patients with infections caused by species of

Fusarium, Trichosporon and *Saccharomyces*. The test does not detect infection with *Cryptococcus* species or Zygomycetes because of the low quantities of BG in the cell walls of those fungi. The BG test (Fungitell; Associates of Cape Cod) was approved by the US FDA for the qualitative detection of BG in the serum of patients with symptoms of or medical conditions predisposing to IFI and as an aid in the diagnosis of deep-seated mycoses and fungemia. The assay has been evaluated in multicenter studies of patients with IFIs and healthy control subjects and for the early diagnosis of IFIs in patients with hematologic malignancies. Sensitivities and specificities ranged from 63% to 70% and from 67% to 96%, respectively (1, 3-5). Although a positive test result for the presence of BG does not identify the infecting fungus, the practical application of this test includes its use as a screening assay (presumptive marker) for invasive fungal infection to allow the earlier initiation of antifungal therapy. Other tests are necessary for the confirmation and identification of the fungal pathogen. BG is ubiquitous in the environment (e.g., in some types of gauze and laundry starch), and false-positive results may be caused by poor specimen handling, hemodialysis using certain cellulose membranes, exposure to certain types of gauze, and recent receipt of albumin or immunoglobulin products. Additional studies are needed in order to better define the clinical application of this test.

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CANDIDA INFECTIONS IN THE INTENSIVE CARE UNIT

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Invasive candidiasis is a life-threatening infection with high mortality rates, especially in immunocompromised and critically ill patient. Mostly nosocomial, it occurs up to 10% of patients in Intensive Care Units (ICU), compared with 1-8% of patients admitted to hospitals. In ICU, it may represent up to 15% of nosocomial infections. In recent decades, nosocomial surveillance data show that *Candida spp*. rank as the fourth most common cause of nosocomial bloodstream infections and the third in ICU patients. Since recent studies have confirmed the predominant role of candidiasis among the invasive mycoses, there is an ever-increasing need to understand the epidemiology of both nosocomial and community-acquired fungemia. This infection adds substantially to the morbidity and mortality rates of seriously ill patients and represents an independent factor for predicting risk of death and prolonged hospital stay. Reviews on *Candida* epidemiology showed that the spectrum of patients at risk of invasive candidiasis is moving from the immunocompromised host (haematology, BMT) to critically ill non-immunocompromised patients. Nowadays, more patients with severe underlying disease or immunosuppression from anti-neoplastic or anti-rejection chemotherapy and at risk for fungal infection are admitted to the ICU. Improvements in supportive medical and surgical care have led to many patients who would previously have died as a result of trauma or disease to survive due to intensive care procedures. There are many studies performed during 1980-1990 underlining the growing importance of candidiasis in surgical and intensive care patients. European data showed that invasive candidiasis accounted for 17% of hospital-acquired infections (1). As will be further discussed, for the difficulty in defining the term *candidiasis* in ICU patients, it is possible that the high rate of fungal infection observed in the EPIC study might have reflected confusion between fungal colonisation and infection. In the US, the NNIS system (National Nosocomial Infection Surveillance), collecting data of 790 ICUs from nearly 300 institutions between 1990 and 1999, evidenced that *Candida spp.* were responsible for 5-10% of all bloodstream infections (2). Although higher rates of candidemia are demonstrated in ICU, trend over time are also important to be considered. Another factor that might be taken into account for the epidemiological trend is the changing of critically ill patients over the last decades. Starting from 1990 and especially in the last five years, an important growth in the pool of patients receiving immunosuppressive antineoplastic or anti-rejection chemotherapy has been registered among patients population admitted to ICU. In addition, improvements in supportive medical and surgical care have led to improved survival rates: this contributed to create a long-term resident group of patients in ICU with increased risk of fungal infection. Moreover, some therapeutic interventions used in critical patients are associated with development of candidiasis, such as broad spectrum antibiotics, intravascular catheters and parenteral nutrition. It is also remarkable that the same fact of being recovered in

ICU is a risk factor for candidiasis, as reported in literature (3). Despite significant geographic and demographic variation respect to the frequency of *Candida spp.* isolates among different institutions, *C.albicans* remained the predominant strain in most countries also among critically ill patients. Isolation of *non-albicans* species, that occurs more frequently in patients with haematological malignancies and bone marrow transplant and reported to be less common among ICU patients (35-55%) recently increases (4). Even if change in the types of Candida spp. isolated has been related to the widespread use of fluconazole in prophylaxis and to increased use of invasive procedures, reasons for the variability in the frequency of the different species and fluconazole rule remain controversial, also in critically ill patients. It is recognized that if the use of azole antifungal agents for prophylaxis has decreased the incidence of invasive candidiasis (e.g. in bone marrow transplant recipients), at the same time it has contributed to the emergence of azole-resistant *Candida albicans* or to a shift in species distribution towards *non-albicans* species. Among the reports on risk factors for *non albicans* candidemia, the studies that identified a highly significant association with prior azole therapy or prophylaxis were performed in cancer and surgical ICU patients. These data strongly suggest that antifungal prophylaxis in critically ill, non-immunocompromised patients could be considered for selected-high risk groups where the incidence of candidiasis is expected to be higher than 10%. This approach may help to limit the quantity of antifungals used for prophylaxis and delay the emergence of infections due to *non-albicans candida* strains seen in immunocompromised patients.

Given high rates of mortality for these infections, definitive diagnosis is imperative. It is often difficult to distinguish colonisation with *Candida spp*. from invasive infection in critically ill patients: among hospitalised patients, only 5–15% of are already colonised at entry, but this proportion irremediably increases with time and exposure to risk factors and as many as 50 to 86% of critically ill patients may become colonised with *Candida spp.* during prolonged ICU stay. However, only 5 to 30% will develop severe candidiasis. Because of the non-specific presentation of systemic *Candida* infections, it would be useful to identify patients who are at high risk of candidiasis in order to initiate antifungal therapy. Sequential spread of candidal colonisation from the abdominal cavity to other body sites before candidaemia occurred was described by Solomkin and colleagues (5) who also demonstrated the role of early antifungal therapy, the pre-emptive approach, in the presence of risk factors for infection and substantial colonisation. Preemptive therapy defines early antifungal treatment given to patients with several risk factors for infection and evidence of significant candidal colonisation.

Recently, Leon and coll. proposed a simple bedside scoring system, named "Candida score" that may assist clinicians in differentiating between *Candida* spp. colonization and proven *Candida* infection. A score > 2.5 helps physicians select patients who will benefit from early antifungal administration (6). Pre-emptive antifungal therapy should be given to patients with well-established risk factors, including a known degree of colonisation with *Candida* spp. In these patients, the risk of severe candidiasis is so high that the benefit of immediate antifungal treatment outweighs its potential side-effects, including the possible emergence of resistant strains.

Alternatively, in cases of suspected candidiasis, the worsening general condition of a critically ill patient with multiple organ failure may also justify empirical antifungal treatment before blood-culture results are available. For patients with some risk factors, but in whom the immediate institution of antifungal treatment is not justified, assessment of the degree of candidal colonisation will allow early identification of those who might benefit from pre-emptive antifungal therapy.

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INVASIVE ASPERGILLOSIS IN ACUTE MYELOID LEUKEMIA: FROM SCIENTIFIC TRIALS TO CLINICAL PRACTICE

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Invasive aspergillosis (IA) remains one of the most frequent infectious complications affecting hematological patients, in particular those suffering from acute myeloid leukemia (AML).
Since '80s, many progresses have been made in the management of such a complication. Several multicenter experiences proved this advance, with IA-attributable mortality rates in acute leukemias (both lymphoid and myeloid) decreasing from 60% during 1987-1988 to 32% during 2002-2003 (p-value 0.019) (1). Both the improvement of diagnostic tools (galactomannan test, polymerase chain reaction tests, and a high-resolution chest computed tomography scan) and the availability of newer antifungal drugs (liposomal amphotericin B at conventional or higher doses, caspofungin, voriconazole, posaconazole) can reasonably explain this success.
In particular several randomized clinical trials (RCTs) have proven the effectiveness and tolerability of newer drugs compared to the former gold standard amphotericin B deoxycolate (2-4). Since their publication, these studies have influenced clinical decision making. Resulting evidence has finally been summarized in clinical guidelines, recently published by different scientific societies (5, 6).
RCTs are more likely to be accepted by physicians generally, as they are the only way to control for confounding variables. However several limits have recently emerged, particularly when focusing on the therapy of invasive mycoses.
Antifungal drugs' efficacy, as reported in RCTs, ranges from 32% to 50% (2-4). However, one should take in mind that the percentage of success reported in clinical trials could be different than that really observed in clinical practice: conventional RCTs usually suffer from the limitations that rely on current disease definitions and outcome criteria (7). That's why, how best patient response can be assessed - whether by clinical/radiological response or by survival analysis - is still debated. For example, the primary determinant of outcome in aspergillosis is host immunity, and a strong relationship between outcome and resolution of immunosuppression does exist. Immune recovery may confound outcome assessment, as occurs in immune reconstitution and inflammatory syndrome, with its transient deterioration related to neutrophils' recovery (8).
Another interesting issue concerns the feasibility of guidelines in hematological series, particularly AML patients. Despite evidence based recommendations, adherence to guidelines could not constitute the best therapeutic choice in each and every patient: subjects' clinical conditions and comorbidities can widely vary and make the "recommended" drug a not-optimal strategy, paradoxally. The introduction of stringent inclusion and exclusion criteria, makes RCTs population extremely different from that daily managed by physicians. Organ dysfunctions, potential interac-

tions with other drugs, childhood age, which are common exclusion criteria in RCT, are normal features in clinical practice and practitioner are daily confronted with such problems (9).

In this context, not only clinical trials and guidelines, but also multicenter surveys and case series could be of help in defining IA optimal management. Local and personal experience could also play a role.

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FUNGI AND ANTIFUNGAL TREATMENTS IN ASTHMA

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Inhalational exposure to environmental fungi occurs ubiquitously and for the vast majority of healthy people is thought to be harmless. Common species such as *Aspergillus spp, Penicillium spp, Alternaria spp and Cladosporium spp,* can be detected in both indoor and outdoor air throughout much of the year, with seasonal and climatic fluctuation. Conidial spore sizes for this group of fungi range in size from 2 μm to 70 μm, leading to impaction on every part of the airway, from the respiratory bronchiole to the sino-nasal passage. More than 80 species of fungus have been associated with symptoms of airways allergy (1).

