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MICOPATOLOGIA UMANA E ANIMALE

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OF MEDICAL MYCOLOGY
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& 2nd Workshop ECMM/CEMM

Università degli Studi di Milano
23-25 September 2010

ABSTRACT BOOK

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UNDER THE AUSPICES OF

Università degli Studi di Milano

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Università degli Studi di Milano

GIOVEDÌ 23 SETTEMBRE 2010

10.30-11.00

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11.00-12.30

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Chaired by: *Stefania Conti (Parma), Flavia De Bernardis (Rome)*

12.30-13.00

KEYNOTE LECTURE

With an educational grant from Gilead Sciences

Chaired by *Francesco Scaglione (Milan)*

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- Candidemia 2009 - *Anna Maria Tortorano, Anna Prigitano (Milano)*
- Infezioni da *Fusarium* - *Anna Maria Tortorano, Anna Prigitano (Milano)*
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Premio Bassi

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Cena sociale

Sala delle Colonne
Museo Nazionale della Scienza e della Tecnologia “Leonardo da Vinci”
Via S. Vittore, 21 Milano

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Museo Nazionale della Scienza e della Tecnologia “Leonardo da Vinci”
Via S. Vittore, 21 Milano

SATURDAY 25 SEPTEMBER 2010

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ACKNOWLEDGEMENTS

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KEYNOTE LECTURES

ASPERGILLOSIS IN CRITICALLY ILL PATIENTS

P. Viale
Clinica di Malattie Infettive, Policlinico S. Orsola Malpighi
Alma Mater Studiorum Università di Bologna

During recent years, a rising incidence of invasive pulmonary aspergillosis in non-neutropenic critically ill patients has been reported. Several risk factors such as chronic obstructive pulmonary disease, prolonged use of steroids, advanced liver disease, chronic renal replacement therapy, near-drowning and diabetes mellitus have been described. Patients with severe chronic obstructive pulmonary disease who are receiving broad-spectrum antibiotics and long term treatment with corticosteroids are becoming one of the main risk groups for IPA in intensive care units, together with chronic immunosuppressed patients.
Diagnosis of IPA in Intensive Care Units may be more difficult than in Haematology Units; in fact, although in many cases the isolation of Aspergillus from lower respiratory tract samples is the first indication of IPA, for a patient, from whom Aspergillus has been isolated, the probability of having IPA is extremely variable, from 72% in severe neutropenia to below 10% in non selected cases. Moreover, just like in haematology patients, in Intensive care patients it is not always feasible to obtain histological demonstration of the fungus in order to meet the gold standard for IPA.
Non-invasive diagnostic tools (i.e. laboratory markers such as serum galactomannan antigen test (GM), 1,3-beta-glucan, and serum Aspergillus PCR), show disappointing results in these patients, probably because of a less invasive disease. The determination of galactomannan antigen on broncoalveolar lavage may be a useful tool for diagnosis of pulmonary invasive aspergillosis in patients admitted to Intensive Care Units, although significant heterogeneity of results in different settings is evident in the literature. Antifungal therapy might be considered in critically ill patients with persistent pulmonary infection who exhibit risk factors together with positive cultures of respiratory specimens or positive galactomannan determination; in these cases the principles of drug choice are similar to those applied in neutropenic patients.

THE TREATMENT OF INVASIVE ASPERGILLOSIS: VORICONAZOLE OR AMBISOME?

L. Pagano
Istituto di Ematologia, Università Cattolica del Sacro Cuore, Roma

Until 2000 deoxycolate-AmB (d-AmB) has been considered the drug of choice for the treatment of invasive aspergillosis (IA). At present new drugs with a less toxicity and a similar efficacy are available and modify the approach to IA in patients with hematological malignancy (HMs). The initial logical alternative to d-AmB have been lipid formulations of AMB [i.e. liposomal AmB (L-AMB); AmB lipid complex (ABLC)], which have a lower risk for nephrotoxicity, generally recommended in patients who develop nephrotoxicity during d-AMB treatment or in patients with renal impairment. Several studies have demonstrated a higher efficacy and improved safety profile with L-AMB compared with d-AMB. Complete or partial responses in clinical trials with the various AmB formulations range from approximately 40% to 70% in patients with IA.

The study that determinates the passage to prefer voriconazole to poliene-based treatments as first choice for IA was that of Herbrecht *et al* (NEJM 2002). In this study voriconazole was compared with d-AmB as first-line therapy in 391 patients with IA, >80% of whom had HMs. A complete or partial response was observed in 76 of 144 (53%) patients treated with voriconazole compared with 42 of 133 (32%) patients treated with d-AMB. This study indicated that voriconazole was more effective than d-AMB, and is effective first-line therapy for IA. Voriconazole has good bioavailability and an acceptable toxicity profile; its clinical use can be limited by its potential for drug interactions (if concomitant use of cytochrome P450 inducers, vinca alkaloids, tacrolimus) and drug-related hepatotoxicity.

Basing on published randomised studies, guidelines by several cooperative groups and International Societies have been redacted, to summarize the current evidences for the treatment of IA. All guidelines are in agreement to recommend voriconazole as 1st choice drug for the primary treatment of IA. L-AMB is also considered as a valid alternative, even if with a different grade of recommendation in the different guidelines. On the other hand, the use of L-AmB is highly indicated for empirical therapy in those patients for which a microbiological finding is not available, and diagnosis is presumed only on the basis of clinical and radiological signs.

L-AMB, ABLC, caspofungin, posaconazole, itraconazole, micafungin are indicated in salvage therapy for patients refractory to or intolerant of primary antifungal treatment.

EPIDEMIOLOGY OF INVASIVE FUNGAL INFECTIONS IN HEMATOLOGY

A. Nosari
SS Terapie ad Alta Intensità, Divisione di Ematologia, AO Niguarda Ca' Granda, Milano

Invasive fungal diseases have been recognized as major causes of morbidity and mortality in patients with prolonged neutropenia. Among patients receiving potent cytotoxic regimens, such as therapy for acute leukaemia, the bowel is the principal portal of entry for candidemia and disseminated candidiasis. Central venous catheters used for administration of chemotherapy or total parenteral nutrition are additional potential sources of candidemia.
In patients with haematological diseases, two major high risk groups for invasive mould infection occur. First are patients in whom the depth and duration of neutropenia predict the risk of invasive mycoses. Examples include patients receiving induction regimens for acute leukaemia, aplastic anemia with prolonged marrow aplasia, and neutropenia following conditioning for hematopoietic stem cell transplantation (HSCT). Second, allogeneic HSCT recipients with significant graft-versus- horst-disease (GVHD) ore those treated with T-cell-depleted allograft are at high risk for both opportunistic fungal and viral diseases. In recent years, large surveillance networks repoted invasive aspergillosis as the most common invasive fungal infection in haematologic patients. In the Italian SEIFEM study, regarding more than 11,000 patients with haematologic diseases admitted in 18 hematology wards in Italy, incidence of moulds accounted for 2.9% in confront to yeasts (1.6%) with a higher incidence in acute myeloid leukaemia (12%). The majority of the mould infections (90%) were caused by Aspergillus SPP, and these infections accounted for 58% of thr IFI analyzed. In these patients the mortality rate attributable to invasive aspergillosis decreased to less than 30%, confirming other reports of a downward trend in this rate. Candidemia related mortality remains within 30-40% range reported in literature although the incidence has decreased probably due to the extensive use of prophylaxis.
Similar results were present in Multicenter Prospective Antifungal Therapy (PATH) Alliance Registry, which collected epidemiologic data from sixteen bone marrow transplant center of North America. Invasive aspergillosis accounted for 59.2% followed by invasive candidiasis (24.8%), zygomycosis (7.2%) and other moulds (6.8%). Antifungal prophylaxis has been successful in reducing the incidence of fungal infections in the preengraftment period. Consequently, fewer fungal infections now occur early during neutropenia, but many centers have reported increasing rate of late (>40 days after HSCT) and very late (>100 days) mould infections. Survival in transplant recipients with aspergillosis seems significantly improved (from 80% of historical data to 35% of PATH registry), while in mycoses due to Candida species and zygomycosis survival was stable and associated with high mortality rates.

EUROPEAN CONTRIBUTION TO MEDICAL MYCOLOGY

L. Polonelli
Sezione di Microbiologia, Dipartimento di Patologia e Medicina di Laboratorio, Università degli Studi di Parma

The European contribution to Medical Mycolgy is chronologically revised along the years from the springtimes of the discipline until today. Since Hippocrates (~460-370 B.C.), the Greek physician considered to be the father of medicine, discussed in his writings candidiasis in the form presently recognized as "trush", fundamental discoveries, representing milestones of the human knowledge that escape the specific scientific field to concern Medical Microbiololgy, Medicine and Biology in general, have regarded European medical mycologists. Some of their seminal pioneering studies have been instrumental to establish "the germ theory of infectious diseases" by G. Fracastoro, to put an end to the pernicious Aristotelian concept of "the spontaneous generation of life" by F. Redi, L. Spallanzani and L. Pasteur, to prove that fungi produced spores that germinated and gave rise to colonies of the species that had produced them by P. A. Micheli, to verify, for the first time, that a microorganism may cause an infectious disease in animals by A. Bassi, including man by J. L. Schonlein, R. Remak and D. Gruby, to dictate that mould diseases may be summarize within the term "mycoses", by R. Virchow.
Mycotic diseases, have been described or depicted in works of art by European artists. Notably, among them were Tinea imbricata by the buccaneer and navigator Sir W. Dampier and favus by the noted painter B.E. Murillo.
The birth of the International Society for Human and Animal Mycology (ISHAM) is radicated in Europe, and precisely in Rome, Italy, as it was founded in September 1953, at the 6° International Congress of Microbiology. The prefoundation of ISHAM started with an informal meeting in a small romantic restaurant "Giardino dei poeti" in the picturesque part of Rome overlooking the river Tevere, called Trastevere. A dozen of enthusiastic mycologists, most European, including R. Vanbreseughem, G. Ainsworth, G. Segretain, F. Mariat, E. Drouhet, J. Lodder, H. Paldrolck, H. Seeliger, were invited by P. Redaelli and R. Ciferri to a very intimate meeting and the idea of a New Society for the study and development of Human and Animal Mycology was advanced by the passionate mycologists present at the dinner. A year later, in July 1954, in Paris, France, at Pasteur Institute, during the 8° International Congress of Botany, ISHAM was officially founded.
On January 15th 1993 in Paris, at the Pasteur Institute, most European countries, endowed with facilities for the study of fungal infections and their agents, either in formally constitued national mycological Societies or in Medical Mycology Groups attached to Societies of Microbiology, Dermatology or Infectious Diseases, decided to found the European Confederation of Medical Mycology (ECMM). Since then, ISHAM and ECMM have promoted Congresses and collaborations amongst members in order to overcome any limit of activity, country and language in Medical Mycology.

LECTURES

FUNGAL STRATEGIES IN HUMAN INFECTIONS

S. Conti
Università degli Studi di Parma, Dipartimento di Patologia e Medicina di Laboratorio, Sezione di Microbiologia

Fungal microorganisms, from environmental saprophytes, which may sporadically infect animals, to opportunistic pathogens, which can benefit of predisposing factors, are able to adopt alternative strategies in their interaction with the human host. Adherence, biofilm formation, dimorphism, germ tube formation, phenotypic switching, interference with the host defense system, synergism with bacteria, production of enzymes or toxic metabolites have been involved as virulence factors.
In host-fungus relationship, however, primary importance should be attributed to individual innate and adaptive immunity.

ROLE OF CELL WALL REMODELLING DURING MORPHOGENESIS AND VIRULENCE IN *CANDIDA ALBICANS*

E. Ragni[1], J. Calderon[1], M. Sipiczki[2], W.A. Fonzi[3], J. Arroyo[4], L. Popolo[1]
[1]Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli studi di Milano;
[2]Department of Genetics and Applied Microbiology, University of Debrecen, Hungary;
[3]Department of Microbiology and Immunology, Georgetown University, Washington D.C., USA;
[4]Departamento de Microbiologia II, Universidad Complutense de Madrid, Spain

Background. Fungal walls are the first interface between the microorganisms and their environment. Cell wall biogenesis is a dynamic process that is tightly linked to morphogenesis. In fungal pathogens, the cell envelope is greatly involved in virulence since it mediates processes such as adhesion, invasion of the epithelia layers and interaction with the immune cells. In *Candida albicans* the cell wall greatly contributes to its virulence potential. *PHR1* is a pH-regulated gene encoding a β(1,3)-glucanosyltransferase involved in cell wall glucan remodelling . *PHR1* is a member of *PHR* family that is composed of five genes, *PHR1-2-3* and *PGA4-5*. All yeast and fungal species so far sequenced have homologs of the *PHR* families. *PHR1* expression is triggered at external pH values higher than 5.5. *PHR1* null mutants exhibit morphological defects, inability to maintain hyphal growth, to adhere and invade epithelia and they are avirulent (J. Calderon, et al., Microbiology, 156, 2010).
Objective. The aim of this work is to investigate the function of Phr1p in *C. albicans* morphogenesis through the study of its localization and a large scale transcriptional response triggered by its absence during hyphal induction.
Methods. *C. albicans* strains expressing a fusion of Phr1p with the Green Fluorescent Protein were obtained using a PCR-based strategy (M. Gerami-Nejad, et al., 2009, Yeast 26: 399-406). For microarray analysis of *C. albicans* strains, RNA was extracted during hyphal induction Hybridization was performed at Washington University in St. Louis (USA) and statistical analysis was done at Universidad Complutense de Madrid (Spain).
Results. During vegetative growth in alkaline pH, Phr1p-GFP was detected in plasma membrane microdomains, in the mother-bud neck, in the septum and in the bud scars. Upon induction of hyphal growth, Phr1p-GFP highly concentrated at the apex of the germ tubes and hyphae and progressively distributed along the lateral sides of the hyphae. Phr1p-GFP also labelled the hyphal septa where it colocalized with chitin. The results suggest that Phr1p promotes cell wall glucan remodelling at sites of wall formation as hyphal apex and septum. A *PHR1* null mutant showed defects in hyphal wall ultrastructure, consisting of loss of compactness and irregular organization of the surface layer, and also a higher accumulation and delocalization of chitin. Moreover in the absence of Phr1p, a wide transcriptional response was activated. In particular, genes involved in chitin synthesis and cell wall remodelling were upregulated suggesting the activation of a cell wall-stress response. These observations indicate that Phr1p plays a crucial role in hyphal wall assembly, a highly regulated process on which morphogenesis and virulence rely.

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C. ALBICANS, C. GLABRATA, C. TROPICALIS AND C. PARAPSILOSIS ISOLATES FROM INVASIVE FUNGAL INFECTION: BIOFILM COMPARISON

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Aim of the study. *Candida* species bloodstream infections (BSI) have become a focus of attention, representing the fourth most common cause of BSI in ICUs, with a crude mortality rates varying from 23% to 53% according to the population studied and the different *Candida* species involved. Biofilm formation is a major virulence factor in the pathogenicity of *Candida*, being *Candida* biofilms difficult to eradicate especially because of their marked antifungal resistance. The aims of this work were firstly to assess the biofilm formation ability of clinical isolates of *C. albicans, C. glabrata*, *C. tropicalis* and *C. parapsilosis* recovered from sterile sites, and secondly, to characterize the difference in biofilm structure.
Methods. A total of 357 clinical strains, recovered from BSI, were used for this study. Candida biofilms were formed on the surface of 96-wells microtitre plates by pipetting standardized cell suspensions. Biofilm forming ability was assessed through quantification of total biomass by crystal violet (CV) staining and through measurement of biofilm metabolic activity by XTT reduction assay. To test genetic basis for variation in biofilm ability among isolates, cell surface hydrophobicity (CSH) was also quantified. Biofilms morphology and architecture were analyzed by confocal laser scanning microscopy (CLSM) after calcofluor staining.
Results and Conclusions. The propensity to form biofilm was shown in 43% of *C. albicans* isolates, 38% of *C. parapsilosis*, 81% of *C. tropicalis*, and in about 12% of *C. glabrata* strains. *C. parapsilosis* and *C. tropicalis* biofilms both showed a higher stability and the unique ability to adhere also to glass surface. Generally *C. glabrata* biofilms had less total biomass and this was less able to form homogeneous biofilm layers. CSH quantification highlighted a good correlation between higher hydrophobicity and effective adhesion. CLSM allowed the visualization of three-dimensional architecture at different depths of biofilm, confirming *C. parapsilosis* and *C. tropicalis* biofilms as the thickest structures, with 31.5 μm and 79 μm respectively.
Biofilm formation propensity and the different biofilm features, especially its antifungal resistance, are important factors to take into account when considering a therapeutic approach. The challenge of the new millennium will be to find early and rapid methods to detect potential molecular targets involved in biofilm formation, thus enabling physicians to adopt the most appropriate pharmacological strategy.

ADHESION OF *CANDIDA PARAPSILOSIS*, *CANDIDA ORTHOPSILOSIS* AND *CANDIDA METAPSILOSIS* TO HUMAN BUCCAL EPITHELIAL CELLS

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Retrospective studies indicate that *Candida metapsilosis* and *Candida orthopsilosis* each represents 1-10% of the infections/colonisations attributed to *C. parapsilosis* by conventional biochemical tests. Little is known on the virulence properties of these fungi and on their role in the establishment/progression of the infection. Of the three species, *C. parapsilosis* has been extensively studied, with a well characterized virulence repertoire including biofilm production, proteinase secretion and adhesion. To date, *C. parapsilosis* is the only species of the complex for which a commensal status has been demonstrated in humans, although a recent metagenomic study showed the presence of *C. metapsilosis* in the oral cavity of healthy human subjects.

The objective of this study was to investigate the adhesive properties of the "psilosis" species, which may influence colonization and successful permanence within the host.

In an assay of adhesion to human buccal epithelial cells (HBECs), 10 clinical isolates of each species were assessed for their ability to adhere following a 45 minute incubation at a HBEC:yeast ratio of 1:1000. Data obtained indicate that, while *C. parapsilosis* and *C. orthopsilosis* strains showed a similar adhesion ability, *C. metapsilosis* isolates displayed a significantly lower ability to adhere to HBECs ($P<0.05$). Representative isolates of the three species were incubated with HBECs obtained from different donors to assess experimental variability. Although minor fluctuations in the mean adhesion values of yeast to HBECs from different donors were found, an overall limited variability was observed. Ectophospatase activity was measured in all isolates *in vitro*, as this enzymatic activity has been previously reported to be linked to adhesion in *C. parapsilosis*. In our experiments, no evidence of a correlation between ectophosphatase activity and adhesion was observed, suggesting that these two mechanisms are independent of one another Ectophosphatase inhibition experiments, performed pre-incubating yeast cells with sodium orthovanadate, an inhibitor of phosphate-transferring enzymes, or different phosphate concentrations, further confirmed that this enzyme does not play a major role in the adhesion of the "psilosis" species to human buccal epithelial cells. These findings clearly indicate that other factors, such as Hyr/Iff-like and ALS-like proteins, are implicated in the adhesion process.

HETEROZYGOSIS, VIRULENCE FACTORS AND PATHOGENICITY IN *CRYPTOCOCCUS NEOFORMANS* AD HYBRID ISOLATES

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Nineteen *Cryptococcus neoformans* AD hybrid isolates were investigated to assess the possible relationship between genomic background and virulence. The level of heterozygosity of each strain was analyzed using primers specific for allele A and D of 12 polymorphic genes. Virulence was tested in a mouse model of systemic infection by inoculating the yeasts in the lateral tail vei and by measuring the survival. In addition, the virulence attributes such as melanin, phospholipase, capsule production, as well as growth at 39°C and UV sensitivity, were also investigated . In eight strains more than 60% of loci were heterozygous, in a further eight <60% of loci, and three strains were homozygous at all loci tested. Isolates in which >60% of loci were heterozygous were significantly ($p<0.01$) more virulent than the other hybrids in the animal model. Virulence was not correlated to mating type loci, any or other allelic patterns, as well as it was not correlated to the level of expression of the different virulence attributes investigated.
The present study confirms that hybridization in *C. neoformans* could represent an important evolutionary driving force in increasing the fitness of this yeast in the environment and in the host.

NEW INSIGHTS INTO THE PATHOGENESIS OF *MALASSEZIA* YEASTS

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Yeast of *Malassezia* genus are component of the normal human and animal skin microbial, but in some circumstances can become pathogenic. This opportunistic behaviour seems to depend on both, the chemical-physical characteristics of the host skin, and the strain virulence (Cafarchia, Otranto, 2008, Parassitologia, 50: 65-67). The phospholipase production, associated with the presence of opioid receptors on the *Malassezia pachydermatis* membrane, seems to be an important virulence factor (Cafarchia et al., 2009, Med Mycol, 48: 1-6). Recently, the *in vitro* formation of *M. pachydermatis* biofilm has been demonstrated (Cannizzo et al., 2007, Med Mycol, 45: 357-61), although its structural analysis has not been performed. Thus, the present study aims to assess the *in vitro* ability of *M. pachydermatis* to form biofilm on polystyrene microtiter plates and on venous polyurethane catheters, and to characterize the biofilm structure. The biofilm formation have also been correlated to the phospholipase production, to the presence of skin lesions on dogs and to *M. pachydermatis* genetic types.

Methods. 62 Strains of *M. pachydermatis* were isolated from asymptomatic (n=30) and symptomatic (n=32) dogs and molecularly characterized as previously reported (Cafarchia et al., 2007, Mol Cell Probes, 21: 229-38). The phospholipase production was assessed using the semiquantitative egg-yolk plate method (Cafarchia, Otranto, 2004, J Clin Microbiol, 42: 4868-4869). Biofilm production was performed on polystyrene microtiter plate wells and quantitatively determined by crystal violet method (adapted from Jin et al., 2003, J Clin Microbiol, 41: 2961-2967). Scanning electron microscopy (SEM) of polyurethane catheter surfaces (Cannizzo et al., 2007, Med Mycol, 45: 357-61) was used to assess the biofilm structure. Statistical differences in quantitative biofilm production in dogs with or without lesions were assessed using Student's t test. The correlation between biofilm formation and phospholipase activity was evaluated using the Pearson's coefficient.

Results. The results show that all *M. pachydermatis* strains isolated from dogs with skin lesions and without skin lesions are capable to adhere on the surface of polystyrene microtiter plate wells forming biofilm. No statistically significant differences were found between biofilm formation and the presence or absence of skin lesions, genetic types of *M. pachydermatis* or phospholipase production ($p>0.05$) considering the quantitative method. At the SEM analysis the biofilm production was strain dependent and revealed structural differences mainly on extracellular matrix production.

Conclusions. These findings indicate that *M. pachydermatis* isolated from dogs with and without lesions may form biofilm. However, the biofilm structure may be related to the origin of *M. pachydermatis*, from lesioned or healthy skin.

INVASIVE FUNGAL INFECTIONS IN ALLOGENEIC BONE MARROW TRANSPLANT

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Invasive fungal infections (IFI) remain an important cause of morbidity and mortality in allogeneic haematopoietic stem cell transplant (HSCT) recipients. The main IFIs affecting these patients are invasive aspergillosis, invasive candidiasis, pneumocystosis, zygomycosis and cryptococcosis. Significant difficulties remain as far as accurate and prompt diagnosis of IFI in HSCT recipients is concerned. However, the development and widespread use of non-invasive serological markers, such as galactomannan and (1, 3) -beta-D-glucan, together with high resolution CT-scan, have improved significantly the possibility of timely diagnosis of IFI.
Moreover, the availability of these diagnostic methods has allowed the introduction of a novel management strategy - pre-emptive therapy, that can replace or complement prophylaxis or empirical treatment. Last but not least, novel antifungal compounds, with different antifungal activity, different toxicity profile and route of administration are currently available.

