Invasive Fungal Infections: new challenges and therapeutic developments
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Abstract

The European Congress of Clinical Microbiology and Infectious Diseases, the annual meeting of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID,) is the most attended infectious disease conference worldwide. This year, more than 10,000 clinicians and scientists attended the congress to present and share the latest research breakthrough in infectious diseases. This article reviews the sessions that addressed the challenges of managing the increasing rates of invasive fungal infections (IFIs) and new diagnostic and therapeutic developments in the area of IFIs.

Key words: invasive fungal infections (IFI), *Candida*, *Aspergillus*, osteoarticular infections, transplantation, antifungals, azole resistance, fungal stewardship, fungal PCR

Optimising antifungal therapy

Working collaboratively to bridge laboratory and clinical expertise optimises antifungal therapy. This was the message from the 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). As part of an educational workshop on antifungal therapy organised with the ESCMID Fungal Infection Study Group (EFISG), Oliver A. Cornely (University of Cologne, Germany), questioned whether clinicians can start or stop antifungal therapy based on biomarkers detection. He discussed the current antifungal strategies – prophylaxis, empirical treatment (which is usually fever driven), pre-emptive therapy (diagnosis driven), and treatment for invasive fungal infection (IFI) (Cornely 2015a). Then Cornely described a new approach entitled '*2 Biomarkers 2 Fungi 2 Decisions*' where the fungi are *Candida* and *Aspergillosis*, the biomarkers are galactomannan (GM) and ß-D-glucan (BDG) and the decisions taken by clinicians will be influenced by the fungi and biomarkers. He pointed out that early exposure to antifungals is a common pattern of all

clinical trials as the aim is to improve survival rates. Cornely believes that reliable diagnostic tests would allow early treatment to be targeted. However, at the moment, diagnostic tools are “too few and are unreliable”. He presented the case of a patient from the AmBiLoad study with strong positive GM and another case where BDG was used as a diagnostic tool.

In the case of IFI, Cornely discussed AmBiGuard monitoring where patients were routinely monitored for signs and symptoms of IFI throughout the study period, and had twice weekly GM and BDG with diagnostic workup if there was one positive GM/BDG antigen assay. Algorithms were then followed for investigation and management of suspected IFI. Cornely recommended that a positive GM should trigger immediate diagnostic work-up; negative GM is a prerequisite, but not sufficient for stopping treatment; positive BDG should not trigger treatment while negative BDG should be used for stopping empiric *Candida* directed treatment. Both tests should ideally be used in the context of clinical judgement, other IVD assays and imaging studies. He added that institutional algorithms should be informed by ESCMID/ European Federation of Medical Mycology (ECMM) guidance.

The role of therapeutic drug monitoring (TDM) of antifungals was discussed by Joseph Meletiadis (National and Kapodistrian University of Athens, Greece) (Meletiadis 2015). He explained that in order to achieve success, the clinician must be aware of the host defences and management of site of infection and consider that appropriate antifungal therapy depends on susceptibility, timing and drug dose. Meletiadis discussed the variation in dose and serum concentrations of antifungals based on pharmacokinetics (PK) variation.

He explained the sources of PK variation, such as age (larger extracellular and total-body water spaces in neonates), gender (women empty solids from the stomach more slowly and have higher gastric pH), physiological factors (body size and composition, gastrointestinal physiology, hepatic status and renal excretion), pathological conditions (renal or hepatic insufficiency), drug interactions, environmental factors (pollutants or diet), chemical properties (e.g., the AUC0-48 and *C*max of [−]-itraconazole were three to four times higher than those of [+]-itraconazole), and genetic polymorphisms (SNPs in drug metabolising enzymes and efflux proteins). He further described the principles of TDM, including measuring drug concentrations in blood and adjusting the dose in order to reduce toxicity, increase efficacy, how to prevent emergence of resistance and avoid breakthrough infections.

There are several methods of TDM, each with their advantages and disadvantages. Meletiadis emphasised that while bioassays are cheap and simple to perform, there is interference from other drugs, including other antifungals and metabolites (e.g., itraconazole); HPLC with ultraviolet fluorescence detection are widely available; in commercially available assays, can test multiple drugs in single sample, but there is interference from miscellaneous substances and the run times may be slow. He explained that while liquid chromatography–mass spectrometry is very sensitive and specific and can test multiple drugs in single sample, it is expensive and not widely available. In his view, a drug assay should be accurate, sensitive, precise, specific, with a short turnaround time, and cost-effective with minimal sample volumes.