Environmental fungi are associated with a number of bronchopulmonary conditions
Fungal antigens can induce a number of different clinical bronchopulmonary disorders, each with a distinct immune pathogenesis. Allergic bronchopulmonary aspergillosis (ABPA) is characterized by type I immediate-type hypersensitivity along with a strong Th2-weighted atopic response to antigens of *Aspergillus fumigatus* in genetically predisposed patients (2). The fungus colonizes the airways in these individuals; hyphus-rich thick mucus casts causing lobar obstruction are a key feature. Other clinical manifestations include airway- and peripheral blood eosinophilia, proximal bronchiectasis and asthma. By contrast, the pulmonary condition *extrinsic allergic alveolitis* can be associated with an IgG-mediated response or type III hypersensitivity reaction to inhaled occupational fungal antigens. Examples (there are many) include 'farmer's lung' (*Thermoactinomyces vulgaris*, *Micropolyspora faeni*) and 'malt worker's lung' (*Aspergillus clavatus*).

Atopy to environmental fungi is associated with severe asthma
Evidence now suggests that those severe asthma suffers without ABPA are more likely to be atopic to fungal allergens than patients with milder disease. The diagnostic label '*Severe asthma with fungal sensitization - SAFS'* has recently been applied to this group (3). This group may be distinguished from ABPA by lower total IgE levels (<1000 kiu/l) and less peripheral blood eosinophilia. A number of studies have shown an association between fungal atopy and hospital admission due to asthma – a key marker of asthma severity. In a UK case-control study, O'Driscoll showed that mould sensitisation among asthmatics was related to hospital admission (4). In the European Community Health Survey, a cross-sectional study revealed that fungal sensitization was related to a composite severity score which included measures of hospital admission, FEV_1 and use of oral corticosteroids (5). These studies help to corroborate previous observations on the seasonality of asthma hospital admissions: in Chicago, admissions due to asthma to intensive care peaked for those patients with *Alternaria spp* sensitization during the peak exposure period for this fungus (6). Furthermore, during thunderstorms, climatic conditions lead to the disturbance and rupture of both grass pollen and fungal spores and occasionally cause asthma exacerbation epidemics. Dual grass-pollen- and fungus-sensitized asthmatics are at

particular risk of emergency admission, with a recent UK study showing the fungal geni *Didymella, Alternaria* and *Cladosporium* to be responsible (7).

Indoor dust reservoirs contain fungus, but how do we measure fungal allergens in the home?

Studies of indoor domestic exposures to non-fungus aeroallergen sources such as the house dust mite and furred pets reveal the existence of household allergen reservoirs in bedding and soft furnishings. For these allergens, one can quite easily investigate the relationships between allergen concentrations, specific allergen sensitization and disease severity. Domestic fungal exposures may also exacerbate asthma: household exposure to *Cladosporium spp.* doubles the risk of an asthma attack (8) and non-specific bronchial reactivity is associated with damp housing (9).

Potential fungal allergen reservoirs do include bedding, soft furnishings and damp walls. However, the relationships among exposure and sensitization are harder to study than for mite and furred pet allergens. β-glucan can give us a measure of pan-fungus exposure, but our main interest is exposure to proteins of the fungal allergome. Its complexity is problematic for researchers; while less than 20 house dust mite allergens are described, this number are known to be produced by *Aspergillus fumigatus* alone (10).

Even if the monoclonal antibodies required for their environmental detection were to be developed, choosing the most clinically-relevant set of allergens will take time. Meanwhile, we still rely on culture methodology to explore potential fungal allergenic reservoirs: pillows recently were shown to be a rich source of fungal growth (11). A further difficulty is the complex aerobiology of fungal allergenic paerticles. Much of airborne fungal biomass is likely to be in the form of hyphal fragments (12) which cannot be easily measured. Hyphal fragments are not inert; allergen expression by the hyphus may be triggered under favourable conditions as may occur in the airways (13). This adds a further variable to be considered when measuring exposure.

Antifungal therapies in asthma

Early studies of patients with allergic broncho-pulmonary aspergillosis showed the therapeutic efficacy of azole medication (15, 16). The first randomised controlled trial of azole medication in patient s with SAFS was recently published (17). Patients were randomised to itraconazole 200 mg *bd* or placebo for thirty-two weeks and were followed up until forty-eight weeks.

There were clinically significant improvements in the active group in both asthma-specific quality of life (AQLQ) and rhinitis score. Morning peak flow improved and total IgE reduced. The benefits in AQLQ and rhinitis score waned at 48 weeks, 16 weeks after withdrawal of the medication. This was a proof-of-concept trial; there is much more work to be done. A significant proportion of individuals in the active arm had reduced early morning cortisol levels, raising the possibility that study medication may have inhibited hepatic catabolism of synthetic inhaled corticosteroids.

The optimum duration of azole therapy in these circumstances remains to be determined as do any long-term sequelae.

Environmental fungi colonize the lungs of otherwise healthy people (14). This raises the possibility of a link between exposure to colonizing fungus and asthma exacerbation in individuals sensitized to these fungi.

We need a better understanding of the human lung microbiome as well as the indoor and outdoor environments. Together with studies of the fungal allergome, this will enable us to interrogate the relationships between fungal sensitization, exposure and asthma and thereby help target future new therapies for this important condition.

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TRANSLATING THE BROAD-SPECTRUM AND FUNGICIDAL PROPERTIES OF LIPOSOMAL AMPHOTERICIN B INTO CLINICAL PRACTICE

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Neutropenic patients are at high risk to develop invasive fungal infections (IFI). Immediate antifungal treatment has been considered necessary for the treatment of these patients. However, only a minority of patients have 'probable' or 'proven' IFI according to EORTC-/MSG-criteria. The majority of the patients present with ongoing fever in neutropenia or 'possible' IFI only. To date, two treatment strategies are used for patients with fever in neutropenia and suspected IFI: empirical or preemptive antifungal therapy. There is no clear evidence as to which strategy is superior. Empirical treatment bears the danger of 'overtreatment' with potentially toxic and expensive drugs, whereas preemptive therapy may sometimes be initiated too late to work efficiently against fungal disease.

Candida and *Aspergillus* spp. are the cause of most infections. These fungal infections are associated with high overall mortality rates. Other yeasts and moulds are being recognized as agents of invasive fungal infection. Different strategies (prophylaxis, pre-emptive treatment, empirical therapy, antifungal combinations, routes of administration) have been tested to improve the prognosis of these invasive mycoses. To achieve this objective it is essential to have new antifungal drugs with a higher spectrum of activity against the fungal pathogens, both classical and emerging, and showing improvements in pharmacokinetic and pharmacodynamic characteristics, ease of administration and acceptability, and lower rates of adverse effects.

The ideal agent for empirical, pre-emptive or targeted, specific therapy is one which has a broad spectrum, acts quickly, and is fungicidal. Amphotericin B is a good example of an antifungal that meets these criteria. Both laboratory studies and clinical response have shown that amphotericin B and its lipid derivatives are the most efficacious in treating a broad range of fungal pathogens.

Time-kill studies indicate how quickly antifungal agents kill the fungus. Liposomal amphotericin B has been shown to have rapid, concentration-dependent antifungal activity. Rapid onset of antifungal activity is crucial in the management of zygomycoses. For example, a time-kill study showed that amphotericin B was rapidly fungicidal against zygomycetes, with 95% killing noted as early as 6 hours and 99.9% killing at 24 hours. Posaconazole showed <70% killing at 6 hours and 99.9% killing at 48 hours These data suggest that amphotericin B is more effective in the early phases of disease, but need to be confirmed by *in vivo* studies. Fungal structure may influence whether a drug is fungicidal or fungistatic. For example, amphotericin B is fungicidal against *Aspergillus fumigatus*, while itraconazole is fungistatic. The antifungal effects of caspofungin against *Aspergillus* are difficult to interpret. It is not known whether echinocandins penetrate fungal mycetomas, for example. The clinical importance of fungicidal activity is unclear.

A greater understanding of the effect of antifungal drugs on fungal structures *in vivo* can inform our clinical decisions. MIC studies can provide useful information. For example, one such study demonstrated that the liposomal formulation of amphotericin B has similar fungicidal activity to conventional amphotericin B. Different formulations of amphotericin B have different *in vitro* activity against fungal species. *In vitro* data indicate that liposomal amphotericin B has broad spectrum activity, while fluconazole does not, and other antifungal agents vary in their activity. Biofilm studies provide further information regarding the mode of action of liposomal amphotericin B. *Aspergillus* biofilms and mycetomas are difficult to eradicate. *In vitro* biofilm models have shown that liposomal amphotericin B can destroy *Aspergillus* biofilms.. Animal studies have shown that the liposomal formulation of amphotericin B is superior to conventional amphotericin B in reducing inflammatory lung activity and fungal burden in corticosteroid-treated mice with pulmonary IA. The survival rate increases as the dose increases, but liposomal amphotericin B can safely achieve higher concentrations than conventional amphotericin B.

In conclusion, a wealth of data from both *in vitro* studies and clinical trials suggests that liposomal amphotericin B is very effective in the treatment of invasive fungal infections. The best choice of drug is always the agent that is the most effective against a given pathogen. The drug should be fungicidal, fast acting, independent of treatment strategy (prophylaxis, empirical, pre-emptive, targeted). The selection may also be influenced by factors such as formulation, tolerability and drug interactions.

WHICH IS THE ROLE OF PK/PD EVALUATION IN THE MANAGEMENT OF FUNGAL INFECTIONS?