POSACONAZOLE PROPHYLAXIS IN ACUTE MYELOID LEUKEMIA. REAL LIFE EXPERIENCE OF A SINGLE HEMATOLOGICAL CENTER

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Introduction. Acute Myeloid Leukemia (AML) patients are at high risk of developing Invasive Fungal Infectious (IFI). Posaconazole, an oral azole with a broader spectrum, was approved in September 2006 for the prophylaxis of IFI in high risk hematologic patients. We report our real-life experience with Posaconazole (POS) prophylaxis of Invasive Fungal Infections (IFI) in Acute Myeloid Leukemia (AML).
Patients and Results. Forty-two unselected and consecutive AML patients (23 females and 19 males) received POS prophylaxis (200 mg three or four times daily) at our Hematologic Division between December 2008 and February 2010. Median age of this population was 47 yrs (range 18-69). All cases were given chemotherapy with anthracyclines and cytosine arabinoside. The POS prophylaxis was started when neutrophil (PMN) absolute count was less than 1000 μL and was stopped at PMN recovery (PMN absolute count over 500 μL). The median duration of severe neutropenia (PMN lower than 500 μL) was 12 days (range 7-30); 13/42 (31%) of cases had an oral mucositis grade II-III ctc (common toxicity criteria) and 95% (40/42) of these patients received a proton pump inhibitor (omeprazole). During the period of severe neutropenia an active diagnostic work up was made in all cases with Galactomannan assay (two times/week), standard chest X-ray (one time/week) and thoracic computed tomographic scan in case of fever of unknown origin (FUO) lasting over 48 hours.
The median duration of POS prophylaxis was 14 days (range 7 to 33 days). Only 3/42 (7,5%) of these patients required parenteral empiric or pre-emptive antimycotic therapy and only 1/42 (2,5%) experienced a proven IFI (breakthrough *Fusarium solanii* fungemia). Mortality IFI related of this population was 0%. POS was well tolerated. In fact, only 10% (4/42) of pts experienced mild drug related side effects (2/42 nausea grade I ctc, 2/4 diarrea grade I ctc). No cases of POS discontinuation, due to the side effects, were reported.
Conclusions. Our real-life experience confirms that POS prophylaxis is feasible, safe, well tolerated and effective (prevention of IFI) in unselected AML population. Only 10% of these high risk patients required parenteral antimycotic therapy.

EFFICACY AND SAFETY OF GRANULOCYTE TRANSFUSIONS FOR THE TREATMENT OF INVASIVE FUNGAL INFECTIONS

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The degree and duration of neutropenia have been recognized as crucial prognostic factors in hematological patients with invasive fungal infections (IFIs). Since the introduction of granulocyte colony stimulating factor (G-CSF), there has been a renewal of interest in granulocyte transfusions (GTX). However there is a large variability in the transfusion practice, and uncertainty about the beneficial effects of GTX as adjunctive measure to antimicrobial therapy.
We designated a retrospective analysis to evaluate feasibility, efficacy and safety of GTX as adjunctive treatment for neutropenic fever unresponsive to antimicrobial therapy.
During a 5 years period (2004-09) 33 courses of GTX were administered to 29 patients with hematological malignancies (HM) and fever during neutropenia (ANC $<500 \times 10^6$/l and anticipated duration >7days), after no clinical response to antimicrobial therapy. Patients were suffering from acute leukemia (20 myeloid and 5 lymphoid), lymphoma (4), multiple myeloma (2). Volunteer donors had received G-CSF (5 μg/kg) 12h before the first of two consecutive collection procedures. All of them had signed an informed consent for G-CSF administration and leukapheresis.
Overall, 162 GTX from 84 donors were administered, with a median of 5 GTX per episode of infection (range 1-20). Infections causing fever were identified in 27 episodes: 10 bacterial sepsis, 16 invasive fungal infections (IFIs) and 1 mixed bacterial/fungal sepsis. Remaining 6 cases were classified as fever of unknown origin (FUO). IFIs included 10 cases of pulmonary aspergillosis, 5 candidemia, 1 invasive zygomycosis and 1 invasive fusariosis.
Donors' mean WBC count at first leukapheresis was 27×10^9/l (range 14-45), while at second procedure the count was lower (15×10^9/l, range 9-33), as expected. The mean yield was 26×10^9 PMN (range 6.7-75.8) at first procedure and 11.7×10^9 PMN at the second one (range 0.6-42). No adverse events occurred in donors. Six patients (18%) experienced a mild allergic reaction. All of them were transient.
The combination of antimicrobial therapy with GTX led to a favourable clinical response in 22 patients (67%); the acute infection-attributable mortality rate was 33% at 30th day after the last GTX. In 75% of HM with IFI a favourable clinical response was obtained, with differences between moulds (91%) and yeasts (40%) (p-value 0.06).
In conclusion, at preliminary analysis GTX may be safe and efficacious in HM with severe infection to bridge the period of deep neutropenia, when antimicrobial therapy have failed. Controlled studies are needed to confirm this datum, to define the proper role of such a procedure and the optimal schedule for HM.

PHARMACOLOGICAL AND CLINICAL ISSUES IN ANTIFUNGAL THERAPY IN INTENSIVE CARE PATIENTS

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The incidence of fungal infections (mostly due to Candida spp) in intensive care unit (ICU) patients is continuously increasing during the last years and nowadays they represent the fourth cause of infections in critically ill patients. Despite the availability of several antifungal drugs, performing an adequate antifungal therapy in ICU patients still represents a difficult task, and mortality attributed to fungal infections remains high. Preclinical data, and, more recently, a few clinical data, link positive outcome from fungal infections with the achievement of pharmacokinetic/pharmacodynamic (PK/PD) targets which are specific for each antifungal class. However, while obtaining such targets might represent a major help for clinicians performing antifungal therapy, because of ICU septic patients' characteristics, such as multiple organ failure, changes in vascular permeability and protein binding, hemodynamic instability - all aspects causing high inter-patient pharmacokinetic variability - these targets are often eluded.
More PK/PD studies with direct measures of antifungal plasma and tissue levels in this special patient population, together with re-elaborations with simulation programmes (such as Monte Carlo simulation) will be needed in order to optimize the clinical cure and contain toxic effects.

COMBINATION TREATMENT OF EXPERIMENTAL ZYGOMYCOSIS

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Zygomycosis is a rare but highly aggressive filamentous fungal infection, occuring primarily in immunosuppressed patients and those with diabetes mellitus. The clinical manifestations include primary rhino-orbital-cerebral zygomycosis in patients with diabetes mellitus and pulmonary infection in transplant recipients and patients with hematologic malignancy. The standard therapy for these life-threatening infections consists of removal of the predisposing factors, widespread surgical debridement, and high doses of intravenous amphotericin B. Nevertheless, the aggressive therapy, mortality is often above 50%.
We evaluated the effect of posaconazole alone and in combination with amphotericin B against *Rhizopus oryzae* and *Lichteimia corymbifera* in an experimental model of neutropenic mice infection. We showed that amphotericin B and posaconazole are both active against zygomycosis, depending on the studied strain. In general, the drug combination was never more effective that the most active drug alone.

ASPERGILLOSIS IN HEMATOLOGIC PATIENTS

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Invasive fungal diseases (IFDs), in particular invasive aspergillosis (IA), are a frequently fatal complication in patients with haematological diseases. The high mortality rate of IFDs results from difficulties in obtaining a reliable and early diagnosis and in establishing a proper treatment.
For these reasons, empirical antifungal therapy has been considered a standard practice in patients with persisting febrile neutropenia, but with the risk of overtreatment with potentially toxic and/or expensive drugs, knowing that only a minority of patients is probably affected by an IFD. Another strategy - the "preemptive approach" - based on a predefined surveillance and diagnostic work-up with laboratory markers, such as serum galactomannan and β-1-3 D glucan and surveillance cultures, and radiological findings, has been proposed in an attempt to fill the gap between over and undertreatment.
However, such strategies do not have standardized criteria, and require time-consuming and expensive laboratory exams, particularly when applied to the entire population of neutropenic patients for a prolonged time period. Recent experiences underline the importance of clinically-driven antifungal approaches based on predefined diagnostic strategies adapted to the various clinical settings of patients with haematological diseases undergoing immunosuppressive therapy, intensive chemotherapy or stem cell transplant. The efficacy of such diagnostic algorithms depends on a multidisciplinary coordination of microbiological, radiological and clinical diagnostic efforts.

CANDIDEMIA AND INVASIVE *CANDIDIASIS* IN CRITICAL PATIENTS

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Candidemia is a major complication of Intensive Care Unit (ICU) stay for a variety of reasons including the underlying diseases (medical and surgical), specific risk factors to the ICU, *Candida spp.* colonization favored by antibiotic treatment and possibly immunesuppression. Invasive candidiasis and candidemia are associated with poor clinical outcomes in ICU patients, with a mortality as high as 40% and related morbidity attributable to the excess of hospital stay and difficult discharge to medical or surgical wards. Strategies of early treatment focused on periodic assessment of risk factors including body site Candida colonization are of great help in reducing attributable mortality, while universal antifungal prophylaxis is not widely accepted, notwithstanding the fact that it is the best studied early antifungal intervention strategy. Antifungal prophylaxis, unless targeted to patients at highest risk, is inefficient. Earlier antifungal intervention strategies such as preemptive therapy and empiric therapy may better improve patient outcomes. Recent data suggests that pre-emptive strategies using a combination of clinical risk factors and Candida colonization parameters require further validation in the ICU. Empiric antifungal therapy is not of benefit when instituted to patients with antibiotic-refractory fever alone.
Aim of the presentation is to show and discuss the epidemiology and microbiology of candidemia in the ICU together with critical issues of antifungal treatment. Such issues are highlighted with a summary of treatment strategies.

EPIDEMIOLOGICAL RELEVANCE OF AN ACCURATE ISOLATE TYPING

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A diagnosis of invasive candidiasis can be achieved using conventional approaches (microscopy, culture, serology), as well as new methods, including antigen detection and polymerase chain reaction (PCR) assays. Most of the conventional approaches lack sensitivity, especially for obtaining a diagnosis of invasive candidiasis in immunocompromised patients.
Antibody detection is useful for the diagnosis of some invasive mycoses, particularly when used in association with microscopic and culture investigations. Serological assays involve both qualitative and quantitative assessment of the circulating antibodies in blood and other biological fluids.
The search for circulating antigens has good specificity, although this test requires frequent sampling due to the rapid clearance of these antigens from the blood. Recent studies have demonstrated a good diagnostic efficacy associating this assay with the search for antimannan in critical, but not in immunocompromised, patients.
PCR assays represent a valid alternative, in terms of their high potential sensitivity and specificity, but these procedures still need to be standardized and evaluated in a large number of patients.

APPLICATION OF MALDI-TOF FOR SPECIES IDENTIFICATION AND INTRASPECIFIC CHARACTERIZATION OF THE GENUS *ASPERGILLUS*

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The genus *Aspergillus* includes approximately 250 species, ubiquitously distributed and associated with a wide spectrum of human diseases, expecially for immunocompromised individuals, that affect skin, ear, lungs, central system nervous, and endocardium. Among these infections, invasive pulmonary aspergillosis (IPA) is the most severe due to high morbidity and mortality. *Aspergillus fumigatus* is the most significant causative agent of IPA, followed by *A. terreus, A. flavus,* and *A. niger.* Recently less frequent species such as *A. ustus, A. alliaceus*, *A. lentulus* and *A. udagawae* have been reported as etiological agents of human aspergillosis.
As the different *Aspergillus* species show different antifungal susceptibility patterns, identification of *Aspergilli* at the species level remains of great importance in order to guide the appropriate antifungal therapy. Misidentification of the *Aspergillus* species by morphological methods often occurs, thus molecular methods have been recommended to provide more reliable identification within the genus. Nonetheless, the latter involve significant cost, are time-consuming, and need the use of universal molecular markers that facilitate the inter laboratory data exchange.
For these reasons, fingerprinting by the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry could offer a reliable, rapid, and unambiguous tool for the identification of clinical *Aspergillus* isolates at both the species and sub-species levels.
To this purpose, we standardized working procedures and generated fingerprint profile spectra to develop an appropriate database that include more than seventy clinical and reference *Aspergillus* isolates. In addition, by using the dedicated MALDI BioTyper software, we generated dendrograms to infer phylogenetic relationships among the *Aspergillus* species and subspecies.
Thus, *Aspergillus* isolates that were grown from clinical specimens routinely processed for mycological examination, were submitted to a short extraction protocol and applied on a sample target plate to obtain spectra profiles. After comparing by the BioTyper pattern matching software the generated peaks with our reference database, we correctly identified at the species level unknown *Aspergillus* isolates, as subsequently confirmed by the DNA sequencing analysis.

PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF MALASSEZIA YEASTS FROM DOGS WITH AND WITHOUT SKIN LESIONS

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Members of the *Malassezia* genus are common commensals of the skin of humans and animals but may become pathogenic under the influence of predisposing factors (Cafarchia et al., 2010, Med Mycol, 48: 73-8). The identification of *Malassezia* species is predominantly based on morphological, physiological and/or molecular characteristics (Batra et al., 2005, FEMS Yeast Res, 5: 1101-13). Currently, 13 species of the genus (i.e., *Malassezia dermatis*, *M. furfur*, *M. globosa*, *M. japonica*, *M. nana*, *M. obtusa*, *M. restricta*, *M. sloffiae*, *M. sympodialis*, *M. yamatoensis, M. caprae, M. equina* and *M. cuniculi*) are recognized as lipid dependent (LD), whereas exclusively *M. pachydermatis* does not require lipid supplementation for in vitro growth (Cabañes C, et al., 2007, Med Mycol, 2010) being designated as non-lipid dependent (NLD). The LD species are usually isolated from normal and/or diseased human skin and *M. pachydermatis* from the skin and/or mucosa of different mammals (Batra R. et al., 2005, FEMS Yeast Res, 5:1101-13). The recent molecular identification of *M. pachydermatis* LD strains from healthy skin sites of dogs spurred the scientific investigations onto the molecular characterization of *Malassezia* LD strains from dogs. In this study lipid-dependent *Malassezia* yeasts from a dog with skin lesions were physiologically, morphologically and molecularly characterized using chitin synthase 2 gene (*chs*-2), first internal transcribed spacer (ITS-1) and the large subunit (LSU) of nuclear ribosomal DNA as genetic markers.

Methods. Three *Malassezia* LD strains (here named 114A, 114B and 114C) isolated from a dog with skin lesions were phenotypically and genotypically identified (Guillot J et al, 1996, J Mycol Med, 6: 103-10; Mayser P et al., 1997, Br J Dermatol, 137: 208-13; Mayser P et al., 1998, Mycoses, 4: 265-71; Cafarchia C et al., 2007, Mol Cell Probes, 21: 229-38).

Results. All the isolates presented ovoid cells and buds formed on a narrow base. Most of the physiological tests were consistent with those of *M. furfur.* Sequencing of the *chs*-2 gene, ITS-1 and LSU rDNA confirmed that 114B isolate was closely related to *M. furfur* (CBS1878 and CBS7019 reference strains). In contrast, the lipid-dependent isolates 114A and 114C were genetically related to *M. furfur* for ITS-1 and LSU differences but not for *chs*-2. In particular, lipid-dependent isolate 114A displayed *chs*-2 sequences similarity (100%) to that of the non-lipid dependent species *M. pachydermatis.*

Conclusion. The results of the phenotypical, physiological and molecular characterization of lipid-dependent *Malassezia* isolates obtained from a dog with skin lesions, might suggest the occurrence of genetic variants of the anthropophilic species of *M. furfur* on dogs. The presence of the genetic and physiological polymorphisms detected in three isolates of *M. furfur* here examined could result from a process of adaptation of this anthropophilic species to a new host.

USE OF MICROSATELLITE MARKERS FOR STRAIN TYPING OF *MICROSPORUM CANIS*

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Microsatellite markers were applied in order to assess the intraspecific genetic variability of *Microsporum canis,* the dermatophyte more frequently associated with cat and dog. Different factors (animal species, breed, age, health state, concomitant pathologies etc.) can participate in affecting clinical presentation and outcome of *M.canis* infection, but the question may arise as to whether animal hosts harbor mixed genotypes of *M. canis* that differ in their degree of virulence and infectivity. In other words, do all lineages of *M. canis* have the same potential to cause different clinical forms and infect humans, or is virulence limited to a subset of isolates within a genetically diverse population? Do these genotypes differ in host predilection and pathogenicity? Methods that can discriminate between dermatophytes at strain level are expected to be useful tools to address these questions. Among the most commonly employed strain typing techniques that have evaluated dermatophyte species are those which involve the amplification of unidentified regions of the genome, relying on a pattern of bands to create a unique fingerprint for each isolate. Microsatellite markers represent a random strategy, by which to differentiate pathogen strains, that have gained in popularity owing to the highly variable nature and rapid mutability of these markers. Multi locus microsatellite typing (MLMT) has the potential to correlate specific genotypes with "phenotypical" features of interest of fungal strains. Actually, the loci under study are unlikely to be based on genes involved in virulence or other features of interest, but, due to the clonal mode of reproduction of dermatophyte fungi, genomes are transmitted to the next generation in unaltered condition and thus associated genes - such as virulence genes and microsatellite markers - are linked (Sharma et al. 2007; Graser et al. 2007).
We used eight microsatellite markers in order to analyse a set of *M.canis* strains of animal origin. Analysis of combined dataset of the polymorphic markers allowed to detect multilocus genotypes, useful to discriminate among geographical unrelated strains. On the other hand, four strains that were recovered from different sites of a single patient (cat) and the bed of its owner yielded identical genotypes for all the loci. In other words, they identified as a single strain isolates collected from a single animal. The use of such markers on a larger collection of *M.canis* strains may allow to:

- *detect genotypes eventually linked with clinical/epidemiological features of intest (e.g particular clinical forms, infectivity for humans, host breed etc.);*
- determine whether multiple strains can be detected in a single patient;
- distinguish relapse from reinfection in patients suffering from recurrent forms;
- obtain tools to detect and trace sources and routes of infection and identification of contaminated spaces, thus contributing to optimize prophylaxis and hygienic regimens.

CURRENT MYCOTIC TOPICAL THERAPIES

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In the treatment of superficial mycoses, particularly at the cutaneous level, topical therapy is of utmost importance. This modality permits a rapid eradication of the etiological agent according to the type of infection due to the fact that it is available in easy to use formulations (creme, lotions, etc.). In most cases, topical treatment alone is sufficient to guarantee complete healing.
In recent years, nail laquer formulations have also been made available which have the capacity to permeate the nail lamina keratin and is therefore extremely efficacious in the therapy of onychomycoses.

LDG7 A CELL WALL PROTEINS INVOLVED IN MICAFUNGIN DRUG RESISTANCE IN *CANDIDA ALBICANS*

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Cell wall of *C.albicans* is very important in the interactions of fungus with host. Many cell wall proteins are involved in modulation of this organelle and changing its expression in presence of environmental stress. The mayor constituents of cell wall of *C.albicans* are the polysaccharides 1,3 β-glucan, 1-6 β-glucan, chitin and mannoproteins and proteins. The cell wall proteins (CWPs) covalently attached to this meshwork of structural fibrillar polysaccharides are in several classes. The first and most abundant class is linked to β 1-6 glucan trough a glycophosphatidylinositol (GPI). 2D analyses shown that in the resistant strain to micafungin CO23RFK, a new cell wall protein of the yeast, identified as LDG7 was more express than in sensitive strain CO23. This protein belonging to a family of related proteins whose function is still unknown, it is also identified as PGA29, putative GPI-anchored protein that localization to the cell wall, its transcription is decreased upon yeast-hyphal switch, transcriptionally regulated by iron and its expression greater in high iron. To confirm that the amount of LDG7 protein was due to micafungin, we havebeen performed experiments in presence of sublethal doses of drug in strains CO23s, CO23RFK, in all cases a little increase of LDG7 were observed in all strains treated with micafungin but in strain CO23RFK is very evident. To confirm the different expression of this protein in sensitive and resistant strains of *C. albicans* we have produced a recombinant protein LDG7. This protein was expressed in a plasmidic vector (pQE30 Qiagen) in *E. coli* for obtained a fusion product containing tail of 6 histidines and purified by affinity chromatography. Antibodies against the purified LDG7 recombinant protein were obtained by immunization of 3 female BALB/c mice. Western blot analysis confirmed the increase of LDG7 in CO23RFK in particular after antimycotic treatment. Moreover, immunogold electron microscopy observations on cryoultrathin sections have been carried out on CO23s and CO23RFK strains, after drug treatment, in order to study the localization of LDG7 epitopes. Further study to value a possible relationship between LDG7 protein and resistance to micafungin will be performed.

MOLECULAR MECHANISMS DRIVING REDUCED ECHINOCANDINS SUSCEPTIBILITY IN *C. PARAPSILOSIS SENSU STRICTO*

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Aims of the study. Although *Candida albicans* remains the most prevalent species, the number of invasive fungal infections (IFIs) caused by non-*albicans Candida* species is increasing worldwide, with *Candida parapsilosis* the most common causative agent in Europe and Latin America. *C.parapsilosis* is responsible for a wide variety of clinical manifestations which are more common in patients admitted to neonatal or surgical intensive care units (ICUs). Since the introduction of the echinocandin drugs in 2001, these antifungals are becoming the preferred choice for invasive candidiasis treatment. Echinocandins target the Fksp subunits of the 1,3-D glucan synthase complex, inhibiting fungal cell wall biosynthesis. *C.parapsilosis* complex showed an in vitro susceptibility reduction, whose underlying mechanism is still poorly understood.
Methods. 136 *C. parapsilosis* isolates were banked in a two-year surveillance Italian study. Echinocandins (anidulafungin, caspofungin and micafungin) susceptibility was assessed by CLSI method. Biofilm producers were screened by both crystal violet (CV) staining and XTT reduction assay. Susceptibility testing assays on preformed biofilm were performed in order to establish the MIC_{50} values for sessile yeast cells. Ten isolates showing high echinocandin MICs (2÷ 4 μg/ml) and ten having MIC ≤2 μg/ml were further studied for FKS1 gene point mutations by means of PCR amplification and sequencing.
Results and Conclusions. The overall frequency of *C. parapsilosis* complex in IFIs was 22%. MIC_{50} values for anidulafungin, caspofungin, and micafungin for *C. parapsilosis* complex were 2, 1 and 2 μg/ml, respectively, whereas MIC_{90} values were 4, 2 and 4 μg/ml. All isolates of the less common species *C. orthopsilosis* and *C. metapsilosis*, accounting for 5% of all isolates, were susceptible to echinocandins. 38% of tested isolates were able to produce biofilm; 61% of those strains were very strong biofilm producers. The MIC_{50} of sessile cells were 16, 4, 8, whereas the MIC_{90} were >16, >16, 16. None of the tested isolates for FKS1 gene mutation showed further amino acids substitutions in the two hot spot regions. Four out of the isolates with a reduced susceptibility were strong biofilm producers, two of them harbored an amino acid substitution downstream the Fksp hot spot 1. On the other hand, half of the most susceptible isolates showed a substitution downstream the Fksp hot spot 2. Biofilm production, when present, surely has a starring role in drug resistance, however we observed high MIC also in non producers isolates. A naturally occurring amino acid substitution in a Fks1 conserved region of the *C.parapsilosis* complex has been reported and linked to reduced echinocandin susceptibility. We could speculate that other amino acids substitution could account for higher MIC of some isolates.

GENE EXPRESSION PROFILE ANALYSIS IN *CRYPTOCOCCUS NEOFORMANS* EXPOSED TO FLUCONAZOLE BY MICROARRAYS

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The basidiomycete *Cryptococcus neoformans* is the aetiological agent of the cryptococcal meningitis, the most common fungal infection of the central nervous system and the third most frequent neurological complication in AIDS patients. To date, fluconazole is the drug of choice for long-term suppressive therapy. During growth in the human host, the fungus survives the diverse stressors it encounters, that include oxidative and nitrosative stress, high temperature, hypoxia, nutrient deprivation, and also antimicrobial molecules.

Therefore, we decided to investigate the global changes in *C. neoformans* gene expression induced by the fluconazole exposure by microarray analysis, in order to elucidate the molecular mechanisms that underpin the response to drug. To do this, Agilent technology was used to design and produce microarrays, using the available genome sequence from the *C. neoformans* reference strain H99. Cryptococcal cells were or not (controls) treated with 10 μg/ml of fluconazole at 30°C for 90 min, and then used to obtain the RNA template. Probe labeling were performed with a one-color system according to the manufacturer's instructions. Quantitative real time reverse transcriptase-PCR was used to confirm the microarray data.