Meletiadis discussed the profile of drugs for TDM, from variable PK in the case of drugs with erratic/saturable absorption (itraconazole, posaconazole), changes in distribution (fluconazole), differential/saturable metabolism (voriconazole), to altered excretion (fluconazole, flucytosine); exposure-toxicity relationship (flucytosine, itraconazole, voriconazole), and exposure-response relationship (voriconazole, itraconazole, posaconazole, flucytosine). In the case of PK variation in absorption for itraconazole, he explained that itraconazole has increased capsule solubility in acidic environment,

manufactures' variability, reduced absorption with PPI and H2-antagonists, and in suspension, it has 20-50% higher bioavailability; and extensive variability.

Posaconazole oral suspension is saturated above 800 mg/day, has better absorption with fatty food and low stomach pH, reduced absorption with mucositis, diarrhoea, and PPIs; with tablet/caps, there is increased bioavailability and large variability.

As in previous years, the topic of antifungal resistance was discussed at the ECCMID. Maiken Cavling Arendrup (Statens Serum Institut, Copenhagen, Denmark) addressed the session by asking whether antifungal resistance occurs everywhere (Arendrup 2015). She explained that it is important to understand what clinicians mean by antifungal resistance, whether it is intrinsic or acquired resistance, related to species or mechanisms, or if it is due to *Candida* or *Aspergillus*. She discussed yeasts resistance, especially the range of *Candida* species with intrinsic resistance, and moulds (*Aspergillus* species remain fully susceptible) that have showed resistance, intrinsic or acquired, to particular antifungals, such as amphotericin B, azoles or echinocandins. Arendrup described the mode of action of systemic antifungals involved in acquired resistance, and the compound target and target gene mutation. She explained how widespread intrinsic azoles resistance has become, especially for *Candida glabrata* and *C parapsilosis*, and discussed the link between increasing antifungal exposure and intrinsic resistance based on data from Denmark and the U.S. Arendrup presented the results of studies on echinocandin use and resistance as well as data on acquired azole resistance to *Candida* from the nationwide surveillance of fungaemia in Denmark. She described the 'high endemic' centres for intrinsic resistance to *Aspergillus terreus* in Houston, Texas, and in Innsbruck, Austria, while intrinsinc resistance to *A flavus* is more common in developed countries where 50-80% of cases of allergic fungal rhinosinusitis are found in India and in the Middle East. Arendrup described data on fungicide use and azole resistance worldwide, and then specifically on azole-resistant *A fumigatus* in azole-naïve patients or in the environment detected in Europe (TR_{34}/L98H), with new resistance mechanisms in the Netherlands (TR46/Y121F/T289A) and in France (G432S). She concluded that intrinsic resistance in *Candida* occurs everywhere, echinocandin resistance in *Candida* is emerging in exposed patients everywhere, acquired resistance in *Candida* does not occur in naïve patients anywhere while acquired *Aspergillus* occurs everywhere.

Sevtap Arikan-Akdagli (Hacettepe University Medical School, Ankara, Turkey) presented the new antifungals in the pipeline, specifically drugs recently approved, antifungals compounds in phase II/III clinical trials, and investigational drugs in phase I/II preclinical trials (SCY078/MK-3118, VT-1161, etc) (Arikan-Akdagli 2015). She discussed the targets and mechanisms of actions of antifungals, the early antifungal pipeline and the changing face of antifungal drug spectrum. Arikan-Akdagli explained that the new drugs were expected to have certain advantages over the established drugs, such as efficacy in difficult to treat IFI, more favourable safety and PK profile that will enable reduce dosing, improved formulation and reduced adverse events. She described the results of the clinical trials on isavuconazole (SECURE, VITAL and ATIVE studies), efinaconazole, luliconazole, albaconazole and other compounds in different stages of development, and emphasised the need for new effective antifungals.

Challenges in fungal infections management

The management of IFI after solid organ transplantation (SOT) is particularly challenging, explained Patricia Muñoz (Universidad Complutense de Madrid, Spain) (Muñoz 2015a). She discussed the complexity of the SOT patient from the perspective of organ insufficiency, immunomodulating coinfections and their high risk of toxicity and drug-drug interactions. The incidence of fungal infections in these patients depends on the type of allograft. Muñoz described some cases of late and uncommon infections, for example, a case of prostatic aspergillosis after a heart transplant. She emphasised the great impact of IFI on post-