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The treatment of systemic fungal infections has been based for many years on the use of polyenes such as amphotericin B, flucytosine and triazoles (i.e. fluconazole and itraconazole). After more than 30 years of clinical use, amphotericin B still remains a drug of choice for the treatment of serious infections, despite its toxicity.
Recently, the antifungal armamentarium has been enlarged by the introduction of new and more potent triazoles characterized by a broad spectrum of activity against both yeasts and moulds (i.e. voriconazole and posaconazole), as well as by the development of newer drugs with a different mechanism of action on the synthesis of the cell wall, namely the echinocandins: caspofungin, micafungin, and anidulafungin (1, 2). Like antimicrobial drugs, the various antifungals display static or cidal activity against pathogenic fungi, and may have both an *in vitro* and *in vivo* post-antifungal effect (PAFE) (3).
Two major PD behaviours of these drugs have been recognized, namely concentration-related and time-related activity. Both the polyenes and the echinocandins are concentration-dependent drugs with predictable dose-exposure relationships, the results obtained from dose fractionation studies suggest that the Cmax/MIC is the best PK-PD parameter with a maximal efficacy for a total drug Cmax/MIC value of 10 and a net inhibitory effect for values near 4 (Table) (3).
For amphotericin B evidence of the concentration-dependent activity has been confirmed *in vivo* in different acute animal models. In the last 15 years, attempts have been made to both reduce its toxicity and improve its solubility without affecting antifungal activity (4). The encapsulation of amphotericin B into liposomes or other lipid complexes has led to reduced toxicity and has improved its clinical efficacy. The PK properties of the various liposomal and lipid formulations are quite different from each other, and the relationship between animal data and results in human studies requires further clarification.
The polyene derivative has been complexed with phospholipid ribbons (ABLC) or cholesterol disks (ABCD), or incorporated into unilamellar liposomes (L-AmB). The daily dose of these new formulations is usually higher than that of AmB-d and L-AmB has significantly higher blood levels than the other lipid preparations or AmB deoxycholate (AmB-d) (4). The echinocandins are lipopeptides that have been synthetically modified from the fermentation broths of various fungi and have demonstrated concentration-dependent killing and prolonged PAFEs, similar to those observed with the polyenes (Table) (3). The predominant PK differences among the three agents are volume of distribution, metabolism and half-life (1).
For both polyenes and echinocandins there is no need for therapeutic drug monitoring during treatment either for efficacy or toxicity purposes, which differs from the requirements for azole derivatives (5).
Azole derivatives are concentration-independent or time-dependent drugs and the

TABLE. Summary of PK/PD relationship of antifungal drugs (3).

Drug	*Time-kill kinetics in vitro*	*PK/PD relationship in vivo associated with effective therapy*[a]
Amphotericin B	Concentration-dependent fungicidal activity against *Candida*, Cryptococcus and *Aspergillus fumigatus*	C_{max}/MIC 4-10
Flucytosine	Concentration-independent fungistatic activity against *Candida*, Cryptococcus spp.	Time/MIC >40%
Triazoles Fluconazole Itraconazole Voriconazole Posaconazole	Concentration-independent fungistatic activity against *Candida*, Cryptococcus Time-dependent and concentration-dependent fungicidal activity against *Aspergillus* spp.	AUC/MIC ≥25 (*Candida* spp.) Not well established for *Aspergillus* but plasma C_{min} >500 ng/ml required for itraconazole and voriconazole efficacy; posaconazole response improves with plasma concentrations from 700 to 1500 ng/ml
Echinocandins Caspofungin Micafungin Anidulafungin Aminocandin	Concentration-dependent fungicidal activity against *Candida* species; concentration-dependent fungistatic vs. *Aspergillus*	C_{max}/MIC >4 (Candida) AUC/MIC >250 (tissue or plasma) C_{max}/MEC 10 (*Aspergillus*)[b]

[a]unless otherwise noted, refers to plasma drug pharmacokinetics; [b]MEC (minimum effective concentration); used in lieu of MIC due to the unique pattern of growth inhibition seen with the echinocandins against *Aspergillus* species.

probability of clinical success is significantly higher when the AUC(free drug)/MIC is ~ 25 (Table) (3).

These agents have different chemical structures, which results in distinct PK and PD properties for each compound, although all are characterized by the presence of at least one triazolic ring (2). The comparative clinical PK data of the triazoles indicate several compound-related differences in absorption, metabolism, tissue distribution and elimination (2).

Fluconazole, itraconazole and voriconazole are available both as intravenous and oral formulations, while posaconazole is, to date, only available as an oral suspension (2). All triazoles inhibit different cytochrome P450-dependent pathways with consequent potential interactions with drugs that are metabolized by the same enzymes and a therapeutic drug monitoring is often recommended (with the exclusion of fluconazole) to ensure either efficacy or to avoid or limit toxicity (2, 5).

From a pharmacological point of view, knowledge of both the pharmacodynamics (PD) and pharmacokinetics (PK) of the antifungal drugs is mandatory in order to evaluate the role of the different agents in the clinical setting, and has gained

increasing importance for the selection and dosing of different antifungal agents (3, 5).

In conclusion, the enrichment of our armamentarium with newer broad spectrum triazoles and echinocandins offers clinicians the possibility of choosing from among many effective antifungal drugs. The determination of both their PK and PD properties has provided important new insights into the safe and effective use of these drugs. Furthermore, PK/PD investigations have been valuable for defining optimal antifungal dosing regimens and developing *in vitro* susceptibility breakpoints. Moreover, the same principles that have been used to characterize PK/PD parameters of single antifungal drugs have begun to be used to examine combination therapy.

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ANTIFUNGAL SUSCEPTIBILITY AND RESISTANCE AGAINST YEASTS AND FILAMENTOUS FUNGI

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During last years the increased incidence of invasive mycoses, based on an enhance in the number of immunocompromised patients, had carried out to a more and more frequent use of antifungal drugs.
This selective pressure of broad-spectrum antifungal agents used for prophylaxis and therapy (empiric and *pre-emptive* therapy) is one of the most important factors responsible of emergence of new strains, as *Mucor*, *Rhizopus*, *Fusarium, Trichosporon,* nevertheless *Candida* spp, *Aspergillus* spp are still the principal nosocomial fungal pathogens.
Until today the incidence of invasive mycosis is very variable centre to centre both to different local epidemiology and different studies. *Candida* spp account for 8-10% of all BSIs but the frequency of *Candida* spp distribution varies according to the geographical setting: *C.albicans* is responsible of 62% *Candida* of BSI in Europe and 44% in Latin America. Although more of 17 species of *Candida* have been identified as responsible of invasive infection, about 95% of *Candida* BSI are caused by *C.albicans, C.glabrata, C.parapsilosis, C.tropicalis, C.krusei* (4).
In particular, after *C.albicans, C.parapsilosis* is the second specie more frequent in Italy, Hispania and Ireland, while *C.glabrata* is the second more frequent in USA, Canada, UK, Switzerland, Island.
The fungal infections are often difficult to diagnose and to manage, even if today a variety of antifungal drugs is available.
Although it's known that the resistance is defined such as the persistence of infection despite however specific therapy, the problem of drug resistance is always very complex. In fact, therapeutic result can depend on several causes: drug dosage, pharmacokinetic and pharmacodynamic, immune state of host and risk-factors. Besides, the correlation between result *in vitr*o and *in vivo* depends on two predominant aspects: factors not related to test *in vitro* and technical difficulty of the used test. So, whether MICs are the best *in vitro* predictor of the *in vivo* response to therapy is still uncertain and the clinical value of MIC as predictor of resistance in fungal infections remains to be established.
In a review of the literature, Espinel-Ingroff compared the data of the *in vitro* antifungal activities of Anidulafungin, Micafungin, Caspofungin, Amphotericin B, Fluconazole, Itraconazole, Voriconazole, Posaconazole, Fluorocitosine determined by CLSI methods for 12,052 yeasts and filamentous fungi isolates. In this study it was observed that the echinocandins have good activity for almost all *Candida* spp but less activity for *C.parapsilosis* and *C.guilliermondii* and no activity for *C.neoformans* and *Trichosporon* spp; they have fungistatic activity for *Aspergillus* spp, *Zygomycetes* and dimorphic fungi while they have activity specie-dependent for the dematiaceous fungi. The triazoles have fungistatic activity for *Candida* spp,

C.neoformans, Trichosporon spp, Zygomycetes and dimorphic fungi; while fungicidal activity for *Aspergillus*, with the exception of Fluconazole. Amphotericine B has fungicidal activity for most of the *Candida* spp and *Aspergillus* and good fungistatic activity for *Zygomicetes* (1).

Lass-Flor et al. studied the activities of antifungal agents against 349 yeasts and filamentous fungi responsible of deep infection according to the methodology EUCAST. The data obtained showed that among yeasts *C.albicans* was the most susceptible and *C.krusei* and *C.glabrata* were the species with the highest MICs against triazoles. Amfotericin B desoxycholate and liposomal had very good activity for almost all isolates and yet 98% of *C.lusitaniae* strains, usually with primary resistance to polyenes, was susceptible. All antifungal drugs had activity against *C.neoformans* with the exception of Caspofungin; while *T.asahii* was resistant to Posaconazole, Caspofungin and Amphotericine B desoxycholate but it was susceptibility to Amphotericin liposomal. Posaconazole and both formulation of Amphotericin had elevated activity against *Zygomycetes*, while Itraconazole, Voriconazole and Caspofungina had no activity (2).

Although today we have several antifungal drugs, the polyenes play an important role to manage invasive and severe mycoses as for broad spectrum activity as for rarity of documented resistance. In particular liposomal formulation is less nefrotoxic than desoxicholate, has elevated activity for *Candida, Geotrichum, Cryptococcus, Trichosporon, Aspergillus, Zygomycetes*, dimorphic fungi.

The wide variety of fungi and their different susceptibility have made the need to identify the etiological agent to level of genera and specie and to know their patterns of susceptibility because they are very variable among different genera and even within species. Besides, because of the lack of standardization of method *in vitro* susceptibility and difficulty of lecture and interpretation of commercial kits, once confirmed the fungal infection, the identification the genera and specie of the pathogen is an effective method for prediction of antifungal susceptibility and appropriate management of infection. Besides, MIC testing can be important in some cases of deep infection, as recommended by IDSA in the Guidelines to management of candidiasis (3).