Among a total of 6823 genes monitored by the gene expression analysis, 231 genes displayed transcription levels that were increased of at least 2-fold, while the other 245 genes had transcription levels that were decreased similarly. Globally, azole exposure led to upregulation of the genes involved in the ergosterol pathway, such as the *ERG* genes (*ERG3*, *ERG13*, *ERG2*, *ERG7*, *ERG5*, *ERG11*, *ERG25* and *ERG1*) and the *SRE1* gene, a well-known regulator of sterol homeostasis in *C. neoformans.* Furthermore, several genes related to other cellular processes, as cell wall maintenance, transport, carbohydrate metabolism, and stress were shown to be affected by the fluconazole exposure. As some of these drug-responsive genes may represent potential therapeutic targets against cryptococcal disease, these results confirm the great importance of the microarray technology in the field of antifungal drug discovery.

ANTIFUNGAL PEPTIDES WITHIN THE ANTIBODIES

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CDRs-related peptides and many decapeptides spanning the variable region of a recombinant yeast killer toxin-like antiidiotypic Ab exerted fungicidal activity *in vitro* against *Candida albicans*. An alanine-substitute of a candidacidal decapeptide displayed increased therapeutic effect *in vivo* against vaginal and systemic candidiasis (Polonelli L Infect. & Immun. 2003). CDR-based synthetic peptides of murine and human monoclonal Abs directed to irrelevant epitopes, showed differential *in vitro* and/or *in vivo* activity against *C. albicans*. Molecular targets to candidacidal CDRs were supposed to be β-glucans, that neutralize their activity, and N-terminal sequence in ALS3 family. Alanine substitutes of synthetic candidacidal CDRs, used as surrogates of natural point mutations, showed differential anticandidal activities (Polonelli L. et al. PLoS ONE, 2008). Some candidacidal CDR-related peptides showed to be characterized in solution by self-aggregation-releasing property, catalyzed by β 1,3-glucan. While the self-assembled state provides protection against proteases and the slow kinetic of dissociation assures a release of the active form over time, the receptor affinity is responsible for targeted delivery (Pertinhez T.A. et al. Mol. Pharmaceut. 2009). A synthetic peptide with sequence identical to V_HCDR3 of a mouse mAb specific for difucosyl human blood group A showed to be taken up by macrophages with stimulation of:
1. proinflammatory cytokine production;
2. PI3K-Akt pathway and
3. TLR-4 expression. V_HCDR3 exerted therapeutic effect against systemic candidiasis without possessing candidacidal properties (Gabrielli E. et al. PLoS ONE, 2009). Selected synthetic peptides possessing sequence homology with fragments deriving from the proteolytic cleavage of the constant region of all classes of Abs, proved to display differential *in vitro* and/or candidacidal and/or immunomodulatory activities. It is hypothesized that, beyond the half life of Abs, some fragments may influence the anticandidal immune response in a way reminiscent of molecules of innate immunity. Inmost candidacidal peptides suggests that Abs, irrespective of the specificity for a given antigen, may be unlimited sources of sequences active against *Candida* and candidiasis. The easy production and low cost of small sized Ab synthetic peptides and the possibility of engineering peptidomimetics, associated to new delivery mechanisms, are expected to give rise to a new generation of antifungal agents.

INTERACTION BETWEEN CLINICIAN AND MICROBIOLOGIST IN THE DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS: SYNERGISTIC OR ANTAGONIST?

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The increase in the immunocompromised population and the incidence of invasive fungal infections (IFIs) are both a challenging issue for the clinician (not only infectious diseases physician) and the microbiologist. The identification of fungi in the medical laboratory requires more expertise now than in the past because laboratory technologists are asked to identify a larger group of fungi. Moreover the distinction between well-known fungal pathogens and "contaminants" is no longer clear since species once considered harmless may now produce life-threatening infections. On the other hand the clinician is facing with different IFIs in the different population of immunocompromised hosts. In this regard the term "immunocompromised host" is misleading since it is used to identify patients receiving hematopoietic stem cells or solid-organ transplants, as well as persons living with HIV/AIDS infection or those admitted in the intensive care units all of whom present differences in the risk factors, clinical presentation, diagnostic and therapeutic approach of the IFIs. Moreover the antifungal drugs armamentarium has been expanded in recent years with the availability of multiple class of drugs that differ in their spectrum of activity, adverse events, drug interactions and costs.

The first problem that need to be addressed is about the training in medical mycology for either the clinician and the microbiologist. However the provision of infection services and training curriculum is not uniform within the European Union countries with wide variation in the roles of infectious disease physicians and microbiologists. Furthermore the ratio of specialists in infectious diseases and microbiology varies across Europe with, for instance a ratio of about 1:4 in the UK (more microbiologists) and 3:1 in Sweden (more infectious disease specialists). In the USA, the American Society of Transplantation Infectious Diseases has recently advocated a new subspecialty of transplant infectious diseases with a distinct curriculum. At the same time in the USA the number of students enrolled in clinical laboratory science programs has decreased from approximately 6,500 in 1980 to 2,500 in 1997. In a survey conducted by the APHL to determine laboratory practices and staff training in mycology only 55,6% of laboratories reported that at least 1 employee attended a formal mycology continuing education program in the 4 years before the survey.

Finally, at the hospital level, it is essential that both services identify at least 1 microbiologist that should investigate the mycological need in term of implementation of modern test technology and 1 infectious diseases specialist familiar with clinical syndromes and causative fungi.

Both should collaborate initially to provide a manual describing the local service and treatment guidelines and to develop continuous interchange of information to realize an effective joint policy in daily routine work.

THE Y238X POLYMORPHISM IN DECTIN-1 IS ASSOCIATED WITH RISK FOR ASPERGILLOSIS IN STEM CELL TRANSPLANTATION

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Immune suppression has been traditionally regarded as a key risk factor for fungal infections in the stem cell transplantation (SCT) setting. However, not all individuals at risk ultimately develop disease, suggesting that additional, likely genetic, factors may also play a role in susceptibility to fungal infections. In this regard, the discovery of genetic variants in TLR genes has highlighted a potential link between genetic variation of the immune system and susceptibility to infections.
Recently, the Y238X polymorphism in the C-type lectin receptor Dectin-1, a receptor recognizing β(1,3)-glucan present in the fungal cell wall, has been demonstrated to lead to diminished receptor activity. We found that the presence of the Y238X polymorphism in either donors or recipients of SCT increased susceptibility to aspergillosis with the risk being highest when it was present simultaneously in both donors and recipients. Functionally, the Y238X polymorphism impaired the production of IFN-γ and IL-10, in addition to IL-1β, IL-6 and IL-17A, by human peripheral mononuclear cells and Dectin-1 on human epithelial cells contributed to fungal recognition.
Mechanistically, studies on preclinical models of infection in intact or bone marrow-transplanted Dectin-1 KO mice revealed that protection from infection requires a distinct, yet complementary, role of both donor and recipient Dectin-1. Overall, our findings disclose Dectin-1 deficiency as a novel susceptibility factor for aspergillosis in high-risk patients and identifies a previously unsuspected role for Dectin-1 in antifungal immunity that is the ability to control both resistance and tolerance to the fungus contingent upon hematopoietic/nonhematopoietic compartmentalization.

NEW STRATEGIES IN THE DIAGNOSIS OF INVASIVE CANDIDIASIS IN NEONATAL INTENSIVE CARE

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Preterm neonates in neonatal intensive care units (NICU) are at high risk of invasive fungal infection (IFI), mostly by *Candida* spp.. In such patients IFI is increasingly leading to high morbidity and mortality and frequent neurodevelopmental disabilities in the survivors. Neonatal IFI is difficult to diagnose, as candidaemia may be transient and difficult to eradicate due to the high rates of end-organ dissemination. Diagnosis is still based mostly on blood cultures; however, blood cultures are thought to be positive for *Candida* in only 24-60%. In septic neonates, phlebotomy may be difficult and the small volume of blood obtained for culture may limit sensitivity. Blood culture detection of candidemia may take 48 hours or more, and some cases of systemic candidiasis are not diagnosed until autopsy. In view of this limitation, other techniques to facilitate a diagnosis of invasive fungal infection have been investigated. Particularly, the use of lysis centrifugation system improves the diagnosis of fungemia especially for the isolation of fungi such as *Malassezia* sp.. Also the Platelia *Candida* antigen kit has been used for early diagnosis of probable candidosis. Detection of *Candida* DNA by polymerase chain reaction (PCR) may provide a rapid and reliable screen for candidemia in high-risk patients.
The aim of this study was to introduce new strategies in the diagnosis of invasive candidiasis in neonatal intensive care. Weekly surveillance cultures from all neonates admitted over a 6-year period were reviewed. For all neonates at risk admitted to our NICU in the period 2005-2007 Platelia *Candida* antigen kit has been used for early diagnosis of invasive candidosis. From 2008 to 2010 we introduced the blood culture with lysis centrifugation system with fungal DNA detection in the same sample. Were included a total of 1541 patients. The overall colonization rate was 15.3% (236/1541). Two hundred twenty-four patients were included in this study, based on the availability of at least three mannan antigen tests during their stay in the NICU. The sensitivity and specificity of the Platelia *Candida* antigen test for all cases of proven and probable candidosis (44 patients) were 88.6% and 93.3%, respectively. The positive predictive value and negative predictive value were 76.4% and 97.1%, respectively. Proven infection, defined in the present study as clinical signs of sepsis and positive blood culture, was observed in 18 of 224 patients, a frequency of 8.0%. Particularly, the frequency in the period 2005-2007 was 7.2% and 9.3% in 2008-2010. Fungal DNA detection performed in 2008-2010 was positive in 22.1%.
The use of serial examinations such as the colonization, the Platelia *Candida* antigen kit and blood culture with lysis centrifugation system with fungal DNA detection in the same sample may represent a new strategy to improve the sensibility and early diagnosis.

MULTIPLEX PCR AND HRMA (HIGH RESOLUTION MELTING ANALYSIS) FOR THE IDENTIFICATION IN BIOLOGICAL SAMPLES OF DIFFERENT *CANDIDA* SPECIES BY USING PRIMERS OF A 65 KDA MANNOPROTEIN

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Invasive fungal infections represent a major public health concern with a significant trend in the emergence of species other than *C. albicans*, intrinsically resistant to common antifungal agents as *C. glabrata* and *C. krusei* and, to a lesser extent, *C. parapsilosis* and *C. tropicalis.* Thus, there is an increasing interest in the development of new technological tools for a rapid diagnosis of invasive candidiasis. In this work, various isolates of *Candida* species and other yeasts were assayed by PCR for the presence of the MP65 gene sequence (a biologically significant 65 kDa β-glucanase adhesin of *C. albicans*). Purified fungal genomic DNAs were used as templates in these experiments and an amplification product of the expected length (476 bp) was observed in all *Candida* isolates, while bacterial, murine, human and protozoan DNAs did not displayed any, and *S. cerevisiae* gave an amplification product similar in size to that of *Candida sp.,* however easily distinguishable by restriction analysis using NcoI enzyme. Besides, we describe the development and evaluation of single-tube multiplex PCR methods for the detection of the five most common *Candida* species causing invasive candidiasis, by the use of different primer pairs selected from MP65 gene sequence: *C. albicans, C. glabrata, C. guilliermondii, C. tropicalis* and *C. parapsilosis.* Six different primer pairs were used in the same PCR reaction with the objective of producing different specific amplicons dependent on the target present in the sample. We developed two types of PCR: in the former, we added a mix of five different *Candida* species DNAs and a single species-specific primer pair, while in the latter, we added a mix of 5 primer pairs and a single *Candida* species DNA, in each reaction tube. Both types of PCR assays detected the five *Candida* sp. All the PCR assays were also performed with DNAs extracted from biological samples (urine and serum).
The total time required for each PCR method was less than 4 h from the extraction to the visualized amplicons after PCR. We then examined the feasibility of a high resolution melting analysis (HRMA), a recent development in real-time PCR technology, for rapid and easy detection of five clinically relevant *Candida* sp. (*C. albicans*, *C. glabrata, C. kefyr*, *C. parapsilosis* and *C. guilliermondii*). HRMA is a closed-tube assay that detects sequence variations within specific genetic loci via melting curve analysis of the amplicons with a saturating dye of double-stranded DNA (LCGreen). The potential resolution of this new approach is much greater than the conventional one because melting curves from different amplicons can be differentiated on the basis of shape, even when they define the same T_m values, and turned out to be sensitive, reproducible and cheap. The major advantage of this method is that the risk of contamination is far lower than in a multi-step procedure, such as RFLP or nested PCR, because the entire process is a single-step closed-tube procedure.

CONTRIBUTION OF THE (1-3) - BETA-D-GLUCAN ASSAY FOR THE DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS

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Diagnosis of invasive fungal infections (IFI) in immunocompromised patients remains a challenge. Unfortunately, delayed diagnosis and therapy for IFI are associated with poor outcomes and high mortality regardless of the therapeutic modalities used, ranging a mortality rate of 50% for candidemia and 80% for invasive aspergillosis. Conventional mycological techniques, including histological and cultural methods that have been used for the diagnosis of IFI are relatively insensitive and time-consuming. Recently, particular emphasis was placed on the detection of fungal cell wall antigens (mannans, galactomannans and glucans) as non-invasive markers for the presumptive diagnosis of IFI.
Several fungal detection kit have been commercialised in the past few years. Two enzyme-linked immunosorbent assay target fungal cell wall antigens: Platelia Aspergillus detects Aspergillus galactomannan antigen and Platelia Candida detects mannan antigens. A chromogenic kinetic test, Fungitell, targets (1→3)-β-D-glucans, which are major cell wall components of various medically important fungi. Galactomannan detection sensitivity in invasive aspergillosis have been reported to be as low as 30% although other studies have reported sensitivities above 90%. (1→3)-β-D-glucans detection assay has the advantage to evidence fungal invasive infections caused by different pathogenic fungi as yeasts and different moulds. We reported the prospective study conducted in our hospital, Ospedale Policlinico of Verona, evaluating the performance of this assay in the diagnosis of IFI, in particular of aspergillosis, mould infections and candidemia.

MOLECULAR DIAGNOSIS OF DERMATOPHYTE INFECTIONS IN DOGS AND CATS BY NESTED POLYMERASE CHAIN REACTION

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Dermatophytes are fungi that can be contagious and cause skin infections of mammals, including humans (Weitzman I, Summerbell RC, 1995, Clin Microbiol Rev, 8: 240-259). Conventional methods like potassium hydroxide (KOH) microscopy and fungal culture lack the ability to make an early and specific diagnosis (Weitzman I, Summerbell RC, 1995, Clin Microbiol Rev, 8: 240-259). In this study a Polymerase Chain Reaction (PCR) using specific primers targeting dermatophyte sequence of entire internal transcribed spacer region (ITS+) of nuclear ribosomal DNA and the chitin synthase 1 (*chs*1) gene (Cafarchia C et al., 2009, Electrophoresis, 30: 3555-3564) has been developed for rapid detection of dermatophyte species directly from skin scrapings of dogs and cats. This method has been followed by a nested PCR targeting ITS1 region to increase the sensitivity.

Methods. A total of 188 animals (i.e., 101 cats and 87 dogs) with and without skin lesions were enrolled in the study. Hair samples were collected (Cafarchia C et al., 2004, Mycoses, 47: 508-513) and divided into three parts. The first part was examined microscopically and the second one was cultured on Sabouraud's dextrose agar with chloramphenicol (0.05 mg/ml) and cycloheximide (0.5 mg/ml) (Liofilchem Diagnostici, Teramo, Italy) and incubated at 25°C for 4 weeks. Colonies grown in the medium were identified at species level as previously described (de Hoog GS, Guarro J, 1995, Atlas of Clinical Fungi, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands). DNA extraction was conducted on the third part (i.e., from 5 to 20 mg) of hair samples using specific protocol procedures (Genomic DNA Purification Kit, Gentra Systems, MN, USA). From each genomic DNA sample, ITS+ of nuclear ribosomal DNA (900-950 bp) and a portion of the *chs*-1 gene (~440 bp) were amplified as previously reported (Cafarchia C et al, 2009, Electrophoresis, 30: 3555-3564). Nested PCR was performed by designing a novel set of primers amplifying a DNA fragment of about 400 bp from the internal sequence of the ITS+ amplicons obtained from the first-round PCR.

Results. Fungal cultures were positive in 21.8% samples from dogs and 39.6% from cats. First-round PCR were positive in 24.1% samples from dogs and 27.7% from cats. Nested PCR were positive in 26.4% samples from dogs and 39.6% from cats. The Nested PCR examination showed a very good specificity (around 95%) and a high sensitivity (100% in dogs and 92% in cats).

Conclusions. The direct DNA isolation from clinical samples and PCR methods employed in this study provide rapid, reproducible and sensitive tools for the detection of dermatophytes from dogs and cats to initiate a prompt and appropriate antifungal therapy.

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ADVANCES IN THE UNDERSTANDING OF THE PATHOGENESIS OF OPPORTUNISTIC FUNGAL DISEASES: CANDIDOSIS

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Candidosis is an opportunistic fungal disease caused by the human commensal yeast, Candida. It is manifested in a diversity of clinical forms, ranging from muco-cutaneous infection to life threatening systemic disease, which affects primarily immunocompromised and debilitated patients. This presentation will overview the state of art of the pathogenesis of candidosis. It is aimed to highlight the current view and general understanding of how candidosis evolves, who is particularly at risk to develop the infection and what is the response to it.
The presentation will focus on the present knowledge in the areas of:

1. specific virulence factors of the fungus, such as biofilm formation, tissue invasion, role of yeast hyphal transition and site specific adaptation;
2. novel models for studying pathogenesis: the Drosophila system - what can we learn from it;
3. epidemiologic, demographic changes in *Candida species* distribution: - *Candida albicans vs non albicans species*; are there explanations for the changes?
4. intra-species genotyping and possible correlations with the various clinical entities: are there differences in the virulence of strains and could it be possible to detect such differences and identify the specific strains with defined virulence attributes?
5. interaction between the fungus and the host: events in the microbe and in the host following interaction - transcriptional changes in the microbe and effects in the host, such as cytoskeletal rearrangements affecting cellular functions and host immune response;
6. who is prone to what type of infection;
7. the host response.

In summary, since it is believed that understanding the pathogenesis of an infection is a basis for a rational approach for diagnosis and therapy, reviewing the advances in the understanding of the pathogenesis of candidosis may contribute to the management of this complex clinical entity.

CRYPTOCOCCOSIS IN SOLID ORGAN TRANSPLANT RECIPIENTS: FROM PATHOGENESIS TO THERAPY

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Cryptococcosis remains a significant opportunistic infection in solid organ transplant recipients. Disease presentation and outcomes in the current era may be affected amongst other factors by the use of calcineurin-inhibitor immunosuppressive agents. It is being increasingly recognized that rapid reversal of immunosuppression in transplant recipients treated for cryptococcosis incurs the risk of immune reconstitution inflammatory syndrome that mimics worsening disease or relapse. This lecture summarizes the current state of knowledge regarding cryptococcosis in transplant recipients and highlights areas where future investigations are needed to further optimize outcomes in these patients.

IMMUNOLOGY OF *ASPERGILLUS* AND ASPERGILLOSIS

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Aspergillus spp. are ubiquitous in nature and the spectrum of disease they cause is myriad, ranging from saprophytic colonization of pre-existing cavities (aspergilloma), allergic asthma, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis (ABPA) occurring as a complication of bronchial asthma or cystic fibrosis and disseminated disease associated with high mortality rate in patients with hematological malignancies, recipients of solid organs and stem-cell transplants and patients admitted to the intensive care unit. While many viral and most bacterial infections can be managed successfully, the relative importance of fungal infections, especially invasive aspergillosis, has even increased. Despite the past decade has witnessed significant progress in the management of invasive aspergillosis, the infection continues to be a deadly disease. The main reasons for this include intrinsic or acquired antifungal resistance, organ dysfunction preventing the use of some agents and the deleterious effect of a deregulated inflammation. The past several years have seen remarkable advances in understanding the basic cellular and immunological mechanisms underlying resistance to the fungus but also organ dysfunction and failure to recovery relating to invasive aspergillosis. Current understanding of the pathophysiology underlying *Aspergillus* infection and disease highlights the multiple cell populations and cell-signaling pathways involved in this complex condition, including the novel findings on the molecular connection between the failure to resolve inflammation, lack of antifungal immune resistance and susceptibility to *Aspergillus* infections and diseases. The intricate cross-talk provided by temporal changes in mediators, metabolites, and cell phenotypes underlines the coordinated processes beyond the dysregulated chaos in which fungal infection and disease is perceived. Applying systems approaches to these complex processes will permit better appreciation of the effectiveness or harm of treatments, and also will allow development not only of better directed, but also of more appropriately timed, strategies to improve outcomes from this still highly lethal infection.

ADVANCES IN THE UNDERSTANDING OF THE PATHOGENESIS OF OPPORTINISTIC FUNGAL DISEASES: ZYGOMYCOSIS

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Zygomycoses are severe angioinvasive infections caused by common filamentous fungi, the zygomycetes.In the last decades there has been a dramatic improvement in the diagnosis of zygomycosis and it is seen with an increasing incidence, especially in patients with various predisposing factors. These ubiquitous opportunistic fungi can cause infections with high lethality in immunocompromised or diabetic patients. Whatever the route of infection (inhalation of airborne spores, ingestion, or direct skin inoculation), the hyphae invade blood vessels, causing tissue infarction and necrosis.

In healthy persons, innate immunity is sufficient to prevent infection, except in cases of massive contamination after traumatic inoculation of contaminated soil. Patients with phagocytic dysfunctions caused by neutropenia or ketoacidosis, as well as patients with high iron serum concentrations, are at high risk of developing zygomycosis These underlying conditions can influence clinical presentation and outcome. The diagnosis of zygomycosis is notoriously difficult. Clinical presentation may suggest zygomycosis, but cultures and histopathology are required for confirmation. New molecular approaches are currently investigated. The identification to the species level of a strain isolated in culture and the identification of a zygomycete in tissues by PCR make the diagnosis much easier.

WHAT HAVE WE LEARNED ABOUT SYSTEMIC ANTIFUNGALS CURRENTLY AVAILABLE ON THE MARKET?

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The armamentarium to successfully treat invasive fungal infections has become broader by the approval of broad-spectrum triazoles, the new antifungal class of echinocandins and lipid formulations of amphotericin B. Well-designed, prospective, randomized clinical trials have elucidated the clinical use of these compounds, reflected in numerous national and international evidence-based guidelines.
For first-line treatment of invasive aspergillosis, voriconazole and liposomal amphotericin B are the drugs of choice, while echinocandins have not been demonstrated to be equally effective. Before antifungal treatment is switched to a second-line ("salvage") regimen, a number of reasons for "pseudo-failure" such as secondary infections, insufficient dosage or treatment duration or immune reconstitution syndrome should be considered. If zygomycosis has been proven or is a serious differential diagnosis, liposomal amphotericin B will be preferred. Invasive candidiasis should be treated according to species and in-vitro susceptibility, but one of the echinocandins should be used in serious cases before the species have been identified. For cryptococcosis, liposomal amphotericin B can now replace conventional amphotericin in order to prevent unnecessary toxicity. The main place for Posaconazole today is prevention of mould infection in severely immunocompromised patients.

THE PHARMACOLOGIST' S REMARKS

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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). 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, 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.
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.
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 3; while azole derivatives are concentration-independent or time-dependent drugs and the probability of clinical success is significantly higher when the AUC(free drug)/MIC is almost 25. The predominant pharmacokinetic differences among the three echinocandins are volume of distribution, metabolism and half-life. Similarly to polyenes a clear pharmacodynamic relationships has been established for this agents and the population pharmacokinetic data available demonstrates relatively predictable dose-exposure relationships. Therefore, for echinocandins there is no need for therapeutic drug monitoring.
The comparative clinical pharmacokinetic data of triazoles indicate several compound-related differences in absorption, metabolism, tissue distribution and elimination. All triazoles inhibit different cytochrome P450 dependent pathways with the consequent potential drug interactions with drugs that are metabolized by the same enzymes. As a consequence, therapeutic drug monitoring of triazoles (with the partial exclusion of fluconazole) is recommended to ensure efficacy and to avoid or limit toxicity.
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.