transplant survival in a retrospective analysis of 502 liver transplant patients with a 12% incidence of IFI and 40% survival at 350 days in the patients with IFI. Muñoz discussed the indications for antifungal prophylaxis in SOT depending on the solid transplant organ and risk factors in accordance with the European consensus on AF in SOT recipients. She specifically described the risk factors for invasive aspergillosis (IA) in SOT, whether early IA or late (more than 3-months post transplant) and a study of IA following heart surgery where despite tailored prophylaxis, there was an outbreak of IA in the ICU caused by the spores in the air of the ICU. Heart transplant recipients are particularly affected, explained Muñoz, with extracorporeal membrane oxygenation (ECMO) being the strongest predictor for fungal infection (OR 29.93 95%CI, 1.51-592.57, p=0.03). The longer the ECMO, the higher the risk of FI. She also discussed the results of the TENPIN study that have showed that in high-risk liver transplant recipients who received in one arm of the trial, micafungin 100mg (n=133) compared with centre standard care, fluconazole (n=62), liposomal amphotericin B (n=59), caspofungin (n=21); both arms were very effective and while adverse events, including liver function, were similar in both arms, kidney function was better with micafungin. Muñoz discussed the challenges faced by clinicians in diagnosis IFI in SOT, the lack of experience and diagnostic tools and the need for specific definitions.

Emmanuel Roilides (Aristotle University, Thessaloniki, Greece) discussed the impact of osteoarticular infections due to *Aspergillus* and other moulds, highlighting the fact that within aspergillosis, osteomyelitis is the fourth most common site of infection following pulmonary, sinus, and cerebral infections (Roilides 2015). The mechanisms of infection are direct inoculation, haematogenous or contiguous. Roilides described the criteria for diagnosis; proven cases require a positive culture and/or histology from bone tissue or metal hardware; probable cases require compatible clinical and radiological features of osteomyelitis and a positive culture of *Aspergillus* and/or histology from a site other than bone tissue or metal hardware; breakthrough or *de novo* cases require patient on (or not on) systemic antifungal agents before or at the clinically apparent onset of *Aspergillus* osteomyelitis. The criteria for outcomes include complete response with the complete resolution of clinical and radiological findings of osteomyelitis or partial response with partial resolution of clinical and/or radiological findings of osteomyelitis, or partial clinical improvement without availability of radiological data.

Roilides presented the IDSA guidelines on the management of *Aspergillus* osteomyelitis that recommend antifungal therapy and individualised surgery based on the site and local complications to achieve a favourable outcome; voriconazole or liposomal amphotericin B are recommended for at least 6-8 weeks of therapy. He discussed the particular cases of vertebral, rib and skull base osteomyelitis. The moulds that cause osteomyelitis include non-*Aspergillus* filamentous fungi with 64.8% of infections due to hyalohyphomycosis, the rest due to phaeohyphomycosis and mucormycosis, explained Roilides. These mycoses are more common in children after injury and in adults after surgery. According to ESCMID and ECCM guidelines, hyalohyphomycosis requires surgery plus voriconazole (400mg) or L-amphotericin B (5mg/Kg), mucormycosis is treated with surgery plus L-amphotericin B (5mg/Kg) and/or posaconazole (800mg); phaeohyphomycosis is managed with surgery plus itraconazole (400mg), voriconazole (400mg), posaconazole (800mg), or L-amphotericin B (3mg/Kg).

Candida and dimorphic fungi can also cause osteoarticular infections and these infections do not always present in immunocompromised hosts, explained Olivier Lortholary (Université Paris Descartes, Paris, France) (Lortholary 2015a). Clinicians should be suspicious in the case of subacute or chronic bone infections with negative bacteriological investigation, look out for the appearance of bone or joint symptoms during or after an IFI, and bear in mind that there is a moderate inflammatory response.

He highlighted the increase in *Candida* osteoarticular infections based on an analysis of IFI incidence trends in French hospitals database from 2001-2010 and a review of 207

published cases of candidemia from 1970-2011. Lortholary described the review in which *Candida* osteomyelitis was the first proven *Candida* site involvement in nearly one half of patients with the remaining half of patients initially had candidaemia or other type of candidiasis. However, 15% of patients had concomitant candidaemia at the time of *Candida* osteomyelitis diagnosis. He pointed out that 71% of cases of *Candida* osteomyelitis were diagnosed before antifungal therapy initiation and the rest of the infection occurred as breakthrough infection during antifungal therapy. He also addressed the diagnosis and management of *Candida* vertebral osteomyelitis, *Candida* osteoarthritis in intravenous drug users, *Candida* non-prosthetic and prosthetic joint infections. The audience was surprised to hear Lortholary describe three cases of *Candida* nosocomial infections due to artificial nails.