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FUNGAL INFECTIONS IN NEWBORNS

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Candida spp are a major cause of morbidity and mortality in preterm neonates (1). Known risk factors for IFI include fungal colonization, high glucose levels, prolonged steroids, antibiotics and H2-blockers use (2).
Key factors for progression from colonization to IFI (occurring in 15-40% of all colonized neonates) are the number and virulence of microrganisms, the degree of prematurity, immunity defects, concomitant gastrointestinal diseases. Also not receiving full enteral feedings, but parenteral nutrition with lipid emulsions is a major risk factor. Dissemination occurs mostly via translocation from the gut reservoir, or from CVC's biofilms dismissing fungal thrombi. In a recent study, CVC fungal colonization (OR 10.8), and colonization in multiple sites (OR 6.15) were the most potent predictive factors of progression to IFI in neonates previously colonized in peripheral sites (3).
The true burden of neonatal IFIs is underestimated as most studies report infection of *Candida spp* from blood. Due to the particular tropism for tissues of *Candida spp*, preterm neonates may have only transient fungemias despite end-organ dissemination. Early diagnosis is challenging also because clinical presentation may be undistinguishable from other pathological conditions. Empirical treatment has been proposed, but not yet assessed in prospective RCTs; however, both empirical and documented treatment does not always prevent the neurodevelopmental impairment and mortality of these infections affecting 73% of preterms with blood/CSF culture-proven infection (4).
Thus, the only effective way to avoid the severe late consequences of IFIs in preterms is via preventative strategies. As avoiding or limiting all the known specific risk factors is a difficult task (most of them are inherent to prematurity and the intensive cares), preventative strategies aim at enhancing the normal composition of the neonatal enteric microbiota, by promoting "healthy" commensal, bifidogenic also via maternal fresh milk feeding, and at evaluating the use of fluconazole. This last strategy is the only one supported by highest-quality evidences coming from 4 RCTs, including a multicenter study (5). All of these trials report significant decreases of colonization and infection by *Candida spp* in the treated infants, with dosages of 3 and 6 mg/kg/every 2nd day. Pooling together their results, fluconazole safely reduced IFI risk by a 75%, and all-causes mortality by a 24% (OR 0.74; 95% C.I. 0.58-0.95) in infants <1,500 g at birth. Fluconazole did not lead in any of these studies to emergence of acquired resistances or to selection of species with intrinsic resistance. Moreover, data coming from 8- and 10-year analysis, respectively, of sensitivities of fungal isolates in NICUs using fluconazole in preterms has recently confirmed that no emergence of resistances occurred (6).
Prophylactic fluconazole is currently the most effective tool to reduce the burden of IFI and Candida-related morbidity and mortality in the nursery. Each NICU should systematically evaluate their practices, analyze their rates and outcomes including

mortality and neurodevelopmental outcomes of all IFI, not limiting to only bloodstream infections, and decide to adopt fluconazole if that burden is high. All neonatologists should never give up the possibility to save even one neonate from the risks of such a devastating disease and to protect them from the risks of lifelong sequelae related to *Candida spp.*

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ASPERGILLOSIS IN THE INTENSIVE CARE UNIT

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Invasive aspergillosis (IA) represents a major cause of morbidity and mortality among severely immunocompromised patients. Most of the literature deals with the classical risk factors for IA, including prolonged neutropenia and stem cell transplantation. However, more recently there is an increase in the number of patients without "classical" risk factors that develop IA in Intensive Care Units (ICU) or are admitted in ICU for IA.

Epidemiological studies on the incidence of IA in critically ill patients without haematological diseases are scant. Often, discrimination between colonization and infection is not clear in these studies; moreover, autopsy examinations are rarely routinely performed in ICU patients, thus not allowing definite evidence on the real burden of IA in the ICU. Finally, characteristic radiological signs of IA are often absent in non-neutropenic ICU patients, and diagnostic laboratory assays, including PCR, are validated only for haematologic patients in units other than intensive care. In the largest epidemiological study on 1850 ICU patients, 127 (6.9%) out of them had microbiological or histopathological evidence of aspergillosis. Of them, 70% (89 cases) had no underlying hamatological malignancy (1). Autopsy studies allowed also to identify cases of IA not previously diagnosed. Roosen J et al. identified 15 cases of IA in 100 autopsies performed in patients in ICU, and in 5 cases diagnosis was missed before death (2). In a recent study on the etiology of septic shock in ICU patients, prevalence of IA was 0.3%; other authors evidenced that 13 out of 67 patients with severe hospital-acquired pulmonary aspergillosis had been admitted to the ICU (3).

Among the risk factors for IA in ICU, the prolonged use of antibiotics, the presence of central venous catheters, and mechanical ventilation are predominant, but are very common in the ICU setting. Other factors including the use of steroids, the presence of chronic obstructive pulmonary disease (COPD), and a generic immune-depression (due to medications, to chronic liver failure, etc.) likely play an important role in causing this infection.

Even though, *Aspergillus* infection may derive from a pulmonary endogenous colonization that is present before entry into the hospital, aspergillosis may be acquired also in the hospital setting. *Aspergillus* spores are indeed common in decomposing material inside the hospital, due to renovation work, wood, improperly cleaned ventilation systems, etc. These spores when are aerosolized can be inhaled thus causing the pulmonary disease.

The manifestation of IA disease in the ICU are several depending on the complex pathogen-host interaction. The commonest interaction is the colonization of the airways, that is very frequent in patients with detective mucociliary clearance and structural changes of the bronchial system, as during mechanical ventilation (5). Indeed, this condition predisposes to colonization and subsequent development of

the invasive disease. Apart the allergic manifestation, the most threatening condition is represented, however, by the invasive disease, that represents a challenge for the ICU physician. Severely immunedepressed patients represent a target population for this manifestation that has a high mortality rate. In more than 90% of cases lungs and sinuses are implicated; cavitating infiltrates are common in patients receiving steroids, with cirrhosis, COPD, and solid organ transplant recipients, mainly lung transplants.

A timely diagnosis is an important tool to reduce the mortality among haematological patients, but among ICU physicians the perception/suspicion of IA could be low because most patients do not belong to the "classical" at-risk populations for IA. In recent years, lung CT has become one of the most important diagnostic tools; however lung CT could be difficult to be interpreted in ICU mechanical ventilated patients, with atelectasis or ARDS. In less than one half of patients with IA, moreover, the culture of respiratory specimens is positive, with a great difficulty to discriminate between colonization and infection. Serological assays investigating on the presence of circulating fungal components (galactomannan and beta-D-glucan) and PCR represent useful diagnostic tools, even though their use has been validated only in haematological patients. More useful is the dosage of galactomannan in the bronchoalveolar lavage fluid, that is a procedure not too difficult to be performed in mechanical ventilated patients in the ICU setting

For several years amphotericin B has represented the cornerstone for the treatment of IA, even though this formulation has been associated with serious adverse events. Lipid-based formulations of amphotericin B, the more recent azoles, including voriconazole - the current first choice option for IA treatment-, and the echinocandins, including caspofungin, micafungin and anidulafungin, nowadays represent effective antifungal drugs with favourable tolerability and safety profiles.

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WHY ANOTHER ECHINOCANDIN? PK/PD ASPECTS

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The echinocandins are a novel class of antifungals. Caspofungin was the first echinocandin agent approved, and anidulafungin and micafungin are recently approved in Italy. The echinocandins are lipopeptides that have been synthetically modified from the fermentation broths of various fungi. Caspofungin is derived from Glarea lozoyenisi. Its molecular formula is 52H88N10O15•2C2H4O2, and its molecular weight is 1213.42. Micafungin is derived from Coleophoma empedri through cleavage of a naturally occurring hexapeptide from the fungus and the addition of a fatty-N-acyl side chain. Its molecular formula is C56H70N9NaO23S, and its molecular weight is 1292.26. Anidulafungin is derived from Aspergillus nidulans.. Its molecular formula is C58H73N7O17, and its molecular weight is 1140.3.
Because of their inconsistent oral absorption, echinocandins are available only for intravenous use. In general, less than 1% of the echinocandins is excreted unchanged in the urine. In addition, the echinocandins are not dialyzable; therefore, supplemental doses after dialysis are not required. None of the echinocandins are inhibitors or substrates for P-glycoprotein.
The predominant pharmacokinetic differences among the three agents are metabolism and half-life. Table 1 provides a summary of the pharmacokinetic parameters for each of the agents.

Caspofungin
The dosage of caspofungin is a 70-mg loading dose on day 1 followed by 50 mg once/day in order to achieve a trough concentration of at least 1 µg/ml. Caspofungin is a triexponential drug with a short a phase immediately after administration, a predominant b phase, and a longer g phase. The b phase exhibits log linearity compared with the g phase, which is nonlinear. The nonlinear kinetics of the g phase appear to be responsible for the accumulation of caspofungin over time. Caspofungin is slowly metabolized in the liver through nonenzymatic peptide hydrolysis and N-acetylation into two inactive metabolites (M1 and M2). There is no apparent metabolism of caspofungin through the cytochrome P450 (CYP) system.

Micafungin
Exhibits linear pharmacokinetics over the therapeutic dosing range of 50-150 mg once/day. No loading dose is required, and doses of 100 and 150 mg provide trough concentrations of approximately 2 and 2.5 µg/ml, respectively, on day 1 of therapy. Micafungin is metabolized into three metabolites: M1, M2, and M5. The M1 metabolite is formed by metabolism of micafungin by arylsulfatase; M1 is further broken down by catechol-O-methyltransferase to M2. The third metabolite, M5, is formed as the side chain of micafungin is hydrolyzed by the CYP isoenzymes (mostly CYP3A). However, the CYP pathway plays only a minor role in the metabolism of the drug.

Anidulafungin
Is dosed as a 200-mg loading dose on day 1 followed by 100 mg once/day; this provides a trough concentration on day 1 of approximately 2.5 μg/ml.79 Anidulafungin undergoes spontaneous degradation into an inactive open-ring peptide, and no liver metabolism has been observed.

Pharmacokinetic/pharmacodynamic (PK/PD
The echinocandins exhibit concentration-dependent killing with a prolonged post-antifungal effect against *Candida* species . In the experimental animal models of candidiasis and aspergillosis, echinocandin activity is optimized when plasma Cmax/MIC ratio approaches 10 or the tissue 24-hour area under the curve to MIC ratio (AUC/MIC) exceeds 250. Supratherapeutic concentrations of the echinocandins may persist in tissue even when plasma concentrations fall below the MIC. This characteristic, along with the safety and concentration-dependent pharmacodynamic properties of this class, has stimulated interest in infrequently administered (i.e.1-3/ weekly), high-dose echinocandin regimens.