ANTIFUNGAL DRUG RESISTANCE: THE IMPACT IN CLINICAL MANAGEMENT

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Failure to respond to antifungal therapy could be due to in vitro resistance (intrinsic or developed during therapy) or clinical resistance; the latter is associated with numerous factors related to the host, the antifungal agent, or the infecting isolate. Antifungal susceptibility testing has been in routine use now for more than 20 years and has become a useful tool for clinicians who are faced with difficult treatment decisions. Although most clinicians order susceptibility testing, much confusion still exists regarding the use of the results.
Sufficient data have been generated to determine susceptibility trends for specific fungi against specific agents, but correlation data are minimal. Azole breakpoints, and the echinocandin susceptible breakpoint, are useful when isolates are tested by CLSI standardized methods; breakpoints are also available by the EUCAST method. Recently, in vitro resistant MIC breakpoints have been assigned for filamentous fungi (moulds) vs. five antifungal agents, but these categories are not based on correlations of in vitro with in vivo response to therapy. Despite the lack of correlation data, antifungal susceptibility testing continues to provide useful information to assist with patient care.

ANTIFUNGAL DRUG RESISTANCE: TOWARDS THE MOST RELIABLE DETECTION METHOD

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Candida and *Aspergillus* species are the most common causes of invasive fungal infections in immunocompromised patients.
The introduction of new antifungal agents and recent reports of resistance emerging during treatment have highlighted the need for in vitro susceptibility testing. For some drugs, there is a supporting in vitro - in vivo correlation available from studies of clinical efficacy. Both intrinsic and emergent antifungal drug resistance are encountered. Various testing procedures have been proposed, including macrodilution and microdilution, agar diffusion, disk diffusion, Etest and commercial test assays.
Each method has its own advantages and disadvantages. E test is easy to perform, useful for the daily routine yet expensive. Disk diffusion is the most attractive alternative method so far, yet we lack sufficient data for aspergilla and other molds. EUCAST and CLSI are reproducible reference testing methods, yet time-consuming. However, early recognition of infections due to pathogens which are resistant to one or more antifungals is highly warranted to optimise treatment and patient outcome.

CAN DIAGNOSTICS IMPROVE THERAPY?

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Human pathogenic fungi include yeasts, moulds, dimorphic fungi and dermatophytes. Dermatophytes cause superficial infections, whereas yeasts, moulds and dimorphic fungi may cause life threatening invasive infections. Outcome is closely related to a triad of factors:

1) virulence and susceptibility of the pathogen;
2) the underlying disease and immunity of the host and
3) the timing choice and dosing of antifungal treatment. In example, mortality increased from 10-15% to 30-40% when antifungal treatment was delayed from day 1 to 3 in two studies of candidaemia.

Thus rapid, sensitive and specific diagnostics are of outmost importance. Microbiological diagnostic approaches include microscopy and culture, histopathology, antigen and antibody detection and molecular diagnostics (PCR). Subsequent tools include various species identification tools including PNA FISH, chromogenic agars, latex agglutination, RTT test, carbon assimilation profiles, sequencing, MALDI-TOF mass spectrometry and susceptibility testing. And finally, therapy may be optimised through therapeutic drug monitoring. For optimal sensitivity of culture and microscopy, the use of fluorescent brighteners and the selective media is important.

This was shown in a recent external quality assessment scheme in the Nordic countries.

A range of new options facilitate rapid species identification as mentioned above – examples of these options will be given during the lecture. Finally, acquired resistance in *Candida* and *Aspergillus* is reported with increasing frequencies and thus reliable susceptibility testing is important to select appropriate therapy.

A range of serologic tests have been marketed over the recent decade. Most important and well studied is the *Aspergillus* galactomannan antigen test which is a rapid and sensitive screening test for invasive aspergillosis in particularly the haematological setting, but the test has also been proven successful in the ICU setting, especially if performed on BAL or homogenised tissue specimens. *Candida* mannan and anti-mannan antibody testing is less widely used although several studies have documented good performance.

Beta-glucan detection as a marker of fungal infection appears promising although price and risk of contamination are issues that so far limit broad routine use. Finally, serology is a cornerstone in the diagnosis of dimorphic fungi. PCR can be used as either diagnostic tests or as a tool for species identification or resistance mechanism detection upon culture.

A range of in house PCRs have been published, several of which with excellent performance compared to culture, particularly regarding time to diagnosis. However, still standardisation of these tests remains an issue. Recently, a few commercial diagnostic PCR tests have been marketed. The performance of these tests in clini-

cal routine settings is awaited with great interest. For several antifungal compounds bioavailability may not be optimal due to either absorption or metabolism variability. These include itraconazole, posaconazole and voriconazole as well as flucytosine. Substantial evidence now exist that sub-therapeutical levels may be associated with failure and the opposite with toxicity, and thus TDM is highly helpful to guide optimal dosing.

In conclusion, the diagnostic options have increased considerably over the recent years and optimal use and interpretation of the results may help the clinician to optimise treatment with respect to timing, choice and dosing of antifungal treatment.

HOW CAN WE PREVENT INVASIVE FUNGAL DISEASE?

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We know that the mortality of proven or probable invasive aspergillosis (IA) in haematopoietic stem cell transplant (HSCT) recipients can be in excess of 80% and so it seems self evident that prevention should be a better strategy than therapy in these patients. The classical approach to infection prevention has been to apply infection control practices/environmental control to exogenous infections, such as IA, and antimicrobial prophylactic agents to endogenous infections such as those with *Candida albicans.*

However, there is overlap with both moulds and yeasts in this respect - *Aspergillus* species may be colonising the respiratory tract on admission for HSCT, for example, and yeast species, particularly *Candida parapsilosis*, may be transmitted by healthcare workers to a patient during a similar event. Consequently, preventative measures for fungal infections would seem to need both approaches. But what is the evidence that they work and how can we deal with the downside of the use of antifungal prophylaxis? This presentation will review recent antifungal prophylaxis studies and guidelines in order to draw conclusions about the patient groups and approaches that are appropriate.

STRATEGIES IN ANTIFUNGAL THERAPY: THE NEUTROPENIC PATIENT

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Morbidity and mortality related to invasive fungal infections remain unacceptably high in cancer patients. In the past, the chance of obtaining a diagnosis was remarkably poor and many patients were treated empirically. Empirical therapy consists in administering an antifungal drug in a persistently febrile and neutropenic cancer patient after a variable period of empirical antibacterial therapy, in absence of any clinical, microbiological or radiological documentation of a fungal infection. The rationale for this practice is based on 2 small, randomized studies, which enrolled less than 200 patients all together. These studies were not double blind and placebo-controlled and actually did not conclude for an unequivocal advantage for the practice under study. In both studies the statistical power of the observed results was very small, especially for subgroup analyses.

Nevertheless, empirical antifungal therapy in persistently febrile and granulocytopenic cancer patients without documented infections has become common practice in many cancer centers worldwide and many drugs have been tested for this indication. In recent years, the availability of new diagnostic tools are making the practice of empirical therapy obsolete and have open to door to a more thoughtful and rationale approach called "pre-emptive therapy". Briefly, pre-emptive therapy is aimed at treating a fungal disease as early as possible, based on the detection of early radiological signs at high resolution CT scan and on the possibility of detecting fungal cell wall components in patient's body fluids.

Whether or not just a pulmonary infiltrate is enough or typical radiological signs are required to start an antifungal is still a matter of debate. Similarly, some centers start an antifungal when both radiological signs and laboratory confirmations are available, while others prefer to be more conservative and start at positive galactomannan or detection of an infiltrate, whichever come first. Despite these differences in interpretation, some sort of pre-emptive therapy is becoming the rule, at least in European centers and at least in those centers in which these diagnostic procedures are available and well organized.

This approach reduces the number of patients unnecessarily exposed to an antifungal drug. There is no consensus on the criteria required and defining the pre-emptive therapy approach and only one randomized clinical trial has compared the 2 strategies, with results somewhat elusive and controversial. For this reason, at the present time, no firm recommendation can be made and the choice is left to every center's experience. Future studies and consensus conferences are required to define criteria for pre-emptive therapy and to test the cost-effectiveness of the 2 strategies.

STRATEGIES IN ANTIFUNGAL THERAPY: THE ICU PATIENT

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Invasive fungal infections (IFI) are frequent life-threatening nosocomial complications. Traditionally observed in neutropenic hematological patients, IFI have been increasingly reported over the last two decades in non-neutropenic hosts, including critically ill, surgical or chronically debilitated patients, in
solid organ transplant recipients and in patients with chronic cellular immunosuppression, e.g. due to
long-term corticosteroid therapy.
Candida is the most frequent fungal pathogen: it accounts for 80-90% of all IFI and belongs to the top ten causes of bloodstream infections. While in North America a peak incidence in the early 1990s has been followed by a progressive decline, an increasing incidence has been recently reported in some European countries. 30-50% of all invasive candidiasis occur in ICU patients: candidemia, intravascular catheter infection, peritonitis and urinary tract infections are the most frequent clinical presentations. Candidiasis classically occurs after a prolonged length of ICU stay: *Candida* colonization at multiple body sites associated with multiple antibacterial therapies, complicated abdominal surgery and other invasive procedures (intravascular catheters, parenteral nutrition, renal replacement therapy) are the most important risk factors. The emergence of azole-resistant *Candida* species, such as *C. glabrata* and *C. krusei*, has been associated with the use of azole prophylaxis in hematological patients. In contrast, in ICU patients, *C. albicans* remains the most frequent species, accounting for 70-80% of all isolates. Invasive candidiasis is associated with an increased morbidity with prolonged hospital stay, substantial health care costs, and a crude and attributable mortality ranging 20-70% and 10-40%, respectively.
Severe sepsis/septic shock/multiple organ failure and dissemination of infection are independent prognostic factors of fatal outcome in patients with candidiasis. Rapid initiation of appropriate antifungal therapy is thus essential for the control of systemic *Candida* infections and has been shown to reduce mortality. In surgical or ICU patients, fluconazole prophylaxis was found to prevent intra-abdominal candidiasis in high-risk surgical patients with recurrent gastro-intestinal perforations or anastomotic leaks and to prevent candidiasis in patients expected to stay in the ICU for more than 3 days or to be ventilated for more than 5 days. In the future early targeted preemptive antifungal therapy based on clinical *Candida* scores, colonization index and new diagnostic fungal blood markers might contribute to reduce the number of patients treated with antifungals by maintaining attributable morbidity and mortality low.
For decades, amphotericin B deoxycholate has been the standard therapy for invasive fungal infections. Unfortunately, amphotericin B deoxycholate is often poorly tolerated and associated with infusion-related acute reactions and nephrotoxicity.

Adverse events have often led to suboptimal dosing and early discontinuation of therapy resulting in a decreased efficacy. In the late 1970s and 1980s, the development of azoles (first miconazole and ketoconazole, and then fluconazole and itraconazole) provided alternative therapeutic strategies to amphotericin B deoxycholate for the treatment of invasive candidiasis. In recent years, several new antifungal agents have become available offering additional therapeutic options.

These include lipid formulations of amphotericin B (colloidal dispersion, lipid-complex and liposomal), new azoles (voriconazole and posaconazole) and echinocandins (caspofungin, micafungin and anidulafungin).

Clinical trials have shown that triazoles and echinocandins are at least as efficacious as and under most circumstances better tolerated than polyenes as first line or salvage therapy of oropharyngeal or eosophageal candidiasis, candidemia or invasive candidiasis. In addition, no cross resistance is observed among these three classes of antifungal drugs. These new agents have been included in the updated 2009 guidelines of the Infectious Diseases Society of America.

While severe underlying conditions substantially contribute to the morbidity and mortality in critically ill patients, management of invasive candidiasis has been improved by the recent development of tools allowing identification of patients at high risk and early diagnosis/therapy and of new broad-spectrum fungicidal agents with favorable efficacy-toxicity profiles.

POSTERS

P01
PHR1P, A BETA(1,3)-GLUCAN ELONGASE CRITICAL FOR HYPHA FORMATION, LOCALIZES TO THE APICAL GROWTH SITES AND SEPTA IN *CANDIDA ALBICANS*

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Background. Glycosylphosphatidylinositol (GPI)-anchored proteins play an important role in the structure and function of the cell wall in yeast and fungal cells. *PHR1*encodes a GPI-anchored β(1,3)-glucanosyltransferase of family GH72. For their activity, GH72 enzymes are also named beta(1,3)-glucan elongases. Their mechanism of chain elongation can be alternative to, or act in synergy with, the activity of the plasma membrane beta(1,3)-glucan synthase (Fks1p). Elongation/ remodeling of beta(1,3)-glucan, the most abundant polysaccharide in fungal cell walls, is essential for fungal morphogenesis as shown by studies of disruption of genes encoding GH72 enzymes, such as *GAS* genes of *S. cerevisiae* or *GEL* genes of *Aspergillus fumigatus. PHR1* belongs to a family of five genes of *C. albicans (PHR1, PHR2, PHR3, PGA4* and *PGA5). PHR1* expression is triggered at pH values of the growth medium >5.5. Loss of *PHR1* induces morphological defects and avirulence which is likely to result from the inability of *PHR1* null mutants to adhere to and invade human epithelia (J. Calderon, M. Zavrel, E. Ragni et al. "*PHR1*, a pH-regulated gene of *C. albicans* encoding a glucan remodeling enzyme, is required for adhesion and invasion". *Microbiology*, in press, 2010).

Objective. The aim of this work was to investigate Phr1p localization, in different morphological states, by exploiting techniques of protein fusion to the Green Fluorescent Protein (GFP).

Methods. *C. albicans* strains expressing Phr1p-GFP were obtained by using a PCR-based strategy (Gerami-Nejad, M., et al., 2009. "Additional cassettes for epitope and fluorescent fusion proteins in *Candida albicans*". Yeast. 26: 399-406).

Results. During induction of vegetative growth, Phr1p-GFP was localized to the periphery of the growing bud and concentrated at the mother-daughter neck region, in the septum and bud scars. Upon induction of hyphal growth, Phr1p-GFP was highly polarized to the tip of the germ tubes and progressively distributed along the lateral sides of the hyphae. Phr1p-GFP also labelled the hyphal septa where it colocalized with chitin. Microtubules were not required for Phr1p-GFP polarization to the tip of the germ tubes but necessary for septa formation whereas actin cytoskeleton was essential for Phr1p polarization. Electron microscopy analysis revealed that the absence of Phr1p causes loss of compactness of hyphal wall and appearance of crenate aspect in the surface layer. These results indicate that Phr1p localizes to sites of wall formation (bud-tip of the germ tube-septum) where it is likely to contribute to glucan incorporation. As hypha elongates, Phr1p also localizes to the lateral walls of the hypha suggesting that continuous remodelling occurs in these sites. Phr1p localization contributes to assist cell wall assembly, a complex process required for proper morphology and virulence traits.

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P02
MACROPHAGE RESPONSE TO *CANDIDA PARAPSILOSIS SENSU LATO* INFECTION

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Aim of the study. *Candida parapsilosis* is the cause of serious nosocomial infections being the second most common *Candida* species isolated from bloodstream infections in many regions of the world, including Portugal. Due to its association with parenteral nutrition and central lines, *C. parapsilosis* affects mainly critically ill neonates, cancer patients and surgical intensive care unit patients. Molecular studies provided evidence to separate this species into three distinct ones, *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*. Little is known about their potential to cause disease, particularly their interactions with phagocytes. Given the complexity of *C. parapsilosis* virulence and the critical role played by macrophages in balancing colonization/ infection caused by this yeast, the analysis of *C. parapsilosis* response to macrophage infection is important to understand the virulence potential of different isolates of this species.
Methods. Clinical and environmental *C. parapsilosis* isolates, *C. orthopsilosis* and *C. metapsilosis* were assayed for their ability to induce macrophage cytotocixity, measured by lactate dehydrogenase release; to activate a pro-inflammatory response, measured by TNF-α secretion; to produce pseudo-hyphae and to secrete hydrolytic enzymes, namely aspartic proteases and phospholipases.
Results and Conclusions. Environmental isolates induced a statistically significant (p<0.0001) higher cell damage compared with the clinical strains, while *C. orthopsilosis* and *C. metapsilosis* induced a lower cytotoxicity. On the other hand, clinical isolates induced a higher TNF-α production compared with environmental strains (p<0.0001). Whereas the amount of TNF-α produced in response to *C. orthopsilosis* strains was similar to *C. parapsilosis* environmental isolates it was lower for *C. metapsilosis* strains. No correlation between pseudo-hyphae formation or proteolytic enzymes secretion and macrophage death was detected (p>0.05). However, a positive correlation between pseudo-hyphae formation and TNF-α secretion was observed (p=0.0035).
Clinical and environmental *C. parapsilosis* strains do not have the same virulence potential; environmental are more resistant to phagocytic host defenses. *C. orthopsilosis* strains behave similarly to *C. parapsilosis* strains, whereas *C. metapsilosis* is the more susceptible to phagocytes.

P03
DIFFERENT INTRACELLULAR FATE OF YEAST CELLS BELONGING TO THE *CANDIDA PARAPSILOSIS* COMPLEX

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Background. *Candida parapsilosis,* the second most common yeast isolated from blood stream infections, has long been considered as a three-groups complex. According to molecular profiling studies, three distinct species have recently been identified, namely *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*. I*n vitro* data provide initial evidence on their ability to produce biofilm and their differential virulence with respect to reconstituted epidermal or oral epithelial tissues, while epidemiological studies reveal that *C. metapsilosis* is the least represented species, among the *C. parapsilosis* complex, within clinical isolates collections.
The aim of the present study was to investigate the pathogenetic potential of different clinical isolates belonging to *C. parapsilosis, C. orthopsilosis* and *C. metapsilosis* species by an *in vitro* infection model.
Methods. Eight different isolates, identified as *C. parapsilosis, C. orthopsilosis* and *C. metapsilosis* (kindly provided by Prof. S. Senesi, Dipartimento di Biologia, Pisa), have been assessed for resistance/susceptibility to phagocytosis and anticandidal activity by microglial cells; in parallel groups, yeast-containing phagosome maturation, cytokine response and LDH release have also been evaluated.
Results. We have shown that *C. metapsilosis* yeast cells are more susceptible than *C. parapsilosis* and *C. orthopsilosis* to microglia-mediated antifungal activity; interestingly, *C. metapsilosis* yeast cells are phagocytosed to a lower extent and, once ingested, the yeast-containing phagosomes become acidified to a higher degree with respect to phagosomes containing *C. parapsilosis* or *C. orthopsilosis*. Furthermore, cytokine response to infection is comparable irrespective of the *Candida* species employed in that comparably high levels of MIP-1α and little or no TNF-α production are consistently observed. Finally, microglial cells infected with *C. parapsilosis* and *C. orthopsilosis,* but not cells infected with *C. metapsilosis,* release high and time-dependent levels of lactate dehydrogenase (LDH), indicating that *C. metapsilosis* inflicts no damage to microglial cells, as opposed to what is observed with *C. parapsilosis* and *C. orthopsilosis*.
Conclusions. Differences occur among *C. parapsilosis, C. orthopsilosis* and *C. metapsilosis* in terms of yeast-host immune cell interaction *in vitro*, being *C. metapsilosis* the least virulent member of the '*C. parapsilosis*' complex. These data underline the importance of achieving species identification among the '*C. parapsilosis*' complex in clinical microbiology laboratories, in order to better tailor patient diagnosis/treatment as well as provide precious epidemiological data for better elucidating the etiopathogenetic role of the three distinct species in clinical settings.

P04
PRESENCE OF CYTOKINES IN THE GRANULOMAS OF RESISTANT AND SUSCEPTIBLE MICE INFECTED WITH A VIRULENT *P. BRASILIENSIS* ISOLATE

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The severe clinical forms of paracoccidioidomycosis present numerous granulomatous lesions and anergy in protective cellular immunity; in contrast, mild forms show few localized granulomas and preserved cellular immune response. This pattern can be reproduced by infecting susceptible (S) and resistant (R) mice with a virulent *P. brasiliensis* (Pb) isolate so allowing the comparison of the granulomas architecture, areas of lesions comprising Pb with preserved or altered morphology, and the presence and distribution of different cell populations, extracellular matrix components cytokines. Regarding these later components we studied relevant cytokines to granuloma formation and host-fungal interactions by determining their concentration in organ supernatant by ELISA, the expression of their mRNAs by RT-PCR and their localization by immunohistochemistry and compared the lesions developed in the omentum of S and R mice to detect eventual differences due to the genetic background of the mice. The total areas of lesions as well as the percentage of these lesions occupied by Pb were significantly higher in S than in R mice, in parallel with the higher viable fungal loads in the former strain. The local production of stimulatory cytokines TNF-α and γ-IFN was always higher in the R strain than in the S one; in contrast, the inhibitory cytokine TGF-β was detected in lower concentrations in the omental tissue of R mice than in those of the S mice, as indicated by the areas of positive imunohistochemical reactions. RT-PCR revealed an 8X higher expression of IFN-γ mRNA and an 11X higher expression of TNF-α mRNA in R than in S strain and immunohistochemistry showed that the number of IFN-γ cells was 2.5X higher in R than in S mice. However, TNF-α positivity was similar in the granulomas of S and R mice. TGF-β and IL-10 mRNAs were respectively 1.2 and 1.0 more expressed in S than in R mice and there were 1.2X more TGF-β cells in the former strain. The total area of granulomatous lesions and the relative areas of lesions containing Pb were respectively 1.2X and 1.9X more extensive in S mice than in the R ones. ELISA of omentum homogenates also revealed high production of regulatory IL-10 and TGF-β cytokines and low levels of stimulatory cytokines TNF-α and γ-IFN. Therefore, infection of R mice by a virulent Pb strain leads to the preferential synthesis of mRNAs and of proteins of TNF-α and IFN-γ that promote macrophage activation, probably enhancing Pb killing and control of fungal dissemination in parallel with development of small granulomatous lesions containing few fungi; whereasinfection of S mice elicits preferential synthesis of mRNAs and protein of TGF-β and IL-10 which deactivate macrophages and may inhibit Pb killing by macrophages, favoring fungal dissemination and formation of bigger granulomatous lesions.

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P05
EFFECT OF THE VIRULENCE OF *P. BRASILIENSIS* ISOLATE ON THE CYTOKINES PRESENT IN THE GRANULOMAS OF RESISTANT AND SUSCEPTIBLE MICE

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Paracoccidioidomycosis is the most prevalent systemic mycosis in Latin America. Of the many clinical manifestations of the disease, severe and mild forms may be determined by the genetic background of the patients; however the importance of variations in fungal virulence cannot be excluded. Indeed, in previous works we showed that 120 days after infection with a highly virulent *Paracocidioides brasiliensis* isolate susceptible (S) mice develop loose disseminated granulomas and resistant (R) mice show compact circumscribed granulomas. On the other hand, infection with an isolate of low virulence, initially elicits formation of active granulomas, which at later stages become residual. Here we analyse the participation of cytokines in the development of granulomas of S and R mice ip infected with the slightly virulent Pb265, in comparison with the granulomas architecture, the areas of lesions comprising Pb with preserved or altered morphology and the presence and distribution of different cell populations. Infection with Pb265 initially developed loose lesions that tended to resolution, as indicated by the reduction of the lesion area and the percentage of the area occupied by Pb, confirming the regressive nature of this infection even in S mice and confirming the role of fungal virulence in the development of different outcomes of Pb infection. In both mouse strains, secretion of the studied cytokines was found locally at 15 days after infection, but at 120 days, very few cells positive to TNF-α and IFN-γ remained, whereas some still produced TGF-β. This phenomenon may eventually be explained by the irrelevance of stimulatory cytokines at the site of a residual infection, but the necessity of regulatory cytokines to eradicate the inflammatory response.
We studied the expression of mRaNAs and the *in situ* presence of IFN-γ, TNF-α, TGF-β and IL-10 in granulomas in the omentum of these mice at 120 days, when the infection was under control in both mouse strains. RT-PCR revealed much higher expression of all studied cytokines in R than in S animals (8, 35, 30 and 20 folds respectively for IFN-γ, TNF-α, TGF-β and IL-10). This finding suggests that although both, R and S animals are able to efficiently control low virulence Pb infection, the mRNAs synthesis of these cytokines is maintained for a longer time-span in the R strain. However, the mRNAs expression was not in parallel with the protein synthesis of these cytokines: immunohistochemistry revealed an extremely low percentage of low intensity positive cells in the granulomas of either S or R animals. This fact suggests that although the mRNAs is still being secreted as the infection is restrained (at a higher level in R mice), there is a further control on the protein synthesis in both mouse strains.
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P06
FIBROSIS TREATMENT IN EXPERIMENTAL *PARACOCCIDIOIDES BRASILIENSIS* INFECTION

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Fibrosis in patients infected with *Paracoccidioides brasiliensis* (Pb) leads to poor quality of life. We used a murine model to evaluate the effect of drugs that influence fibrosis: the cytokine γ-IFN; the antibiotic Tetracycline and the anti-inflammatory drug Lumiracoxib comparing with controls only infected with Pb. We collected organs of the mice and evaluated *in situ* the overall architecture of the granulomas, the local presence of collagen and its degradation product hydroxiprolin (HPro), levels of NO and of relevant cytokines to granuloma formation and maintenance and also quantified Pb with preserved or altered morphology.