William Hope (University of Liverpool, UK) gave an engaging “esoteric” presentation on making clinical use of an increased understanding of antifungal PK/PD in the past 10 years (Hope 2015). He examined the advantages and limitations of using tissue concentrations for a more complete understanding of antifungal effects, starting with determining where is the pathogen, for example, epithelial lining fluid (ELP), lung tissue or pulmonary alveolar macrophages. *Aspergillus* is both an intracellular and extracellular pathogen. Hope explained that while ELP is very useful to determine efficacious antifungal exposure in prophylaxis and early infection (first 24 hours) and triazoles and polyenes achieve measurable levels in ELP, it is unlikely to offer more information in the case of advanced pathological changes. He reminded the audience of the concept of hysteresis and gave examples of PK and pharmacodynamics (PD) of different antifungals. For example, caspofungin achieves very high concentrations in the kidney and has a long mean residence in the tissue which explains its persistent antifungal effect. Hope touched on the antifungals dosage problem and whether clinicians should deviate from the drug licence based on clinical practice.

Prophylaxis or treatment of invasive fungal infections

Invasive fungal infections continue to be difficult to treat as clinicians consider whether prevention or treatment might be the cornerstone of IFI management. In his presentation on this topic, Rafael Duarte (Madrid, Spain) discussed whether novel diagnostic tools help improve survival and queried the possible confrontation between prophylaxis and novel diagnostics in IFI (Duarte 2015). He explained that when talking about the impact of effective prophylaxis on diagnostics, we need to bear in mind that a valid test can be unreliable in some clinical scenarios, and that, for a given sensitivity and specificity, the main driver to test performance is the pre-test prevalence of the largest event. Duarte presented examples on the use of the diagnostic tool calculator to showcase probability pre-test. One study he mentioned used serum GM-based early detection of invasive aspergillosis (IA) in haematology patients receiving effective antimould prophylaxis. In this study, serum GM test results determined the distribution of the high-risk episodes which confirmed a very low incidence of breakthrough IA of less than 2%; there were false-positive events to be accounted for, but serum GM early detection of IA remains an important asset to diagnose persistently febrile symptomatic patients.

Duarte discussed the ICO experience in prophylaxis and diagnostics, specifically the data on breakthrough IF disease (IFD) as per European Organisation of Research and Treatment of Cancer (EORTC) criteria, and the impact of international guidelines and novel agents. He commented on the results of the AmBiGuard trial and of other antifungal studies and the 2013 recommendations for the management of AML patients undergoing chemotherapy as well as recommendations for antifungal prophylaxis for allogeneic haematopoietic stem cell transplantation (HSCT) recipients. Duarte discussed trial results of posaconazole oral suspension 300mg or tablet 300mg, and novel agents such as isavuconazole (SECURE trial) and voriconazole. He emphasised the need for a new posaconazole formulation that will have better predictability of blood levels and be used in

patients in critical conditions. There is also a need for novel diagnostic tools to drive treatments that are based on prophylaxis. The responsibility is to obtain improvements in prophylaxis and treat efficiently. Duarte emphasised that clinical practice needs to reconcile prophylaxis of IFI with diagnostic tools and treatment.

Patients at risk of IFD benefit from posaconazole intravenous (IV) solution due to its specific PK and safety profile, explained Oliver Cornely (University of Cologne, Germany) (Cornely 2015b). He presented the risk factors for IFD, including prolonged neutropenia following chemotherapy and graft versus host disease (GVHD) after allogeneic HSCT. Posaconazole oral suspension (OS) is an extended-spectrum triazole with demonstrated efficacy in prophylaxis and treatment. However, some patients with mucositis, nausea or diarrhea have problems taking the oral formulation and will benefit from posaconazole IV solution. Cornely explained that this new formulation is an aqueous solution which has a solubilizer, sulfobutyl ether betacyclodextrin. It is designed to ensure adequate exposure in patients unable to tolerate or absorb oral posaconazole.

He presented the results of the 2-part phase 1B/3 study evaluating the PK and safety of posaconazole IV solution (300mg) in 237 patients at risk of IFD that included neutropenic patients with AML (n=147; 62%), MDS (n=8; 3%) or post-allogeneic HSCT (n=82; 35%). In the study, patients received posaconazole IV 300 mg bid on day 1, followed by 4-28 days of posaconazole IV solution 300mg qd, followed by posaconazole oral suspension 400mg bid or 200mg tid to complete a 28-days of posaconazole dosing. Cornely explained that the primary PK parameters of interest were C_{avg} steady state average plasma concentration and C_{min} trough levels. The target exposure in the study was C_{avg}≥500 and ≤2500ng/dL in 90% of trial subjects. There was a subset of 49 PK evaluable patients with ≥10 days of IV dosing of which 46 (94%) attended the steady state exposure target of C_{avg}≥500ng/dL and ≤2500ng/dL with similar steady C_{avg} across the groups. The posaconazole IV solution was well tolerated; the most common adverse events were diarrhoea (8%), nausea (5%), rash (5%), vomiting (4%) and hypokalaemia (4%). Cornely pointed out that infusion site/phlebitis/thrombosis were each reported in ≤1% of patients receiving posaconazole IV solution.