Conclusion
Because of their novel mechanism of action and excellent safety profile, echinocandins have become a preferred treatment option for invasive candidiasis and are frequently used in combination with polyenes or triazoles for refractory aspergillosis. Each of the agents is relatively safe; however, a higher rate of adverse reactions has been reported for caspofungin compared with micafungin and anidulafungin. Finally micafungin only has demonstrated efficacy and safety profile in children and neonates and approved in this stage of age.

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FROM LABORATORY TO BEDSIDE. WHY A NEW ECHINOCANDIN

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Over the last 5 years, three different echinocandins have been studied in phase III clinical trials and have been registered in Italy.
Undoubtedly, the availability of the new class of antifungals significantly increased therapeutic options for invasive fun gal infections. The well documented *in vitro* cidal activity of echinocandins in invasive candidiasis, together with encouraging results of the clinical registration trails, were reflected in the recommendations of the guidelines of the Infectious Diseases Society of America - echinocandins are considered the first line treatment, either as an empirical or targeted therapy, both for patients in critical clinical conditions and in those with cultures positive for *Candida krusei* or *glabrata* (1). As far as invasive aspergillosis is concerned, the particular target of action of echinocandins, which is completely different from the one of azols and polyens, make these antifungals particularly suitable for a combination therapy with one of the aforementioned classes (2).
On the other hand, the choice of prescribing a single compound proves to be much more difficult. The recent introduction of anidulafungin and micafungin causes and will continue to cause particular problems for clinicians and for the authorities responsible for drug prescription handbooks of single hospitals or regions, who will have to decide whether to consider only the long-time available compound, i.e. caspofungin, or to introduce two or all the three echinocandins.
The pharmacological properties and the spectrum of activity, together with pharmacokinetics and pharmacodynamics are of little help, since the differences between the three compounds are only minor and do not justify the choice of one of them (3). This is further confirmed by data from the only clinical trial where two echinocandins were confronted (caspofungin and micafungin) for treatment of invasive candidiasis and where the results were practically identical in both groups (4).
Thus, the only factor that can guide the choice of one echinocandin over another is the type and the setting in which the drug has been developed and tested in clinical trials. Moreover, caspofungin and micafungin have been widely used in haematological setting. The former was successfully studied as rescue therapy for invasive aspergillosis and as empirical therapy of febrile neutropenia, where it resulted equivalent to liposomal amphotericin B, whereas the letter was registered for prophylaxis in haematopoietic stem cell transplant. Additionally, micafungin has been the most extensively studied, both in vitro and in vivo, as a part of antifungal combination therapy, even though there is still no clear evidence for this indication (3).
It is possible that the present indications change as a result of new data, such as pharmacokinetics of the compounds in different settings, thus every decision might be subject to modifications. The same seems true as far as the pharmacoeconomics is concerned, as it may change as a result of different price negotiation strategies for single compounds.

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FUNGAL ENDOCARDITIS

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Fungal endocarditis has been considered for a long time as a rare and often fatal infectious disease that usually presents similarly to subacute bacterial endocarditis. Until recently, most of the studies were case reports or very small series (1). Recently, the International Collaboration on Endocarditis – Prospective Cohort Study (ICE-PCS) in a five year prospective multicenter study, observed only four cases (0.27%) of fungal infection in 1622 cases of infectious native valve endocarditis (2).
On the other hand, during almost the same study period the ICE-PCS observed 23 (4.1%) fungal infections in 556 prosthetic valve endocarditis (PVE). Fungal PVE represented 9.4% of "early PVE", 4.3% of "intermediate PVE", and 3.3% of "late PVE" (3).
Endocarditis from filamentous fungi is mainly caused by *Aspergillus* and is more rare and lethal than infections caused by yeasts[1]. However, in solid organ transplant recipients *Aspergillus* etiology is reported in approximately 20% of infectious endocarditis cases with an associated mortality as high as 87% (4). Recently an emerging role of *Aspergillus* has been considered for pace-maker associated endocarditis, where the infection usually presents as a fever of unknown origin, with negative blood cultures, that is correctly diagnosed only after pace-maker removal or at autopsy (5).
Optimal treatment of *Aspergillus* endocarditis requires a combined approach of valvular surgery, with debridement of infection including of removal of the infected valve, and antifungal medical therapy with voriconazole or amphotericin B (1, 5).
Candida endocardits is also rare, but it real incidence may be underestimated. A recent prospective one-year survey of *Candida* bloodsteam infections (BSIs) in seven university hospitals of western France has been performed in order to assess epidemiology and clinical features of *Candida* endocarditis (6). Out of 190 cases of *Candida* BSI, 7 (3.7%) were complicated by endocarditis that was located on biological valves and on a pacemaker in 2 and 1 instances, respectively. On the other hand, the ICE-PCS recently indicated that *Candida* endocarditis accounted for 33 (1.2%) out of 2749 cases of definite infectious endocarditis.
Patients with *Candida* endocarditis were more likely to have prosthetic valves, short-term indwelling catheters, and have an HCA *Candida* infection (7). It should be underlined that during the last decades the number of patients undergoing open heart surgery and carrying prosthetic implants has increased. Moreover, many patients who undergo such operations are critically ill and require prolonged ICU monitoring, with many risk factors of developing nosocomial candidemia (1).
Moreover, most of these patients undergo postoperative splanchnic hypoperfusion, leading to intestinal intramucosal acidosis and increased intestinal mucosal permeability, or may develop ventilator associated pneumonia (VAP) requiring antibiotic therapy with associated endotoxemia that may damage the intestinal mucosal barrier which favours *Candida* translocation.

Implantation of a contaminated allograft can also occur. All the aforementioned factors might predispose to the spread of *Candida* species into the systemic circulation and development of candidemia (8).

Thus, the scenario of a patient with a prosthetic heart valve or other intravascular prosthetic device who develops candidemia is becoming more common. The risk of developing prosthetic valve endocarditis (PVE) in candidemic patients with prosthetic heart valves was addressed in a retrospective study of Nasser et al. (9).

Overall, *Candida* PVE was documented in 11 of 44 (25%) patients: 7 patients were diagnosed at the same time of candidemia (group 1), while the remaining 4 (group 2) several weeks after an episode of early postoperative candidemia. Reviewing these published data, it is possible to note that in both groups PVE was diagnosed late after valve replacement (after a mean of 270 days in group 1, and after a mean of 246 days in group 2) (9), a fact suggesting that all the episodes had a similar pathogenic mechanism.

Thus, *Candida* PVE may be a two-step process: the first step is represented by a postoperative transitory candidemia occurring during the ICU stay, which leads to colonization of prosthetic valve and subsequent biofilm formation. After the initial colonization, the fungus, slowly growing on the prosthesis surface, becomes more resistant to antifungal agents. This speculation lends support for pre-emptive antifungal therapy with agents that display activity in the biofilm in patients with prosthetic heart valves while at risk of candidemia (8).

The Infectious Diseases Society of America 2009 guidelines on the treatment of candidiasis recommend intravenous amphotericin B with or without 5-fluorocytosine or an echinocandin in addition to valve surgery as first-line treatment for *Candida* endocarditis (10).

However, recent *in vitro* studies have shown reduced activity of amphotericin B against *Candida* biofilm, and poor penetration into vegetations and blood clots in experimental models of infectious endocarditis (8).

On the contrary, echinocandins, display potent *in vitro* activity against sessile *Candida* cells within biofilms, and caspofungin has been successfully used in anedoctal cases of *Candida* endocarditis (8). Thus, these drugs may have a favourable impact on the management of this infection.

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PNEUMOCYSTIS PNEUMONIA: A FUNGAL DISEASE NOT TO BE NEGLECTED

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Pneumocystis jiroveci pneumonia (PJP) is still the most frequent opportunistic infection in HIV-infected patients. PJP can occur not only in AIDS patients, but also in other immunocompromised patients (i.e. those being given steroids or other immunosuppressive medications, those affected by haematological malignancies) where its incidence is rapidly growing.
PJP occurs in HIV patients with less than 200 CD4 cell/ml, representing an AIDS-defining condition.
PJP is an interstitial-alveolar pneumonia, with a consistent mortality rate, estimated in 10-20% in HIV patients and in 30-60% in non-HIV patients. As with any immunocompromised host with infection, early diagnosis and therapy are critical for a good clinical response to PJP.
Pneumocystis jiroveci was first considered a protozoan, based on morphologic appearance, proposed life cycle and antimicrobial susceptibilities. Subsequent phylogenic analyses using rRNA sequences suggested that the organism was more closely related to the fungi despite the absence of ergosterol in the cell wall. Conversely, *Pneumocystis* cell wall contains (1, 3)-β-d-glucan (BG), a component of many fungal organisms. The clinical features of PJP include dyspnoea on exertion, non-productive cough, fever, chest X-ray finding of bilateral interstitial infiltrate, arterial PO_2 less than 70 mmHg. Usually, in non-HIV patients the clinical course is more severe, with marked hypoxemia, perhaps due to the greater inflammatory infiltrate, which leads to an acute respiratory insufficiency. Therefore, it is important to recognize that the clinical presentation of patients with PJP in HIV-negative individuals may differ from that in patients with AIDS. Indeed, the progression of PJP in patients with AIDS tends to be subacute, often evolving over several weeks.
Due to the lack of specificity of clinical and radiological findings, the frequent coinfections in immunocompromised patients and the potential toxicities of the agents used for the treatment of PJP, the diagnosis should require histopathologic confirmation. In general, noninvasive testing should be attempted initially, but invasive techniques should be used when necessary and clinically feasible. Bronchoalveolar lavage made by fiberoptic bronchoscopy is the usual method of choice for diagnosing PJP, but it is often very difficult to be performed due to the frequent severe respiratory failure of affected patients.
Other diagnostic procedures - such as polymerase chain reaction (PCR) - have been reported to be both sensitive and specific, although none of these procedures turned out to be a reliable method in the analysis of biological samples - sputum, pharyngeal swabs - taken with non-invasive techniques.
BG is a component of the cell wall of many fungal organisms and its presence in serum had been shown to be a reliable marker of invasive fungal infections (IFI), both in clinical and autoptic studies. Its clinical usefulness had been demonstrated also

in haematologic patients, either used alone or in combination with serum galactomannan. BG is also a component of *P. jiroveci* cell wall but no method for detecting BG in serum has been so far validated for diagnostic purposes in PJP, despite some encouraging reports. In our experience, BG showed to be clinically reliable in presumptive PJP and therefore may be suggested as an adjunctive test for PJP diagnosis in those patients unable to undergo invasive procedures.