At 15 days after infection, γ-IFN treatment caused decrease in the number of viable Pb in parallel with increased inflammatory cells influx and NO, IL-12, γ-IFN and TGF-β production, and the deposition of thick collagen fibers in an organized, concentric pattern, delimitating the lesion. Tetracycline administration elicited reduction of viable fungal load and increase in NO, GM-CSF and IL-12 levels. Anti-inflammatory treatment increased collagen synthesis, deposited in a disarrayed pattern and decreased production of NO, which resulted in fungal dissemination in spite of increased TNF-α production.

At 120 days, γ-IFN significantly reduced viable fungal load but increased cellular influx, NO and IL-12 levels, eliciting the formation of compact granulomas. Tetracyclin increased the synthesis of HPro, NO and IL-12 as well as cellular influx and caused intense collagen deposition in a well organized pattern of concentric fibers, forming compact granulomas and providing confinement of the reduced numbers of Pb present. Lumiracoxib treatment resulted in infection exacerbation, with histological suggestion of unhindered dissemination of numerous Pb, due to the disorganized deposition of abundant collagen fibers in the granulomas, few inflammatory cells, low levels of NO and in spite of high concentration of γ-IFN. At this infection time, all treatments reduced TGF-β production.

Our results suggest that the best indicators of control of paracoccidioidomycosis as expressed by successful local Pb lysis are the presence of compact granulomas, delimited by a continuous deposit of collagen type 1 arranged in concentric orientation to contain the fungi, and the production of high concentration of cytokines IL-12 and γ-IFN as well as of NO. Based on these parameters, we can conclude that therapy with either γ-IFN or Tetracycline seems promising, reducing the fungal load, increasing the production of NO and of the stimulatory cytokines γ-IFN and IL-12, decreasing that of the inhibitory cytokine TGB-β and altering the granulomas architecture towards a compact structure that provides Pb containment without excessive fibrosis.

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P07
EFFECT OF HENE LASER IRRADIATION ON EXPERIMENTAL PARACOCCIDIOIDOMYCOTIC LESIONS

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The pathogenic fungus *Paracoccidioides brasiliensis* is the etiological agent of Paracoccidioidomycosis (PCM) the most prevalent systemic mycosis in Latin America. The infection is thought to take place firstly in the lungs and then may disseminate to other organs and tissues. Cutaneous lesions are extremely painful and sensitive, and difficult to be cured by currently available antimycotics because of the multi drug resistance that some *P. brasiliensis* isolates present. Also, conventional treatment is frequently toxic at therapeutic dosages and induces side effects to patients. In this perspective, the helium-neon (HeNe) laser emerges as an excellent alternative of treatment since its microbicidal action and wound healing properties are already well known. Previously, we have established an experimental model to evaluate the effects of HeNe laser irradiation on cutaneous inflammatory lesions caused by the inoculation of yeasts cells into the back foot-pad of Balb/c mice.
Our results showed that laser-treated mice present reduction of foot-pad edema, faster cutaneous wound healing, and confluent granulomas. Here, by using the same experimental model, we analyzed the gene expression of pro- and anti-inflammatory cytokines and CCL3, CCL5 e CXCL10 chemokines. Data show decreased levels of pro-inflammatory IL-17 and TNF-α, and of anti-inflammatory IL-10 cytokines, and of chemokines CCL3 and CXCL10. Besides, fungi that were harvested from laser-treated animals presented no capability of growth *in vitro* as compared to those obtained from non-treated mice.
Data presented herein show that HeNe laser can speed up the lesion resolution most likely to influence local cytokines and chemokines production contributing for fungal clearance and tissue regeneration. We believe that HeNe laser is a new non-harmful strategy that may be used as an adjuvant tool to be combined with anti fungal agents for improving the treatment of PCM lesions.

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P08
ENVIRONMENTAL SURVEILLANCE OF FILAMENTOUS FUNGI IN AT-RISK HOSPITAL DEPARTMENTS IN SOUTHERN SARDINIA

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The epidemiology of fungal infections has considerably changed in recent years. Fungal diseases determined by filamentous fungi such as *Aspergillus, Mucorales, Fusarium and Scedosporium* are becoming more common. The use of invasive diagnostic and therapeutic systems may improve the management of difficult diseases, while leading in immunocompromised patients to the invasion of tissues and organs by microorganisms such as fungi, ubiquitous and usually harmless. The hospital may influence the incidence of such infections and therefore air quality is of great importance for their prevention.
The aim of our study was to evaluate the degree of environmental contamination by filamentous fungi in at-risk departments of hospitals in southern Sardinia.
Materials and Methods. The study was carried out from January 2009 to June 2010. Air samples of 1000 L volume were collected with a SAS (Surface Air System) loaded with plates containing Sabouraud agar with chloramphenicol (CAF). Air samples were taken at the center of rooms, approximately 1.3 m above the floor.
Surfaces were sampled using contact plates containing Sabouraud agar with CAF held pressed for 10'' on the surfaces to be examined. Plates were incubated at 25 °C, the reading was done after 4-6 days. Suspicious colonies were isolated, incubated at 25 °C, and daily examined. The identification was based on macroscopic observation of colony and microscopic with lactophenol blue.
Results. From a total of 1,344 air samples, 83.5% tested positive. Of which 70.4% had a charge between 1-50 cfu/m^3, 11.0% between 50-100 cfu/m^3; 4.9% between 100-150 cfu/m^3; 2.30% between 150-200 cfu/m^3; 11.4% >200 cfu/m^3.
From a total of 2708 samples taken from the surfaces, 35.8% resulted positive. Of which 73.8% had a charge ≤12 ufc/24 cm^2, the 26.2% had a charge >12 ufc/24 cm^2.
The fungi found belonged to the genera *Penicillium, Paecilomyces, Cladosporium, Fusarium, Mucorales, Aspergillus, Rhodotorula* and other yeasts.
Fungi belonging to the genera *Aspergillus* and *Mucorales* were prevalent in lower load ranges. Furthermore, our investigation pointed out their frequent presence in at-risk areas such as Intensive Care, CTMO, Haematology, Oncohematology, Paediatric burn center and surgery rooms.
Conclusions. Since there is no "threshold of acceptability" for environmental contamination in areas at risk, we considered both the total charge and the species of the isolated fungus. Although the overall contamination by fungi was rather low, the frequent presence of *Aspergillus* and *Mucorales* species may increase the risk of infection in severely immunocompromised patients. It is evident, therefore, the need to maintain an adequate level of attention and a continuous monitoring.

P09
ENVIRONMENTAL ISOLATION OF *CRYPTOCOCCUS GATTII* IN SOUTHERN ITALY

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Until recently *Cryptococcus gattii* was believed to be endemic in tropical regions such as Australia where it is most associated with *Eucalyptus* trees. To date, for reasons that are not yet fully understood, *C. gattii* has acquired the ability to adapt to new climatic conditions, such as those existing in Canada, where this yeast has unexpectedly emerged as a primary pathogen causing, since 1999, infections mostly in immunocompetent individuals. Moreover, several clinical autochthonous cases have also been described of patients who live in Mediterranean countries, showing that this fungus is more widespread than was previously thought.
Earlier attempts to isolate *C. gattii* from our environments were unsuccessful but this time, 18 years after the last environmental screening for *C. neoformans,* we isolated *C. gattii* in Reggio Calabria, Italy. A total of 34 samples of *E. camaldulensis* debris, including leaves and barks were examined. Yeast strains suspected of being *Cryptococcus* spp. were identified by phenotypic and molecular methods.
Twelve isolates with a presumptive identification of *Cryptococcus* spp. were recovered from 8 of 34 processed *Eucalyptus* samples. Seven isolates were identified as *C. neoformans* by using ID32 C system whereas the remaining 5 isolates were identified as being *C. laurentii* (3 isolates) and *C. albidus* (2 isolates). Surprisingly, 4 out of 7 *C. neoformans* isolates were confirmed as being *C. gattii.* These strains were serotype B, mating type α and were assigned to the VGI genotype.
The emergence of *C. gattii* colonization in Canada and the environmental isolation reported here, indicates that this fungus has evolved and adapted to new environmental conditions. In 1992, we examined 198 samples of *E. camaldulensis* but no *C. gattii* isolates were recovered. Eleven year thereafter, the possible relationship between *C. gattii* and *E. camaldulensis* in Italy was further analyzed by others but the yeast was still absent. Therefore the idea that this species colonized our environments was abandoned and it was also believed that the only environmental isolation of *C. gattii* in 1997 in Apulia (Italy) was due to the presence of exotic animals, some of them from Australia. One European study showed that C. *gattii* caused only 1% of clinical infections and to our knowledge, there have been no cases of cryptococcosis due to *C. gattii* in Reggio Calabria e Messina. This indicates that, *C. gattii* infections are rare in European countries but the presence of the yeast in our environments merits more attention because as revealed by genetic analyses the hypervirulent genotype may have been present in the North American environment for more than 30 years before causing the recent outbreak. Therefore further investigations are needed to reveal the extent of *C. gattii* prevalence in Europe especially in the Mediterranean area where several clinical cases have already been described, but still few environmental studies have been conducted.

P10
WHAT IS THE SOURCE OF CRYPTOCOCCOSIS IN CUBAN PATIENTS?

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Aim of the study. An autopsy study carried out in Cuba showed that systemic or central nervous system cryptococcosis was the (contributing) cause of death in 29% of cases. Its causative agent, *Cryptococcus neoformans* species complex, is ubiquitous in the environment, where it is usually associated with avian guano or vegetative debris. We conducted an epidemiological investigation in order to shed light on the possible infection source of this mycosis in Cuba.

Methods. We analyzed 377 isolates from clinical samples (n=122), pigeon droppings (n=68) and plant debris (n=187) previously identified as *C. neoformans* by conventional methods. All isolates were studied using molecular techniques. DNA was isolated by MagNA Lyser/MagNA Pure protocol. Serotype/mating type was determined using PCRs that specifically amplify the mating-type α or alpha allele of the *STE20* locus for either serotype A or D; AFLP analysis was performed using EcoRI and MseI restriction enzymes; genotyping was done with a panel of 9 microsatellite (STR) markers: (CT)n, (TG)n, (TA)n, (CTA)n, (TCT)n, (CCA)n, (TTAT)n, (ATCC)n and (TATT)n and DNA sequence analysis was carried out on the ITS1-5.8S-ITS2 and the D1-D2 regions.

Results. Isolates from clinical samples and pigeon droppings were confirmed as *C. neoformans* Aα. Only one from 187 isolates from plant debris involved *C. neoformans* var. grubii, The most prevalent species among the plant isolates was *C. heveanensis* (33%) and no less than 53 unidentifiable isolates segregated into 7 potentially novel cryptococcal species. AFLP of the plant isolates yielded 6 clusters with 75, 23, 17, 9(2) and 6 isolates respectively, 3 isolates with similar genetic patterns and 16 unique patterns. STR analysis showed 11 cluster and 116 genotypes; the largest AFLP cluster was segregated in two groups corresponding with the separate clinical and environmental isolates.

Conclusions. Molecular analyses need to be applied to confirm identification of environmental isolates as such. No *C. gattii* isolates were recovered in this Cuban study. The genotypic segregation between clinical and environmental isolates suggests additional source(s) of human cryptococcal infections in Cuba. The selected panel of microsatellite markers is an excellent tool to study the epidemiology of *C. neoformans*.

P11
CHARACTERIZATION OF A *CRYPTOCOCCUS GATTII* ISOLATE FROM *ACINONYX JUBATUS* (CHEETAH) AT THE NATIONAL ZOO, HAVANNA, CUBA

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Aim of the study: *Cryptococcus gattii* has emerged as an important pathogen of humans and animals on Vancouver Island and the Pacific Northwest in North-America. This species posed new diagnostic and treatment challenges to microbiology practitioners. We describe the characterization of a *C. gattii* strain isolated from an *Acinonyx jubatus* (Cheetah) from the National Zoo in Havana.
Methods. The strain was isolated from respiratory secretions and post-mortem from the lungs and identified by conventional techniques. Biotyping was performed by culturing the isolates on L-canavanine-glycine-bromothymol blue medium and D-proline assimilation. The obtained results were confirmed with serological (Iatron kit) and molecular techniques (PCR for serotype/mating type and AFLP for genotype). Multilocus sequence typing (MLST) was performed using the following seven loci: *CAP10*, *GPD1*, *IGS1, LAC1*, *MPD1*, *PLB1* and *TEF1*alpha. Susceptibility to seven antifungal drugs (amphotericin B, flucytocine, fluconazole, itraconazole, voriconazole, posaconazole and isavuconazole) was carried out by the broth microdilution method (NCCLS).
Results. According to the obtained patterns and MLST results it was a *C. gattii* strain serotype B, mating type α and genotype AFLP4/VGI. Minimal inhibitory concentration was 0.125 µg/mL for amphotericin B, and posaconazole; 8 µg/mL for flucytocine, 2 µg/mL for fluconazole, 0.016 µg/mL for isavuconazole and 0.031 µg/mL for itraconazole and voriconazole.
Conclusions. This is the first reported *C. gattii* isolate from Cuba. However genotype analysis corresponded with genotypes found in South-Africa. The animal was imported 6 months previously from South Africa, suggesting that the *C. gatti* isolate originated from that region and not from Cuba. Isavuconazole, a new azole antifungal drug, demonstrated the lowest MIC for this isolate.

P12
ASYMPTOMATIC CARRIAGE OF *CRYPTOCOCCUS SPECIES* AND OTHER YEASTS IN STRAY CATS

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Yeasts of the *Cryptococcus* genus are distributed in nature associated to animal and vegetal organic residues and cryptococcosis is a fungal disease found worldwide in human and animal populations.

Different Authors report that infection in animals and humans is the result of inhalation of the airborne environmental fungi and subsequent colonization of the nasal cavity and paranasal sinuses (Duncan C., et al. Med Mycol., 2005; 43: 511-516). In this study stray cats were used as a natural biological sampler to understand the presence and prevalence of *Cryptococcus* spp and other yeasts species in the nasal cavity of asymptomatic animals.

Materials and methods: 385 deep nasal swabs (i.e., 1 swab for each cat) were obtained from stray cats of Verona municipal shelter undergoing general anaesthesia for chirurgical sterilisation in 2009/10 period. Culture swabs were plated onto Sabouraud dextrose agar (SDA) and incubated at 25°C and 37°C degrees. Yeast identifications were achieved using API ID 32C strips (BioMerieux®) and/or by PCR and direct sequencing on D1/D2-26S rRNA amplicons. Prevalence differences of yeast isolated were evaluated using chi-squared test.

Information about the general health status and signs suggestive of respiratory tract disease were collected.

Results: out of the 385 cats, 55 (14.3%) had culture positive for at least one yeast strain. The 57 isolated yeasts belonged to 3 major genera and to 21 different species, as reported in table 1. Prevalence of *Cryptococcus* species (9.87%) was significantly higher than prevalence of *Candida* species (3.38%) ($p<0.01$).

Table 1: Yeast genera, species, number of isolates and prevalence (%)

Yeast genera	**N°**	**%**	**Yeast genera**	**N°**	**%**	**Yeast genera**	**N°**	**%**
***Cryptococcus* spp.**	**38**	**9.9**	***Candida* spp.**	**13**	**3.4**	***Rhodotorula* spp.**	**3**	**0.8**
Yeast species			**Yeast species**			**Yeast species**		
C. albidus	6	**1.6**	*C. albicans*	1	**0.3**	*R. lysinophila*	1	**0.3**
C. carnescens	4	**1.0**	*C. deformans*	1	**0.3**	*R. graminis*	1	**0.3**
C. laurentii	9	**2.3**	*C. intermedia*	1	**0.3**	*R. sloffiae*	1	**0.3**
C. luteolus	1	**0.3**	*C. sake*	1	**0.3**			
C. magnus	7	**1.8**	*C. zeylanoides*	2	**0.5**	**Other genera and species**	3	**0.8**
C. mycelialis	2	**0.5**	*Debaryomyces hansenii*	7	**1.8**	*Sporobolomyces roseus*	1	**0.3**
C. neoformans	5	**1.3**				*Hannaella coprosmaensis*	1	**0.3**
C. oeirensis	1	**0.3**				*Cystofilobasidium infirmominiatum*	1	**0.3**
C. stepposus	1	**0.3**						
Cryptococcus spp.	2	**0.5**						

Two cats had positive culture for 2 different yeast species: *Cryptococcus carnescens* and *Hannaella coprosmaensis* the first one, *Cryptococcus stepposus* and *Candida zeylanoides* the second one.

Conclusions. The prevalence and the range of yeast species obtained confirmed cats as efficient carriers of environmental yeasts as reported by C. Duncan, *et al*. (Med Mycol., 2005; 43:511-516). In general all cats were in good health conditions. Rhinitis was reported in 11 cats but only three cats had positive culture for *C. laurentii* (2 cats) and *Cryptococcus* spp. (1 cat). One cat was Filv positive, but any yeast was isolated. The high prevalence of *Cryptococcus* species than the other genera of fungi doesn't allow to understand if exists a sub-clinical infection but suggests that cats can carry *C. neoformans* and other yeasts in their upper respiratory tract asymptomatically and, according to Malik R. et al. and Connolly JH. et al. (Aust Vet J. 1997, 75: 483-488; Med Mycol., 1999, 37: 331-338), that nasal colonization may be much more common than clinical disease as previously reported.

P13
RECURRENT CRYPTOCOCCAL INFECTION IN NON-HIV-INFECTED PATIENT WITH A COMMON VARIABLE IMMUNODEFICIENCY (CVID)

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Introduction. Cryptococcal infection is frequent in HIV-infected patients but may occur also in presence of other conditions of immunodepression and, in these cases, diagnosis is often delayed.
Case report. A 65 year old Caucasian female was admitted in our ward on 17 October 2008 with a diagnosis of cryptococcal meningitis.
Medical history. A localized cutaneous cryptococcosis, following traumatic inoculation, was diagnosed in 1979 by isolation of *Cryptococcus neoformans (C.n.)* from an ulcerated nodule (ø 4 cm) while serum cryptococcal antigen was repeatedly negative (Berti et al., Bull. Soc. Mycol. Med. 1981; 10: 207-12). CVID was diagnosed in 1998. The patient presented a history of one month fever. Chest X-ray was negative. Autoantibodies screening, HIV-1 and 2, and other serologic tests were negative. An empirical antibiotic therapy was started without clinical benefit. C.n. was isolated from five blood and one cerebrospinal fluid (CSF) cultures. Serum and CSF cryptococcal antigen titres were 1:256 and 1:128, respectively. A brain MRI showed multiple inflammatory thalamic lesions. Conventional amphotericin B (1 mg/kg) was started and after a week, because of nephrotoxicity, was changed to liposomial amphotericin B (3 mg/kg) given for 6 weeks with remission of fever after 4 weeks. Due to diagnosis of cryptococcal infection, the immunological status was investigated: NK deficit and T lymphocyte deficit were diagnosed, with 140 CD4/mm^3 (40.5%) and 77 CD8/mm^3 (22.3%). After discharge, the patient continued secondary prophylaxis with fluconazole 200 mg qd and started a therapy with intravenous immunoglobulins every 3-4 weeks. Serum cryptococcal antigen progressively decreased to 1:8 (March 23, 2010).
Molecular typing. Two strains isolated from skin in 1979 and one isolated from blood in 2008 were investigated. The major molecular type and mating type were identified by two different multiplex PCRs as previously described (Cogliati et al., Med. Mycol. 2000; 38: 97-103; Esposto et al. Clin. Microbiol. Infect. 2004; 10: 1089-104). The patient resulted infected by two varieties during the two episodes, namely the strains isolated in 1979 were *C. n.* var. *neoformans*, genotype VNIV, mating type alpha, whereas that isolated in 2008 was *C. n.* var. *grubii*, genotype VNI, mating type alpha.
Discussion. The present case, initially thought to be a relapse of a 30 year apart infection, was proved to be a new infection caused by a strain belonging to a different variety. In this patient, in addition to hypogammaglobulinemia, a reduced T-cell number with a very low CD4 and CD8 T-cell count has been detected. Subjects with such panel biomarkers are usually more prone to develop opportunistic infections and have a reduced survival. Since the T-cell number cannot be restored, as it happens in other immune deficiency conditions such as HIV infection, a long-life maintenance therapy with fluconazole and monitoring of serum cryptococcal antigen is suggested.

P14
NOSOCOMIAL CANDIDEMIA IN THE ELDERLY: A NEW PATIENT POPULATION?

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Objective. In recent years, a dramatic increase in the incidence of candidemia was found among patients admitted to an Italian tertiary care hospital.The aim of this study was to evaluate the incidence, potential risk factors for candidemia, causative pathogens, treatment and outcome of patients with candidemia.
Patients and Methods. We retrospectively evaluated the clinical charts of all consecutive patients with ≥1 blood culture positive for *Candida* spp after 48 hours following the admission to any ward of this hospital during the period 2008-2009. Our hospital did not include obstetrician, gynaecological, pediatric and transplant units.
Results. Over the period 2008-2009, a total of 94 episodes of nosocomial candidemia occurred in 90 patients (median age 81 years, range 33-96 years), accounting for an average incidence of 1.74 episodes per 10,000 patient-days per year. Most of the patients were admitted in medical wards (64%) and the other patients in surgical wards (22%) and intensive care units (14%). The underlying diseases that caused hospitalization included cardiovascular diseases (16.7%), abdominal disease requiring surgery (14.4%), neurovascular diseases (13.3%), solid neoplasia (11.1%), and other medical or surgical illnesses (54.4%). Potential risk factors for candidemia included antibiotic therapy (88.3%), urinary catheter (62.8%), total parenteral nutrition (51.1%), central venous catheter (48.9%), diabetes mellitus (27%), surgery (21.3%), mechanical ventilation (11.7%). One patient was HIV-positive and no patient was neutropenic. *Candida albicans* accounted for 51% of episodes; other species were *C. parapsilosis* (25%), *C. glabrata* (13%), *C. tropicalis* (5%), and other *Candida* spp (6%). Among the 46 candidemia episodes occurring in patients with CVC, 24 (52.2%) were CVC-related candidemia. Thirty-six (78.3%) episodes underwent CVC removal following a median time of 2 days (range 1-19) after the diagnosis of candidemia. Antifungal treatment (fluconazole 89.4%, caspofungin 9.4%, amphotericin-B 1.2%) was adequately administered in 68.1% of episodes. The 30-day crude mortality rate was 51%.
Conclusion. In the present study, nosocomial candidemia was quite frequent, especially among elderly non-neutropenic patients admitted to medical wards. Despite of potentially adequate antifungal therapy and early CVC removal, the overall mortality was high. A case-control study is warranted to evaluate risk factors for candidemia in this patient population.