Dimitrios P. Kontoyiannis (Houston, USA) discussed the impact on serum levels when switching from posaconazole oral suspension to tablet (Kontoyiannis 2015a). He mentioned that the epidemiology of fungal infections has evolved over the past 20-30 years due to organ transplantation, leukaemia, antifungal resistance and mould infections with *Aspergillus*. While guidelines have agreed that posaconazole is the preferred drug in AML/MDS and high-dose costicosteroids in alloHSCT, Kontoyiannis explained there are gaps in antifungal coverage because in real life the patient doesn't present like in the guidelines. He discussed the 12-year experience of treating breakthrough IFI in 261 patients with AML/MDS undergoing cytotoxic chemotherapy at Royal Melbourne Hospital. Patients on posaconazole/voriconazole showed significant reduction in premature discontinuation (46% vs 22%, $p<0.001$) and in empirical treatment (31% vs 8.5%, $p<0.001$). However, those on posaconazole had fewer courses requiring CT (43% vs 25%, $p<0.001$). The number needed to treat (NNT) for posaconazole prophylaxis was 6.

Kontoyiannis presented the incidence rates of documented mould and yeast IFI 120 days after first remission-induction chemotherapy (RIC) in 152 patients with newly-diagnosed AML that underwent prophylaxis from 2009-2011. Then in late 2013, when posaconazole tablets became available, nearly all patients in leukaemia service who were on posaconazole oral suspension (n=12) were bridged to the new formulation (300mg daily), of which 3 patients received posaconazole as prophylaxis and 9 as treatment. In the 9 patents who switched formulation, there was a significant increase in median posaconazole concentration.

Cornelius Clancy (Pittsburgh, USA) discussed the facts behind *Candida* and *Aspergillus* susceptibilities, *Candida* susceptibility testing and *FKS* mutation (Clancy 2015).

He described the *Candida* species included in the ESCMID guidelines for the diagnosis and management of *Candida* diseases 2012 in adults with haematological malignancies and alloHSCT and presented US data of *FKS* mutation of *C. glabrata*. For example, in Pittsburgh from 2007-2014, there were 4-8% FKS mutant *C. glabrata*, 18% in Houston (2009-2012) and 8% in Duke (2001-2010). Prior echinocandin exposure is the key risk factor for *FKS* mutation, followed by duration of exposure.

Clancy explained that *FKS* mutations are rare among other *Candida* species - 1-5% for *C. albicans*, and less for *C. tropicalis*, *C. krusei*, *C.* parapsilosis. He described the hidden reservoirs of antifungal resistance: only 3% of *Candida* recovered from intra-abdominal candidiasis (IAC) undergo susceptibility testing, while 24% of patients with IAC who have received an echinocandin are infected with *FKS* mutant *Candida* of which 50% were breakthrough (83% *C. glabrata*), 7% due to distant exposure and prolonged exposure. Clancy explained that *FKS* mutant candidaemia is often preceded by IAC, and the mortality rate for *FKS* mutant IAC is 100% despite source control intervention. He presented his own take on echinocandin resistance was to keep it in perspective – FKS mutations are hard to induce in the clinic, they are rarely seen without prior exposure and very rarely encountered in non-*C. glabrata* species. Most treatment failures are not due to microbiologic resistance, but are due to clinical resistance, biofilms, local PK, host immune function, and/or underlying conditions. Clancy recommended that other agents should be used in patients with breakthrough candidaemia, or recent, extensive prior echinocandin exposure. Echinocandin susceptibility testing may be a useful management tool in treatment failures or in patients with prior exposure. He explained that assays for *FKS* mutations may be useful in certain cases while routine susceptibility testing and *FKS* mutation screening are important for epidemiological purposes and surveillance for resistance. However, caspofungin MICs and CBPs by reference broth dilution methods are not reliable.

Paul E. Verweij (Nijmegen, Netherlands) presented the facts behind azole-resistant *Aspergillus*, a growing challenge for the management of IFI (Verweij 2015). He explained that acquired azole resistance is increasingly recognised in *A. fumigatus*. In the Netherlands, azole resistance prevalence varies per institute with an overall prevalence of 7.8%; environmental prevalence is 82% with 58% TR34 and 24% TR46. Verweij described the data on azole resistance from Leiden University Medical Center where in the period 2011-20133, there were 38 patients with *A. fumigatus* culture positive IA in the ICU of which 10 (26%) patients were azole resistant; at Utrecht University Medical Center, during the same period, the frequency of patients with azole-resistant isolates was 16.2%, of which 24.6% were in the haematology department and 4.5% in ICU. He presented a case study of a 71-year-old male who developed possible IA in ICU following a kidney transplant and was prescribed voriconazole initially but then died of respiratory failure. The autopsy showed proven IA, multiple fungal lesions in the lung and one fungal lesion in the transplant kidney. Verweij used the case study to explain the clinical implications of IA. In the case of the environmental route of IA, it can be any *Aspergillus* disease, 64% of patients would have no previous azole resistance. It has high mortality, specific mutations and azole R and azole S co-infection. He recognised that timely diagnosis of azole resistance is difficult – it should be prevented if possible with posaconazole; treatment options include L-amphotericin B, high dose voriconazole and possibly posaconazole IV.