Pneumocystis pneumonia therapy can rely on few treatment options. Trimethoprim–sulfamethoxazole is the most effective agent against *Pneumocystis*. However, therapy with high-dose trimethoprim-sulfamethoxazole is often associated with significant adverse effects, including potentially severe hypersensitivity reactions, dose-related bone marrow suppression, and hepatotoxicity. Thus, such a choice - without a diagnostic confirmation - should be carefully weighed on a risk/benefit ratio, considering the poor clinical conditions of many patients at risk for PJP.

Adjunctive therapy with corticosteroids is effective in AIDS patients with PJP with hypoxemia (partial pressure of arterial oxygen in room air under 70 mm Hg) Such patients should receive prednisone at a dose of 40 mg bid for five days, then 40 mg qd on days 6 through 11, then 20 mg qd on days 12 through 21.

In patients without AIDS with severe PJP, a dose of 60 mg of prednisone daily can result in a better outcome than lower doses.

Second-line therapies include intravenous pentamidine, atovaquone, the association clindamycin - primaquine, although none of them has been proved to be as effective as TMP-STX. Anedoctical reports show some efficacy, either alone or in combination, of caspofungin, albeit further, randomised studies are needed to confirm these preliminary data.

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ASPERGILLOSIS: COMMUNITY ACQUIRED OR HEALTH CARE ASSOCIATED INFECTION?

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Aspergillus is a well recognized mould causing human infections both community acquired and health care associated. Because it is widespread in the environment (water, soil, air, debris, etc.) and the spores are easily aerosolized, prevention and control of the related infections are still a challenge for Health Care Organizations (HCO). Greater survival of critically ill patients, increasing number of invasive procedures, improved diagnostic techniques, use of drugs for fungal prophylaxis (voriconazole, fluconazole) or as corticosteroids are among the main reasons for the present epidemiological situation.

Definition of Aspergillosis had an evolution in the last ten years. At the moment the 2008 consensus from EORTC/MSG (European organization for Research and Treatment of Cancer/ Mycoses Study Group) is the reference (1). This consensus gives infection definitions based on host factors, mycological criteria and clinical signs but, at the same time, adopts three levels of certainty: infection proven, probable, possible. While this routinely adopted classification includes almost all patients, it still remains to better define when the infection is healthcare associated or community acquired. Many studies adopt different temporal criteria with respect to how long the patient should stay in hospital before to consider the infection hospital acquired [i.e. 7 days (3) or 10 days (2)]. Certainly all studies include the category of "undetermined" when it is impossible to clearly define the origin. This explains part of the variability in incidence and mortality data. Aspergillosis accounts for 3-5 infections /100.000 patients days in large tertiary French hospitals (2), for 3.8 hospital admissions /100.000 inhabitants and 0.03% of the total hospital discharges in USA (4). Length of stay increases over 12 days and related costs over $ 50,000 for each admission (4). The vast majority of Aspergillosis (50%) are community acquired, the "undetermined" range from 12 to over 30%; those healthcare associated are the remaining. Looking at single patient categories the figures can change significantly: for instance in transplant recipients the reported incidence of invasive Aspergillosis varies from 1% to 26%; in allogenic stem cells transplants attributable mortality ranges from 60 to 100% (5).

Outbreaks in health care settings are not unusual and often they occur related to ongoing works of construction and renovation.

Risk factors are mainly related to the host conditions and to the surrounding environment. Identification of at risk patients is essential. Haematopoietic stem cell recipients, neutropenic patients with leukaemia treated by intensive chemotherapy, solid organ recipients, critically ill patients, preterm babies admitted to neonatology units, patients receiving chronic treatment with corticosteroids, patients with acquired immune deficiency syndrome are universally considered categories to be protected. Recognized environmental risks in hospitals are: not filtered air, water, ventilation systems, construction and renovation activities, presence of carpets, moquettes as

well as ornamental plants. While risks related to dust production in construction and renovation activities are well known, it should be stressed the recent evidenced role of hospital water systems contamination (6). Prevention and control of hospital acquired Aspergillosis need a multifaceted approach involving clinicians, hospital management, engineering and building departments, infection control teams; furthermore this approach is essential during construction and renovation activities. Standard precautions are normally recommended with adjunction of Contact Precautions and Airborne Precautions if massive soft tissue infection with copious drainage and repeated irrigations is required (7).

Management and control of renovation works, control of traffic flow to minimize high risk patient exposure, appropriate spatial separation of patients, number of isolation rooms, ventilation for isolation rooms, control of potable water systems are among the main recommended measures in HCO to reduce the risk of fungal infections (8).

More specifically a document of the Canadian Centre for Infectious Disease Prevention and Control (9) proposes an effective approach to risk reduction based on a matrix where 4 types of construction activities (from type "a" (inspection and non invasive activities) to "d" (major demolition, construction and reconstruction projects) are matched with 4 groups of risk areas (from group 1 lowest risk (offices, public areas, etc.) to group 4 highest risk (ICU, operating theatres, transplant units, etc)). Four classes of recommendations are then proposed: the simplest (class I i.e. inspection in lowest risk areas), the more complex (class IV i.e. demolitions in highest risk areas). Each class mentions duties for engineer/maintenance staff & contractors, environmental services, infection prevention and control personnel, medical/nursing staff.

Special attention should be put on settings where hematopoietic stem cell transplant patients are managed. Some of the control measures recommended (10) for these patients include:

- for ventilation: use of HEPA filtration, maintain positive pressure, maintain >12 air exchange per hour, check of window seals, stairwells, vents, ducts. Although use of HEPA filtration is recommended, it should be noted that in case of building renovation it is necessary check very strictly the efficiency of the system;
- for personal hygiene: patients should wear masks (N-95) when out of protected environment, possible use of protective environment (mobile tents), avoid unprocessed food such as raw fruits;
- for environment: eliminate carpets, flowers and potted plants, avoid dust generating activities, remove wet and damp areas, repair water leaks, keep windows closed, develop and maintain strict cleaning procedures;
- for water supply: monitor water contamination if an Aspergillosis suspected as nosocomial infection occurs, consider the use of filters in highest risk areas when the pipelines are not easy to control;
- for construction: develop policy for construction and renovation, seal off construction areas and place them under negative pressure, use rigid dust proof barriers, during outdoor construction seal intake air, minimize the traffic through construction areas, exhaust construction dust;

- for surveillance: define disease and find cases of nosocomial infection, monitor air and/or environment for *Aspergillus* species if suspected nosocomial infections occur;
- for cleaning rooms: use thorough cleaning procedures to ensure daily decontamination and cleanliness of the room.

Aspergillosis prevention and control is becoming a very important risk for HCO. Often it is not easy to understand the origin of the infection but certainly for HCO it is necessary to do all the efforts to adopt the recommended good practices and to reduce the risk.

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ANTIFUNGAL PROPHYLAXIS: PRO & CONS

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Invasive fungal infections (IFI) represent a severe clinical condition with high morbidity and mortality, and therefore they may be worth prophylaxis.
Low birth weight neonates and patients undergoing abdominal surgery and treated in intensive care units are at high risk of developing IFI. In these conditions fluconazole prophylaxis has been demonstrated an effective approach.
The risk of IFI in patients undergoing antineoplastic chemotherapy or hemopoietic stem cell transplant (HSCT) varies according to the aggressiveness of chemotherapy and the donor source for transplant. Aggressive treatment of acute leukemia and allogeneic HSCT, especially from alternative donors, are the conditions associated with the highest risk of IFI. Previous studies demonstrated that fluconazole prophylaxis reduced the incidence of invasive candidiasis in allogeneic HSCT and in some subgroups of leukemic patients. More recently, posaconazole resulted effective in preventing IFI in adults receiving chemotherapy for acute leukemia or myelodisplasia or in presence of severe acute or chronic graft vs. host disease following allogeneic HSCT. Some concerns exist on these results. In fact, the number needed to treat for preventing one IFI (or a death related with an IFI) varies according to the incidence of the disease and therefore varies in different centers, being very high in centers with a low incidence of IFI. Moreover, posaconazole may present interactions with other drugs administered for the management of the underlying disease, with the risk of toxicity or reduced activity of both compounds. Finally, posaconazole is available only for oral administration and needs food for its absorption; these conditions may reduce the compliance of the drug in patients with intestinal problems or who cannot swallow. All these aspects must be kept in mind when a program of antifungal prophylaxis is activated. In this case, it must be remembered that the use of physical barriers, like filtered face masks and HEPA filters for all the hospital rooms, during all the treatment periods of acute leukemia or HSCT might result as effective as antifungal drug prophylaxis with less side effects and without interactions with other drugs.
Immunocompromised patients are frequently at risk of P.jiroveci pneumonia. Prophylaxis with low-dose cotrimoxazole (or other drugs in case of adverse reactions) is effective in reducing this disease and should be ever considered.
Finally, no clinical trial has evaluated the efficacy of secondary antifungal prophylaxis. However, its administration is recommended in patients with previous IFI who are still immunosuppressed. In these cases the choice of the drug must be driven by the identified (or most probable) pathogen, by the site of the IFI, and by the pharmacological characteristics of the antifungal drugs (administration, toxicity, interactions).