P15
NOSOCOMIAL CANDIDEMIA: EPIDEMIOLOGY AND ANTIFUNGAL SUSCEPTIBILITY PATTERNS AT THE UNIVERSITY HOSPITAL OF MODENA

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Objective. Aim of the present study was to evaluate the incidence of candidemia in an Italian tertiary care hospital, and the antifungal susceptibility patterns of isolates of *Candida* spp. The antifungal agents tested were fluconazole, itraconazole, voriconazole, posaonazole, amphotericin B, caspofungin, anidulafungin and micafungin.
Materials and Method. A retrospective, observational study was conducted at the University hospital of Modena. Data were collected from January 2007 to July 2010. Candidemia diagnosis was defined as the detection of at least two consecutive positive blood cultures yielding *Candida* spp. during the same hospital admission. MICs were determined according to the CLSI guidelines (M27A-3 protocol).
Results. During the study period a total of 142 episodes of candidemia occurred in 129 patients, accounting for a global incidence of 12.1 episodes/10,000 in-patients. Median age was 56 years and the 58% of the patients was male. The most common species isolated was *C. albicans* (79 episodes, 55.6%), followed by *C. parapsilosis* (26 episodes, 18.3%), *C. glabrata* (18 episodes, 12.7%), *C. tropicalis* (10 episodes, 7.0%), *C. guilliermondii* (4 episodes, 2.8%), *C. krusei* (3 episodes, 2.1%), *C. dubliniensis* and *C. norvegensis* (1 episode, 0.8%). Among the study period the incidence of candidemia increased from 7.1 to 18.5 episodes/10,000 in-patients ($p<0,01$). *C. glabrata* and *C. krusei* fungemia occurred more frequently in patients admitted in intensive care units ($p=0.04$). All the isolates resulted susceptible to voriconazole, posaonazole, amphotericin B and to the three echinocandins The susceptibility rate of fluconazole and itraconazole was 99.3 and 72.5%, respectively. The antifungal agents tested had the following MICs90: 8 μg/dl fluconazole, 2 μg/dl itraconazole, 0.125 μg/dl voriconazole, 0.5 μg/dl posaconazole, 1 μg/dl amphotericin B, 0.5 μg/dl caspofungin and 2 μg/dl anidulafungin and micafungin.
Conclusions. Candidemia is still a frequent complication in hospitalized patients and we observed a progressive increase during the study period. This trend could be due to the increase of immunocompromised patients admitted to our institution, in particular haematological patients, solid organ transplanted and post-surgical patients admitted to intensive care units. Nevertheless, *C. albicans* remains the prevalent species and the resistance rates to antifungal agents very low.

P16
TRIAZOLE CROSS-RESISTANT *CANDIDA ALBICANS*: A CASE REPORT

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Candida spp. are common causes of bloodstream infections among hospitalized patients in ICU and
mortality rate is 10-49%. Fluconazole remains a first-line therapy for candidemia, but empirical therapy with fluconazole most likely to be inadequate. Voriconazole is also approved for the treatment of candidemia in non neutropenic patients. We present one case of invasive candidiasis and candidemia due to a Candida albicans resistant to all currently available triazole antifungals in a pediatric immunocompetent host.
A 3 years old girl was admitted in our Hospital for a septic shock and acute renal failure. Two days before she underwent surgery for intususception. Because of severe renal failure she started haemodialisys through subclavian and femoral catheters. On the 11 day a C. albicans was isolated from bloodstream and venous catheters (Vitek-Bio Merieux). L-Amb was started 5/mg/kg/die i.v. The clinical conditions improved after 15 days and the dialysis was discontinued on the 28 day. At the last follow-up after four months from admission the renal function was normal.
One of ten bloodstream Candida albicans isolates collected during the first 6-month of 2010, wase resistant to all triazole. Susceptibilities to triazole of Candida albicans isolated from bloodstream in our Hospital ICU until 2004 was 100%. Resistant C. albicans is an emerging problem. Early diagnosis followed by prompt appropriate treatment improves prognosis. Is required to survey the susceptibility of infective strains, since some of them appear soon to be resistant to fluconazole. Our data suggest that Voriconazole should be avoided as initial therapy in unstable patients with invasive candidiasis (1). Triazole cross-resistence suggests new implications for antifungal therapy, our strains were all susceptible to amphotericin B.

Reference

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P17
BLOODSTREAM INFECTION DUE TO *TRICHOSPORON ASAHII* IN A NEUROLOGIC PATIENT: CASE REPORT

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Introduction. *Trichosporon* spp is a fungus found in soil, water, plants, mammals and birds. In humans it can be found as an opportunistic yeast on the skin or in the mouth. *Trichosporon* spp can cause superficial and deep-seated infections, referred to as trichosporonosis. *T. asahii* is one of the causative species and thus represents an emerging pathogen which can cause severe life threatening infections in immunocompromised hosts.

Case report. A 70 year old woman was admitted to the General Hospital Pula on September 25, 2008 because of symptoms due to intracerebral and subarachnoidal hemorrhage. The bleeding was caused by a rupture of a cerebral aneurysm, confirmed by a computed tomography scan. The patient was transferred to the Clinical Center Rijeka for neurosurgery.

On October 30, she was returned to the General Hospital Pula. At that time she was febrile (39^{+}C) and in poor general conditions, with central vascular and urinary catheters. Sepsis caused by *Pseudomonas aeruginosa* was diagnosed and then piperacillin + tazobactam therapy was administered. Yeast colonies grew in cultures from two blood samples and from a catheter tip, all taken on November 19. The yeast was identified as *Trichosporon asahii* by the API ID 32C system (BioMerieux), and the identification was confirmed by ribosomal DNA sequence determination of ITS 1-5.8S-ITS2 regions. According to the susceptibility testing results, fluconazole (200 mg twice a day) was added to the antibiotic therapy. The patient's clinical condition gradually improved. Blood cultures taken on November 25 and 26 were negative, as were cultures of stool and urine. On December 5 the patient was transferred to a nursing centre.

P18
FOUR CASES OF INVASIVE ASPERGILLOSIS IN PEDIATRIC PATIENTS

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Immunocompromised children are at heightened risk for invasive aspergillosis, rare in immunocompetent children but contribute to significant morbidity and mortality. Aspergillosis has a very high mortality rate of up to 100% (1).
Autors describe four cases: two immunocompetent with fungal infection mediastinitis by aspergillus, after cardiosurgical sternotomy; the first is suffering for a congenital neoplasia charged to the left ventricle from the first week of live, the second a girl of 5 years with complex congenital hearth disease in natural history; two immunocompromised: the first a girl of 11 years with AML arose on myelodysplasia with monosomy of chromosoma 7, the second a boy of nine years with ALL. Both of developed a pulmonary aspergillosis.
Diagnosis was post microbiologically mediastinal swab colture on Sabouraud agar and biopsy of the mediastinum in the first two cases, in the second two cases with CT-Scan and detection of serum galactomannan antigen (Platelia Aspergillus). Patients had a fatal outcome despite the immediate deployment to the first clinical suspicion of antifungal therapy with L-Amb at dose of 3 mg/kg/die i.v.
A timely and adequate antifungal therapy is not always able to stop the progression of invasive fungal diseases, especially aspergillosis. Hence the need for continuous and accurate monitoring in the hospitals to try to reduce the number of CFU. Further research is required to better establish optimal approaches to the management of pediatric patients with invasive aspergillosis. In patients who do not benefit from initial antifungal therapy options include switching to another agent with a different mechanism of action or combination therapy (2).

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P19
WHEN TOO MUCH IS NOT ENOUGH

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Introduction. Describing the case of a pulmonary aspergillosis in a child with relapsed neuroblastoma treated with a very high dose of voriconazole in order to reach the therapeutic blood level.
Case. A 7 years old child was diagnosed with IV stage suprarenal neuroblastoma (N-Myc positive) with the involvement of the bone marrow, the L2 vertebral body and the sacral wing. Two years later, after complete disease remission, she developed a second relapse involving pleura, lung and femur. An aggressive polychemotherapy with ifosfamide, carboplatin and etoposide (ICE) was started. At the end of the first cycle, while in neutropenia, she developed low fever, cough, coryza; the serum CRP levels were risen while her physical examination was normal. The empirical antibiotic therapy, firstly with amikacin and ceftazidime then with teicoplanin, was not successful. Child's clinical conditions progressively worsened and she started to complain pain at the left hemithorax and diarrhoea. Diffuse crackles were present at the left pulmonary base. The chest radiography showed a left pneumonia in the inferior lobe and the galactomannan antigen was positive in two subsequent dosages. The thorax CT was compatible with aspergillosis and therefore, after seven days, a therapy with i.v. voriconazole (7 mg/kg bid; 14 mg/kg/day) was started. At that moment she was also treated with Phenobarbital, with a prophylactic dosage, for a previous convulsive episode but she was not receiving a therapy with proton pump inhibitor. Child's clinical conditions started to improve and after four days the fever resolved. The thorax CT showed an evolution compatible with aspergilloma (half moon sign) and the serum CRP level became normal. A shift to oral therapy was started eleven days later at 10 mg/kg/day (5 mg/kg bid). After the second ICE cycle, almost a month later, an extremely low voriconazole blood level was detected. Firstly we tried, unsuccessfully, to low the phenobarbital dose. Then we administered an higher total dose, subdivided in three doses (7.5 mg/kg tid; 22.5 mg/kg/day). After another month, the blood voriconazole levels resulted again under the therapeutic level and an increased dose (6.25 mg/kg 4 time per day; 25 mg/kg/day) resulted necessary. The voriconazole therapy was stopped four months later when the thorax CT showed a marked reduction of the pulmonary lesions and the child had completely recovered.
Conclusion. It is very important to control an invasive fungal infection to maintain a therapeutic voriconazole trough blood level (therapeutic interval 1-5.5 mg/l) and, therefore, to keep it monitorized during the treatment. As suggested in several studies, the drug blood level is not always directly related to the dose administered, especially when the patient is a child treated with multiple drugs and voriconazole is administrated orally. In our case, a higher total dose and higher number of doses were the keys to maintain an appropriate drug blood level.

P20
ASPERGILLUS EVERYWHERE

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Introduction. Describing the case of invasive aspergillosis in a 3-year-old child with ALL.
Case. In December 2007 to the child was diagnosed common ALL. At the end of the induction phase of the ALL-R2006 AIEOP protocol while in neutropenia, he developed diarrhoea, fever, cough, abdominal distension, bladder globe, paraplegia with risen serum CRP levels. The antibiotic therapy was unsuccessful; the hemoculture and the galactomannan antigen were negative. The vertebral column MR and thorax CT showed respectively a vertebral spondylitis (flogosis of L3-L5 vertebral bodies and swelling of the medullary cone) and a rounded lesion (with air inside) in the left superior lung. Given the high suspicion of aspegillosis, liposomal amphotericin was started. After few days, he developed several intestinal perforations that were surgically treated. Voriconazole was added and liposomal amphotericin was substituted by caspofungin. Despite the treatment, the infection disseminated to stomach (with subsequent perforation), spleen, liver, both kidneys, right shoulder (scapular-humeral arthritis with scapular fracture), vertebral bodies (L3-L5 with involvement of the paravertebral space, the psoas muscles and the spinal space) and opposite lung. He underwent several interventions: gastrectomy, splenectomy, hepatic resection, left lung lobectomy; drainage was located in the paravertebral space. The fungal iphes and the galactomannan antigen were detected in the spleen, liver and kidney tissues; two Aspergillus species (*Aspergillus terreus* and *Aspergillus fumigatus*) grew in the culture from lung parenchyma. Because of the extremely invasive infection, a deficit of the monocytes killing capacity was suspected. For this reason we excluded a CGD and started INF γ (25μg every 2 days sc for 2 months) and G-CSF (50 μg/day ev for 3 months), as suggested in literature in some cases of invasive fungal infection. The aspergillosis dissemination stopped and child's clinical conditions improved. After 5 months, the caspofungin was suspended and a maintenance ALL therapy was started with daily oral purinethol and methotrexate. The child underwent an orthopedic intervention of distraction and arthrodesis L1-S1 after 8 months and then a periodic treatment (every 8 weeks) with vincristine and prednisone ev was initiated. 15 months later, with the insertion of a Ommaya reservoir, the intrathecal prophylaxis with methotrexate was also possible.
Conclusion. At the present time, after 2 years, the voriconazole therapy, that had been maintained during the chemotherapy as prophylaxis, has been stopped. The child is in complete remission and out of treatment, but has still several problems: flaccid paraparesis, neurologic bladder, osteoporosis, malnutrition and recurrent bacterial infections. Even though we did not find any dysfunction of the monocytes killing capacity, the therapy with INF γ and G-CSF seems to have a fundamental role in stopping the Aspergillus dissemination in our case.

P21
FUSARIUM SOLANI SINUSITIS IN REFRACTORY ANEMIA WITH EXCESS BLASTS

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Fusarium spp are important plant pathogens causing a broad spectrum of infections in humans. Immunocompromised patients with prolonged and profound neutropenia and/or severe T-cell immunodeficiency are at risk for disseminated fusariosis.
Case report. A 56 year old female, suffering from refractory anemia with excess blasts myelodysplasia was admitted for an evaluation of a severe (<500/mm^3) and prolonged (>100 days) cytopenia. On admission (Jan 31, 2008), the patient had 100 WBC/mm^3, feveret and sinusitis, both not considered of infectious nature. A computed tomography (CT, Feb 14) showed partial involvement of inferoposterior portion of the right genyantrum, and a less severe involvement in the left one. AmBisome was started on Feb 28 as aspergillosis was suspected (serum galactomannan 0.5-0.6), but, due to the lack of improvement after one month, the therapy was changed to posaconazole (200 mgx4), given for 43 days. *F. solani*, in vitro resistant to itraconazole, posaconazole, voriconazole and amphotericin B, was cultured from purulent nasal discharge (May 12). Voriconazole (200 mgx2) was started on May 14 as the patient was yet febrile. On June 3, the patient underwent surgical exeresis and histological examination confirmed the diagnosis of mycetoma. On July 7, she was submitted to allogeneic haematopoietic stem cell transplant. From July 21, AmBisome was added to voriconazole and, four days later, voriconazole was replaced by posaconazole due to liver toxicity. The post-transplant period was complicated by *Corynebacterium striatum* and *Serratia marcescens* sepsis. On August 11, while WBC count was still 100/mm^3 and *Stenotrophomonas maltophilia* was isolated from blood cultures, the patient died.
Discussion. Fusariosis is a highly aggressive disseminated fungal infection associated with high mortality in heavily immunocompromised patients. In our patient, even in presence of multi-resistant *F. solani* aetiology and of profound neutropenia prolonged till death, the infection remained localized for a long period of time. No dissemination occurred either after the allogeneic haematopoietic stem cell transplant, as demonstrated by the absence of growth of filamentous fungi in the several blood cultures performed by both Bactec and Isolator Systems. In the present case, the surgical debridement of the infected tissues associated with the antifungal therapy resulted in the prevention of dissemination despite the reduced in vitro potency of the antifungals and the delayed start of antifungal therapy. This in agreement with the results of a recent report (Wiederhold et al. AAC 2010; 54: 1055-9) showing the extent of efficacy of posaconazole dependent on the infecting inoculum in neutropenic murine model of disseminated fusariosis.

P22
PRIMARY LOCALIZED CUTANEUS INFECTION CAUSED BY *FUSARIUM OXYSPORUM*

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We report a case of primary localized cutaneus infection caused by Fusarium oxysporum, observed in a 29-year-old woman diagnosed at the Mycology Unit of Siena University. The patient presented a bright red elastic asymptomatic nodular lesion on the volar surface of the first finger of the left hand. The lesion was partially ulcerated with signs of infiltration and a scab on the lower margin.
The woman was a gardener and not use the gloves during gardening activities. A biopsy specimen was obtained for histological and mycological examination. Hematoxiln-eosin showed hyperkeratosis, acanthosis, spongiosis, ulceration and inflammation in the upper dermis. Perivascular inflammatory infiltrate was prevalently composed of mononucleate cells. Hyphae and conidia were abundant in the stratum corneum and were detectable in the dermal inflammatory infiltrate.
Grocott and PAS after diastase showed conidia and medium-large septated and branched hyphae in the dermis. Some hyphae formed right angles, others acute angles. There were no granulomas in the dermis.
Mycological examination of biopsy fragments coltured on Saboraud dextrose agar with chloramphenicol (CAF), produced cottony colonies, initially withish than pink above, purple below, identified as Fusarium oxysporum by microscope and scanning electron microscope (SEM) examination. The culture was deposited in the culture collection of the Mycology Section of IHEM, Brussels (IHEM21984 n. 125).
The patient was healthy and refused antimycotic therapy. She was treated with surgery after checking that other organs and systems were not involved. Clinical recovery was confirmed one year after surgery.

P23
RHINOCEREBRAL MUCORMYCOSIS, AN UNUSUAL LONG COURSE

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Introduction. Zygomycosis is the 3rd invasive mycosis, like importance, after candidosis and aspergillosis. With highly variable incidences and prevalences in different parts of the world (rarely reported in Europe), zygomycosis has as etiological agents fungi of the orders Mucorales and Enthomophtorales. Mucorales fungi are ubiquitous, which, once inhaled, may give fulminant diseases rapidly fatal (1-2 weeks) with rhinosinusitis onset (and brain extension) or lung onset at immunosuppressed patients, especially by blood malignancy or by diabetes mellitus unbalanced.
Case report. A 71 years-old patient, with chronic lymphatic leukemia (Sept 2009), treated with prednisone under which developed steroid diabetes, is diagnosed in Oct 2009 with intracranial tumor with rhynosinusal origin, with palatal expansion and in the left orbit, proved to be mucormycosis by biopsy. He didn't follow any specific antimycotic or surgical treatment until Feb 2010, when he was hospitalized for super-infection of the facial injuries (Morganella spp, Enterococcus spp, Geotrichum and several Candida spp). The isolated Mucor spp was resistant to Posaconazole.
Cranial CT scan shows massive extension of the bone lesions with high bones damage, meningeal contiguity at frontal level, compromised left eye, large palatine communication. It was achieved a metabolic control (remission of the hyperglycemia), antibiotic and antifungal treatment: Posaconazole - initially, based on empiric criteria and because of the lack of Amphotericin B. The evolution was slightly favorable by the reducing of the bacterial component. The patient was_addressed to several clinics for the surgical treatment, with the refusal of two clinics because of the large extension of the lesions. Finally he received surgical therapy at Coltea ENT Clinic, consisting in total resection of the left maxillary, exenteration of the left orbit, ligature of the left carotid artery, the cure of the sphenoidal and frontal sinusis (March 2010). The operation was very large and the results were spectacular. He received Amphotericin B deoxicholate, 7 days preoperative and after surgery till the total dose of 3.5 g. The evolution was favorable, to be continued with facial reconstruction.
Topics. Although a rare entity, mucormycosis should be evoked in differential diagnosis of the rapidly extensive facial massif tumors, at the immunosuppressed patients. The mortality of the rhinocerebral Mucormycosis being extremely high, the cure must be done quickly with doing aggressive surgery (with the removal of as much as possible of the nechrotic infected tissues), under adequate antifungal protection (Mucor spp are constantly susceptible to Amphotericin B and may be also susceptible to Posaconazole), and controlling the immunosuppressing conditions.
The particularity of this presented case was the very long term of the survival (five months) in the absence of an appropriate treatment.

P24
INVASIVE FUNGAL INFECTIONS IN LYMPHOPROLIFERATIVE DISORDERS

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Patients with lymphoid malignancies display multiple cellular and humoral deficiencies, which increase with the use of immunosuppressive chemotherapies and monoclonal antibodies (MAB), constituting an important predisposing factor for opportunistic infections.
We reviewed the records of invasive fungal infections (IFI) in 40 patients (pts) affected by lymphoproliferative disorders, admitted to our hematologic department between 2003 and 2009; among them, 29 were affected by lymphoma, 8 by chronic lymphocytic leukemia and 3 by Waldenstrom macroglobulinemia. The median age was 62 years (r: 17-71). The majority (80%) of pts had progressive or relapsed hematological disease and 60% was treated with multiple lines of chemotherapy.
Additional risk factors were: immunosuppressive therapy for solid organ transplants (2 pts), autologous bone marrow transplantation (1), high dose steroid therapies (2), MAB administration with rituximab (16), alemtuzumab (4) and 90Y-Ibritumomab Tiuxetan (Zevalin, 1). According to the revised diagnostic criteria of EORTC, we recorded 40 IFI. Four infections by Candida (3 blood cultures, 1 culture from freshly placed biliary drainage), 33 by moulds, and 3 by mixed yeasts (2 C. Glabrata and 1 C. Albicans) and moulds (2 probable and 1 proven aspergillosis documented by autopsy) were observed. Among mould infections (IMI), 36 had lung involvement, 2 had sinusal localizations and 1 showed disseminated infection with pulmonary and cerebral localizations. Fifteen were possible mycoses, with a positive chest CT scan. Nineteen pts showed probable infection with suggestive CT scan associated to serum galactomannan antigen positivity (10), BAL galactomannan antigen positivity (2), 1 to both serum and BAL fluid antigen positivity, 7 positive cultures (BAL fluid 5 pts, 3 A. Fumigatus, 1 Fusarium, 1 Scedosporium; sputum 2 pts, A. Fumigatus; nasal swab 1, A. Fumigatus). Two pts had proven IMI due to Aspergillus spp. documented by lung biopsy and autopsy respectively.
Yeast infections were treated with caspofungin (6) except 1 case (positive blood culture for C. Albicans) treated with fluconazole. One pt didn't receive any therapy because the positivity of blood culture was known after death.
Possible IMI were treated with amphotericin B (AMB) or its lipidic formulations (10) and caspofungin (5); probable and proven IMI received AMB or its lipidic formulations (8), voriconazole (8), and caspofungin (5). Two pts were lost to follow up. Twenty-one pts were cured. Seventeen pts died within 90 days from the beginning of the infectious episode, all with not controlled hematological disease.
Eleven pts died due to progression of IFI, 3 due to Candida and 8 due to moulds, 7 of them showing probable or proven mycosis, and 2 with mixed fungal infection. The remaining 6 pts died because of hematological disease progression, after resolution or improvement of their mycotic disease. Fungal attributable mortality was 27.5%.

P25
AN UNUSUAL CASE OF DERMATITIS WORSENED DURING PREGNANCY

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We report a case of a 40-year-old woman presenting a chronic, relapsing dermatitis. Her dermatological history started 7 years ago with erythematous scaly lesions on thighs and gluteal region. She was evaluated by many dermatologists with a diagnosis of eczematous dermatitis. For this reason the patient received cycles of topical steroid treatments, without improvement of her conditions. During pregnancy, in 2009, the patient decided to consult a new dermatologist. A diagnosis of psoriasis was performed and the patient was treated with Mometasone cream for the entire period of her pregnancy with a progressive worsening of her condition and extension of cutaneous lesions.
In February 2010 she arrived to our clinical observation for the first time. She has given birth to a baby 15 days before and she was steel breastfeeding. The patient presented large, merging erythematous plaques, with thinly scaling surface and polycyclic edges, covering the entire areas of her thighs, pubic and gluteal regions. Her abdomen, right forearm and back of feet were spotted with few lesions of the same aspect, sharply marginated with raised edges. A mycologic examination revealed mycelial elements at light microscopy and the culture grew up *Trichophyton rubrum*.
Her newborn baby was previously evaluated by the paediatrician for a scaly dermatitis of the scalp with a diagnosis of seborrhoeic dermatitis. At our clinical observation she was 15-day-old and she presented scaly and squamous lesion on her scalp and a circular element on her abdomen. Also in this case the mycological examination was positive for *T. rubrum*.
It's important to observe that dermatophytoses are rarely reported before 2 years of life. A diagnosis of *T. rubrum tinea corporis* was performed for both patients.
At the moment of our observation the patient was brestfeeding so we prescribed only topical therapy with Econazole cream. After 1 month of therapy the woman and the baby presented a complete clinical remission of cutaneous lesions. Unfortunately they had a relapse after 4 weeks of therapy suspension. Even in this case we decided to prescribe only topical therapy with Terbinafine cream in order to allow the woman to carry on the brestfeeding.
We reported this case for the unusual clinical presentation with remarkable extension of cutaneous lesions that underline the importance of a correct and early diagnosis.