Evolving epidemiology of invasive fungal infections

Paul Verweij (Nijmegen, Netherlands) chaired a lively interactive panel discussion on the management of IFI in the face of evolving epidemiology. He explained that the epidemiology of IFI is changing and resistance is emerging in some pathogens such as *C. glabrata*. Diagnosis is very difficult, as sometimes clinicians are unable to get adequate samples. Tests might not be available in some centres due to cost; the assays do not have the right sensitivity and specificity. There are issues around identifying the organism causing the IFI

as well as the treatment. Treat patients as quickly as possible, but in comparison with antibiotic fungal, it is unclear what the benefits are of combination antifungal therapy, Verweij added.

The aim of the session was to engage the audience and the expert in the field in an interactive session about the impact of various antifungal treatment strategies on the epidemiology of IFI, the current and future state of antifungal susceptibility testing, the role of antifungal stewardship in the management of IFI and antifungal sequencing using a clinical case to demonstrate the potential approaches.

Olivier Lortholary (Université Paris Descartes, Paris, France) discussed epidemiological trends in IFI and asked the audience which organisms have shown increased incidence in recent years in the delegates' working place (Lortholary 2015b). He explained the risk factors of IFI, for both endogenous and exogenous IFI, and the French Hospital Database experience of IFI from 2001-2010. There were 35,876 incidents of IFIs registered of which 43.4% candidaemia, 26.1% *P. jirovecii* pneumonia, and 23.9% IA. Lortholary discussed data from the prospective surveillance of IFI in France (RESSIF) from 2012-2014, which analysed results from 25 microbiology laboratories in university hospitals. There were 3990 IFI episodes registered in this period; 48.7% were due to fungaemia, 19.8% *P. jirovecii* pneumonia, 16.4% IA, 2.6% cryptococcosis and 2.2% mucormycosis. In France, the main risk factor for IA was haematological malignancies (78% of IA). In the U.S., in HSCT patients, Mucorales comprised 62% of non-*Aspergillus* mould infections. Aspergillosis remains the number 1 killer in haematological malignancies while in some ICUs, there has been an increased incidence of candidaemia due to *C. albicans*, *C. glabrata* and mortality; increased frequency of yeasts and mortality in septic shock complicating cirrhosis. Lortholary explained that aspergillosis has also become a major killer in alcoholic hepatitis.

Cristina Toscano (Universidade Nova Lisboa, Lisbon, Portugal) discussed evolving role of antifungal susceptibility testing, including the pros and cons of reference methods for yeasts and molds (EUCAST, CLSI) as well as commercial methods for yeasts such as Etest-caspofungin susceptibility testing *Candida* species, Vitek 2, Vitek 2 YS07, Sensititre Yeast One, SensiQuattro *Candida* EU; and, commercial methods for moulds such as disk diffusion, 4-well plate/VIP check, Etest, Sensititre Yeast One (Toscano 2015). Toscano reminded the audience that Etest cannot be recommended for *in vitro* susceptibility testing of Mucorales. She emphasised that while commercial methods are easy to perform, they have limited scope, do not mirror reference breakpoints, and produce variable inter-laboratory results, so she emphasised the need to test your method first. Future promising methods include PCR for mutation detection, MALDI-TOF for *Candida*/*Aspergillus* and microcalorimetry. However, these methods need standardisation.

Patricia Muñoz (Universidad Complutense de Madrid, Madrid, Spain) presented an interactive session on antifungal stewardship –prevention and control. She asked the audience whether their hospital has an antifungal stewardship programme (Muñoz 2015b). Interestingly, 32% of delegates had such a programme and found it useful, while 44% of delegates do not have it but would like to have it. In the attendees' centres, 57.1% of delegates have controlled prescription of certain antifungals, 47.6% had received education on antifungal therapy in the last year, 41.3% have written guidelines for the management of fungal infections, and 34.9% had access to monitoring voriconazole levels. Muñoz described antifungal stewardship as the practice that assures the optimal selection, dosage and length of antifungal therapy, which is one of the most important reasons for inadequacy in their centres. She explained that most common problems with antifungal therapy are too much

empirical therapy in ICU, colonisation, combination therapy or excessive prophylaxis and no adjustment for microbiology results.