ANTIFUNGAL TREATMENT AND DIAGNOSTIC TESTS OF NEW GENERATION

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Major advances in healthcare have led to an increase in the number of life-threatening infections due to opportunistic fungi. Immunosuppression and breakdown of anatomical barriers such as the skin are the major risk factors for fungal infections. A variety of recognised high-risk groups, such as neutropenic cancer patients and recipients of bone marrow or solid organ transplants, are increasingly found in the hospital. Outside these very high-risk groups it is possible to identify specific risk factors which predispose patients to systemic *Candida* infection. Long-term and high density colonisation has been shown to lead to candidemia. Multiple studies showed that the use of central venous catheters, parenteral nutrition, multiple blood transfusions and artificial ventilatory support were significant risk factors for invasive candidiasis. Well-established risk factors for invasive aspergillosis include underlying lung disease, prolonged neutropenia, immunosuppressive therapy, corticosteroid therapy, allogenic haematopoietic stem cell transplantation (HSCT) and graft-versus-host diseases (GVHD) and its treatment. The management of fungal infections has greatly improved in recent years, with the advent of new drugs very active against both *Candida* and *Aspergillu*s. These include drugs belonging to the triazole and echinocandin families. In addition, lipid amphotericin B compounds remain widely used, because of their very large spectrum of activity and improved tolerability. Together with new drugs, new strategies are also being tested, with the aim of decreasing the number of patients unnecessarily exposed to antifungals, but also to treat as early as possible patients likely to have the fungal infection developing. As a consequence, there is a general trend in many hospitals to abandon empirical antifungal therapies, and to use a more watchful therapeutic approach. This is mainly due to the availability of new diagnostic tools and validated clinical prediction rules. The detection of galactomannan in serum and other body fluids, accompanied by imaging studies and clinical evaluation has become essential for managing leukemic and transplant patients with aspergillosis. For *Candida*, provisional data coming from several authors (including ourselves) seem to show that the beta-D-glucan test used in patients selected by clinical prediction rules might be able to identify and treat pre-emptively patients at a very early stage of infection. Combining the prediction algorythms with laboratory tests and imaging seems to be a logical conclusion to drive early therapy and avoid overtreatments.

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IMPACT OF CENTRAL VENOUS CATHETER REMOVAL ON OUTCOME IN PATIENTS WITH CANDIDAEMIA

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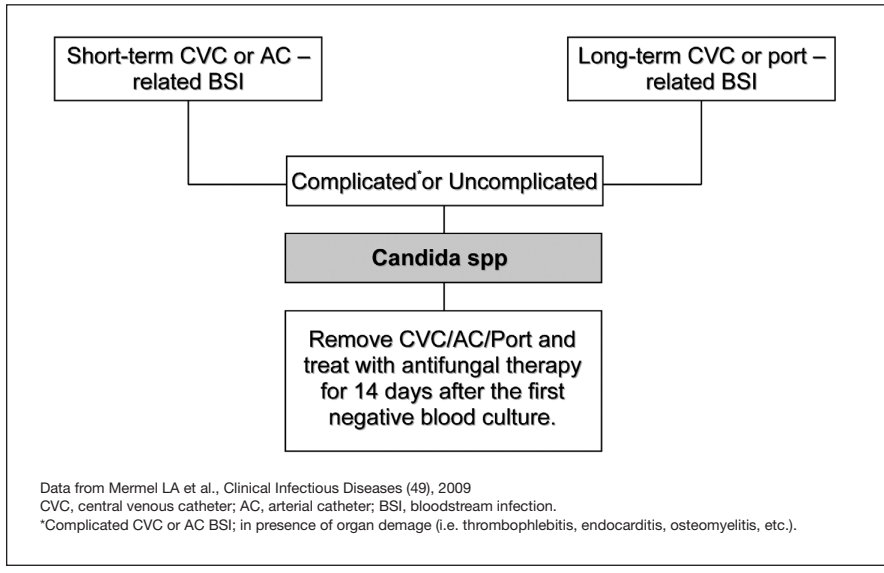
Candidemia has increased worldwide over the last few decades with an estimated annual incidence in the United States of 6-10 episodes of candidemia per 100,000 population, resulting in a relevant public-health problem (1). Even though adequate antifungal treatment has been shown to reduce mortality, recent studies indicate that candidemia-attributable mortality remains 19%-49% (1, 2).
Central venous catheters (CVCs) appear to be the most common risk factor for the development of candidemia in patients without neutropenia or major immunodeficiences, thereby more than half of BSIs, including candidemia, are considered to be catheter-related in intensive care unit (ICU) patients (1). In addition, *Candida* spp. are able to form biofilms on catheters and other implanted devices and the formation of biofilms, particularly of *C. parapsilosis*, is promoted by high glucose media through enhancing the microorganism's capacity to colonize indwelling CVCs. The *Candida* biofilm lifestyle results in antifungal drug resistance and protection of the fungus from host defenses both of which carry important clinical repercussions; in particular, biofilm production by *Candida* has been recently associated with an increased mortality in hospitalized patients with candidemia, probably by preventing complete organism eradication from the blood (2).
For all these reasons, catheter-related infections are difficult to treat and affected

FIGURE 1 - *Treatment recommendations for intravascular catheter-related infection, according to current Infectious Diseases Society of America (IDSA) guidelines.*



devices often need to be removed; however, catheter removal can be hazardous for some patients and a multifaceted approach for the management of intravascular catheter-related bloodstream infections should be used. Depending on the causative organism, removal of the CVC or retention in combination with antimicrobial catheter lock therapy must be considered, as well as the type and duration of systemic antimicrobial therapy.

In the case of *Candida* spp. infection, recommended treatment, according to the Infectious Diseases Society of America (IDSA) guidelines, includes removal of the CVC and administration of appropriate antifungal agents for 14 days from the last blood culture showing the presence of the candidemia (3) (Figure 1).

However, in clinical practice there are some circumstances in which removal of the venous catheters must be weighed carefully, in particular for long term CVCs or surgical implanted devices.

In addition, the gastrointestinal origin in a relatively high number of cases of candidemia, the costs and the possible onset of complications associated with catheter replacement, and the problems that could be encountered in patients with difficult vascular access, can be considered arguments against the mandatory recommendation to remove all catheters from selected patients with candidemia. It is not completely clear which patients benefit most from this practice, or whether the timing of removal is critical to improved outcome in all patients.

In general, removal of an indwelling CVC is reported to be associated with a clear benefit in terms of a significant reduction in duration of candidemia and of attributable early and late mortality rates and these findings are the basis for recommendations in IDSA guidelines (3).

However, other studies do not support this consensus. A large observational study, including 404 consecutive patients with cancer and CVCs who developed candidemia, showed that catheter removal ≤72 h after onset of candidemia improved response to antifungal therapy in patients with catheter-related candidemia only; in addition, they analysed the clinical characteristics of patients and found that disseminated infection, previous chemotherapy, previous corticosteroid therapy, and poor response to antifungal therapy were factors independently associated to a non-catheter source for the candidemia; the sub-analyses of prognostic factors conducted on patients included in the study who had ≥1 of these predictors showed that catheter removal at 72 h did not improve outcome (4).

In addition, in a literature review of selected studies investigating CVC removal as a prognostic factor in patients with candidemia, Nucci et al. (5) highlighted the paucity of studies that specifically evaluated outcome of candidemia in relation to vascular catheter removal and the complete absence of a randomized trial in a defined patient population that was designed to specifically address the effects of catheter removal versus retention in patients with candidemia.

Finally, in a recent prospective population-based surveillance for candidaemia evaluating the impact of early (≤48 h) removal, Rodriguez et al. demonstrated that, after stratifying study patients according to characteristics associated with early or delayed CVC removal, exclusively high severity of illness category, and not early CVC removal, was a significant risk factor for mortality, suggesting that patients are likely

to benefit most from a careful and individualised analysis of risks and benefits of the timing of catheter removal (6).

In conclusion, on the basis of current evidences, CVC removal has to be included as part of the treatment for patients diagnosed with candidemia. However, further researches should focus on specific characteristics of patients who can obtain the major benefit from CVC removal in particular settings.

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PROPHYLAXIS AND TREATMENT OF INVASIVE FUNGAL DISEASES IN ALLOGENEIC STEM CELL TRANSPLANTATION: RESULTS OF A CONSENSUS PROCESS BY GITMO (Gruppo Italiano Trapianto di Midollo Osseo)

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In the last years, prospective studies have been conducted to assess the role of prophylaxis and treatment of invasive fungal diseases (IFD) in allogeneic hematopoietic stem cell transplantation (allo-HSCT). Although results of these studies have been encouraging, they have been unable to generate a consensus for optimal prophylaxis and treatment of IFD in the complex scenario of allo-HSCT. A consensus process was undertaken to describe and evaluate current information and practice regarding key questions on IFD management in allo-HSCT recipients; these questions were selected according the criterion of relevance by group discussion.

The Panel produced recommendations for risk stratification, prophylaxis, monitoring and therapy of IFD, and identified top priority issues for further investigation. The definition of the level of risk for IFD, in the various types and phases of transplantation and the implementation of surveillance and diagnostic strategies are the critical determinants in the antifungal prophylactic and therapeutic approach in allo-HSCT recipients.

POSTER

ALS1 AND ALS3 GENE EXPRESSION IN SESSILE AND PLANCTONIC YEAST CELLS OBTAINED FROM CLINICAL BLOODSTREAM ISOLATES OF *CANDIDA ALBICANS*

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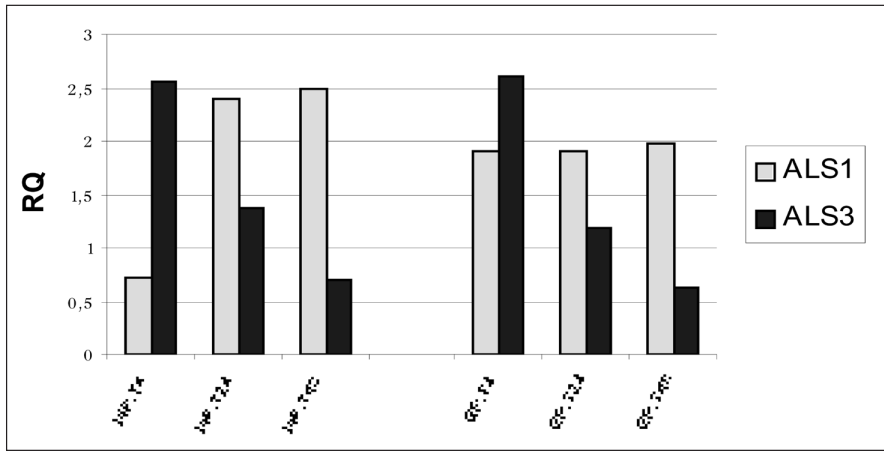
Invasive fungal diseases represent one of the major life-treating infections of the immunocompromised hosts and/or critically ill patients due to their high morbidity and mortality rates. Candidemia is the most common fungal nosocomial bloodstream infections and is frequently associated with *Candida* spp. colonization of central venous catheters (CVC), and endotracheal tubes or prosthetic heart valves.
These indwelling devices provide a surface for the formation of an adherent fungal population or biofilm and represent an important route through the host barrier defenses for systemic invasion.
The *ALS* (agglutinin-like sequence) gene family encodes a set of differentially regulated cell surface glycoproteins that promote fungal adherence and are involved in biofilm formation.
Using XTT assay, the ability to form biofilm has been investigated in 60 bloodstream isolates of *C. albicans*, 16 of them from CVC. *In vitro*, 35 (58%) of clinical isolates showed a moderate or good biofilm production.
Moreover, the goal of the present study was to monitor ALS1 and ALS3 gene expression during *C. albicans* biofilm formation (on polystyrene surface), to better understand the role of these adhesins.
Preliminary experiments have been conducted on three clinical isolates: moderate, good and non biofilm producers. Gene expression analysis were performed using RealTime PCR and SyBr Green chemistry (Figure).
We showed that ALS1 and ALS3 gene expression levels vary between different stag-