P26
STEROID HORMONES LEVELS IN CATS' HAIR IN PRESENCE/ABSENCE OF *MICROSPORUM CANIS* INFECTION

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Aim. The persistence of M. canis infections in asymptomatic subjects, even after treatments or vaccination, is a well-known phenomenon in cats. The failure of some commercial vaccine could be related to a weak activation of cell-mediated immune response (Lund A, 2010, Parassitologia, 52, 131-133). Since host steroid hormones are involved in the modulation of the immune response, we carried out a survey to assess their levels in cats' hairs, in presence/absence of M. canis infection.
Material and Methods. The study was conducted on 316 cats, mostly (n=273) sourced from shelters. A hair sample was collected from each animal with plastic brush technique. The plastic brush was then infixed in Mycosel agar (BBL) Petri dishes, incubated at 26°C $\pm$2° for at least 15 days. Dermatophyte colonies were counted in all positive plates. Another sample of hairs was simultaneously collected from each cat with clippers to assess the concentration of cortisol, testosterone and progesterone by radioimmunoassay technique (RIA) (Accorsi PA et al., 2008, Gen. Comp Endocrinol, 155: 398-402; Gaiani R et al. 1984, J reproduct. Fert, 70: 55-59; Seren E. et al., 1974, Arch Vet. It., 25:1-20). X^2 test and ANOVA were used for statistical analysis.
Results and Conclusion. One hundred and forty three cats (45.2%) were positive for M. canis. Only 12 (8%) of the positive cats had skin lesions, the other ones were asymptomatic. The number of colonies of M. canis isolated in agar plates varied between one and more than 20: in particular 43 of the positive animals (30%) showed full plates. In subjects that were positive for M. canis, irrespective of the number of colonies isolated, the level of cortisol was higher than in negative ones. However, if we arbitrarily consider cats as really "infected" only when more than 10 colonies were isolated, these "infected" subjects showed significantly higher levels both of cortisol (11.39$\pm$2.02 vs 4.55$\pm$0.82 pg/mg; $p<0.01$) and testosterone (1.56$\pm$0.11 vs 1.35$\pm$0.44 pg/mg $p<0.05$) than "not infected" ones.
No differences in the hormones average levels were found in presence/absence of skin lesions. If we consider three categories of positive cat (a: from 1 to 9 colonies; b: from 10 to 19 colonies and c: more than 20 colonies) the average levels of cortisol and testosterone increased across categories from a to c, but only in cats with more than 20 colonies the average cortisol levels (14.7 pg/mg, $p<0.01$) were significantly different from those of the other positive categories and the negative one. In conclusion, even if no useful parameters based on culture results to differentiate cats as actually infected or mere vehicle are described, we observed an association between a high number of colonies of M. canis (also in asymptomatic cats), and the presence of high level of both cortisol and testosterone. High level of cortisol could be related to a decrease in host cell immune response, whereas the role of testosterone has to be defined.

P27
A NEW CASE OF MYCOTIC DISEASE DUE TO *PHIALOSIMPLEX CANINUS* SP. NOV. (SIGLER ET AL. 2010) IN A DOG

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Recently, Sigler and co-workers (2010) investigated the identity of six isolates (five from dogs and one from a human source) that resembled *Sagenomella* species based on morphological analysis. Phylogenetic analyses of internal transcribed spacer (ITS) and small subunit (SSU) region sequences revealed that all of the canine-associated isolates were distinct from *Sagenomella* species. The new anamorphic genus and species *Phialosimplex caninus* was described to accommodate the clinical isolates (Sigler at al. 2010, Med Mycol 48: 335-345). This report describes a new case of deep infection in a dog caused by *P. caninus* showing highly pleomorphic forms in affected tissues. A 3 year old, female, crossbreed dog was presented to visit for anorexia and depression. The clinical examination revealed generalized lymph-adenomegaly and mild hyperthermia. Fine needle aspiration cytology (FNAC) from enlarged lymph nodes revealed marked fungal macrophagic lymphadenitis. The fungal elements, variable in shape and dimensions, included monocellular yeast-like elements, cigar-shaped and big forms with a thick wall resembling chlamydospores, and septate, branched hyphae showing single phialides sometimes bearing conidia in chains. Culture on Sabouraud Dextrose Agar (SDA) at 25°C yielded grey-yellowish, fast growing colonies. At microscopy, septate, branched narrow hyphae were present. Conidiogenous cells were simple phialides borne laterally on the vegetative hyphae or occasionally on short, unbranched conidiophores. Long chains of conidia were present. According to cytological and cultural findings, a presumptive identification of *Paecilomyces* spp. was made. The dog was treated with itraconazole 10 mg/kg/die for 3 months. A slight improvement was observed, afterwards a relapse occurred. The dog was euthanized and a necropsy was declined by the owner.
At a later date the fungal isolate was subcultured for further analyses. After extraction of genomic DNA, sequences of the rRNA gene internal transcribed spacer (ITS) region were obtained using the sequencing primers ITS1 and ITS4 (Lindsley et al. 2001, J Clin Microbiol 39: 3505-3511).
Following a GenBank BLAST search, DNA sequences showed a sequence similarity ranging from 97% to 99% to *P. caninus* strains described by Sigler at al. (2010). A retrospective morphological study of the isolates allowed recognizing the cultural features reported by the same authors (phialides narrow, cylindrical to slightly swollen at the base or below the midpoint and taper to a narrow neck with indistinct collarette; conidia borne in long chains or aggregate in heads, hyaline, smooth, subglobose, pyriform, obovoid or ovoid with a truncate base). The present report confirms this new species as another potential agent of canine infection. It also represents the first description of the morphological features of *P.caninus* in tissues, in which, as above described, this fungal species was shown to develop with highly pleomorphic elements.

P28
YEASTS FROM HEALTHY ANIMALS: IDENTIFICATION AND SUSCEPTIBILITY TO SOME ANTIFUNGAL DRUGS

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Domestic and wild birds, as well as domestic mammals, are known to be possible carriers of fungi pathogenic to humans and others animals. Opportunistic fungal infections resistant to antifungal agents have been increasingly documented in recent years, and their frequency will likely continue to increase. The aim of the present survey was to investigate the antifungal drug susceptibility profile of some yeasts isolated from farm animals and pet birds.
Fifty-two samples were collected from healthy animals and cultured for yeasts, including conjunctival swabs from 17 cattle (*Bos taurus*) and 15 swine (*Sus scrofa domesticus*), and faecal matter from 16 canaries (*Serinus canaria*), 3 grey parrots (*Psittacus erithacus*) and 1 house sparrow (*Passer domesticus*). Macro and microscopic features of colonies grown on Malt Extract Agar, micromorphology on rice agar, presence of capsule, and assimilation of carbon sources were evaluated for identification. Susceptibility to four antifungal drugs was determined using Etest strips (AB Biodisk) containing amphotericin B, caspofungin, fluconazole and voriconazole. Tests were performed on RPMI 1640 agar plates as recommended by the manufacturer. *Debaryomyces hansenii*, *Pichia etchellsii*, *Candida albicans*, *Candida catenulata*, *Candida krusei*, *Cryptococcus laurentii*, *Trichosporon cutaneum*, *Candida colliculosa*, *Candida parapsilosis*, *Pichia membranaefaciens*, *Candida tropicalis* and *Candida pelliculosa* were recovered and identified. Twenty six isolates were resistant to at least one of the four tested antimycotic agents; one isolate of *D. hansenii* cultured from a canary sample was found to be resistant to all the drugs, while 22 isolates were fully susceptible. These latter included 8 isolates of *D. hansenii* from cattle and canaries, 4 of *P. etchellsii,* 2 of *C. catenulata,* 2 of *C. krusei* and 2 of *C. colliculosa* from swine, and finally 3 of *C. albicans* and 1 of *T. cutaneum* from birds. Three *D. hansenii* and one *C. catenulata* isolates resulted dose dependent susceptible to fluconazole. The present results indicate the occurrence of a variety of yeasts in different animal anatomical sites. Among yeasts belonging to *Candida* genus, both *albicans* and non-*albicans* species were recovered. *Cryptococcus neoformans* was never isolated. During the past decade, there has been an increasing trend of human systemic and fatal diseases due both to non-*albicans Candida s*pecies and to non-*neoformans* cryptococci. Most of the isolated organisms are known to be widespread environmental contaminants and saprophytic commensals of different animal species with worldwide distribution. As many of them are documented human pathogens, animals could play a role in the zoonotic transmission of fungal agents sharing the same environment with humans. Furthermore, healthy animals seem to harbour potentially zoonotic yeasts with variable antimycotic susceptibility pattern within the same species.

P29
UTILITY OF CT- SCAN/PET FUSION IN DIAGNOSIS AND THERAPY OF CHRONIC PULMONARY ASPERGILLOSIS (CPA)

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Objective. 200 out of 315 patients affected by CPA have been followed and studied through Walsh- Stevens (1) and Schiraldi (2) guidelines. Diagnosis and therapy duration is often a problem; diagnosis has been usually established- through the analysis of clinic data, thorax cT- scan (T) with contrast medium, presence of
Aspergillar antibodies in the blood and cultural positivity for Aspergillus on BA/BAL. CT/PET Fusion (concurrently cT- scan and Positron Emission Tomography) represents a diagnostic method able to highlight parenchymal areas endowed with a metabolic activity potentially suggestive of an Aspergillus infection. In fact, the simple cT-scan with contrast medium often proves to have significant limits in the distinction between simple fibrotic areas and areas with active aspergillosis. In suspected cases based on clinical history and aspergillar positive precipitins- despite a negative BA/BAL, it is useful to perform a Ct-guided thin needle aspirate in order to formulate an undoubted aspergillar diagnosis by means of cytological and cultural examination of the sampled material. T/PF has a key role in evidencing the metabolically active areas in which the biopsy should be performed.-Moreover, T/PF is useful for monitoring CPA evolution - requiring a 3-12 months or longer treatment period - through the metabolic uptake of radioactive fluorodeoxyglucose used as a short-lived tracer attenuation. This technique proves to be optimal for predicting the disease evolution and the course of the relative pharmacological treatment.
Methods. In our observational study we have examined 28 patients (aged from 37 to 80; average age 50) by subjecting them to T/PF. All patients were previously examined by common techniques. The results exclude the diagnosis of CPA in 4 patients: since one of them was affected by lung cancer and saprophytic contamination and the other 3 from ABPA with non concomitant CPA. Among the 24 remaining patients (14 males and 10 females) 9 were also affected by concomitant aspergilloma. In 17 patients T/PF was used to confirm the diagnosis and to focus the area for effecting a cT-guided thin needle aspiration in 9 patients; cultural mycological examination was positive in 6 patients. Only in 7 patients T/PF has been performed at the beginning and at the end of the antimycotic therapy: in all these patients both precipitins and T/PF proved as negative at the end of treatment, with T/PF agreeing with antibody negativisation.
Conclusions. T/PF permitted: 1. active confirmation of CPA within apparently simple fibrotic areas; 2. guided thin needle aspiration of samples from areas with suspected Aspergillus infection; 3. useful tool in recovery therapy to confirm eradication of aspergillosis.
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P30
ANTI β-GLUCAN ANTIBODIES: PROTECTION AGAINST FUNGAL PATHOGENS IS ASSOCIATED TO A RESTRICTED SPECIFITY FOR β 1-3-LINKED GLUCAN SEQUENCES

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The persistently high morbidity and mortality due to fungal infections and the increasing fungal resistance to chemotherapy have strongly prompted novel approaches to the control of these diseases based on antifungal vaccines or antibodies (Ab). For these immunological treatments, β-glucan is an ideal target since it is essential for the growth and the survival of fungal cells and is represented in the cell wall of almost all pathogenic fungi with a similar basic molecular structure, mainly consisting of β-(1-3) and β-(1,6)-linked repeating units of D-glucose cross-linked together, and variously complexed with chitin and other glycoproteinsis.
We have recently demonstrated that the anti-β-glucan Abs elicited by a β-glucan (laminarin-CRM197) vaccine and an anti-β-glucan monoclonal IgG2b (mAb 2G8) can confer significant protection, in animal models, against different experimental fungal infections, including invasive or mucosal (vaginal) candidiasis and disseminated cryptococcosis or aspergillosis.
To characterize the protective β-glucan epitopes, we comparatively investigated the protective mAb 2G8 and a non-protective anti-β-glucan mAb (1E12) with identical complementarity-determining regions. Competition ELISA and microarray analyses showed that the protective IgG2b selectively bound to β1,3-linked (laminarin-like) glucose sequences whereas the non-protective IgM bound to β1,6- and β1,4-linked glucose sequences in addition to β1,3-linked ones. In addition, only the protective IgG2b recognized β-glucan motifs associated to two important, virulence-related proteins, ALS3 and HYR1, in fungal secretion.
Then, we generated novel, CRM197-conjugated β-glucan-vaccines, using either linear, natural (Curd-CRM 197) or syntetic (15 mer-CRM197), β-(1,3)-oligosaccharides, or β-(1,6)-branched, (17 mer-CRM 197) β-(1,3)-glucan sequences. When these vaccines, formulated with the human-acceptable adjuvant MF59, were used to immunize mice, we found that the Curd-CRM197 and the 15 mer-CRM 197 coniugates induced high titres of anti β-(1,3)-glucan IgG but no Abs against β-1,6-glucan and conferred protection to mice sistemically challenged with *C.albicans*. In contrast, the 17mer-CRM197 coniugate, which induced anti β-(1,6)-glucan antibodies in addition to the anti-β-(1,3)-glucan IgG, was non protective.
Overall, these results indicate that competence for antifungal protection by anti-β-glucan Abs, either pre-formed or vaccine-elicited is strictly associated to exclusive specificity for β-1,3-linked glucan sequences and open the way to a more rationale design of β-glucan vaccines and anti-β-glucan therapeutic Abs for the treatment of fungal diseases.

P31
PERSISTENCE OF POSITIVE BETA-D-GLUCAN TEST AFTER CLEARANCE OF CANDIDEMIA IN HSCT RECIPIENTS

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Background. Beta-d-glucan (BG) is a fungal cell wall component circulating in the blood of patients with fungal infections and high sensitivity of BG was reported in candidemia (1). Positive BG was reported to precede the onset of IFI-related fever or diagnosis of IFI by other methods (1-3), and BG levels decreased in patients who responded to antifungal treatment[1,3]. In order to study the relation between the time to positivity and negativity of BG compared to blood cultures, kinetics of BG was analysed in HSCT recipients with candidemia.
Materials and Methods. HSCT recipients that developed candidemia were identified and BG testing (Associates of Cape Cod, Inc., Falmouth, MA) was performed. All the samples were stored in -20°C. Levels of ≥80 pg/ml were considered positive. The day of the first blood culture positive for yeasts was considered day 0.
Results. Six patients with candidemia were identified. All the patients had at least one sample positive for BG, but only one patient had positive BG before the day of candidemia. The median time between positive BG and culture was 2.5 days (range:-7;14). BG positivity persisted long after blood cultures became sterile (in 3 patients in whom BG became negative, the median time between the first negative blood culture and the first negative BG was 48 days, range: 17-102). The kinetics of BG is outlined in figure 1. Patients who responded to antifungal therapy had BG levels diminishing slowly overtime, while patient number 5 who had persistently high BG was diagnosed with splenic candidiasis.

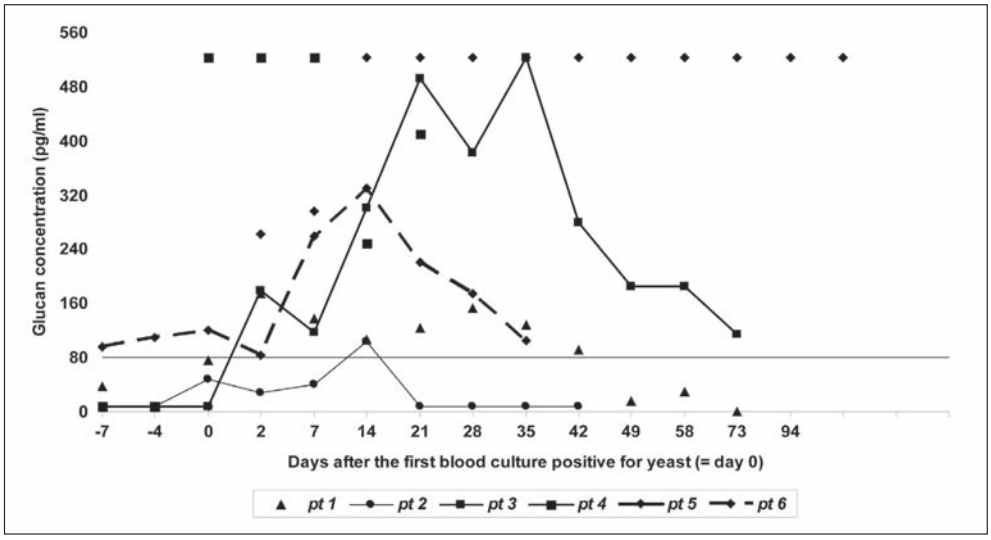

Figure 1

Discussion. BG resulted positive in all patients with candidemia, however in most cases it did not precede the positive blood culture. BG persisted long after cultures became negative, even in patients clinically responding to treatment.
Despite low number of cases reported, BG seems useful for diagnosing candidemia in HSCT recipients, particularly because the results can be obtained in 2 hours, compared to at least 48 hours of traditional microbiological cultures. The time to BG negativisation in patients responding to treatment was long, but the persistence of repeatedly high levels of BG might indicate that the infection has become chronic, even if blood cultures are negative.

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P32

COMPARISON OF GALACTOMANNAN ENZYME IMMUNOASSAY AND QUANTITATIVE REAL-TIME PCR ASSAY IN BRONCHO ALVEOLAR LAVAGE FLUID FOR DIAGNOSIS OF INVASIVE ASPERGILLOSISS

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Purposes. To evaluate and to compare the galactomannan enzyme immunoassay (GM EIA; Bio-Rad), quantitative real-time PCR (qPCR) and quantitative cultures, and to examine the possibility that the application, singly or together, of the GM EIA and the qPCR assay in BAL fluid would enhance rapid diagnosis and accuracy for invasive aspergillosis.
Patients. Between 2007 and 2008, 98 patients at the Molinette Hospital-Turin underwent bronchoscopy for evaluation of pulmonary disease. BAL fluid that was left over after conventional microbiologic valuations (quantitative cultures and GM EIA test) was stored at -70°C to perform qPCR. According to the criteria of the EORTC/MSG, 14 of 98 patients were classified as probable IPA, and the 88 remaining patients were considered as "no-IPA" patients.
Methods. BAL supernatant was used to perform the Aspergillus GM antigen test (Platelia Aspergillus EIA, BioRad®), using index ≥1,5 as positive. The qPCR amplified the fungal 18S rRNA gene with a commercial kit (Nanogen, Buttigliera Alta, Italy), PCR inibitor control was performed by co-amplification with human beta-globin gene. A standard curve was generated by running eight positive standards containing *A. fumigatus* genomic DNA ranged from 100 to 100.000 genome/reaction; as positivity cut-off the manifacturer suggests 77 copies/reaction (616 genome/ml BAL).
Results. 11 sample were culture positive, 20 showed a galactomannan index >1.5. Aspergillus DNA was undectectable in 17 patients. To quantify the threshold for fungal DNA positivity we classified the sample DNA burden in four categories compared with IPA: <10 genome/ml: 17 patients, none with IPA, between 10 and 100 genome/ml: 50 patients, one with IPA and 49 no-IPA, between 100 and 1.000 genome/ml: 22 patients, 4 with IPA and 18 no-IPA, >1.000 genome/ml: 9 patients, all with IPA. Probably the correct cut-off to define a BAL positive is between 100 and 1.000 genome/ml, and the value suggested by manifacturer could be used as diagnostic cut-off. 4 patiens with IPA were colture negative (sensibility 71%), 1 patient no-IPA was culture positive (specificity 93%). All patients with GM negative were no-IPA showing a very high predictive negative value of this test. All IPA patients had GM index >1.5. but we found 6 BAL with index >1.5 in no-IPA patients (specificity 93%). We had 10 samples GM positive and qDNA <616 genome/ml: four were IPA patients and the DNA level was 29, 309, 204 and 471 genome/ml, the other six were GM false positive results. We found no false-positive PCR (cut-off 616 genome/ml) in patients without IPA.
Conclusions. GM has high sensibility to detect IPA, but low specificity, whereas qDNA has high specificity with the cut-off used in our study. The use of galactomannan detection together with qPCR assay in BAL may enhance diagnostic accuracy for invasive aspergillosis in patients suspected of having IPA. Further studies are needed to evaluate the qDNA cut-off.

P33
DETERMINATION OF PROCALCITONIN SERUM LEVELS IN IFI HIGH RISK PATIENTS

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Introduction. Procalcitonin (PCT) is a prohormone of calcitonin, synthesized in the C cells of thyroid gland, increasing in patients with sepsis or septic shock. Although many authors consider PCT as a valid marker in systemic bacterial infections, its role in IFI is still controversial. Aim of our study was to value whether PCT might serve as prognostic marker for the early diagnosis of IFI and to verify the data agreement by two commercially systems.
Material and Methods. The study was carried out in patients admitted to Onco-haematology (HAE) and Neonatal Intensive Care (NICU) Units of University Hospital of Bari (Apulia, South Italy).
Eligible patients in OHAE had AML or ALL, Hodgkin's or non-Hodgkin's lymphoma or multiple myeloma, candidate for bone marrow transplantation; PCT levels were determinate at admission (T0) and T7 - T14 - T28 days. Eligible patients in NICU were preterm infants (gestational age <37 weeks) and full-term babies presenting respiratory stress, hypothermia and tachycardia; PCT was valuated at T3 (3rd day of life), T7 and T10. Serum PCT levels were determined by two methods: VIDAS® BRAHMS PCT (bioMérieux, France) and KRYPTOR® BRAHMS PCT (DASIT, Germany). In parallel, all enrolled patients were also investigated to circulating antigens (1,3-ß-D-glucan [BDG], galattomannan and mannan).
Results. 141 patients were enrolled and 14 IFI episodes (9.9%) were documented. In NICU, 6 (42.8%) babies developed BSI (3 *C.albicans* and 3 *C.parapsilosis*); PCT values were slightly increased in 4 cases either 2 days before and at diagnosis but one of them had also a severe bactaeriemia. At diagnosis, BDG test was positive in all neonates, while the mannan antigen only in 3 cases with *C.albicans* infection.
In HAE 8 (57.1%) patients developed IFI: 5 probable pulmonary aspergillosis, and 3 BSI (2 *C.krusei* and 1 *C.parapsilosis*). PCT values remained very low except for one patient with severe renal injury in the third day of enrollment. At diagnosis, BDG and galattomannan tests were positive in the patients with invasive aspergillosis, while mannan values were negative in the patients with *C.krusei / C.parapsilosis* BSIs. Over all, no statistically significative difference was observed between two systems in every time of monitoring (p>0,643).
Conclusion. Our data suggest that the PCT significance in serum depending on examined population and its clinical setting. Its diagnostic value in IFIs, supported by other parameters (i.e. circulating antigens, daily monitoring), could provide important additional information to exclude bacteraemia and better follow-up of infection. It is known PCT can be enhanced in no infectious disorders, so its serum levels must be evaluated with clinical context. So, other studies on larger numbers of cases (especially in NICU patients) are warranted to analyze the predictive value of PCT in fungal diseases.

P34
IN VITRO PRODUCTION OF DERMATOPHYTES ARTHROCONIDIA/CHLAMYDOSPORES

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Aim. Arthroconidia are produced by the fungus in its parasitic form; their production *in vitro* is reported in few works only for the genus *Trichophyton*. As the antifungal susceptibility test are usually performed on the saprophytic macro- microconidia or hyphae, the purpose of this study was to find a suitable technique for the *in vitro* production of arthroconidia to test the antimycotic drugs.
Material and Methods. Four different techniques were used on 11 dermatophytes strains (4 *M. canis*, 3 *M. gypseum*, 2 *T. mentagrophytes,* 1 *T. rubrum* and 1 *T. equinum*): A) incubation of the agar plate in jar according to Bibel DJ et al., (1977, Infect Immun, 15: 958-971) modified using a candle to obtain 2-3% of CO_2; B) cultural plates covered with 3 ml of sterile distilled water and sealed with parafilm (Hashimoto J and Blumental Y, 1977, Infect Immun, 18: 479-486); C) growth on membranes in accord to Hashimoto e Blumental (1978, Appl Env Microbiol, 35: 274-277) modified using steril polycarbonate membrane with pore size of 10µm instead of dialysis membrane. The plates were incubated in a jar with a base of wet tissue paper to achieve a saturation moisture; D) growth on Malt Extract Agar at pH 7.5 in thermostat with 5% CO_2 (Coelho et al., 2008 J Antim Chem, 62: 758-761). All the cultures were incubated at 37°C. The plates were checked regularly in order to evaluate the production of arthroconidia and/or chlamydospores.
Results. With the test C there was a lack of growth on the membranes; in the test D, there was a significant dehydration of the medium, not allowing fungal development. With the test A and B there were different performance according to the strains tested. *T. mentagrophytes* produced arthroconidia efficiently with both techniques. *M. canis* generally revealed a lower production of arthroconidia only after a long time of cultivation (~30 days), and a higher prevalence of chlamydospores with both methods. Concerning *M. gypseum*, method B was not suitable for the mycelial growth or the production of arthroconidia; method A allowed instead an abundant production of chlamydospore but only few arthroconidia. *T. equinum* showed growth of chlamydospore and arthroconidia already after 17 days with both methods. *T. rubrum* did not produce arthroconidia and chlamydospore with method A, while method B revealed a higher prevalence of chlamydospore in comparison with arthroconidia.
Conclusions. The data reported in literature concerning the *in vitro* production of arthroconidia mainly refer to the genus *Trichophyton*, while the genus *Microsporum* is not mentioned, so the results we obtained are particularly interesting and may be used as a basis for further studies and practical applications. Furthermore, these results lead us to presume that, as reported in literature, not only cultural conditions influence the production of arthroconidia (moisture, temperature, pH, CO_2), but also the fungal species and the individual strains.