Muñoz's antifungal strategy has three pillars: education, diagnosis and therapy. In her centre, she organised a collaborative group with official support, included the most important prescribers, provided leadership, and gained prescriber acceptance and institutional support. The next step was to identify the knowledge gaps and prescription problems through an audit, followed by initiating educational activities and producing local consensus guidelines. Muñoz recommended multidisciplinary interventions in the pharmacy department and clinical microbiology as well as bedside interventions. She discussed what would be compulsory in a stewardship programme versus non-compulsory aspects, advised delegates to select and share their goals and to told them to always celebrate their successes.

Dimitrios Kontoyiannis (University of Houston, Texas, USA) discussed passionately the case of a long-term survivor with disseminated mixed mould infection and shared with the audience the lessons he learned that "no trial has addressed or could address" (Kontoyiannis 2015b), The patient was a 24-year-old Caucasian female with AML, admitted for neutropaenia, new fever and diarrhoea. She had an autologous SCT followed by AML relapse and then allogenic MRD SCT, complicated by skin and ocular GVHD. She had a second AML relapse and salvage chemotherapy. The patient was on a cocktail of prophylactic antibiotics and antifungals (itraconazole suspension) and had persistent fever on broad-spectrum antibiotics. Kontoyiannis described the stages in investigations and management - antifungal therapy typically triggered by symptomatic or radiographic evidence of breakthrough infection. The patient's condition worsened in the next days and a repeat chest CT showed progressive multifocal nodular pneumonia, and she developed sudden bilateral visual loss. Voriconazole was added and the L-amphotericin B dose increased. An emergent brain MRI was performed. Emergency left occipital craniotomy and open biopsy was performed and found angioinvasive, branching septate hyphae in brain tissue followed by right occipital craniotomy and evacuation of abscess that found angioinvasive hyphae in necrotic brain. The patient had re-exploration of left occipital craniotomy and evacuation of residual abscess that found hyphae necrotic brain. Posaconazole 200mg qid suspension was added to L-amphotericin B; voriconazole and caspofungin were discontinued. Immunotherapy was commenced. Kontoyiannis described how the patient improved, went in remission and continued on posaconazole 200mg qid as maintenance. However, at a later stage the patient requested a break from posaconazole and stopped treatment, despite being cautioned against a treatment holiday. She had a clinical relapse a few months later. Currently, she remains on posaconazole after more than 10 years with no toxicity and will continue on the drug for life. Kontoyiannis shared the lessons he learnt from this, especially that knowledge is better than ignorance and the importance of a specific diagnosis. There was no evidence of posaconazole resistance after 10 years of use, and there was no change in posaconazole levels over time. He advises delegates to be reluctant to stop secondary prophylaxis in the setting of cavitary lung lesions.

Fungal PCR for invasive aspergillosis

IFI such as invasive aspergillosis (IA) are important causes of morbidity and mortality in immunocompromised patients, explained Lena Klingspor (Karolinska Institutet, Stockholm,

Sweden) (Klingspor 2015). It is well known that *A. fumigates* is the most common cause of IA in humans, which pose a diagnostic and therapeutic challenge. Klingspor discussed the current diagnosis methods (culture-based methods), which lack sensitivity and delay diagnosis; and emphasised the need for more rapid, practical and reliable tests with high sensitivity and specificity for detecting *Aspergillus* directly from blood, sterile fluids and tissue. It is hoped that molecular methods such as DNA detection by PCR will improve diagnosis. She described the types of clinical specimens most commonly used for molecular diagnostic testing in patients at high risk of IA, including serum, plasma, whole blood, broncho-alveolar lavage (BAL) and fresh or paraffin-embedded tissue from affected sites. However, it remains to be determined which blood fraction is best to test for early detection of fungaemia. Klingspor explained that there is a lack of standardisation of *Aspergillus* PCR assays that has limited its acceptance as a diagnostic tool. It is hoped that the standardisation for *Aspergillus* PCR assay in blood, serum and plasma that has now been proposed by the European *Aspergillus* PCR initiative (EAPCRI) will help.

Another controversial area mentioned by Klingspor was the choice of primers, species-specific vs. pan-fungal, with pros and cons for each choice. The specificity of the primers is crucial for the detection of *Aspergillus*-specific DNA. She explained that pan-fungal primers can give rise to false-positive results due to environmental fungal DNA contamination and may hybridise with non-targeted fungi (in specimen types such as BAL) and also with human DNA. Furthermore, the specificity of the amplicon cannot be determined if non-specific probes are used. *Aspergillus* species-specific primers miss infections caused by untargeted fungi (such as fusarioses, mucormycoses, candidosis etc.), and *A. fumigates* specific primers can miss infections caused by other *Aspergillus* spp.