FIGURE 1



es of biofilm formation and between producers and non producer clinical isolates. ALS3 was found to be overexpressed in the initial stages (4h and to a lesser extent at 24h), whereas at later stages this gene was also downregulated relative to the gene expression level in the start culture.
The role of ALS1 is less clear, even if its expression was higher in both producer strains as compared to non producer isolate. For the good producer, the ALS1 expression is time-stable, whereas in the moderate one seems to be more involved in mature stage of biofilm.
On the whole, *C. albicans* biofilm production seems to be relatively frequent in clinical isolates, and correlates with an higher ALS3 expression in the early stages. The study of ALS1 and ALS3 gene expression profiles will be extended to other clinical strains to further confirm the hypothesis of a direct involvement of these genes in biofilm production. Moreover, we aim to monitor the effect of fungicidal drugs on ALS3 expression during biofilm formation and on pre-formed biofilm.

PREVALENZA DELLE FUNGEMIE IN UN OSPEDALE POLISPECIALISTICO

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Obiettivi

Con il presente studio si vuole valutare la prevalenza delle infezioni fungine sistemiche in un Ospedale polispecialistico di Milano-centro privo di un reparto di Malattie Infettive.

Materiali e Metodi

Abbiamo valutato retrospettivamente 16084 emocolture aerobie pervenute al Laboratorio di Microbiologia da UO del nostro Ospedale nel periodo 1/04/2007 - 1/04/2009. Le emocolture, prelevate da pazienti di età compresa tra 8 giorni e 88 anni ricoverati in reparti di Medicina, Chirurgia, Terapia Intensiva (TI), sono state processate con sistema automatico Bactec (BD). Nel nostro Ospedale vengono effettuate inoltre emocolture mirate per la ricerca dei miceti utilizzando sia flaconi Bactec Mycosis sia il metodo di lisi e centrifugazione Isolator (Oxoid). I miceti sono stati identificati con metodo morfologico (*C. albicans* e *Fusarium spp*.) e biochimico: Fumouze (Techno-Genetics) e ID32C (BioMerieux). E' stato considerato un campione non ripetuto per paziente e i dati sono stati estratti con Virtuoso Plus (Metafora Informatica).

Risultati e conclusioni

I microrganismi più frequentemente isolati sono stati CoNS (43.9%), enterobatteriacee (10.7%), enterococchi (8.6%), *S.aureus* (7.8%), Streptococchi viridanti (4.2%), *Pseudomonas spp* (3.9%). Miceti sono stati isolati nel 3.3% delle 1780 emocolture positive. I pazienti chirurgici hanno presentato la maggiore prevalenza di fungemia (14.5% delle emocolture positive), mentre i pazienti ricoverati in Medicina e in TI rispettivamente il 4.4 % e il 2.9 %. Si è verificata candidemia nel 93.9 % dei casi. *C.albicans* rappresenta il 34.1 %, C. parapsilosis il 24.4%, *C.glabrata* e *C. tropicalis* ciascuna il 14.6%, altre specie 6.3 %. Abbiamo osservato 3 criptococcosi sistemiche (3.6%), un caso di fusariosi e un caso di infezione da *S. cerevisiae*. *C.albicans* è il micete più frequentemente isolato nei pazienti di Medicina e di TI, in età pediatrica e tra i 17-64 anni, mentre nei pazienti di età superiore ai 65 anni non si è osservata una distribuzione significativamente diversa delle varie specie. Sono state processate 142 provette Isolator e tra queste solo 3 positive per miceti concordemente a flacone Bactec aerobio. I flaconi Bactec Mycosis pervenuti nel periodo in studio sono stati 336 di cui 18 positivi e di questi 17 concordi con i Bactec aerobi. In un caso una batteriemia da catetere mascherava la presenza di *C. albicans* che solo il flacone Bactec specifico ha permesso di rilevare. In conclusione si evince che, nonostante l'esiguità dei casi riscontrati, *C. albicans* rimane la specie più frequentemente isolata e non sono state osservate fungemie da *C. krusei*. In età pediatrica non si sono riscontrate positività per *C. glabrata*. La nostra esperienza suggerisce inoltre, nonostante i miglioramenti tecnologici dei sistemi automatici per emocoltura, l'utilità di affiancare un terreno o un metodo specifico per miceti nei casi sospetti.

OCCURRENCE OF YEASTS IN DROPPINGS FROM SOME HEALTHY EXOTIC PETS AND IN VITRO SUSCEPTIBILITY PROFILE TO FOUR ANTIFUNGAL AGENTS: PRELIMINARY REPORT

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The number of non conventional animals kept as pets in households has strongly increased during the last years. More and more people are discovering exotic and wild animals because they like to possess unusual pets or regard them as status symbols. Persons with weakened immune system are often advised to give up their pets to avoid getting various diseases from them. However, many of them decide to keep their companion animal. In these cases, the patient and the families must be aware of the potential risk for diseases that can be passed from animals to humans. Although some guidance for the care of animals entering shelters and for persons handling them are provided (Wong SK, Feinstein LH, Heidmann PJ Healthy pets, healthy people Am Vet Med Assoc. 1999; 215: 335-8), the evaluation of the role of exotic and wild species in spreading and transmitting potential pathogens such as opportunistic yeasts is still poorly investigated. Aim of he present survey was to characterize the yeasts present in the cloacal content of healthy reptiles kept in a pet shop, and in the faeces of healthy psittacine birds and canaries from the premises of breeders and private households. Some isolates were essayed for *in vitro* sensitivity to four antifungal drugs commonly recommended to treat human systemic mycoses (Hay RJ, Antifungal drugs used for systemic mycoses Dermatol Clin 2003; 21: 577-87). Three-hundred twenty five parrots, 284 canaries and 218 reptiles were culturally examined for fungi. Faeces were collected with a sterile cotton swab from the inner cloacal wall or from droppings immediately after shedding, dipped in saline additioned of 0.5% gentamicin, seeded on Malt Extract Agar and incubated at 25°C. Yeast colonies were identified by morphological, physiological and biochemical features. *In vitro* susceptibility to amphotericin B, fluconazole, caspofungin and voriconazole was determined by Etest. Yeasts were cultured from 49.2% of parrots, 18.7% of canaries and 75.2% of reptiles, respectively. Nineteen species of *Candida*, 4 of *Pichia* and 2 of *Cryptococcus* were obtained. *Geotrichum*, *Trichosporon* and *Saccharomyces* species were also identified. A great number of recovered fungi represent opportunistic agents of invasive fungal infections in immunocompromised patients (Sahin GO, Akova M Treatment of invasive infections due to rare or emerging yeasts and moulds. Expert Opin Pharmacother 2006; 7: 1181-90), confirming the role of exotic animals as a potential hazard to human health. Caspofungin and voriconazole were the most active drugs against *Candida* species, while fluconazole showed a lower activity. Amphotericin B was effective against *Rhodotorula* spp. It is noteworthy to observe that the same species cultured from different animals often showed discordant *in vitro* patterns. It is important for both veterinarians and physicians to educate pet owners about zoonoses and to work together to gather further information and develop evidence-based recommendations.

USE OF DIVERSILAB FOR C. PARAPSILOSIS MOLECULAR CHARACTERIZATION IN AN OUTBREAK SETTING

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Epidemiological surveys indicate that 7-20% of *Candida* bloodstream infections (BSI) is caused by *C. parapsilosis*, species for which exogenous acquisition from the patient's skin, the hands of healthcare workers or the hospital environment is implicated. During the 2-year ECMM-FIMUA survey of fungal infections in Intensive Care Unit (ICU) patients, a participating centre emerged among the others because of the high percentage of *C. parapsilosis* BSIs, namely 28 out of 42 episodes (66%). This induced to suspect an outbreak of *C. parapsilosis* BSIs.

The aims of the present study were to review the epidemiological data concerning the BSI episodes occurred in this centre and to assess the genetic relatedness among *C. parapsilosis* isolates in order to distinguish a true outbreak from a pseudo-epidemic.

Blood isolates from 25 patients were available for the analysis. The ITS1-5,8S-ITS2 region was sequenced to differentiate among the three closely related distinct species (*C. metapsilosis, C. orthopsilosis, C. parapsilosis sensu stricto*), and the DiversiLab semiauthomated rep-PCR was performed to type the isolates.

C. parapsilosis BSI occurred in 13 medical and 15 surgical adult (mean age 60.2 years, range 23-85) patients hospitalised in the unit in the period June 2006 - February 2008. All episodes were considered ICU-acquired as diagnosed after a median of 21 days (range 8-113) of stay in the ward.

All the isolates were identified as *C. parapsilosis sensu stricto*. Three different genotypes were distinguished among 24 typed isolates. One genotype caused eight episodes of BSIs that were diagnosed from July 2006 to November 2007, another genotype caused only two episodes that occurred one year apart. Other two highly related genotypes (>95% of homology) were responsible for a total of 14 episodes occurred from August 2007 to February 2008.

The DiversiLab System resulted useful in this epidemiological investigation supplying evidence of the circulation of three genotypes in the ICU. The conclusion that most of the BSIs were caused by epidemic strains justified the exclusion of the data concerning this centre from the analysis of *Candida* species causing infections in ICU patients in Italy and to stress recommendations for control measures for catheter-related infections focused on improving hand hygiene compliance.

AUTHORS INDEX