P35
THYMUS VULGARIS L. ESSENTIAL OIL ENHANCES THE INTRACELLULAR KILLING OF *CANDIDA ALBICANS* BY HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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The increasing recognition and importance of fungal infections, the difficulties encountered in their treatment and the increase in resistance to antifungal agents have stimulated the search for new therapeutic alternatives. The essential oils and products of plant secondary metabolism had a wide application in folk medicine, fragrance industries, food flavouring and preservation but only in recent years they have started to be recognized for their potential antimicrobial role. Clinical experience has shown that the efficacy of antimicrobial agents depends not only on their direct effect on a given microorganism but also on the functional activity of the host immune system. The literature reports evidence suggesting that a larger number of plants and their constituents could show beneficial therapeutic effects, including anti-oxidant, anti-inflammatory and immunomodulatory activity, which still need to be further investigated.
Since data on the effects of essential oils on innate immune system are scanty and fragmentary, in this study the interaction of *Thymus vulgaris* L. essential oil (EO), known for its antibacterial and antifungal activity, with human polymorphonuclear leukocytes (PMNs) was evaluated, focusing on intracellular killing towards a clinical *Candida albicans* strain.
Intracellular killing was investigated by incubating the yeast cells (10^6 blastoconidia/ml) and PMNs (10^6 cells/ml) at 37°C for 30, 60 and 90 min with ½ x MIC of EO. EO-free controls were also included. Killing values were expressed as the survival index (SI), which was calculated by adding the number of surviving yeast cells at T_0 to the number of survivors at T_x, and dividing by the number of survivors at T_0. According to this formula, if fungal killing was 100% effective, the SI would be 1. Preliminary results showed that ½ x MIC of EO significantly increased the intracellular killing by PMNs, in comparison with EO-free controls. The mechanism of such enhancement is still unknown, although EO direct damage to the yeast cell may, at least in part, be responsible, resulting in changes that make the yeast cells more susceptible to PMN lytic mechanisms. These encouraging results require to be further confirmed, to better elucidate this issue.

P36
ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA PARAPSILOSIS* COMPLEX BLOOD ISOLATES

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Aims. *Candida parapsilosis* is the most common causative agent in Europe and Latin America for candidemias due to non-*albicans Candida*. The present study was undertaken to determine the distribution of *Candida parapsilosis* complex species and the antifungal susceptibility of clinical isolates collected during an Italian surveillance study of yeast invasive fungal infections (IFIs) in intensive care units.
Methods. MICs were determined using the Clinical Laboratory Standards Institute reference broth microdilution method. Results were interpreted according to the established clinical breakpoints for susceptibility for echinocandins and triazoles. *Ban*I digestion patterns of the secondary alcohol dehydrogenase PCR products were used to identify *C. parapsilosis sensu stricto*, *C. orthopsilosis*, and *C. metapsilosis*.
Results. 144 *C. parapsilosis* isolates were banked (January 2007-December 2008). The overall frequency of *C. parapsilosis* complex in IFIs was 22%. Of 138 tested isolates, 95% were *C. parapsilosis sensu stricto*, 3.6% were *C. orthopsilosis*, and 1.4% were *C. metapsilosis*. MIC_{50} and MIC_{90} values were, respectively: amphotericin B 0.5 and 1 mg/L; anidulafungin 2 and 4 mg/L; caspofungin 1 and 2 mg/L; fluconazole 1 and 4 mg/L; itraconazole 0.5 and 1 mg/L; micafungin 2 and 4 μg/ml; posaconazole 0.5 and 1 mg/L; voriconazole 0.015 and 0.06 mg/L. All isolates of the less common species within the *C. parapsilosis* complex (*C. orthopsilosis* and *C. metapsilosis*) were susceptible to echinocandins.
Conclusions. This study, the most comprehensive study conducted to date to evaluate the frequency and antifungal susceptibility profiles of *C. parapsilosis* complex isolates from critically ill patients in Italy, highlights the low prevalence of *C. orthopsilosis* and *C. metapsilosis* in IFIs.

P37
ANTIFUNGAL SUSCEPTIBILITY TESTING OF *TRICHOPHYTON MENTAGROPHYTES* AND *TRICHOPHYTON RUBRUM* ISOLATED FROM SKIN

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Objective. Dermatophytosis is a common human mycosis, and dermatophytes of the *Trichophyton* genera are the most common causative agent. Many antimycotic agents are safe and highly effective for the treatment of dermatophytosis, and are available for clinical practice. The aim of this work was to determine the MICs of four antifungal drugs (fluconazole, itraconazole, terbinafine and griseofulvin) recognized for dermatophytosis treatment caused by *Trichophyton* species, especially *Trichophyton mentagrophytes* and *Trichophyton rubrum*.
Method. We used the standard broth microdilution M38-A2 method adopted by the Clinical and Laboratory Standards institute (CLSI) for testing of antifungal susceptibility of filamentous fungi, including the dermatophytes. We tested 28 *T. mentagrophytes* and 18 *T. rubrum*. Two reference strains, *T. mentagrophytes* ATCC MYA 4439 and *T. rubrum* ATCC MYA 4438, were included as quality control isolates, as recommended by the CLSI. Each organism was tested in duplicate. The inoculated plates were incubated at 35°C for 4 days. MICs were determined visually using an inverted reading mirror and were defined as the lowest drug concentration that caused 80% inhibition of the growth in comparison to the growth control.
Results. MICs for quality control *T. mentagrophytes* ATCC 4439 and *T. rubrum* ATCC 4438 were within the recommended ranges. The MIC range, median and geometric mean MIC values of antifungal drug against *T. mentagrophytes* and *T. rubrum* isolated are shown in Table 1.

Table 1 - The MIC range and geometric mean MIC values (GM) for the *T. mentagrophytes* and *T. rubrum.*

Isolates	Fluconazole		Itraconazole		Terbinafine		Griseofulvin	
	Mic range	GM	Mic range	GM	Mic range	GM	Mic range	GM
T. mentagrophytes (28)	0.25-32	9.75	0.002-1	0.05	<0.001-0.03	0.004	0.125-0.5	0.40
T. rubrum (18)	0.25-32	1.08	0.001-1	0.08	<0.001-1	0.01	0.25-1	0.70

Conclusions. Terbinafine was the most potent agent tested in this study, with MIC values for both tested species lower than the other agents tested, suggesting a possible correlation to data that support the use of terbinafine to treat dermatophytic infections. Given the wide MIC range and the high value of the geometric mean for *T. mentagrophytes*, the use of fluconazole, expecially in the presence of a poor clinical response, would require the *in vitro* determination of MIC.

P38
USE OF CONTACT TESTS TO ASSESS THE IN-VITRO ACTIVITY OF TWO PRODUCTS CONTAINING CHLORHEXIDINE AGAINST *MALASSEZIA PACHYDERMATIS*

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Aim of the study. Chlorhexidine (CHX) is a biguanide antiseptic frequently used in topical therapy of dermatological diseases of dogs. It is contained in numerous marketed products at various concentrations and associated with different ingredients. The aim of this study was to assess the in vitro effectiveness against *Malassezia pachydermatis* of two commercial products containing CHX respectively at 0.45% (product A, Clorexyderm spot gel® - ICF) and 0.3% (product B, Clorexyderm spot gel + Tris EDTA® - ICF).
Materials and Methods. Antimicrobial activity was evaluated by contact tests according to guidelines of the European Standard EN 1275, which specifies a suspension test for establishing whether a chemical disinfectant or antiseptic (biocide) does or does not have a *basic* fungicidal activity. The activity is evaluated as the capability of a product to reduce the number of viable fungal cells under defined conditions. Five strains of *M.pachydermatis* were tested for each product, and tests were repeated twice. A sample of each product was added to a test suspension of the yeast. The mixture was maintained at 20 °C for 15 min (obligatory test conditions). At the end of this contact time, an aliquot was taken, and the fungicidal activity in this portion was immediately suppressed using a neutralizer solution (lecithin 3 g/l; polysorbate 80 30 g/l). The resulting suspension was then cultured on Sabouraud Dextrose Agar and the colony forming units counted after 72 h incubation. Products were considered effective (fungicidal) if a 4 decimal log (lg) reduction of the germ after a 15 minute contact time was observed (UNI EN 1275 - Quantitative suspension test for the evaluation of basic fungicidal or basic yeasticidal activity of chemical disinfectants and antiseptics).
Results and Conclusions. A 4 decimal log reduction of viable yeasts was obtained in all the experiments performed using five strains of the yeast. Both products can therefore be deemed effective against the target microorganism under in-vitro defined conditions, strictly fixed according to guidelines of European Standard UNI EN 1275. It's the authors' opinion that such an approach can represent an useful model able to take into account the several factors which are known to influence the activity of the active ingredients in biocides. Actually, the efficacy of antimicrobial products which are used topically depends on, and varies significantly with the period of contact and formulation effects, which can often enhance activity despite the presence of lower levels of the biocide. The mentioned factors are important in evaluating the modes of action of, and mechanisms of resistance to, biocides and are often regrettably overlooked (Russell and McDonnell 2000. J Hosp Infect 44: 1-3). This study confirms the effectiveness of CHX against *M.pachydermatis* and underlines the usefulness of contact tests when evaluating the antimicrobial effectiveness of topical therapy.

P39
ENUMERATION OF YEASTS AND MOULDS IN SAMPLES OF COMMERCIAL BREADCRUMBS AND SUBSEQUENT ASSESSMENT FOR THE CONSUMER AND THE PRODUCER

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Objective of the Study. The operators of the bakery industry are looking with increasing attention to moulds that can contaminate bread, baked goods and raw materials. Their presence in fact involves not only problems of sanitation and potential toxicity, but also economic ones. They can cause discolouration, odour and change texture of the food. The most common mould in bread and bakery products belong to the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Monilia sitophila* and *Mucor*. In most cases the fungal contamination was not caused by raw materials, but a secondary contamination of the baked product. The aim of this study was to evaluate the presence of yeasts and moulds in samples of prepared and packaged breadcrumbs from industries or bakeries chosen by a random criteria. There are only a few data reported in scientific literature relating to the contamination limits acceptable for yeasts and moulds from breadcrumbs, and there are no normative references.
Methods used. in the laboratories of the IZS Catania in Sicily 50 samples of sealed packages of breadcrumbs were analyzed. These samples were taken from nine supermarkets in the province of Catania. Of these, 34 were industrial products and 16 local bakeries. The analytical method used followed the standard ISO 21527-2:2008 "Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of yeasts and moulds-Part 2: Colony count technique in products with water activity less than or equal to 0,95. The most significant isolated colonies of moulds were then typified with Biolog (AES).
Results and Conclusions. Literature data (Marshall J.P. 1986 and Guidelines of the Lombardy Region for hospital catering-2001) report a limit of contamination of yeasts and moulds in breadcrumbs at a value of 1x10^3 CFU/g. This limit was considered appropriate and guarantor of good hygienic conditions of the product, in the evaluation of the results of this study. Of the 50 samples analyzed, 15 (30%) were positive for moulds, of these, 9 samples (18%) had a value above the limit considered, while the remaining 6 samples (12%) were within the limits of contamination. Moreover from the typification of fungal colonies the presence of the prevailing predominant genera *Penicillium* spp. (*P. citrinum*, *P. crustosum*, *P. chrysogenum*, *P. brevicompactum, P. roqueforti*) and *Aspergillus* spp. (*A. niger*) was found. It should be noted that the highest values were found primarily in samples from local bakeries (7 to 9 or 78%), this is perhaps because, unlike industry, these structures do not have forced ventilation, air filtration, environmental control of temperature and humidity, factors leading to unfavourable conditions for secondary contamination of bread. It is therefore preferable that the consumer chooses packs of industrial breadcrumbs or bakeries which ensure the daily packaging of a fresh product not a stale one.

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ENUMERATION OF YEASTS AND MOULDS IN GRATED CHEESE SAMPLES AND SUBSEQUENT CONSIDERATIONS

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Objective of the Study. Cheeses are characterized by the presence of numerous species of micro-organisms, some of which contribute to the aging process of the product. Their development depends on parameters such as light water, pH, potential redox, and quantity of nutrients, ecc.., from the expected production and aging processes and storage conditions. Unlike blue cheeses such as Gorgonzola, Roquefort and Camembert where moulds are harmless and contribute to giving the typical flavour to the cheese, they may also be contaminated by unwanted moulds and yeasts, that if present in excess can cause product defects. Reg. (EC) n.1441/2007 amended by Reg. (EC) n. 2073/2005, and the former DPR 54/97 does not refer to the evaluation of the level of yeast and mould from cheese. Guidelines of the Lombardy Region for hospital catering-2001, Appendix. Title IV Ig Loc Reg. BURL 15/05/1993, indicate which are the limit value for moulds in grated cheese 1x10^2 CFU/g. The objective of this study was to monitor the amount position of yeast and moulds in samples of grated cheese.
Methods used. 50 samples of mature, grated cheese, packed in a modified atmosphere in sealed bags, were analyzed, the samples were chosen by a random criteria, from nine supermarkets in the province of Catania. The types chosen were: Grana Padano, Parmigiano Reggiano, Pecorino and Mix (Provolone, Emmental, spicy provolone, parmesan, parmesan cheese). The reference used was the standard ISO 21527-2:2008 "Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of yeasts and moulds- Part 2: Colony count technique in products with water activity less than or equal to 0,95".
Results and Conclusions. Of the 50 samples analyzed, all were free of mould and 25 (50%) contaminated with yeast. For the 25 positive samples the following charges were recorded: six of them in the order of 10^ 3 CFU/g; for fourteen 10^4 CFU/g; for the other six 10^5 CFU/g. The types concerned were for the first group 2 gran mix and 4 Grana Padano, and for the second 7 gran mix, 4 Grana Padano and 2 Parmesan, (parmigiano reggiano); for the third, 1 gran mix 3 Grana Padano and 2 Parmesan (parmigiano reggiano). Overall, 25 samples of the contaminated cheeses were not represented by a particular type among the various samples. The absence of mould growth indicates that the cheese used by the dairy industry for marketing in the form of pre-grated, ready for domestic use, are not cheese scraps.
The presence of yeast on the other hand could lead to the assumption that the physiological contamination of mature cheese and the possible use of forms that have defects of a commercial impact. In the case of higher charges it may also suggest a cross contamination during production of the grated cheese by the instrumentation and the environment, increased by conditions that may develop inside the package.

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LOOKING FOR *CANDIDA NIVARIENSIS* AND *C. BRACARENSIS* AMONG A LARGE COLLECTION OF *C. GLABRATA* ISOLATES: RESULTS OF THE FIMUA WORKING GROUP

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Introduction. Two new recently described pathogenic *Candida* species, *C. nivariensis* and *C. bracarensis,* share many phenotypic characteristics with *C. glabrata* and are easily misidentified as such (1, 2). *C. nivariensis* and *C. bracarensis* have carbohydrate source assimilation profiles with the API ID 32C and the VITEK 2 system which are similar to those of *C. glabrata*. However they yield white colonies on CHROMagar, in contrast to the pink colonies usually exhibited by *C. glabrata*. Molecular approaches for the rapid detection of these species have been reported (1, 2, 3). The aim of this study was to screen Italian isolates classified as *C. glabrata* for the presence of the two cryptic species *C. nivariensis* and *C. bracarensis*.

Materials and Methods. A total of 540 yeast isolates, collected between January 2009 and June 2010 from 32 medical centers in 13 Italian regions, were included in this study. These isolates had been identified as *C. glabrata* by phenotypic and biochemical methods. Isolates were collected from: sterile body sites including blood (105 isolates; 19.4%), vaginal exudate (138 isolates; 25.5%), other biological sites (214 isolates; 39.6%). For 83 isolates (15.3%) the origin was unknown.

A total of 468 isolates were screened on CHROMagar and colony color scored as either pink or white. The remaining 72 isolates were analysed by a multiplex PCR using four primers targeting the ITS1 region and 5.8S ribosomal RNA gene (3). The combination of these primers allows discrimination between *C. glabrata, C. nivariensis* and *C. bracarensis* (3).

Results and Discussion. Among the 540 isolates examined none was identified as *C. nivariensis* or *C. bracarensis*, despite the nationwide distribution and the variety of biological origin of the isolates.This result is consistent with the results of a recent analysis of a global collection of 1598 isolates reporting a prevalence of 0.2% (4). However, because of the documented increase of these cryptic species in some European countries and their propensity to exhibit antifungal resistance, it would be prudent to continue to monitor for these emerging pathogens.

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CANDIDEMIA 2009: PRELIMINARY REPORT OF THE FIMUA WORKING GROUP

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Ten years after the FIMUA-ECMM survey on candidemia performed in Lombardia a new survey was launched in order to verify epidemiological changes.
The present survey was carried out prospectively during 2009 involving 35 Microbiology Laboratories, 30 of which in Lombardia.
A total of 468 cases were notified and 461 isolates collected. The most representative etiologies were *Candida albicans* (51%), *C. glabrata* (18%), *C. parapsilosis* (17%) and *C. tropicalis* (8%). Candidemia was associated mainly to surgery and intensive care treatments, 49 and 38% of the cases, respectively. Comparison of 1997-99 and 2009 data concerning Lombardia evidences a decrease of *C. albicans* etiology from 58 to 52% and an increase of *C. glabrata* from 13 to 20%, and a decrease of both surgery and intensive care treatments as predisposing factors, from 56 and 45% to 52 and 37%, respectively. In the centres other than Lombardia *C. albicans* represented 45% and *C. parapsilosis* 23% of the etiologies and candidemia occurred associated with intensive care and surgery in 42 and 40% of the cases, respectively.

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ECMM-FIMUA EPIDEMIOLOGICAL SURVEY ON INFECTIONS DUE TO *FUSARIUM* SPECIES IN ITALY: PRELIMINARY REPORT

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Fusarium species cause a variety of infections in humans, including superficial, locally invasive, and disseminated infections. The clinical presentation largely depends on the immune status of the host and the fungal portal of entry. The main purpose of this study is to understand the epidemiology of fusariosis in Italy collecting information on the patients infected by *Fusarium* and on the infecting isolates.
Study design. Cases of fusariosis, deep seated as well as superficial infections, for which the infecting isolate is available, have been recorded on a questionnaire and the isolate collected and identified by molecular methods**.**
Results. From January 2007 to July 2010 a total of 110 cases of fusariosis were recorded by twelve Microbiology Laboratories, namely 12 disseminated infections in patients affected with haematological malignancies; 18 respiratory tract infections, mainly in oncohaematological patients (11), or in patients with chronic lung diseases (5) or submitted to intensive care treatments (2); two infections localised to the urinary tract occurred in hematological patients; two cases of peritonitis in patients undergoing peritoneal dialysis; four cases of keratitis, three of which associated with contact lens and one following trauma; five cases of skin infection developed on traumatized tissue (3), or in patients with autoimmune disease or leukemia (2). A total of 67 *Fusarium* isolation from nail infections were also reported.
The species most frequently encountered were *Fusarium oxysporum* (34%) and *F. solani* (32%). However *F. verticillioides* predominates (55%) among isolates causing disseminated infections and *F. oxysporum* (48%) among nail isolates. Three rare species - *F. andyiazi, F. brachygibbosum, F. dimerum* - were isolated from respiratary tract samples. A cluster of *F. solani* was suspected as this species was isolated from three patients submitted to bronchoalveolar lavage in a restricted period of time.

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SIMIFF STUDY: A MULTICENTRE SURVEY ON INVASIVE MOULDS INFECTIONS IN ITALY, 2009-2010

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Objectives. Invasive mould infections (IMIs) are associated with significant morbidity and mortality in critically ill patient. SIMIFF study**,** an Italian prospective multicentre survey, aims to assess the epidemiology of proven/probable IMIs in Italy from 2009 to 2010.
Design and Methods. The survey is on-going in 22 Italian hospitals. For each episode, participating centres are required to complete a patient data form about clinical features, underlying diseases, risk factors, laboratory findings, therapeutic approaches and outcome 90 days after diagnosis.
Results. After 18 months of surveillance, 39 cases were enrolled from 10 Centres: 10 (25.6%) proven and 29 probable (74.4%) diseases. Median age of patients was 52.2 years (range 0-81 y) with a prevalence of males (69.2%).
The main underlying diseases were malignancies (61.5%, mainly haematological), respiratory diseases (15.4%) and solid organ transplantation (7.7%).
The most common site of infection was lung (84.6%): in 30 cases it represented the only localization but in 3 patients it was associated with central nervous system, paranasal sinus and liver respectively. Other involved sites were blood (7.8%), corneal tissue, peritoneum and mediastinum (2.5% respectively).
Aspergillus spp was the most frequent isolate (77%), followed by *Trichoderma viride* (7.7%)*, Fusarium solanii*, *Fusarium dimerum*, *Paecilomyces* spp, *Scedosporium apiospermum* (3.8% respectively). In 30 patients diagnosis was carried out by cultural and/or histological surveys (18 patients were positive also for galactomannan test); the remaining 9 patients resulted positive only to galattomannan test.
Crude mortality was 39.4%.
Conclusion. Preliminary data of this study underline the majority of IMIs are caused by the well-known opportunistic pathogens (*Aspergillus* spp) and occur mainly in immunocompromised patients; however, less common moulds are emerging as further causes of diseases. These survey could provide useful propositions for future epidemiological studies in Italy.

Study participants: Ancona (F. Barchiesi, E. Manso); Bari (C. Buquicchio, M. Delia, G. Donvito, N. Lopatriello, A. Matarrese, G. Specchia); Catania (S. Oliveri, L. Trovato); Catanzaro (R. Masciari, P. Scervo); Genova (C. Viscoli, M. Mikulska); Palermo (S Giordano, R Monastero); Perugia (A Carotti, V De Angelis, L Pitzurra); Roma (L Pagano, M Sanguinetti); Torino (A.M. Barbui, R. Serra); Udine (A. Candoni, C. Scarparo)

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ANTIFUNGAL COMBINATION THERAPY IN ITALIAN HEMATOLOGICAL CENTERS: THE SEIFEM-COMBO STUDY

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This observational Clinical Trial (NCT 00906633) evaluated the feasibility, efficacy and toxicity of Antifungal Combination Therapy (Combo) as treatment of proven or probable IFI in Hematological patients (pts). Between Jan 2005 and Dec 2009, 76 cases of Combo were reported from 18 Hematological Dpt in Italy. Median age of pts was 33 yrs (range 1-73) and 41% had less than 18 yrs. Acute Leukemia was the most common underlying hematologic disease (60/76; 79%). Status of hematologic disease: onset 18/76 (23%), remission 18/76 (24%), refractory/relapse 40/76 (53%). The main site of infection was lung with or without other sites. Fungal pathogens: *Aspergillus* sp 60 cases (79%), *Candida* sp 5 cases (7%), *Zygomycetes* 4 cases (5%), *Fusarium* sp 4 cases (5%). The most used Combo were: Caspofungin+Voriconazole 33/76 (43%), Caspofungin+Liposomal Amphotericin B (LAmB) 19/76 (25%), and LAmB+Voriconazole 12/76 (16%). The median duration of Combo was 21 days (range 3-180). The overall response rate (ORR) was 79% (60/76 responders) without significant differences between the Combo regimens. The most important factor that significantly influenced the response was PMN recovery during Combo (P 0,001). Only one pt discontinued therapy (voriconazole related neurotoxicity) and 18% experienced mild and reversible adverse events (hypokalemia, ALT/AST increase, creatinine increase). The IFI attributable mortality was only 15%. This study indicates that:

1) Combo was rarely used in Italian Hematological Dpt;
2) Combo was effective and well tolerated in both children and adults pts.
3) The most used Combo regimens were Caspofungin+Voriconazole and Caspofungin+L-AmB.
4) The ORR was 79% and the mortality IFI related was 15%. PMN recovery during Combo predicts a favourable outcome.

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