The pros and cons of pan-fungal PCR were discussed by Rosemary Barnes (Cardiff University, UK) including the differences between real-time and pan-fungal PCR (Barnes 2015). She explained that for species-specific PCR, a particular infection needs to be suspected for the test to be diagnostically useful. Pan fungal PCR is more suited to multiplex reactions where a range of potential pathogens can be targeted. Barnes added that while in the past, the addition of multiple primers and probes limited sensitivity, newer genomic and proteomic approaches such as PCR-electrospray ionisation mass spectrometry are emerging with the potential to detect all known (and some unknown) pathogens. She described the pros of pan-fungal PCR, including its broad-range detection, and it being ideal for screening as it reduces the chance of “missed” fungal infection. Pan-fungal PCR can detect emerging infections and outbreaks, and it is more suitable for multiplex syndromic diagnosis. Barnes mentioned that pan-fungal PCR has improved turnaround times and reduced cost compared to multiple monoplex reactions. It is also more suited to emerging genomic and proteomic technologies such as PCR electron spray ionisation/mass spectrometry, and provides limited validation in different specimen types. However, there are cons. Barnes explained that the range of potential pathogens implicated limits the commercial interest of pan-fungal PCR. It is susceptible to false positives due to contamination from environmental fungi as well as compromised sensitivity at the limit of current PCR detection. She added that species specific probes are needed as melt curve analysis lacks specificity across the range of potential pathogens. Other cons were: sensitivity can be compromised by commensal flora, while the low prevalence of non-*Candida* non-*Aspergillus* infections limits its clinical utility and increased the chance of hybridising with human DNA.

Choice of antifungal in IA

Sebastian Heinmann (University Hospital of Cologne, Germany) presented the results of a study that looked at direct treatment costs for IA (Heinmann 2015). The researchers analysed data extracted from the Cologne Cohort of Neutropenic Patients (CoCoNut), which

was split into patients receiving liposomal amphoptericin B (LAmB), voriconazole (VCZ) or caspofungin (CFG). Cost factors were analysed from the German societal perspective and included treatment on general ward and intensive care unit, anti-infective treatment, diagnostic measures, radiological findings, and laboratory tests. Costs were expressed in EUR (€), year 2013 values. Discounting of costs was performed with an annual discount rate of 5%.

Heinmann and his colleagues identified 166 patients with underlying haematological disease, who were treated with LAmB, VCZ, or CFG due to IA; of which 155 (63.3%) had AML or ALL as primary underlying disease and were included into pharmacoeconomic analysis. Thirty-five patients (33.3%) were treated with LAmB, 31 (29.5%) with VCZ and 39 (37.1%) with CFG, whereby distribution of treatment with LAmB, VCZ, and CFG due to possible, probable, or proven IA was as follows: 31 (88.6%), 3 (8.6%) and 1 (2.9%); 29 (93.5%), 2 (6.5%), and 0 (0%); 33 (84.6%), 4 (10.3%) and 2 (5.1%). Patients in the LAmB, VCZ, and CFG group had a mean overall length of stay of 55.6 days (95%CI: 48.7–62.6), 56.5 days (95%CI: 46.8–66.1) and 53.9 days (95%CI: 44.7–63.0, P= 0.907), were neutropenic for 22.8 days (95% CI: 16.5–29.2), 25.2 days (95% CI: 18.9–32.1) and 22.2 days (95% CI: 17.8–26.7, P= 0.696) and were treated with LAmB, VCZ, and CFG for 20.8 days (95% CI: 16.5–25.1), 22.4 days (95%CI: 14.7–30.1) and 21.3 days (95% CI: 17.0–25.6, P= 0.666). Treatment of patients in the LAmB, VCZ, and CFG group caused in mean overall daily treatment costs of €732 (95%CI: 658 – 807), €654 (95%CI: 654 – 743) and €717 (95%CI: 645 – 789, P= 0.333) and mean overall treatment costs of €41,312 (95%CI: 34,634 – 47,900), €35,805 (95%CI: 28,195 – 43,416) and €38,157 (95%CI: 31,664 – 44,659, P= 0.535). Twenty-nine (82.9%), 27 (87.1) and 32 (82.1%) patients in the LAmB, VCZ, and CFG group survived hospitalisation.

The pharmacoeconomic evaluation showed comparable results in length of treatment, treatment costs, and outcome of AML and ALL patients with IA treated with LAmB, VCZ, and CFG. Heinmann concluded that the choice of antifungal did not appear to be a main cost driver of overall treatment costs.

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